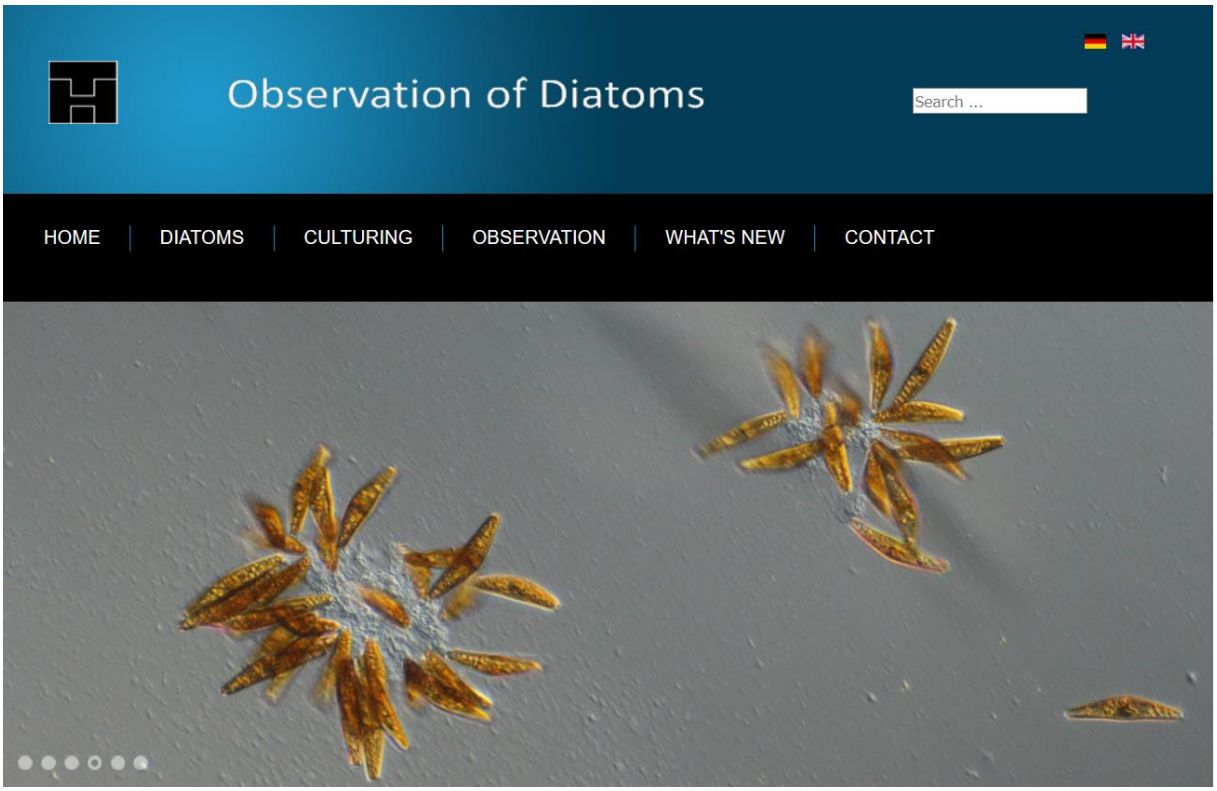


Dear visitor,

You may already have seen images of diatoms with their diverse forms and fine structures. Perhaps you also had the opportunity to observe live diatoms under the microscope and noticed that some are capable of moving smoothly or jerky. They often change the direction of movement. The mechanism of movement is not visible.

One of my favourite pastimes is observing diatoms with the focus on species that are motile. I am fascinated by the reaction of diatoms to environmental conditions and external stimuli, such as obstacles or light. In cultures of colonial diatoms, I try to understand the link between motility and the formation of colonies.

I would like to mention that I studied physics and spent my working life with typical activities of an engineer in the field of telecommunications and the automotive area. Since a few years I am retired.

The purpose of this homepage is to encourage you to make your own observations on diatoms. If you like to microscope and follow this suggestion, you will hopefully find useful information on these pages.

You will get information about the following aspects:

- Cultivation of diatoms
- Observation and creation of video recordings of the movement of diatoms in different environments
- Analysis of the resulting images and videos

- Hypotheses and conclusions from the observations

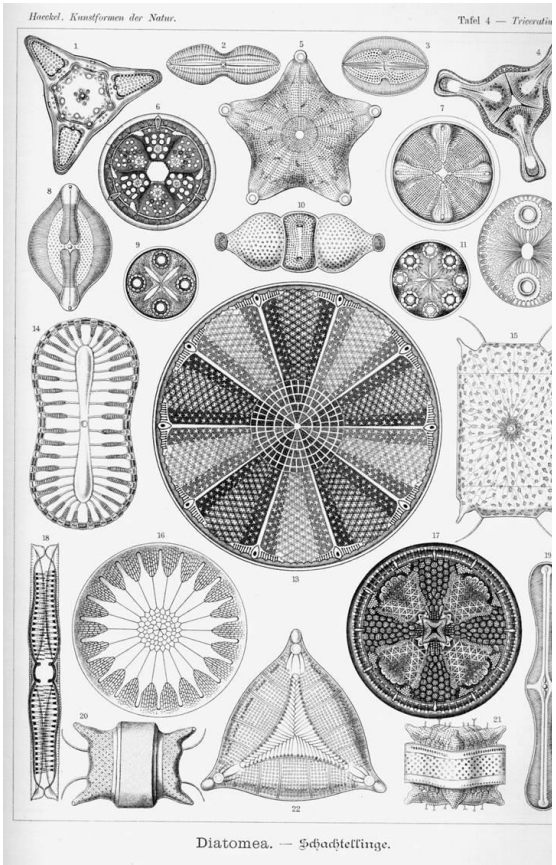
As there is a comprehensive literature and many web pages to the biology of the diatoms, I restrict myself to some remarks, links and literature references.

From time to time the site will be supplemented by observations and hypotheses. If you are interested in the subject, please feel invited to visit this website now and then. I am grateful for content discussion and suggestions for improvement.

Please note that the content is protected by copyright. If you quote something from this page, please refer to my name and add a link to this page. Please contact me if you want to make use of pictures or videos. I do not pursue any commercial interests.

The site contains many short videos. To view them, a browser is required, which masters HTML5. If a video on a browser is not running correctly, it is recommended to try another browser.

As an introduction to the topic, I show a picture from the book "Kunstformen der Natur" by Ernst Heinrich Philipp August Haeckel (16.02.1834 to 09.08.1919):

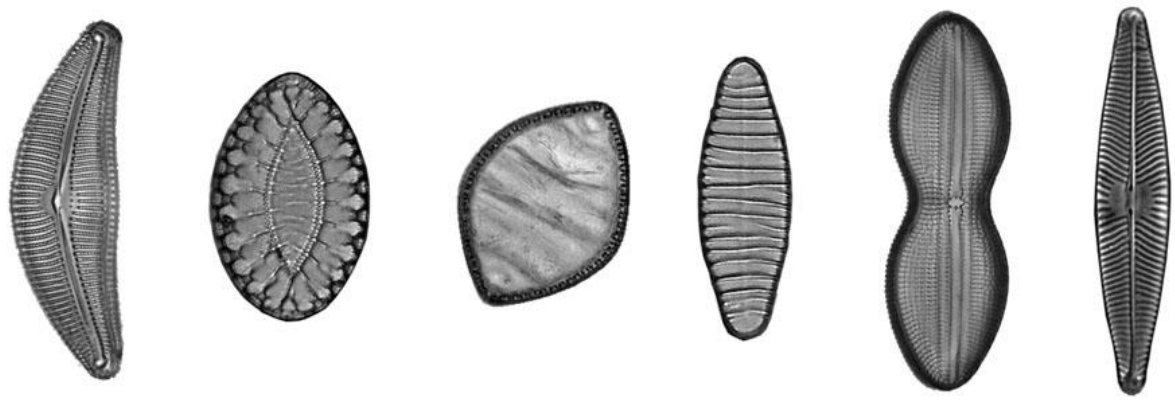


Diatomea. Schachtellinge.

Stamm der Urpflanzen (Protophyta); — Hauptklasse der Algarien; — Klasse der Diatomeen (Schachtel- oder Kiesel-Algarien).

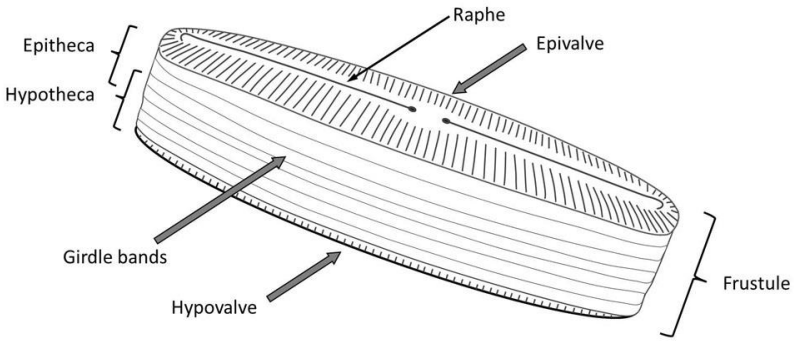
Die Diatomeen oder Schachtellinge bilden eine formenreiche Klasse von einzelligen Urpflanz
welche massenhaft sowohl im Süßwasser als im Meere leben; über 2000 Arten sind bekannt.
zeichnen sich vor anderen Protophyten durch die Bildung einer zierlichen, zweiklappigen Kieselschale au
die beiden Hälften oder Klappen derselben verhalten sich wie eine Schachtel und ihr Deckel. Die obe
etwas größere Hälfte, die Deckelklappe, greift mit einem breiten Rande, dem Gürtelbande, über den Ra
der unteren größeren Hälfte, der Schachtelklappe, hinüber. Daher hat jede Schale zwei sehr verschiede
Ansichten, die parallele (horizontale) Boden- oder Hauptseite (Fig. 1, 4 ꝛc.) und die ringförmige (ver
kale) Gürtel- oder Nebenseite (Fig. 20, 21 ꝛc.). Die erstere ist meistens durch sehr zierliche Skulpt
ausgezeichnet: Rippen, Leisten, Felder, Körner ꝛc. Sie ist von sehr feinen Poren durchbrochen.
meisten Diatomeen sind sehr klein, schweben frei im Wasser und bilden einen wichtigen Bestandteil d
Plankton; andere Arten sind durch Gallertstiele am Boden befestigt. Viele Arten bilden Cönobien od
Zellvereine, indem die durch Teilung entstehenden Tochterzellen in Zusammenhang bleiben. Alle auf die
Tafel abgebildeten Arten gehören zu den einsam lebenden (Monobien) und frei schwimmenden. Ihre Schal
sind meist durch eine sehr regelmäßige geometrische Grundform ausgezeichnet: zweiseitig (Fig. 2, 3, 1
dreistrahlig (Fig. 1, 4, 22), vierstrahlig (Fig. 7, 9, 11), fünfstrahlig (Fig. 5), vielstrahlig (Fig. 1
Der lebendige, weiche Zellenkörper, welcher in der Schale eingeschlossen ist (Fig. 15), enthält in der Mi
einen Zellenkern; von der feinen Plasmaschicht, die ihn umgibt, strahlen verzweigte Plasmafäden au
welche die strömende Bewegung der lebendigen Zellsubstanz zeigen. Im Plasmanetz zerstreut liegen vi
Chromatellen oder Farbkörner; ihre grüne Farbe (Chlorophyll) wird meistens durch einen gelben od
braunen Farbstoff verdeckt (Diatomin).

Fig. 1. Triceratium digitale *(Brun).*
" 2. Navicula lyra *(Ehrenberg).*
" 3. Navicula excavata *(Greville).*
" 4. Triceratium mirificum *(Brun).*
" 5. Triceratium pentacrinus *(Wallich).* Vgl. Fig. 21.
" 6. Actinoptychus constellatus *(Brun).*
" 7. Aulacodiscus mammosus *(Greville).*
" 8. Navicula Wrightii *(Meara).*
" 9. Auliscus crucifer *(Brun).*
" 10. Biddulphia pulchella *(Gray).*
" 11. Auliscus craterifer *(Brun).*
Fig. 12. Auliscus mirabilis *(Greville).*
" 13. Aulacodiscus Grevilleanus *(Norma*
" 14. Surirella Macraeana *(Greville).*
" 15. Denticella regia *(Max Schultze).*
" 16. Asterolampra eximia *(Greville).*
" 17. Actinoptychus heliopelta *(Brun).*
" 18. Plagiogramma barbadense *(Brun,*
" 19. Pinnularia Mülleri *(Haeckel).*
" 20. Biddulphia granulata *(Smith).*
" 21. Triceratium pentacrinus *(Wallich* Vgl. Fig. 5.
" 22. Triceratium moronense *(Greville).*



What are Diatoms?

Let me begin with a few statements about diatoms. In biological terms they form a class of single celled algae. It is characteristic of them that they live in a transparent house made of hydrated silica (SiO_2 + n H_2O), which is coated with an organic material. This exoskeleton (frustule) consists of two halves, the epitheca and the hypotheca. The structure is similar to a petri dish, with the epitheca overlapping the hypotheca. Each theca comprises of a more or less arched valve and the cingulum, a number of associated siliceous bands, the so-called girdle bands (see following picture).



According to their shape, diatoms are divided into centric diatoms that are radially symmetric and pennate diatoms that are bilaterally symmetric. Among the pennate diatoms there are many species which are able to glide over a substratum (see next page).

The size of diatoms ranges typically from a few microns up to about 2 millimetres. However in most cases diatoms are microscopic and require at least a light microscope to observe.

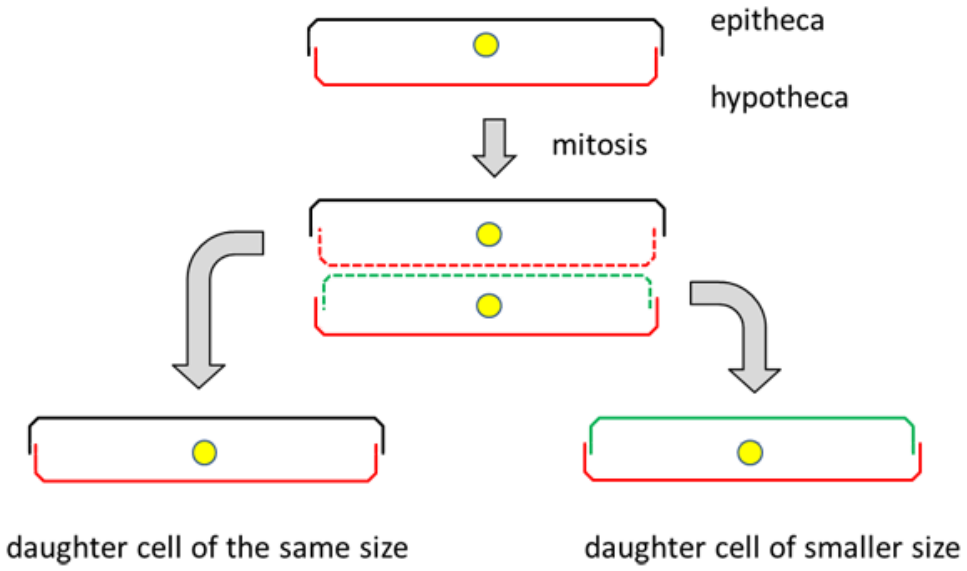
Diatoms are widespread and can be found in almost all fresh and saline waters like brooks, rivers, lakes and sea. Even moist soil serves as a possible habitat. Some diatoms are floating, others live at the bottom of a body of water.

Diatoms show an immense variety of shapes and structures. Their casing exhibit pores allowing them to exchange nutrient and waste. The valves of some diatoms have a slit, the so called raphe allowing them to move over surfaces of grains of sand, stones or the surface of aquatic plants.

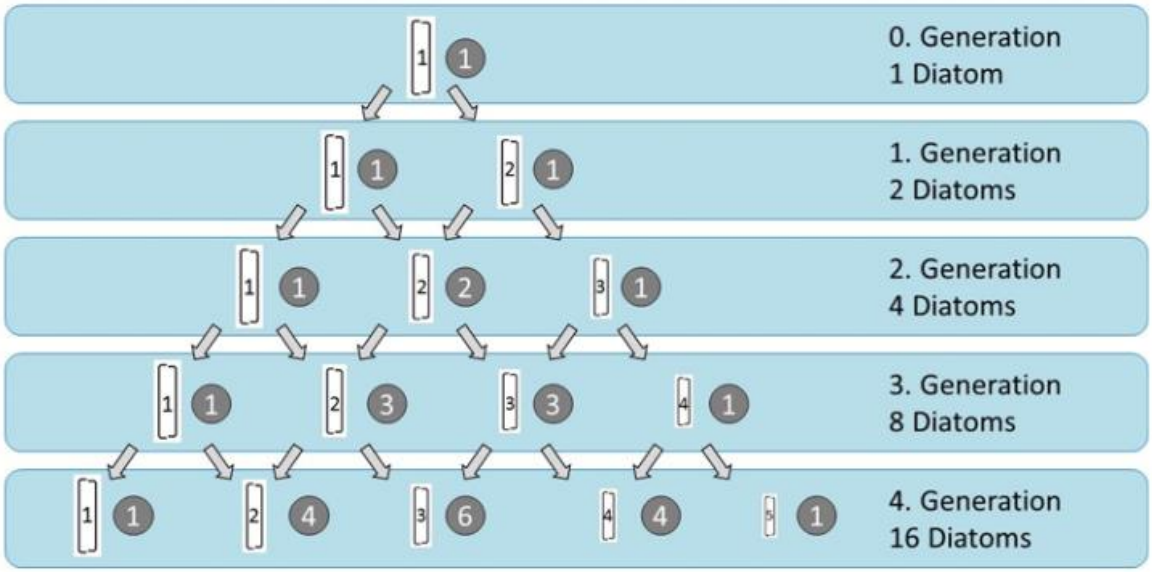
Like all plants diatoms use photosynthesis to gain solar energy. Apart from chlorophyll a and c, fucoxanthin serves as photosynthetic pigment which gives diatoms a golden-brown colour.

There is a huge taxonomic diversity with hundreds of diatom genera. One finds different estimates on the number of recent species. It may be 100,000 or even more.

Diatoms reproduce by asexual (vegetative) and sexual reproduction. When a cell divides (mitotic division), a smaller valve is re-formed, so that after the division one has one cell of the same size and a smaller one:



The following picture illustrates the vegetative reproduction over 5 generations:



Statistically, therefore, the size decreases (MacDonald-Pfitzer rule). When the smallest diatom reaches a minimal size, sexual reproduction is required to gain a cell of maximal size. In rare cases, there is also a vegetative cell enlargement.

The sexual reproduction of pennate diatoms has a great variety of variants. Diatoms are diploid, so they have a double chromosome set. By reduction division (meiosis) one or two haploid gametes are formed in each diatom, that is to say, germ cells with a simple chromosome set. In pennate diatoms isogamy prevails, in which the gametes are of the same size and are not flagellated. By fusion of the gametes a zygote or two zygotes with a double chromosome set are produced. Finally, each zygote grows to the auxospore and forms a new vegetative cell (initial cell) with two valves, which has the maximal size.

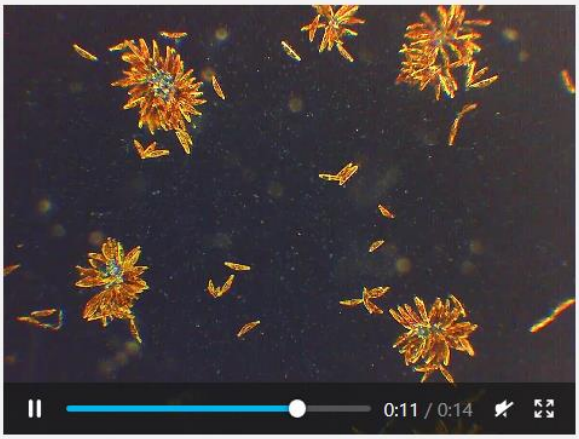
It is also possible that two gametes of the same gametangium will fuse into a zygote, from which the auxospore and the initial cell are formed. This self-fertilization is called automixis.

Furthermore, it was observed that an auxospore can mature without reduction division (asexual reproduction). In addition conjugation was discovered in pennate diatoms.

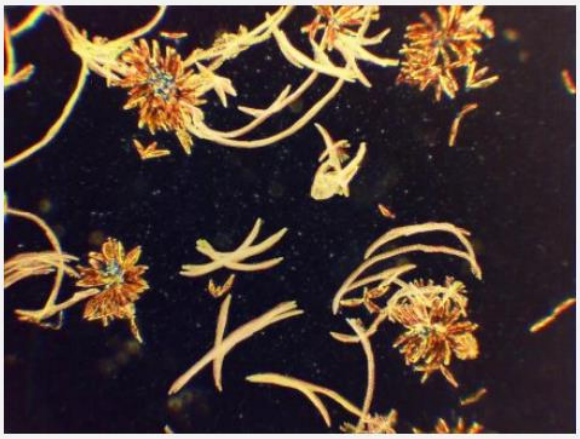
For details on the steps, see Round et. al. (2007). Terminology and pictures can be found in Irena Kaczmarska et. Al (2013).

F. E. Round; R. M. Crawford; D. G. Mann (2007), Diatoms: Biology and Morphology of the Genera, Cambridge University Press; 1 edition (2007)

Irena Kaczmarska , Aloisie Poulíčková , Shinya Sato , Mark B. Edlund , Masahiko Idei , Tsuyoshi Watanabe & David G. Mann (2013): Proposals for a terminology for diatom sexual reproduction, auxospores and resting stages, Diatom Research, DOI:10.1080/0269249X.2013.791344



Cymbella spec. in dark field (40x time lapse)



Visualization of the motion of the diatoms from the video by calculating the maximum over all frames (click to enlarge)

Movement

A number of benthic species have the ability to move. Nearly all of them have a raphe. On smooth ground they glide in straight or curved paths, the shape of the paths depends on the curvature of the raphe. They also show complex movements such as sudden reversing, turning around the apical axis, erecting, horizontal rotation about one point of the cell, pirouettes in the erected state, etc. Overall, the movements seem to be randomly and it is not necessarily clear what benefits are generated.

Possible benefits are in particular:

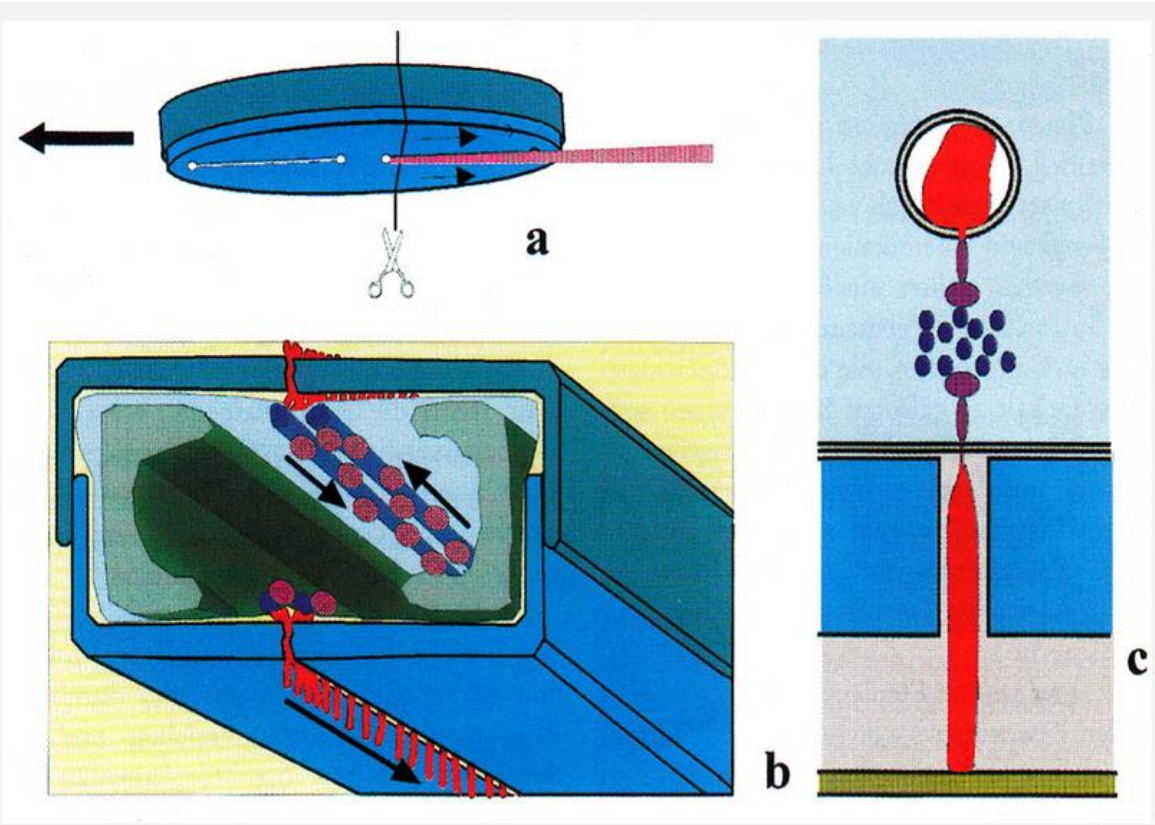
- Optimization of light conditions, because many motile species show positive or negative phototaxis. A photophobic response can also be observed in which diatoms react strongly on local changes in the light intensity with reversal of the direction of motion.
- Periodic vertical migration of diatoms inhabiting sand deposits in particular in intertidal zones. These sediments can be disturbed by tides and currents (see review article Harper (1977))
- Search for places with better nutrient concentration or other favorable chemical environmental conditions (chemotaxis). In the publication of Karen Grace V. Bondoc et al (2016) it is shown that *Seminavis robusta* moves towards a silicate source.
- Colonization of new habitats
- Search and approach to a partner for sexual reproduction. The structure and function of sexual pheromones has been elucidated for *Seminavis robusta* (Frenkel, Johannes. PhD Thesis (2014) and Bondoc et al. (2016))
- Leaving the copulation envelope in certain species (see post about sexual reproduction)

An approach to the target of the movement achieves a diatom by varying the movement activity, in particular by controlling the duration of the movement in one direction.

At this point I would like to make a comment from my point of view. It is common to all mentioned advantages of the movement that they are due to a change of location. When you look at the movement of some species, you can doubt whether this can always be the motivation. *Cymatopleura*

elliptica usually rotates slowly around a vertical axis, interacts uncontrolled with the environment and comes hardly from the spot (see video). In many cases, the benefit of mobility is only temporary, as in sexual reproduction. Nevertheless, the majority of the diatoms in a culture is independent of their size always in motion. In certain cases, the benefits could also have a physiological background such as the regulation of energy balance.

Since the discovery of the movement of diatoms, one struggles for an understanding of the mechanism of motion. As early as 1838, Ehrenberg described a snail-like foot for creeping (Die Infusionsthierchen als vollkommene 0rganismen. 1838 p. 175), which was not proved to be correct. Up to this day, there are numerous theories; none of them is definitively confirmed. They range from a movement due to capillary effect to a cytoplasm flow and to the recoil principle. Today a secretion of mucilage through the raphe and a movement of the mucilage along the raphe are usually used as an explanation, whereby muscle proteins serve as a drive. The prevailing hypothesis is given by Edgar, L.A. & Pickett-Heaps J.D. (1984). A compact representation was created by Menzel, D. and O. Vugrek (1997) and is reproduced below with the original illustration and translated text:



Schematic representation of the sliding motion in patented diatoms.
(a) Bottom of a migrating cell. A mucilage trail is formed from the posterior raphe.
(b) cross-section along the line in (a). The silicified cell wall consists of two parts (blue-gray), which, like the two halves of a petri dish, are on top of each other. The chloroplasts are shown in green; the nucleus in the center of the cell is omitted for the sake of clarity. Underneath the raphe is a pair of actin filaments (blue), which are used as pathways for the transport of mucilage vesicles (pink). The vesicles fuse at the ends of the raphes with the plasma membrane. Each vesicle unloads a mucilage trail (red) to the outer side of the membrane where it swells and is pressed through the gap of the raphe. One end of the thread adheres to the substrate, the other remains bound to the cell membrane, and it is assumed that this end is connected with a motor molecule (dark violet), which transports the thread along the actin bundles. Upper and lower raphe produce mucus filaments at the same time.
(c) detail of b.

Two known problems of this theory are mentioned here:

- The movement of myosin on actin filaments always takes place in one direction. The movement of the diatoms, however, changes the direction of motion, with no difference in speed. Diatoms have one or two raphe systems on one valve. Each raphe system allows movements in both directions. In some species one can even observe that particles are transported in the opposite direction along the same raphe and collide (Nultsch, W. (1962)).
- The raphe of many motile diatoms is not simply an open cleft. Some move in spite of an almost closed tongue in groove slit. Other mobile species do this by means of a canal raphe, which is located on the valves and is connected to the interior of the cell only by a series of pores.

At least amendments are necessary to explain the observations by the described actin-myosin transport.

J. Wang, S. Cao, C. Du and D. Chen have investigated the movement at *Navicula* sp. and propose an updated model. In this model the diatoms are moved by pseudopods protruding out of the valves. One can not necessarily assume that this model can be applied to other genera as well.

On this homepage, observations will be added to the already existing countless ones on the movement behaviour of diatoms. According to my means, these are always observations with the light microscope and one can ask what is still to be observed at all after 200 years of light microscopic examination. In fact, much is being reproduced. Other observations perhaps encourage the reader to his own observations. In several cases I could not check how far these are described in the literature. I would be grateful for hints. Such observations cannot yield a new explanation of the mechanism of motion but may allow a critical look at the described model conception.

Edgar, L.A. & Pickett-Heaps J.D. (1984), Diatom locomotion., Progress in Phycological Research Vol. 3: 47-88

Frenkel, Johannes. PhD Thesis (2014). Struktur und Funktion von Sexualpheromonen der Diatomee *Seminavis robusta*. Friedrich-Schiller-Universität Jena, Biologisch-Pharmazeutische Fakultät

Harper, M.A. (1977). Movements. In: The Biology of Diatoms, (D. Werner, ed). 224-249, Blackwell, Oxford

Karen Grace V. Bondoc, Jan Heuschele, Jeroen Gillard, Wim Vyverman & Georg Pohnert. Selective silicate-directed motility in diatoms. Nature Communications http://dx.doi.org/10.1038/ncomms10540

Bondoc, Karen Grace & Lembke, Christine & Vyverman, Wim & Pohnert, Georg. (2016). Searching for a Mate: Pheromone-Directed Movement of the Benthic Diatom *Seminavis robusta*. Microbial Ecology. 72. 10.1007/s00248-016-0796-7.

See also:

Jeroen Gillard, Johannes Frenkel, Valerie Devos, Koen Sabbe, Carsten Paul, Martin Rempt, Dirk Inzé, Georg Pohnert, Marnik Vuylsteke, Wim Vyverman: Metabolomik unterstützt die Strukturaufklärung eines Sexualpheromons von Kieselalgen., Angewandte Chemie, DOI: 10.1002/ange.201208175

Nultsch, W. (1962) Über das Bewegungsverhalten der Diatomeen., Planta 58: 22.

Wang, J., Cao, S., Du, C. & Chen, D. Underwater locomotion strategy by a benthic penate diatom *Navicula* sp. Protoplasm 250, 1203–1212 (2013).

Links

Useful links to English sites:

Wikipedia, Diatom https://en.wikipedia.org/wiki/Diatom
International Society for Diatom Research: http://www.isdr.org/
University College London, Micropalaeontology Unit, Diatoms: http://www.ucl.ac.uk/GeolSci/micropal/diatom.html
The Phycology Section: Ecology and Taxonomy of Freshwater Algae, particularly Diatoms: http://diatom.ansp.org/

Databases, Diatom Identification:

Diatoms of the United States: https://westerndiatoms.colorado.edu/
Algae World: diatoms: http://rbg-web2.rbge.org.uk/algae/diatoms.htm
Introduction to Diatom Identification: http://rbg-web2.rbge.org.uk/ADIAC/intro/intro.htm

Useful links to German sites:

Wikipedia, Kieselalgen: https://de.wikipedia.org/wiki/Kieselalgen
Diatomeen – Kurzeinführung: http://www.diatomeen.de/
Diatomeen-Homepage von Dr. phil. nat. E. Alles: http://www.kieselalgen.com/JSIndex.html

Books

The following compilation is a tip to the very extensive literature.
Further references to publications are given in the appropriate place.

Books in English:

Diatoms: Biology and Morphology of the Genera
F. E. Round; R. M. Crawford; D. G. Mann
Cambridge University Press; 1 edition (2007)

The Biology of Diatoms (Botanical Monographs)
Dietrich Werner (Editor)
University of California Press, Berkeley, California (1977)

Identification of Freshwater Diatoms from Live Material
E.J. Cox
Springer (1996)

Algal Culturing Techniques
Robert A. Andersen (Editor)
Academic Press (2005)

Books in German:

Diatomeen im Süßwasser-Benthos von Mitteleuropa : Bestimmungsflora Kieselalgen für die ökologische Praxis;
über 700 der häufigsten Arten und ihre Ökologie
Horst Lange-Bertalot (Editor)
Koeltz Scientific Books

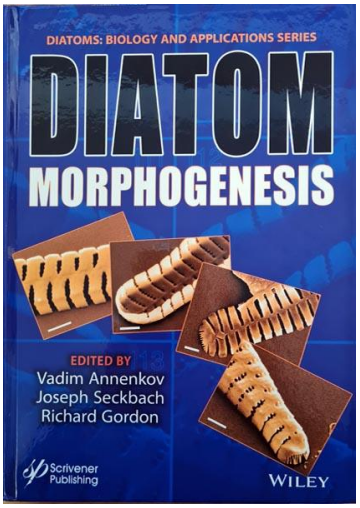
Kieselalgen in Binnengewässern
Lothar Kalbe
VerlagsKG Wolf (2005)

Kieselalgen : Biologie, Baupläne d. Zellwand, Untersuchungsmethoden
Kurt Krammer
Kosmos Verlags-GmbH (1990)

Algenreinkulturen, ihre Herstellung und Erhaltung
E. G. Pringsheim
Jena Fischer (1954)

Articles relating to the homepage:

Harbich, T. (2021), On the Size Sequence of Diatoms in Clonal Chains. In *Diatom Morphogenesis* (Diatoms: Biology and Applications) Vadim V. Annenkov (Editor), Richard Gordon (Series Editor), Joseph Seckbach (Series Editor), Wiley-Scrivener; First published: 29 October 2021, https://doi.org/10.1002/9781119488170.ch3

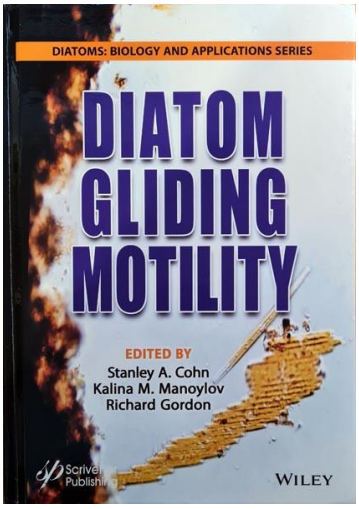


Harbich, T. (2021) Some Observations of Movements of Pennate Diatoms in Cultures and Their Possible Interpretation. In *Diatom Gliding Motility* (Diatoms: Biology and Applications) S.A. Cohn (Editor), K.M. Manoylov (Editor) and R. Gordon (Series Editor), Wiley-Scrivener, Beverly, MA, USA; First published: 20 July 2021, https://doi.org/10.1002/9781119526483.ch1

The sections are:

- Kinematics and Analysis of Trajectories in Pennate Diatoms with Almost Straight Raphe along the Apical Axis
- Curvature of the Trajectory at the Reversal Points
- Movement of Diatoms in and on Biofilms
- Movement on the Water Surface
- Formation of flat Colonies in Cymbella lanceolata

Harbich, T. (2023) Pattern Formation in *Diatoma vulgaris* Colonies - Observations and Description by a Lindenmayer-System. In *The Mathematical Biology of Diatoms* (Diatoms: Biology and Applications) Janice L. Pappas (Editor), Richard Gordon (Series Editor), Joseph Seckbach (Series Editor), Wiley-Scrivener; Wiley-Scrivener; First published: 21 April 2023, https://doi.org/10.1002/9781119751939.ch10

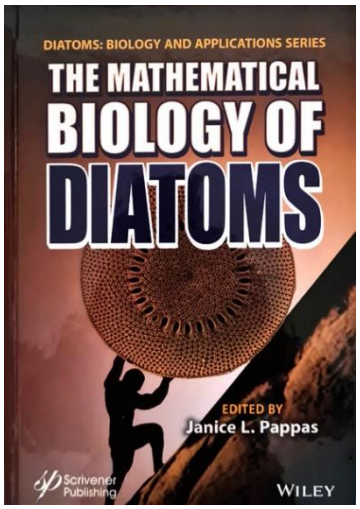


Please also have a look at these publications:

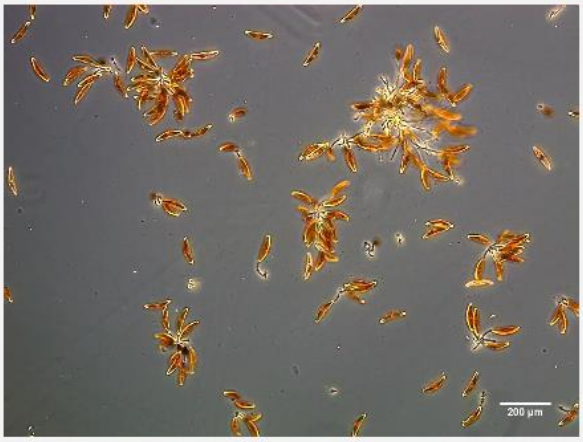
Alicea, B., Gordon, R., Harbich, T., Singh, U., Singh, A., & Varma, V. (2021) Towards a Digital Diatom: image processing and deep learning analysis of Bacillaria paradoxa dynamic morphology. In *Diatom Gliding Motility* (Diatoms: Biology and Applications) S.A. Cohn (Editor), K.M. Manoylov (Editor) and R. Gordon (Series Editor), Wiley-Scrivener, Beverly, MA, USA; First published: 20 July 2021, https://doi.org/10.1002/9781119526483.ch10

Preprint: https://www.biorxiv.org/content/10.1101/2019.12.21.885897v2

Gebeshuber, I. C., Zischka, F., Kratochvil, H., Noll, A., Gordon, R., & Harbich, T. (2021), (2021) Diatom Triboacoustics. In *Diatom Gliding Motility* (Diatoms: Biology and Applications) S.A. Cohn (Editor), K.M. Manoylov (Editor) and R. Gordon (Series Editor), Wiley-Scrivener, Beverly, MA, USA; First published: 20 July 2021, https://doi.org/10.1002/9781119526483.ch11



Harbich, T. (2023) Modeling the Synchronization of the Movement of *Bacillaria paxillifer* by a Kuramoto Model with Time Delay. In *The Mathematical Biology of Diatoms* (Diatoms: Biology and Applications) Janice L. Pappas (Editor), Richard Gordon (Series Editor), Joseph Seckbach (Series Editor), Wiley-Scrivener; Wiley-Scrivener; First published: 21 April 2023, https://doi.org/10.1002/9781119751939.ch8



Colonial *Cymbella cistula* attaced to the substrate by mucilage stalks (30x time lapse)

Craticula cuspidata in darkfield and brightfield (20x time-lapse)

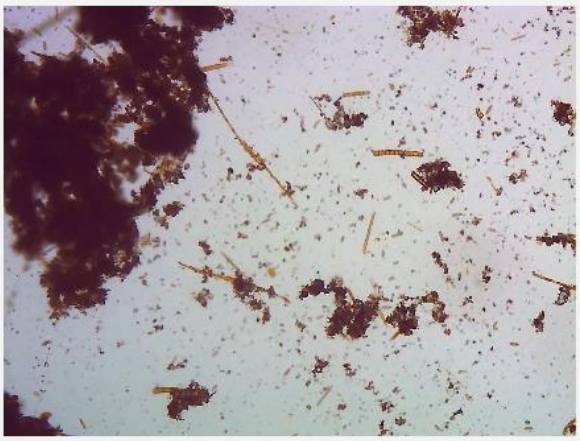
Purpose of cultivation

Why is it useful to cultivate diatoms that you want to observe? From our point of view, the advantages are as follows:

- Compared to an observation on a fresh sample, the genus and possibly the species can be identified with greater certainty and assigned to the observation.
- The identification can take place independent of the observation.
- The use of just one species is prerequisite for the reproducibility of experiments.
- A large number of diatoms of a certain species is available. This allows investigations in which a statistical statement is the goal, such as in the case of population dynamics.
- Other organisms and impurities do not interfere with the observations (see videos below).

Thus, the conditions under which one observes become well controllable. The development of a population can be investigated as a function of external parameters such as temperature, light conditions or composition of the culture medium.

Moreover, the culture vessel (e.g. petri dish) itself can be used for observation.

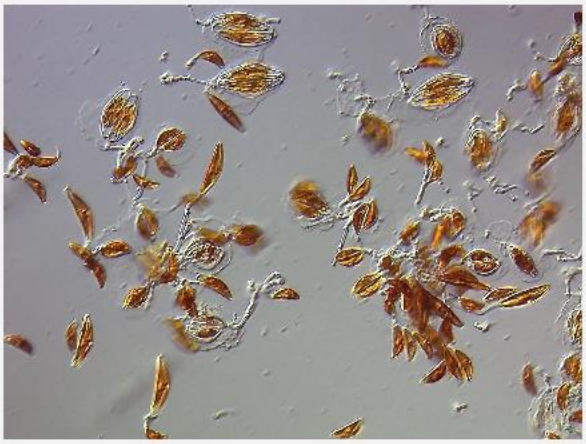


A sample from the river Neckar. Most striking are colonies of the diatom *Bacillaria paxillifera* (*Bacillaria paradoxa*). These colonies exhibit a unique movement based on the motility of the diatoms relative to the neighbouring diatoms. The observation is particularly affected by detritus. (4x time lapse).

View into a culture that was created with *Bacillaria paxillifera* from the Neckar river. The quality and the possibilities for observation are significantly improved compared to the observation in the sample. (4x time lapse).

On the pages on the subject of cultures we briefly describe what Mr. Kurt Schneider and I can convey from our experience. On this occasion, I would like to thank Mr. Oliver Skibbe (http://www.larger-than-life.de/micropage/start.html) for his valuable advice.

Cymbella aspera (Objectives: 5x, 10x and 20x; 150x time lapse)

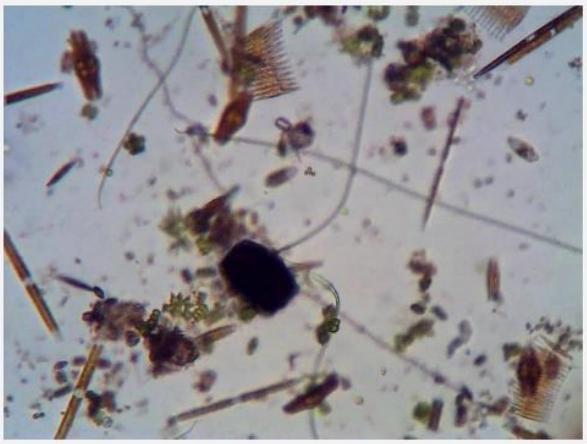


Cymbella cistula: cells of minimal size, initial cells and auxospores (60x time lapse; PlasDIC)

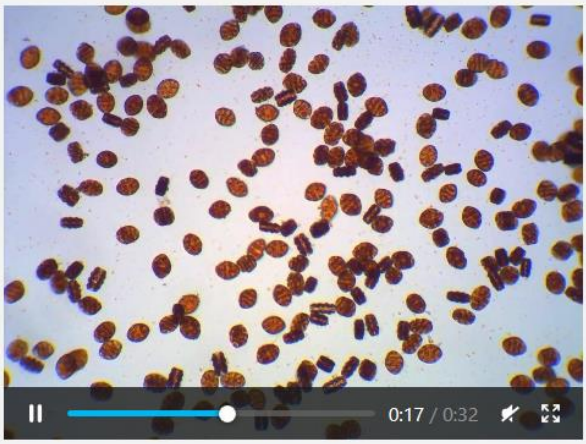
Forms of cultures

For our purposes, it is useful to distinguish between the following forms of cultures:

- A culture should be referred to as a pure culture if it contains only one species of diatoms.
- An axenic culture, on the other hand, is present when there are no other organisms besides the desired ones. In particular, it must be free from bacteria. This is only possible in a professionally equipped laboratory and has never been our personal goal.
- Clonal is a culture when it has emerged from a single cell, so that in the case of exclusively asexual reproduction all the diatoms are genetically identical.
- In a batch culture the nutrient medium is added once. The growth of culture comes to a standstill if one of its components has a limiting effect.
- Often one understands by a permanent culture a culture with a continuous supply of nutrient medium. A permanent culture is in this context to be understood as a culture which exists over an extended period of time, this means, over many generations. A series of batch cultures (repeated culturing of new cultures from parent cultures) can make this possible.



Sample from a lake (4x time lapse)



Successfully cultivated *Cymatopleura elliptica* (5x time lapse)

Creating cultures and care

Culturing diatoms

As my interest is above all the locomotion of the diatoms, I limit myself to cultivating benthic pennate (bilaterally symmetric) diatoms with few exceptions.

At the beginning of a culturing process is the collection of samples. Fortunately you can find diatoms in practically all waters. It is sufficient to place a small stone or parts of a plant in a sample container with water and to examine them later at home. For this purpose, the sample can be kept in a petri dish filled with water from the place where the sample was taken. The diatoms are carefully brushed from the sample with a soft brush.

Not only diatoms will be rinsed into the petri dish, but in addition flagellates, ciliates, rotifers, amoebae or species of single-celled algae. Sand grains, particles of the sample and organic detritus are also found in the petri dish. After a short time, the diatoms are sedimented and easily recognizable. An impression of a rich sample from a nearby small lake is given in the top left video.

It is advisable to first examine the sample at low magnification. For this purpose an inverted microscope or stereo microscope can be used. Diatoms, which one would like to cultivate, are removed with a pipette from the bottom of the petri dish and transferred into a prepared culture vessel with a nutrient solution, such as a petri dish or culture tube. If the sample contains other organisms in high concentrations, it is convenient to wash the diatoms by transferring them into an intermediate bath (drop of nutrient solution on a microscope slide) and to bring them into the culture vessel in the next step.

As only a part of the isolated species is expected to reproduce, it is advantageous to put different species together in a culture vessel.

This first rough culture should be checked after a few days with regard to increase of contained organisms. It is not unlikely to find unwanted organisms that multiply faster than the ones you have been looking for. Single-celled green algae or small species of diatoms (eg *Nitzschia*) can reproduce in a few days to such an extent that isolation of the desired diatoms becomes difficult. In this situation one has to act quickly and transfer the desired diatoms into a new culture vessel. In the case of heavy contamination, it is necessary to wash the diatoms in intermediate baths. However this is not always successful, because on the often sticky surfaces of diatoms, for example, single-cell green algae adhere very well. It is recommended to set up new cultures in parallel each inoculated with one or a few diatoms.

The video on the left (180x time lapse) shows an example of contamination. It is the first culture from a sample with several *Pinnularia* contaminated unintentionally with diatoms of the genus *Fragilaria* and flagellates (time lapse). Examples of contamination with epiphytic diatoms and amoebae can be seen by clicking on the links in this sentence.

If a reproduction occurs of one or more species, the desired candidates are transferred into the next culture dish. Starting a new culture with a single diatom creates a clonal culture. The risk that this culture is not developing well is significantly higher than with cultures that are launched with several diatoms.

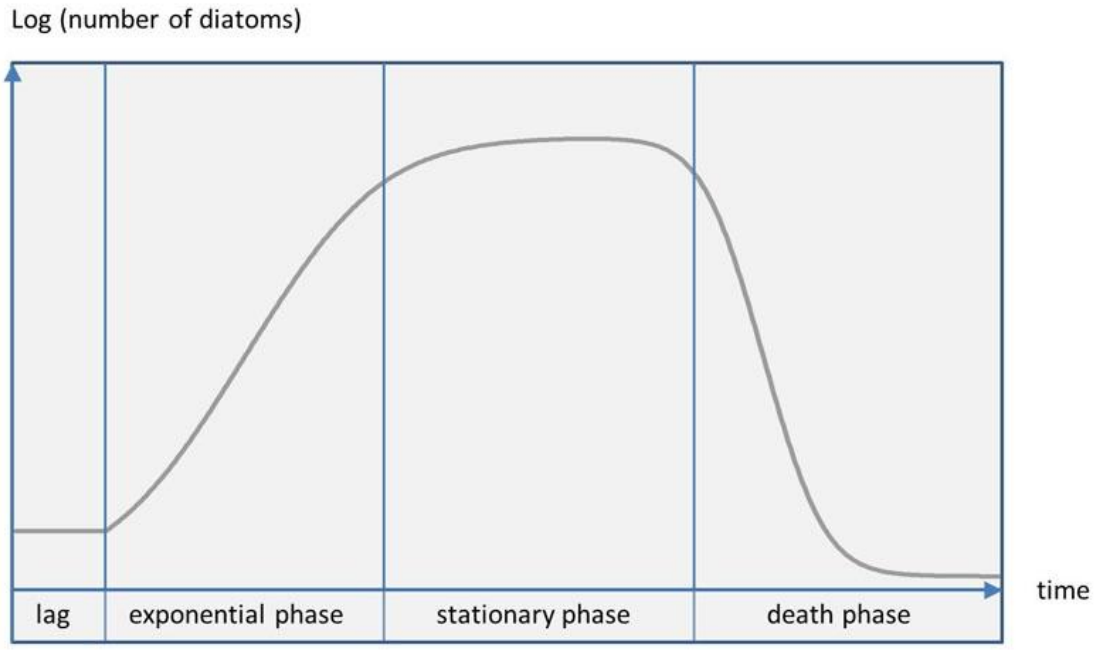
Temperature

Our cultivated species are from local Central European waters. They thrive well at room temperature. If one intends to cultivate diatoms from arctic regions, one must keep them at lower temperatures.

Dynamics of growth

Batch cultures of diatoms undergo the typical phases, which are also known from other microorganisms.

After a lag phase without multiplication of the cells, exponential growth follows. When resources (in particular nutrients and carbon dioxide) get limited, cell division slows down and a stationary phase is reached. Here no increase takes place. Finally, death phase (decline phase) starts.



The temporal duration of the phases is strongly dependent on the species and the environmental conditions. While some small *Nitzschia* species have already undergone the cycle after two weeks, some *Rhopalodia* or *Pinnularia* species can be kept successfully in a small petri dish with moderate light for several months. Some diatom species exhibit a stable phase over weeks without striking cell divisions or dying out.

Observation of the cultures

The cultures should be checked regularly. A weekly inspection has proved to be a good thing for me. It is possible to determine whether they are suitable for removing diatoms for observation or "repotting". Negative developments such as sudden death of Diatoms or the prevalence of bacteria are not to be missed.

As soon as one cultivates more than a few cultures, it is important to keep the overview. Culture vessels must be labeled for identification, for example with a sequence number. As a contemporary lab book, one can use a spreadsheet (e.g., MS-Excel) or a database. With each new culture an entry is created, which is maintained and supplemented with every check-up. At the beginning, the following data should be included at least:

- Date on which the culture was prepared
- The genus or species, as far as it is known
- The culture from which it was inoculated, unless it was freshly prepared from a sample
- Information on the nutrient medium used (type, concentration, pH)
- Environmental conditions such as light source and light intensity

For the reviews, the information may be supplemented by:

- Date of the last inspection
- Growth phase (for instance “exponential growth”)
- Rough estimation of the density of diatoms (for instance "well developed")
- Identifiable contaminations with other organisms
- Removal of diatoms for observation, if this is relevant
- Observations, such as the detachment of a biofilm
- When a culture is disposed, it is recommended to enter the corresponding date.

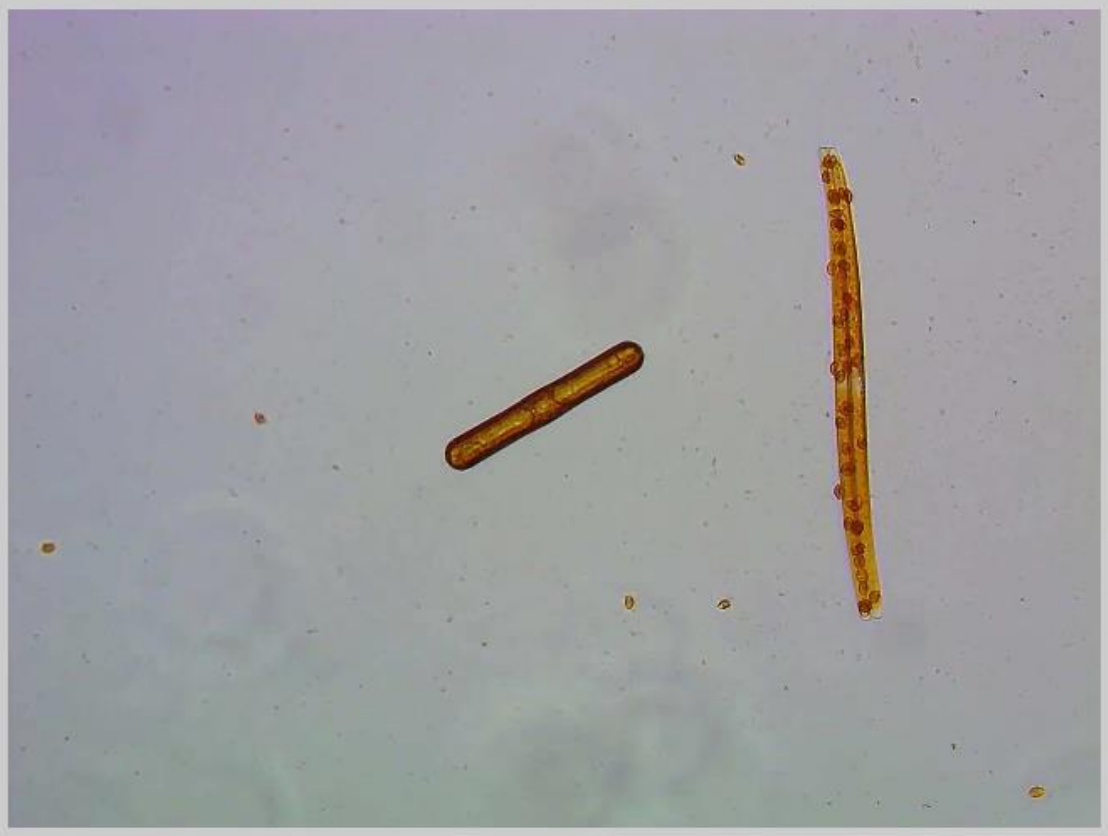
Finally, a few hints are given:

- As not every culture develops successfully, it is recommended to set up cultures in parallel. The medium and environmental conditions may be varied.
- If a species is to be maintained for a long time, it is necessary to transfer live diatoms into a new culture vessel before the death phase. It is advisable not to wait too long, but to remove diatoms when the culture shows a good appearance.
- Culture vessels should be kept closed except for withdrawals. This protects them against contamination.
- Pipettes can be reused if they have been cleaned. It is usually sufficient to rinse them with hot distilled water.

Observation of Diatoms

Epiphytic diatoms

A raw culture was prepared with diatoms of the genus *Nitzschia* and *Pinnularia*. Small epiphytic diatoms (possibly *Amphora pediculus*) came into the culture together with *Nitzschia* and proliferated strongly. They grew attached to the substrate and the diatoms of the genus *Nitzschia* but not on *Pinnularia*. The latter can be easily transferred from the raw culture to a new culture. The following video (120x time lapse) shows the culture at different magnification levels.



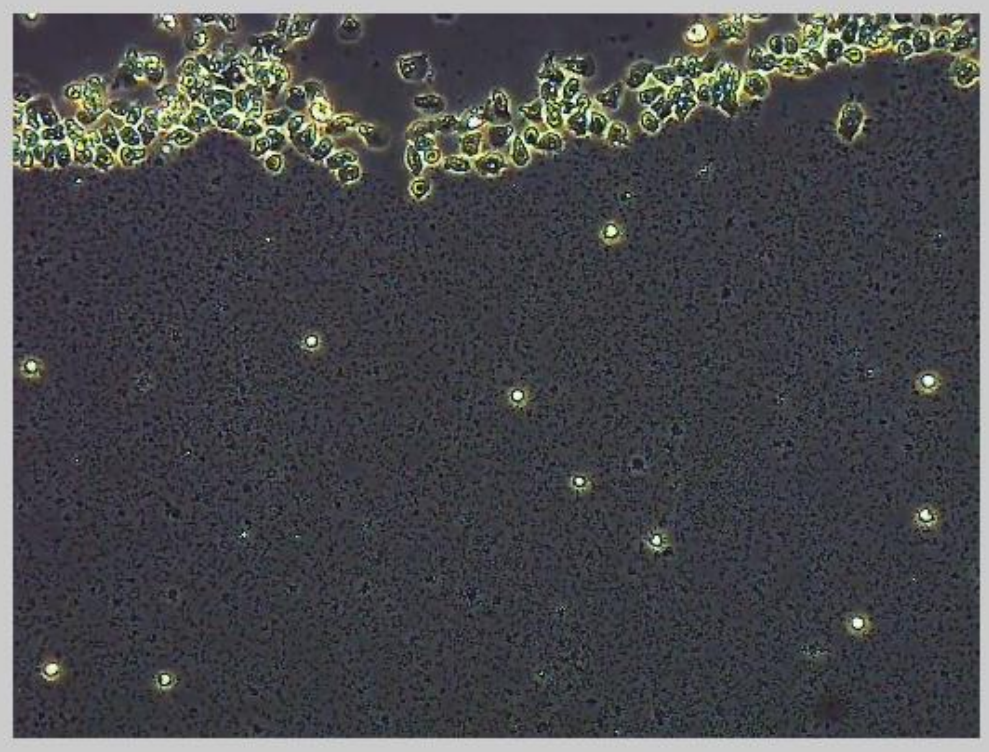
Thomas Harbich http://diatoms.de/en/culturing/creating-cultures-and-care

Amoebae

In a raw culture with diatoms of the genus *Cymbella*, bacteria quickly spread over the substrate. A short time later, amoebas with a typical size of 17 µm (diameter including ectoplasm) could be observed, which quickly proliferated. The culture can be seen in the following video (150x time lapse) in phase contrast.

Below you can see the appearance of amoebae in a raw culture of *Meridion circulare* (60x time lapse).

Although it does not contribute anything to the topic of diatoms, a video (150x time lapse) is shown below in which numerous amoebas move over the bottom of the petri dish and eat bacteria.



They form a dense chain. This appears to be caused by the fact that the amoebas move more slowly in a region of high bacterial density and systematically move further in the direction in which they were successful. Chemotaxis could play a role. In the area behind the chain, which is almost free of bacteria, the amoebae move in a kind of random walk.

Biofilm with gas bubbles (oxygen), which becomes detached from the bottom of the petri dish. This biofilm consists of diatoms and mucilage extruded by the diatoms.

Nutrient solution

Diatoms can be cultivated in water or on agar. Agar is often used for the raw culture, because the diatoms which have reproduced on it can easily be isolated. For unknown reasons, we were not successful in cultivating on agar. For the observation of diatoms in the culture vessel, water is preferable, because mechanical devices can be used for observation in the culture vessel and the environment is more natural. In particular, there is no additional reduction in the mobility of the diatoms in water.

In cultivation, diatoms are transferred into a suitable nutrient solution. Apart from water, this medium typically consists of various salts, vitamins, trace elements, phosphate, nitrate and silicate. Marine salt is to be added in case of marine diatoms. Often in recipes soil extract is included, which in my opinion is also a concession that the ideal ingredients are not exactly known.

Procurement

One can produce nutrient solutions according to classical or modern recipes by themselves. A variety of recipes for algae in general and diatoms in particular for both freshwater and seawater can be found in "Algal Culturing Techniques" (Robert A. Andersen [Editor], Academic Press). It is more convenient to purchase ready nutrient solutions on the market. They are obtained as a medium ready to be used or in the form of a stock solution or a powder.

As we were mainly concerned with cultivating some larger species from local waters, we did not bother to search for media suitable for diatoms that are difficult to cultivate. For enthusiasts of such species there are recipes in literature.

We usually use two commercially available media, which both have proven themselves:

Alga-Gro® from Carolina Biological Supply Company (www.carolina.com). In Germany it is marketed by the subsidiary Carolina Science GmbH (www.carolina-science.com). The composition of Alga-Gro has not been released. Silicate for diatoms is included. Alga-Gro is available as a sterile medium ready for use or as a sterile concentrate (stock solution). If one wishes to limit the growth of the cultivated diatoms, one can, according to our experience, reduce the concentration by half as compared with the manufacturer's specification.

Cell-hi WP from Varicon Aqua Solutions Ltd (www.variconaqua.com). It is the well-known Walnes medium. Cell-hi WP is delivered as a powder ("all-in-one powder"). From this, either a stock solution (concentrate) or the finished medium is prepared by addition of water. Sodium metasilicate is added for diatoms in accordance with the manufacturer's instructions.

Varicon Aqua Solutions also provides Cell-hi F2P, which is based on the Guillard F/2 medium and differs from Walnes medium by other amounts of ingredients. Our first experiences show that for our cultivated species the media according to Walnes and Guillard are comparable well suited. However it is to be expected that the media differ in particular in their suitability for certain species, the duration of the lag phase, the achieved cell density and the growth rate. A comparison of the two media with respect to the duration of the Lag phase, the achieved cell density and the growth rate in *Skeletonema* sp. is given in Vivi Endar et. al (2012). However, the investigated alga is not a diatom.

Nutrient solution for marine diatoms

Enriched seawater is used for the cultivation of marine species.To reproduce the natural environment well, filter sterilized seawater is desirable. If this is not available, sea salt can be added, which is offered for aquariums.

Salinity

For successful cultivation, the salinity, i.e. the amount of salt dissolved in a body of water, must be adapted to the conditions at the location where the diatoms are found. For this purpose, it is necessary to measure the salinity of the nutrient solution, which can be done using different measuring methods:

- Measurement of the density with a hydrometer, also called an areometer (right picture). Here the immersion depth of a floating body is measured.
- Determination of the refractive index with the refractometer (see below picture of the instrument and view through the eyepiece of the refractometer).
- Measurement of the conductivity, which is proportional to the salinity of the water.

If only small volumes of nutrient solution are needed, a large (precise) hydrometer is less recommendable, because it requires a measuring cylinder with a volume of approx. 250 ml. A refractometer works with only a few drops of nutrient solution, which also allows monitoring the salinity of long-term cultures in small Petri dishes (increase of salinity by evaporation).

When measuring salinity, attention must be paid to temperature dependency. The few drops that are placed on a refractometer quickly take on the temperature of the refractometer. Since it is not the salinity but the refractive index that is measured, many refractometers have temperature compensation, so that the salinity can be displayed on the measuring scale.

Common hydrometers have a built-in thermometer. Tables are used for conversion to a reference temperature.

Conductance meters can also have automatic temperature compensation. In this case, conversion is made to a reference value of 25° C.

Note on the preparation of the medium

- Carolina recommends to combine the concentrated medium with Carolina spring water. Distilled or demineralized water is not recommended. We use the (relatively mineral-poor) mineral water Volvic®. Correspondingly, we use Cell-hi WP with Volvic®, even if this has an influence on the composition of the nutrient medium.
- After the nutrient solution has been prepared, its pH value (increase with KOH, decrease with HCl for example) and its salinity can be adjusted for marine species.
- It is recommended to filter the finished nutrient solution.
- To avoid contamination, the finished nutrient solution is autoclaved or at least pasteurized. The manufacturers provide recommendations for this.
- It is recommended to store opened containers of nutrients cool, dry and in the dark.

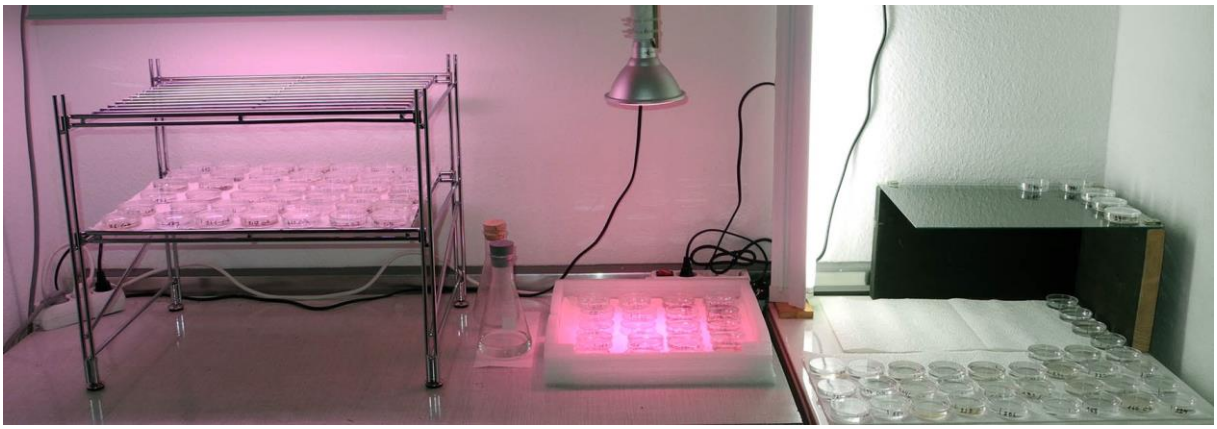
Marine diatoms of the species *Bacillaria paxillifera*, which were kindly provided to me by Matt P Ashworth (University of TX Austin) and originate from Florida.

The nutrient solution was f/2 according to Walnes with a salinity of 32 ppt.

At the time of recording, sampling was already 19 months ago, so that a strong shortening of the apical length occurred.

(4x time lapse)

Vivi Endar , Sarjito, Johannes Hutabarat and Budi Prayitno, 2012. EFFECT OF USING GUILLARD AND WALNE TECHNICAL CULTURE MEDIA ON GROWTH AND FATTY ACID PROFILES OF MICROALGAE *Skeletonema* sp. IN MASS CULTURE. Journal of Coastal Development Volume 16, Number 1.



Light and Lighting

Like all algae, diatoms photosynthesize. They assimilate inorganic carbon dioxide for conversion into organic substances. For this reason, it is necessary to cultivate diatoms in the light.

Collected samples can be kept for a few days at a shady window. Light stress due to direct sunlight should be avoided. Diatom cultures however are better cultivated with an artificial light source. You are able to control the conditions, brightness and lighting can be varied and one is not dependent on the weather or the season. This is advantageous, even if no larger biomass is to be obtained. For the selection of the illuminants, a few remarks are made here.

Spectrum

The following illustration shows the absorption spectra of the pigments (1 mM in acetone, a) and a light-harvesting complex (FCPa from *Cyclotella meneghiniana*, b):

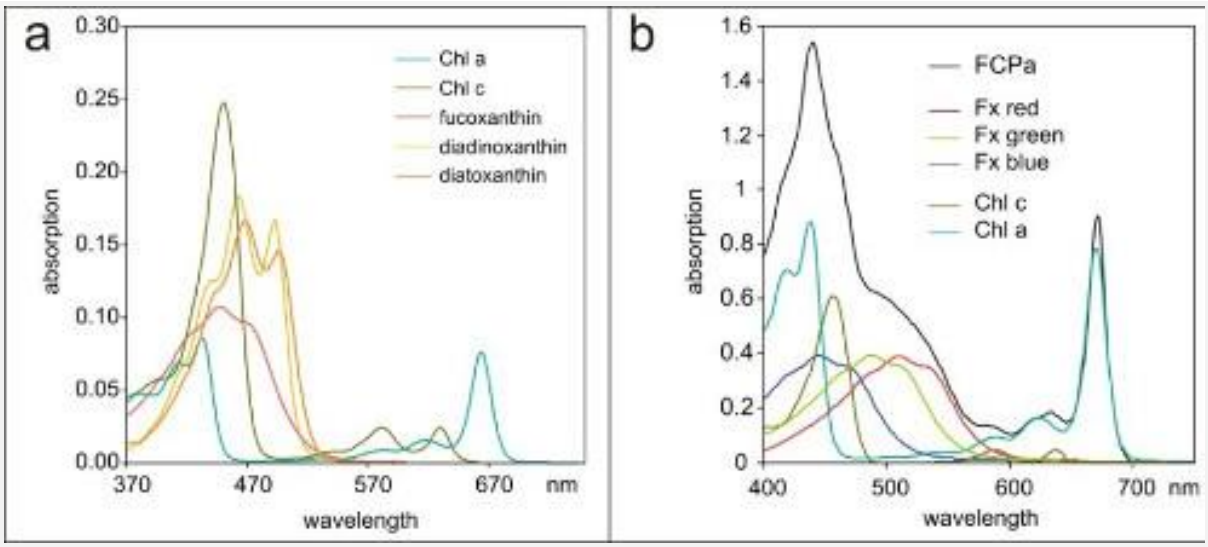
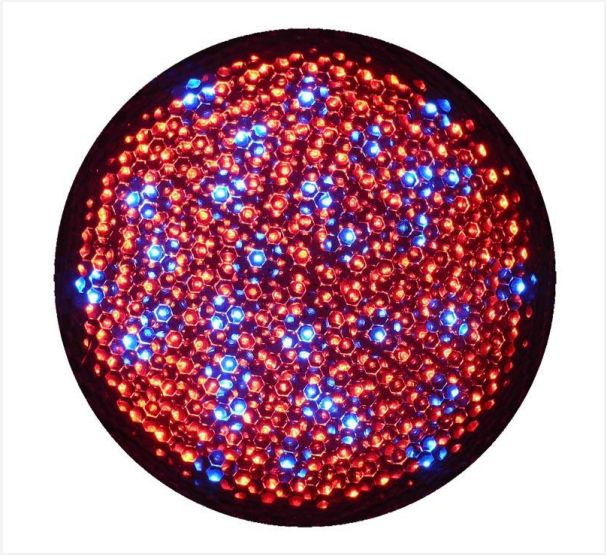
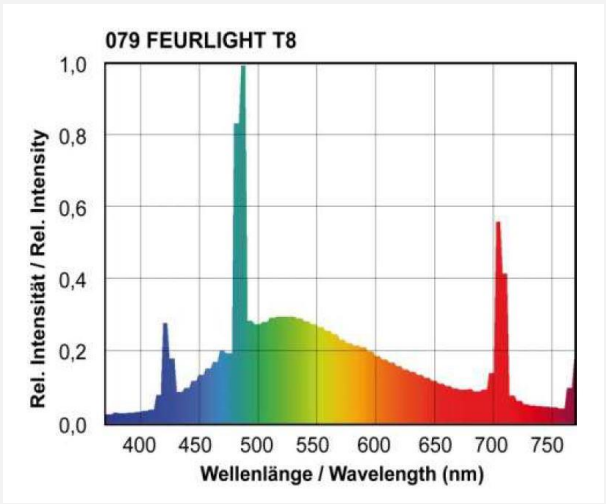


Figure from “Untersuchungen zur Struktur und Funktion von photosynthetischen Proteinen in Eukaryoten" (www.bio.uni-frankfurt.de/43967656/forschung) by courtesy of Prof. Dr. Claudia Büchel, Institute for Molecular Bioscience, Goethe University, Frankfurt am Main, Germany

As with green algae and land plants, the absorption spectrum shows maxima in the blue and red portions of the spectrum. Carotenoids such as the mentioned fucoxanthin also make it possible to use the light even in the green. This ability gives them an advantage over green algae. It is therefore quite useful to offer diatoms green light.





In nature, diatoms receive the sunlight filtered through the water. Several meters below the water surface, the red portion is already quite small. Planktic living species do not possess motility and have to cope with unfiltered sunlight as well as blue light in different brightness. You can conclude from this that the requirements for lighting the cultures cannot be very critical. We also do not focus on optimizing the yield.

It is very unfavorable to keep diatoms in exclusively or predominantly red light. In red light they are not able to measure the light intensity. Their reproduction is severely limited (Schellenberger, Costa et al. 2013). Apparently the blue sensitive aureochrome has to be stimulated, which interacts with the red sensitive phytochrome, so an irradiance measurement and adjustment of the metabolism can take place.

Our attempts of cultivation in the light of white LED lamps with low color temperature were not successful, which should be due to the barely usable blue spectral component. There are, however, LED lightings for aquariums that should be well suited.

It would be possible to use a conventional bulb with a blue filter for cultivation. If one uses a filter of in water dissolved copper sulfate, the spectrum of sunlight in a few meters of water depth can be reproduced well (Davis, Harrison, Dugdale (1973)). In view of the immense waste of primary energy, this does not seem to be a practicable and up-to-date method for cultivation.

The picture at the top shows in the middle a LED grow lamp (Lunartec FAST GROW PRO Ø 125 mm - blue: 460 - 465 nm, red: 625 - 630 nm), which has blue and red LEDs (See picture on the left) and is mainly offered for land plants, but has proven itself well for diatoms. Apparently the manufacturer specifies a range around the maxima of the red and blue LEDs. Below the lamp the spectrum is shown, which was recorded by us with a spectroscope. The width of the red and blue bands is so large that the red LED can also contribute to photosynthesis of the diatoms.

Well suited are conventional fluorescent lamps with a high color temperature of approximately 6000 Kelvin as offered for use in an office. This is the low-cost way of illumination.

On the left side of the picture at the top you see cultures illuminated by the fluorescent lamp for plants LT - T8 18 W FLEURLIGHT by NARVA. The corresponding emission spectrum can be seen on the left (by courtesy of NARVA Lichtquellen GmbH + Co. KG).

I am also very satisfied with the fluorescent lamp Biolux 965 from Osram. Meanwhile general lighting products of OSRAM are outsourced to LEDVANCE GmbH. Unfortunately LEDVANCE did not agree to reproduce the emission spectrum here. You can find it at this link. This fluorescent lamp simulates the daylight and is often used in keeping reptiles, but also birds and fish. The color temperature is very high with 6500 Kelvin. As the spectrum shows, a considerable proportion of UV-A (380 nm to 315 nm) is also present. Petri dishes made of polystyrene absorb a high degree of UV light, so that there is hardly UV-Irradiation to be present in the cultures. I could not observe a harmful influence compared to the other lamps.

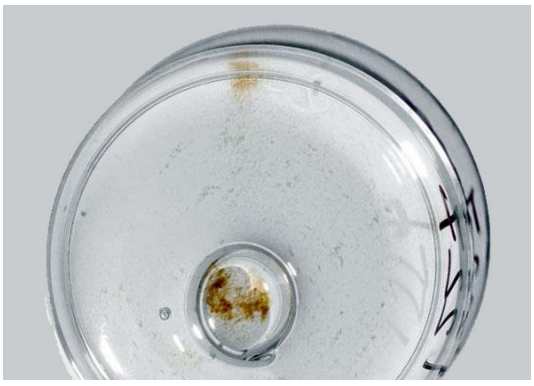
Light intensity

As mentioned already, diatoms must be able to adapt to changing light conditions. As the lifetime of the culture and not the yield of biomass is the focus of our applications, we keep the light intensity relatively low.

In general, the dimensioning of lighting depends strongly on the type of cultures. For larger culture vessels and high cell densities, a higher brightness of the illumination is required in order to penetrate sufficiently through the culture. From the cultivation in petri dishes over erlenmeyer flasks to large vessels the required intensities increase (see www.fao.org/docrep/003/w3732e/w3732e06.htm#b12-2.3.1.2.%20Light).
In addition, the mixing of culture is becoming increasingly important. Incidentally, for large cultures aeration is required.

At low light intensities the growth rate vs. intensity increases linearly (observed at *Ditylum brightwellii*). At higher intensities the function flattens (Paasche (1968)). We cultivate diatoms between 200 lux and 600 lux. This is well above the light compensation point and probably in the linear regime. The brightness depends on the location where the petri dish is placed and can be selected as required.

It has been shown that the light requirements for the different species are not the same. As is well known some motile species show phototaxis. They control the duration of the movement in the same direction in a way that they reach favorable conditions. At low light intensities a positive phototaxis is expected and at high intensities a negative. This can be used to find a favorable light intensity (hint by Dr. Oliver Skibbe). If a fluorescent lamp is placed vertically (in the upper right corner of the image at the top), the diatoms can move towards or away from the light source. In addition, there is a brightness gradient



within the petri dish. The picture shows a culture of *Nitzschia sigmoidea*, in which a ring of thin steel plate was inserted. It touches the ground only at a few points so that diatoms can easily migrate between the outer and inner region. In the selected lighting direction, the bottom of the tray is shaded within the ring. As can be seen, the diatoms preferentially accumulate within the ring. A small portion of Diatoms can be seen on the upper edge of the Petrischale, which is turned away from the light source. A light intensity of only 200 lux seems unpleasantly bright for this species. Unfortunately only a few species show a clearly recognizable phototaxis under our light conditions. In our collection this concerns particularly species of genera *Nitzschia* and *Cymbella*.

When the population reaches its highest density, a high light intensity quickly leads to the enrichment of the diatoms with reserve materials.

Light-dark Cycle

At least at high light intensities, there is a reduction in the growth rate in the case of permanent illumination (Paasche (1968)). Some species seem to prefer a light-dark cycle. This is in particular true for freshly isolated cells (Andersen, Kawachi (2005)).

We use a daytime cycle with about 12 hours light per day. For mass cultivation at least 18 hours of light are recommended (http://www.fao.org/docrep/003/w3732e/w3732e06.htm#b12-2.3.1.2.%20Light).

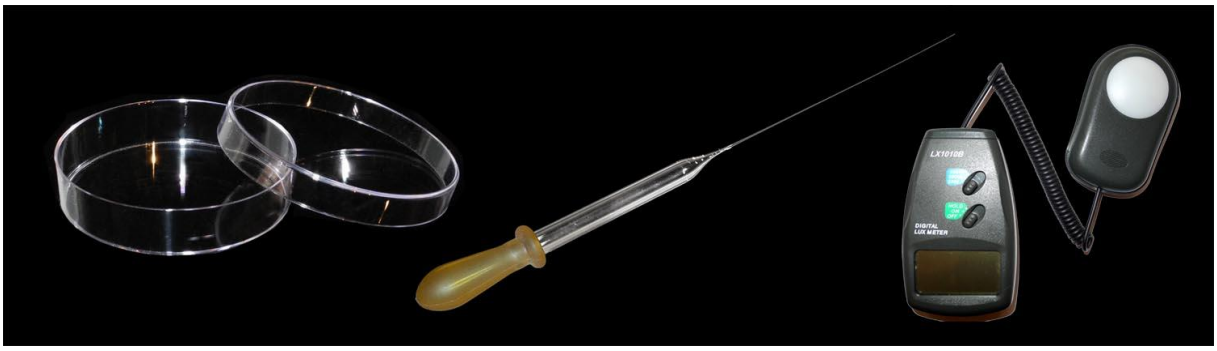
In terms of light intensity, diatoms are quite robust. They stand a postal dispatch of a few days easily.

Schellenberger, Costa et al., Aureochrome 1a is involved in the photoacclimation of the diatom *Phaeodactylum tricornutum*. Plos One 8: e74451, 2013.

Davis C.O., Harrison P.J. & Dugdale R.C. (1973) Continuous culture of marine diatoms under silicate limitation I. Synchronized life cycle of *Skeletonema costatum*. J. Phycol. 9, 175-80.

Paasche E. (1968) Marine plankton algae grown with light-dark cycles. II *Ditylum brightwellii* and *Nitzschia turgidula*. Physiologia Pl. 21, 66-77.

Andersen R.A., Kawachi M. (2005) Traditional Microalgae Isolation Techniques. Algal Culturing Techniques, Robert A. Andersen (Editor) Academic Press



Auxiliaries for cultivation

There will be a few hints for equipment and tools that are important for cultivation. It has already been written about suitable nutrient solutions and lamps.

Stereomicroscope

A stereomicroscope is particularly suitable for selecting diatoms and for transferring them into cultures. Especially in dark field, diatoms can be easily found and pipetted. A high-contrast dark field is achieved by using an inclined mirror in the stand of the stereomicroscope. As the mirror leads to a large height of the stand, a hand rest is required for pipetting. If such a device is not available, one can make use of a matte, black plate under the Petri dish. Care should be taken that the Petri dish does not rest on the black plate but is at such a distance from it that its surface structures are not in focus with the diatoms. For most cases, a magnification of 10x to 30x is sufficient. Ring illumination is well suited for illumination, and switchable segments are advantageous. A camera port enables documentation and also allows observations with a large field of view. The image on the right shows the used Zeiss Stemi 305 with the K LAB stand. Video cameras can be used via a C-mount connector, and a reduction with a factor of 0.5 is built in.

Inverse microscope

For checking cultures a simple inverse microscope is preferable to a stereomicroscope. With it one can quickly detect impurities due to the higher magnification.

To assess cultures, brightfield and objectives with small magnifications, such as a 5x, 10x and possibly a 20x objective are sufficient. An object-guide is useful for capturing images and videos, but is a hindrance to the fast-paced viewing of many petri dishes.

The requirements for live observation in petri dishes are higher. Comments are made in the post on observation devices. An appropriately equipped inverted microscope can be used for both purposes. The picture on the left shows the older inverse routine microscope Wilovert (Helmut Hund GmbH) used by me. It has also been used for many of the videos shown as it has phase contrast and objectives with suitable magnifications. The images were taken using an eyepiece adapter attached to one of the two eyepieces. In connection with observation accessories an inverse microscope with more extensive equipment is presented.

Petri Dishes

As our goal is not the achievement of a large biomass, petri dishes are well suited as culture vessels. We recommend polystyrene (PS) petri dishes for one time use. Reusable petri dishes made of glass allow observation in differential interference contrast (DIC) but the effort for cleaning is significant. We use sterile bacteriological petri dishes with a diameter of 55 mm. Therein enough diatoms can be cultivated and the space requirement for cultivation in one's own home remains acceptable. In order to reduce the risk of aerial contamination and to avoid water loss by evaporation, we prefer petri dishes without ventilation ribs. As the cultures are kept at room temperature and are not incubated, there is hardly any danger that the water condenses on the lid and makes observation more difficult.

Pipettes

In order to pick diatoms from a fresh sample a micropipette with a sufficiently small tip is needed, so that the probability of contamination is low. Its diameter should be about twice as large as the diatoms to be taken, that means between about 20 µm to 100 µm depending on the size of the diatoms.

A pipette with a long, thin capillary creates a strong pull of water for a few seconds, which can usually detach diatoms from the substrate without contact. However, the longer the pipette, the more difficult the handling.

When taking diatoms from a pure culture, pipettes with a larger capillary diameter can be used in most cases.

Micropipettes can easily be produced from a glass or quartz tube. For this purpose, a glass tube is heated above a Bunsen burner or a soldering lamp. Roll it carefully between your fingers. Once the glass is soft enough remove the tube from the flame and pull the two ends apart. The faster you pull, the thinner the capillary becomes. After cutting through the halves one has two pipettes with the same opening.

Luxmeter

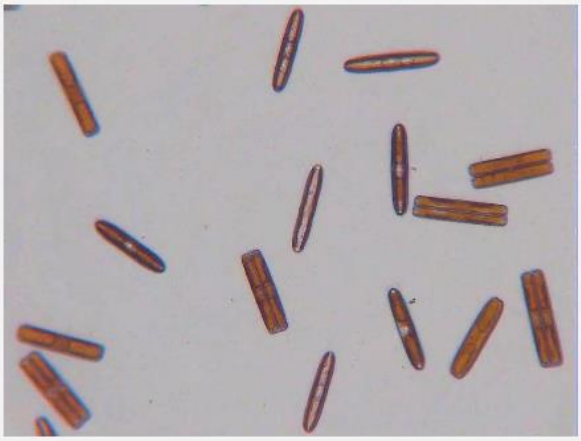
There are no high requirements on the measuring accuracy of the luxmeter. A comparison of cheap with expensive devices has shown that cheap devices are sufficient for cultivation. There are also luxmeter apps for smartphones. Unfortunately, trying to use one of these apps was not successful. It is quite possible that there are apps with sufficient accuracy available.

Measuring device for salinity

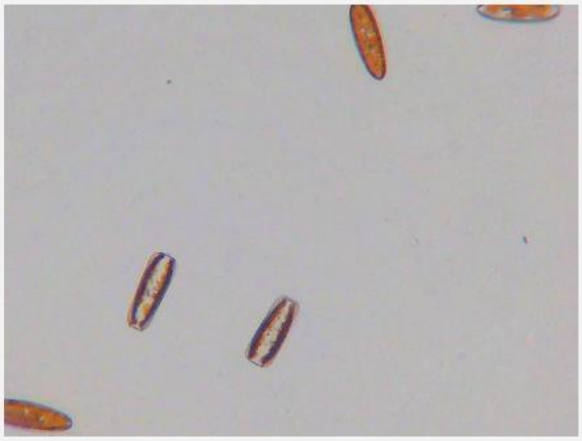
For cultivating marine diatoms, a measuring instrument for measuring salinity is required, such as a hydrometer, refractometer or conductivity meter. Information was given in the section on nutrient solutions.

Others

To prepare the nutrient solution, an Erlenmeyer flask, sufficiently accurate scales, a graduated pipette, a thermometer and possibly Ph test strips are sufficient.



Pinnularia spec. (60x time lapse)



Surirella robusta (different time lapse rates from 90x to 30x)

Identification of genus and species

The cultivation of diatoms makes it possible, to assign observations to a genus or even species. For this they must be identified. It is a difficult area with an extensive literature that is only mastered by experts. This is is the reason why the information on the videos shown here is to be questioned occasionally. Often we have intentionally limited ourselves to the assignment to the genus.

At this point only a few general hints for preparation and determination are given.

The morphological identification is based on the shape and structure of the valves and is traditionally based on a light microscopic examination. However, it is probably not possible to distinguish two different species morphologically in general. The method of DNA barcoding, which uses a molecular genetic fingerprint for taxonomic classification, is therefore considerably more sensitive (see „Revolutioniert DNA-Barcoding die Gewässergüteanalyse?“ https://www.bgbm.org/de/pr/revolutioniert-dna-barcoding-die-gewaesserguteanalyse). In view of the high number of diatom species, the practical applications of the method are presumably dreams of the future.

Although the structures of the valves in living diatoms are not discernible in detail, certain genera or species of diatoms can well be identified. If features are added such as chloroplast types and arrangement in valve and girdle view, or colony formation, the possibilities of determination enlarge. This approach is followed in "Identification of Freshwater Diatoms from Live Material" (E. J. Cox).

In most cases, however, the determination is carried out using a microscopic image of a diatom frustule. It must be free from interfering cell components. In aged cultures one usually finds suitable valves of dead diatoms, so that one often gets along without preparation. If no such valves are available, preparations from living cells can be made. There are various methods for this. The use of strong acids (sulphuric acid) or oxidizing agents (hydrogen peroxide) is usually described.

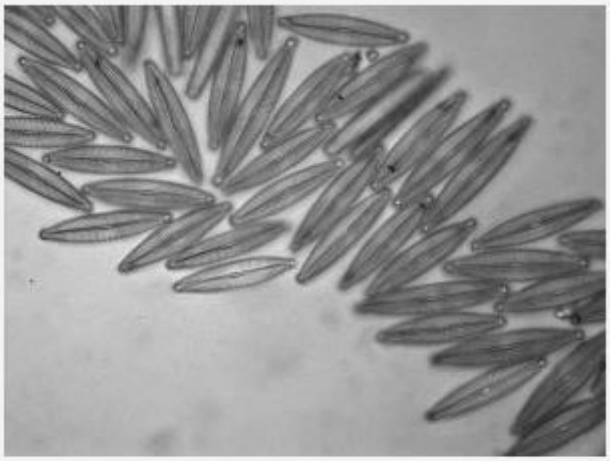
A very old method, which is no longer recommended by experts, is the annealing of samples. As I do not like to deal with dangerous chemical substances and do not have a fume hood, I have taken up this very simple and fast procedure to take pictures of the valves. Durable preparations are not

produced in this way and these photos do not win a beauty contest, but they do their job well. The process is described below.

First of all, drop droplets from a culture onto several clean cover slips and allows them to dry thoroughly. Then place them on a hotplate and heat them up. It has proved useful to initially increase the temperature slowly in order to prevent the bursting of cells with a high remaining water content. When approximately the hotplate begins to glow, the cover slips are removed from the hotplate bit by bit. Thus, it is more likely to find a cover slip with good results. If the heating is too low, unburnt black organic residues are found. Too strong heating leads to the destruction of fine structures. There is also the risk that the cover slips will deform considerably.

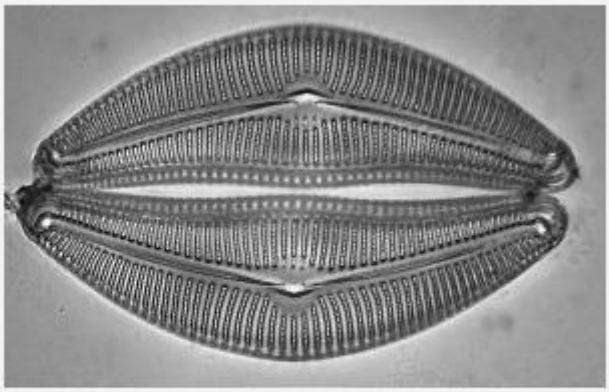
The cover slips are turned carefully, so that the diatoms are located on the underside and are placed on a microscope slide. With the upright microscope the glass thickness is obtained for which the objective lenses are optimized. The difference in refractive index between air and silicate is so large that a high-contrast image is obtained in the bright field. If immersion oil is used, the cover glass must be fixed to the slide beforehand.

As a large number of diatoms of one species can be prepared simultaneously, the probability of finding intact and well cleaned valves is high. With a bit of luck, an overview looks like this (Click to enlarge):



(Click to enlarge)

This image shows two diatoms of the genera *Cymbella*, which have been cleaned this way (100x oil, stacked image):



(Click to enlarge)

Now one can look at the identification literature (identification keys) or the databases on the internet.

A characteristic feature of the annealing process compared to the use of acids is that the frustules of diatoms are almost never separated. The images of the diatoms therefore appear darker in transmitted light. At high depth of field (especially at low magnifications) the structures of both valves can superimpose, which worsens the visual impression and the photographic image. If you put together an image from a stack of images at different focus levels, you have to be careful to consider only the required layers.

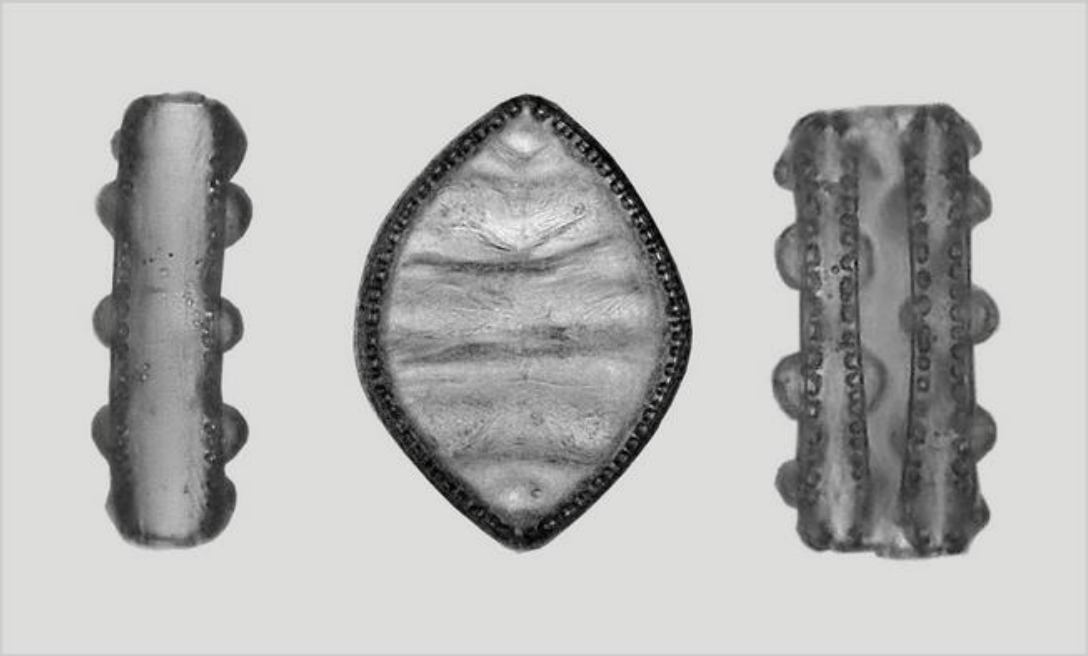
If the diatoms are located in a suitable spatial position, this is an advantage as it can help to gain a better spatial impression of the shape of the diatoms. However, this does not help to identify them. As an example, photographs of *Cymatopleura elliptica* are shown.

Cox, E.J. (1997) The Identification of Freshwater Diatoms from Live Material. Chapman & Hall, London

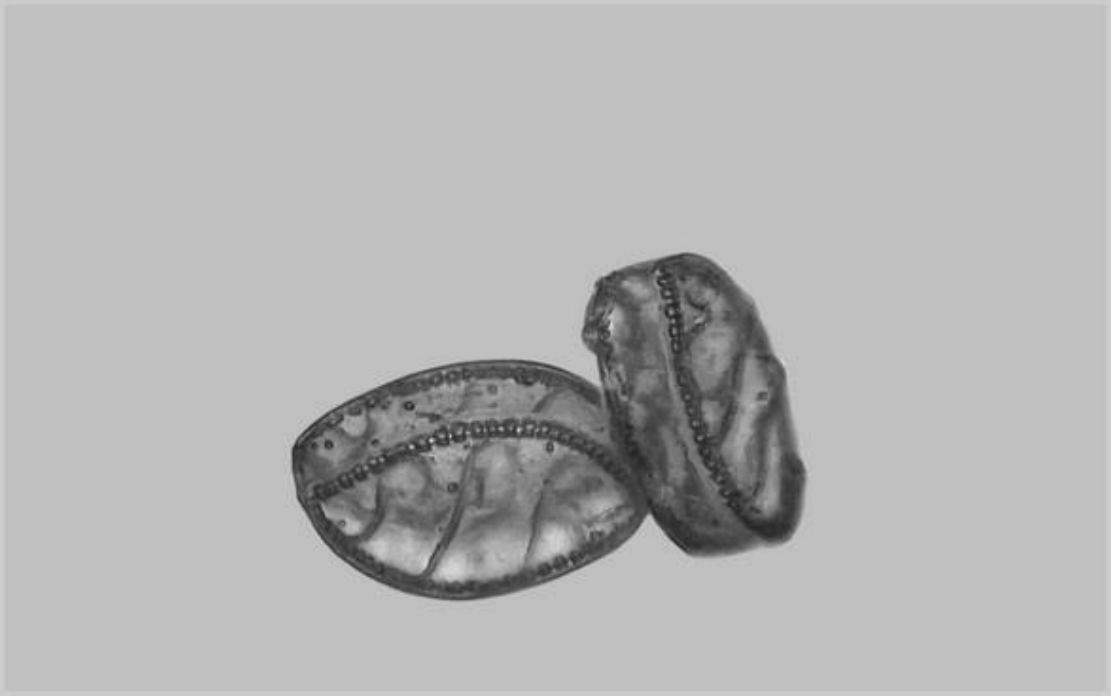
Observation of Diatoms

Pictures of *Cymatopleura elliptica* after annealing

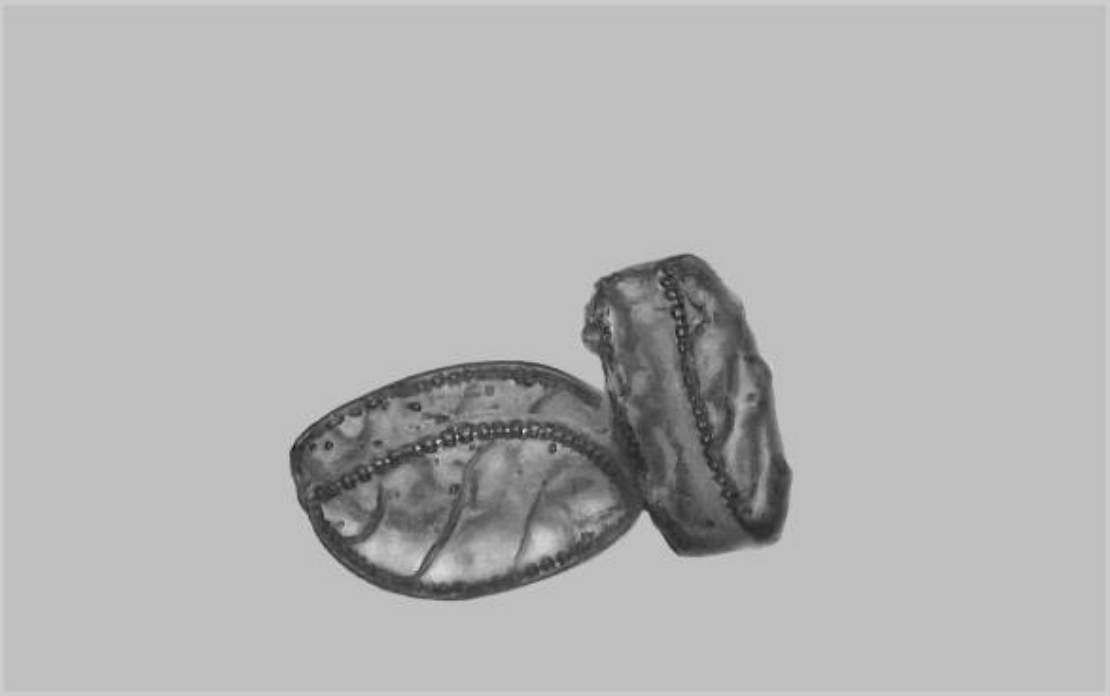
The species *Cymatopleura elliptica* shows a very characteristic appearance in valve view and belt view. Below you can see some diatoms of this species in both views. The diatom on the right side of the picture is currently in division. The images were composed of image stacks and placed in front of a homogeneous background.



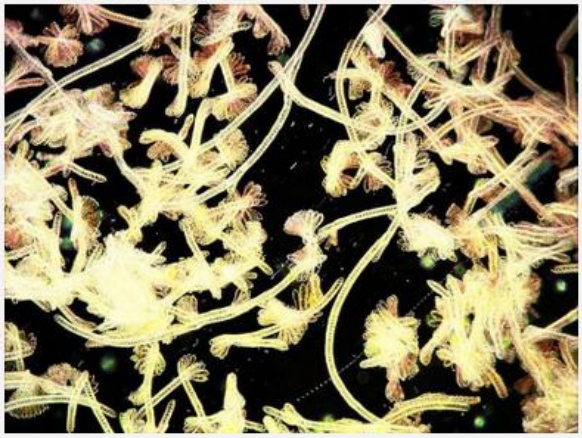
It is difficult to gain a spatial impression from the projections into a plane. This is easier if the diatoms are located by chance in a position that lies between valve view and belt view. Such a situation is found in this picture:



This image was also created using a stack of images, with the focus planes approximately equidistantly spaced. Therefore, a depth map of the captured structures can be calculated. This is accomplished by the software (freeware) "Picolay" from Heribert Cypionka (http://www.picolay.de/). The following animated gif image was created with it, which shows the view of the right and left eye alternately.



Cymatopleura solea in dark field (20x time lapse)



Visualization of the motion of the diatoms from the video by calulating the maximum over all frames (click to enlarge)

Challenges

All in all, it does not require much effort or special knowledge to cultivate some species and keep them alive for a period of months. Cultivation of various species of the genera *Navicula*, *Nitzschia*, *Pinnularia*, *Cymatopleura*, *Cymbella* or *Rhopalodia* proved to be quite unproblematic. The nutrient solution was never adapted specifically for one species. Only in light intensity did we occasionally consider an individual requirement.

At this point, however, it should not be concealed that one can run into difficulties with the cultivation of diatoms. From our experience these are above all:

- Some species are difficult to cultivate. It often requires some trials until the cultivation succeeds (see example). For others, it seems hardly possible by simple means. If you do not want to cultivate a particular genus or species, you can see that relaxed.

- In the case of asexual reproduction, the average length of the diatoms is reduced in each generation (Pfitzer and MacDonald). This phenomenon has already been pointed out in the introduction. If a sequence of batch cultures is generated by regularly creating new cultures, one always transfers a sample of diatoms of different lengths. The subsequent culture can produce no larger diatoms than its largest diatom without the sexual reproduction. The dying of the largest diatoms also leads to the fact that the lengths of the diatoms in culture are becoming smaller and smaller.

  Sexual reproduction (or vegetative cell enlargement) is now required but is difficult to achieve in vitro. We could not recognize sexual reproduction in our cultures with only a few exceptions. (We would be very grateful for hints on how to create suitable conditions for sexual reproduction.)

  The gradual reduction in the length of the diatoms thus led to a limited durability of the sequence of cultures. Whereas species of *Navicula* have been preserved for only a few months, it has been possible to cultivate lines of *Pinnularia* or *Rhopalodia* for years.

In addition, malformations of the frustules frequently occurred in long-term cultivation. This can be caused in particular by decreasing valves or by symptoms of deficiency (examples: *Surirella* and *Nitzschia*, *Cymatopleura solea*).

- At the beginning of a series of batch cultures, contamination with other diatoms, green algae, flagellates, or bacteria may occur. If the cultures are handled without care, this can happen even later. Here a very clean process helps in the cultivation. The sterilization of pipettes, the choice of a favorable diameter of the pipettes and the washing of diatoms in intermediate baths were addressed. Furthermore, one should avoid leaving petri dishes open for a longer period of time.

The difficulties mentioned should not discourage you, because a good looking culture compensates for some trouble.

One of the rare cases of sexual reproduction in our cultures occurred several times in a *Stauroneis* culture (probably *Stauroneis phenicenteron*).

The diatoms of the culture were already reduced to a typical length of 126 µm 8 months after collecting the samples. The length of the initial cells is about 270 µm. In addition to the two auxospores, which have almost developed into initial cells, the four valve halves of both parent cells are also visible.

The image was generated from a stack of images with photos of different focal planes.

(click to enlarge).

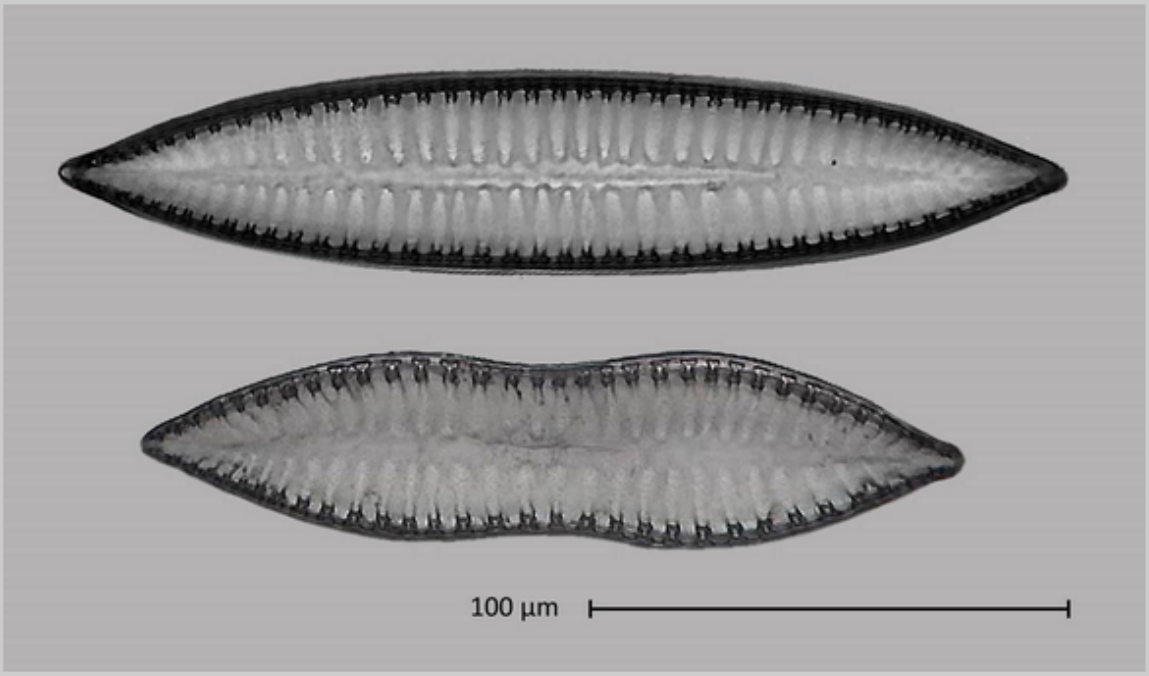
Observation of Diatoms

Malformations in *Surirella biseriata*

Repeatedly I observed deformations and other abnormalities in cultures. Anomalies of the silicate walls of the valves can have various causes, such as contamination with heavy metals (Falasco et al. (2009)), water pollution with herbicide (Debenest et. al. (2008)) or artificial growth conditions (deficiency symptoms) in cultures. In addition, malformations can also occur if diatoms are close to the minimum length after vegetative divisions or if vegetative divisions take place beyond this number of generations (Locker F. (1950)). An overview can be found in the paper by FALASCO et. al. (2009).

The diatoms of the species *Surirella biseriata* (Brébisson) were convex in valve view at the beginning of cultivation. The following video (450x time lapse) was made about 6 months after starting cultivation. It can be seen that the outline of the valves is often concave, as there are more or less pronounced waists. There seems to be a smooth transition from the shape of normal cells to malformed cells. Symmetry with respect to the apical plane is not always maintained.

The following picture shows an early generation valve with a length of 211 µm. Underneath it is a malformed diatom with a length of 176 µm. All diatoms with concave contours are about this size. The malformation seems to be restricted to the outline.



Cultures with diatoms showing such deformities do not thrive well. In the case of vegetative reproduction of diatoms with malformation, malformations also occur in daughter cells.

I exclude contamination as a cause. It could be a deficiency symptom due to an unsuitable nutrient solution or a consequence of reaching or undercutting the length required for sexual reproduction. As the malformation occurred in two different nutrient solutions to the same extent and only the small diatoms and their daughter cells are affected, I tend to believe that the small size is the cause.

It should be noted that these observations are not entirely in line with Hofmann et al. (2011). There the concave forms are assigned to the larger forms. A transition between these forms in the course of a succession of generations is not mentioned. Also the frequently observed asymmetries (different radii of curvature of the concave sides) are not taken into account. (The length range is given by Hofmann et al. (2011) as 80-400 µm.)

DEBENEST T., SILVESTRE J., COSTE M., DELMAS F. & PINELLI E. 2008. Herbicide effects on freshwater benthic diatoms: induction of nucleus alterations and silica cell wall abnormalities. Aquatic Toxicology 88: 88-94.

Falasco Elisa, Bona Francesca, Ginepro Marco, Hlúbiková Daša, Hoffmann Lucien, Ector Luc. Morphological abnormalities of diatom silica walls in relation to heavy metal contamination and artificial growth conditions. Water SA [Internet]. 2009 Oct.

Falasco, E., Bona, F., Badino, G. et al. (2009). Diatom teratological forms and environmental alterations: a review, Hydrobiologia, 623, pp 1-35.

Hofmann, G & Werum, Marcus & Lange-Bertalot, H. Diatomeen im Süsswasser-Benthos von Mitteleuropa. Lange-Bertalot, H. (ed.), Gantner Verlag, Ruggell, Liechtenstein, (2011)

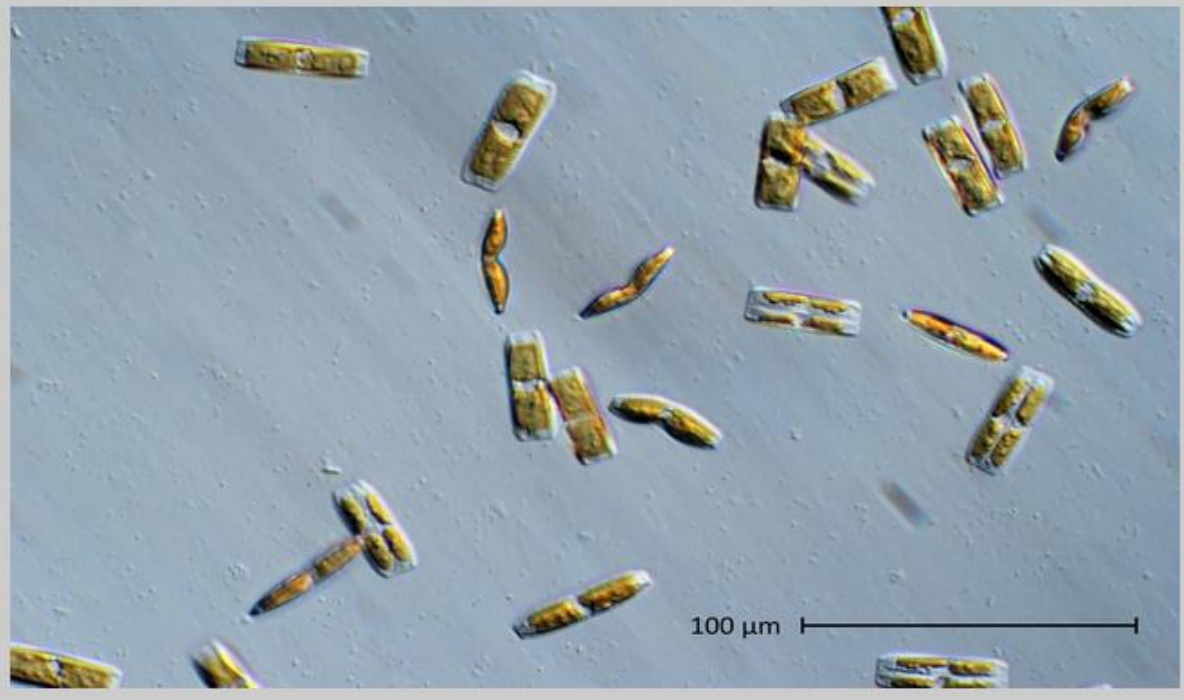
LOCKER F (1950) Beiträge zur Kenntnis des Formwechsels der Diatomeen an Hand von Kulturversuchen. Österr. Bot. Z. 97 (3-5), 322-332

Observation of Diatoms

Malformations in *Nitzschia* sp.

Malformations and their causes were discussed in connection with *Surirella biseriata*.
I observed very pronounced abnormalities in a *Nitzschia* species. At the start of cultivation in February 2016, the average cell length was 146 µm. Because of the short lifetime of a culture of only two to three weeks, I inoculated a new culture every week. The generation time was very short, so that the bottom of the petri dish was already densely populated after just one week. The following video (25x time lapse) shows a look into a culture shortly after the beginning of cultivation:

After 18 months of cultivation there was much less motility and the cultures did not thrive well. The following picture (PlasDIC) of a culture shows significant abnormalities:



The mean length of the diatoms reached only 38 µm. It was thus only 26 % of the original length.

On the left there are three diatoms in larger magnification. Shortly after these pictures were taken the culture was given up. As in the case of *Surirella biseriata*, I suspect that the cause of the malformations is the very small size. Unfortunately, as in nearly all our cultures, there was no sexual reproduction.

Thomas Harbich http://diatoms.de/en/culturing/challenges

Observation of Diatoms

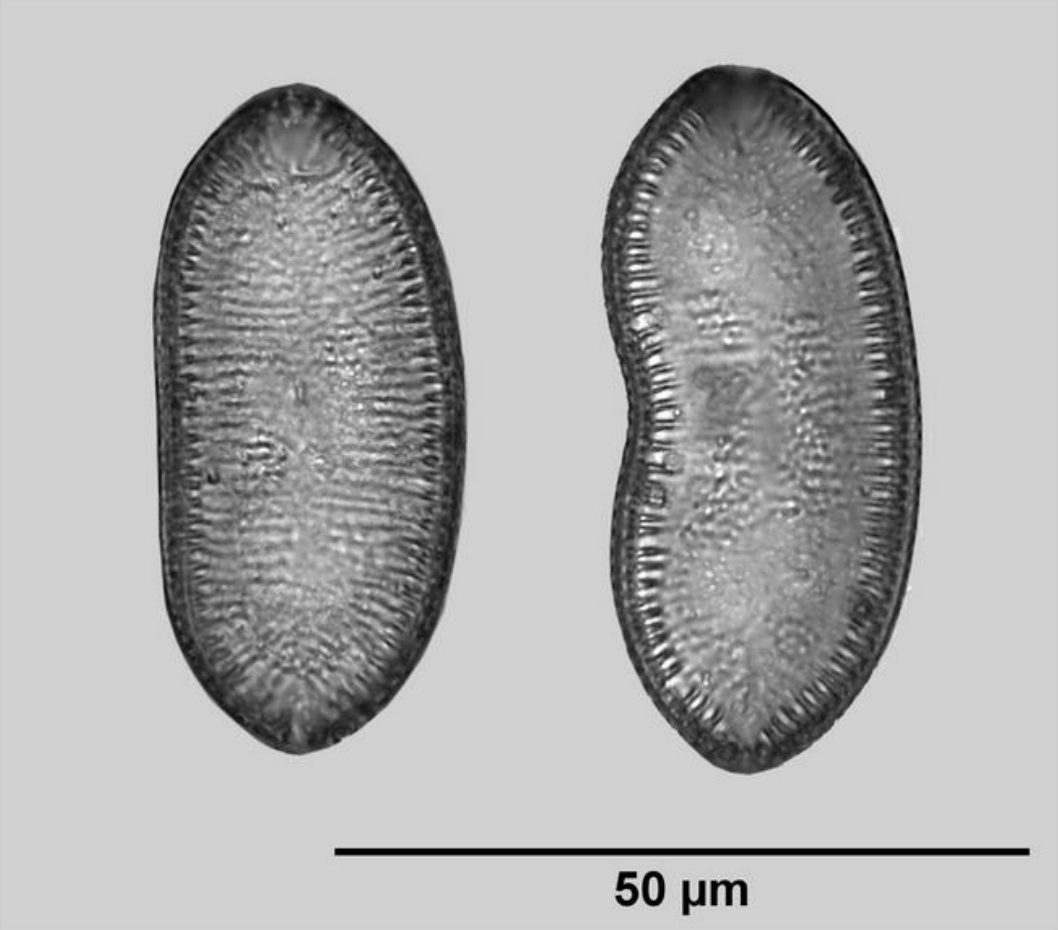
Malformations in *Cymatopleura solea*

Cymatopleura solea will serve as a further example of malformations that occur in older cultures. Diatoms of this species were isolated from Lake Ebnisee (48°55'25.5"N 9°36'32.8"E) on 25 March 2017 and kept in clonal culture. About every other week diatoms of a parent culture were inoculated into a subsequent culture. The video at the top shows (30x time lapse) a view into such a culture taken on 16 May 2017. At that time, the diatoms still had an almost uniform length of about 160 µm.

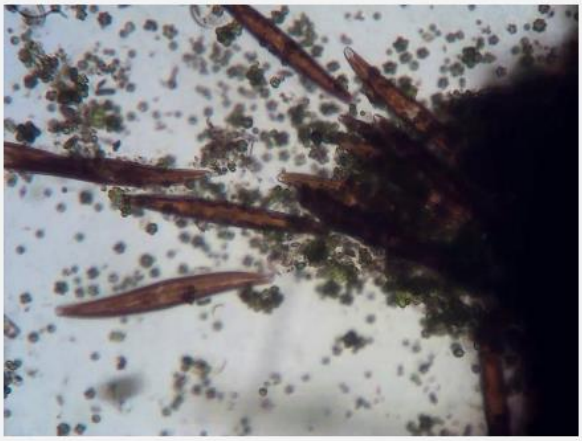
In the following months a gradual reduction of the diatoms was observed. Malformations occurred in larger numbers from August 2018. The following video is taken on November 7, 2018:

(20x time lapse when using 10x objective; 10x time lapse at higher magnifications)

The length of the diatoms was typically in the range of 55 µm to 75 µm. The arched deformed diatoms, however, had a length of only about 45 µm (distance from apex to apex). The following picture gives an impression of the valves of such diatoms:



The left valve shows only a slight asymmetry with respect to the apical axis.

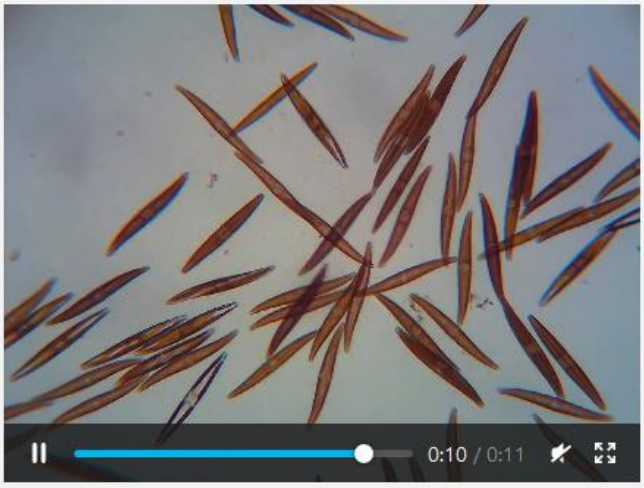


Detritus flake with *Gyrosigma* sp. (40x and 60x time lapse)

Gyrosigma sp. and *Nitzschia sigmoidea* in culture (4x time-lapse)

Example of a difficult cultivation (*Gyrosigma*)

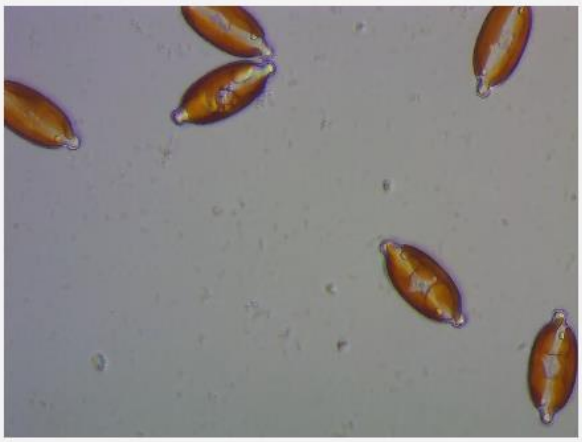
Diatoms of the genus *Gyrosigma* are often found in samples from brooks and lakes. It is noticeable that these diatoms usually move to and fro in flakes of detritus. Presumably, they absorb substances from the detritus.



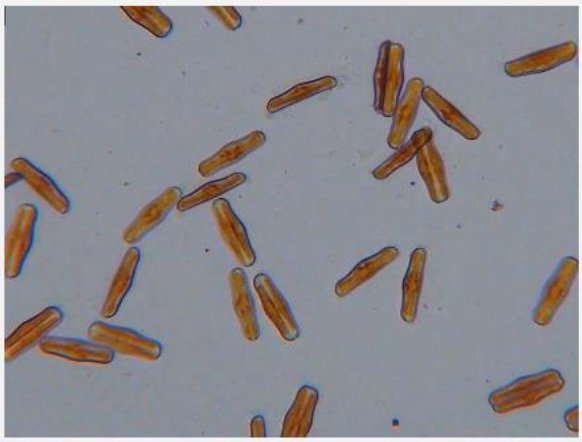
Isolated diatoms of genus *Gyrosigma* usually did not multiply in our raw cultures. It proved successful to cultivate these diatoms with the detritus. Some detritus flakes together with green algae, ciliates, *Gyrosigma* sp. and other diatom species were pipetted into a petri dish containing nutrient solution and a favorable light exposure was selected. In the video on the upper left you can see a flake of detritus (from the brook "Schwarze Rot") in which *Gyrosigma* sp. developed well, first under the stereomicroscope and then under the inverse microscope at two different magnifications. This has nothing to do with a pure culture, but the conditions seem to be favorable.

Occasionally one can be lucky and the cultivation succeeds. A raw culture was inoculated with Nitzschia sigmoidea and some specimens of the genus *Gyrosigma* from a pond near Hohenheim (Stuttgart). Both reproduced well, as the video in the upper right shows. It is very easy to separate the two species in the following culture and so the video was created on the left (4x time-lapse). Unfortunately, the culture proved to be unstable and after several successive cultures the increase stopped.

Sometimes I add *Gyrosigma* to a raw culture. Unfortunately the successful cultivation could not be repeated so far.



Caloneis amiphsbaena (30x time-lapse)



Rhopalodia spec. (120x and 180x time lapse)

Devices for observation

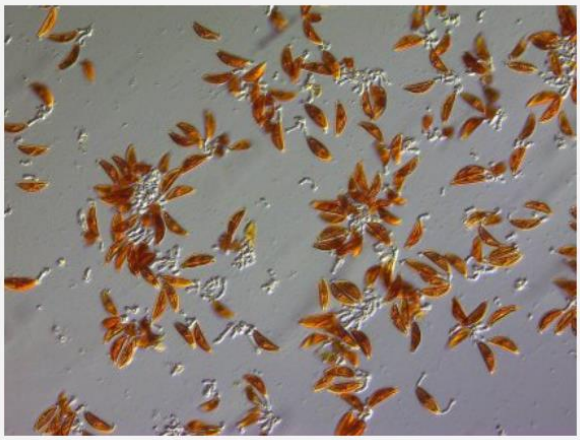
Microscopes

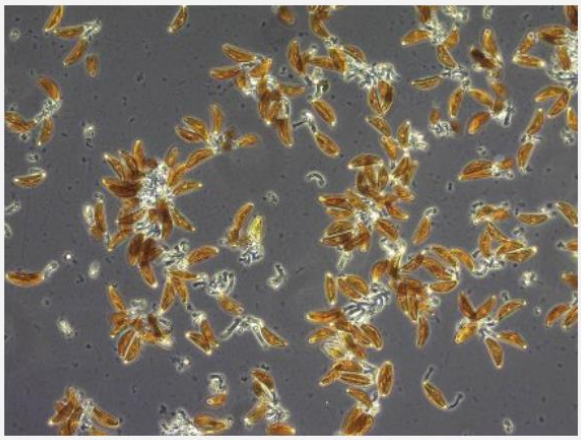
The inverted microscope and the stereomicroscope were already mentioned in the context of cultivation. They are also used for the observation of diatoms, especially when they are cultivated in petri dishes. The requirements for an inverse microscope in live observation can vary depending on the object and the observation goal. Higher magnifying objectives with 20x and 40x magnification are often useful. For documentation a trinocular tube or a dedicated photo tube is recommended.

An object guide with a petri dish holder makes work easier. This enables a uniform tracking of moving diatoms at long term observations. If you mark a point on the bottom half of a petri dish, you can place it in the holder again in the same orientation and find locations whose coordinates you have written down.

If you want to observe the movement activity of diatoms over a longer period of time, LED lighting is recommended. With halogen lighting and blue filters, it is difficult to achieve a usable spectrum. In addition, the colour temperature is highly dependent on the light intensity of light bulbs.

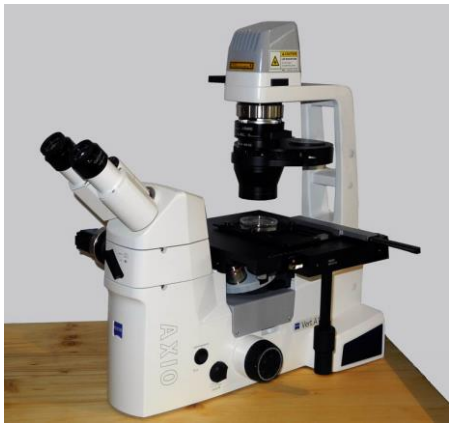
Living diatoms are good amplitude objects and can therefore easily be observed in the brightfield. However, the mucilage stalks that some *Cymbella* sp. produces, are mainly phase objects. The deformation of water surfaces in the vicinity of floating diatoms can only be observed well with phase-sensitive contrast methods. Phase contrast is suitable for such tasks. In principle, differential interference contrast (DIC) is desirable, but the preferred plastic petri dishes are not suitable for this because of their active optical properties. Zeiss offers the PlasDIC contrast method (Differential Interference Contrast for Plastic Receptacles), in which an optical anisotropy in the petri dish material and in the object itself is not disturbing. The slit diaphragm in the condenser can lead to stripe-shaped artefacts, which I regard as acceptable. For comparison, a culture with colonial *Cymbella* in PlasDIC and phase contrast is shown below (click to enlarge):





In both contrasting techniques, the almost transparent mucilage stalks are clearly visible. However, the impression is very different. Certain fine structures on the mucilage stalks are barely visible in phase contrast, whereas they can be recognized in PlasDIC.

With the same camera settings, PlasDIC requires a significantly higher intensity of the illumination compared to phase contrast (additional polarizer). If you don't have a very sensitive camera, phase contrast is recommended for long term observations.



Since August 2017 I have been using a Zeiss Axiovert A1 with phase contrast and PlasDIC. The microscope can be seen on the left (click to enlarge). Earlier recordings on this homepage were made with the Wilovert (Helmut Hund GmbH) shown elsewhere.

I will not go into other contrast methods that I do not use, such as VAREL contrast or Hoffman modulation contrast.

Furthermore, an upright microscope is required, with which a higher resolution can be obtained. Here too, observation in the brightfield is sufficient in most cases. This allows you to observe details of movement or photophobic response, for example. In principle, brightfield is sufficient for the determination of genus and species on the basis of a sample. However, since only the valves are observed, phase contrast or differential interference contrast (DIC) are advantageous. These contrasting methods are furthermore required for the phase objects mentioned above, such as mucilage stalks. In the past I used only an older Zeiss standard microscope for brightfield and phase contrast with trinocular tube. A strongly magnifying dry objective (e.g. 63x with cover glass correction, NA = 0.9) and a 100x immersion objective were helpful. At the end of 2019, an older Zeiss Axioplan was added, which has Plan-Neofluar objectives and DIC. It can be seen in the picture on upper right (click to enlarge).

Macroscope

For long-term observations (long time lapse) at low magnification, I use a self-constructed macroscope, which consists of a a stand, bellows, camera lens in retro-position or macro lens and an eyepiece. You can see it on the left with the video camera attached and a simple lighting device. On the right you can see the device with a Zeiss-Luminar (25 mm focal length). This requires an adapter with RMS thread.

It turned out that such a macroscope is more light sensitive than a conventional stereomicroscope. It is possible to position it between the other cultures without additional illumination, so that the observed petri dish receives sufficient light for reproduction and for taking pictures. The dimmable illumination (LED) irradiates the diatoms from underneath with a few lux at night, which allows observation even in the dark phase.

Camera

These pages are primarily about the observation of the movement of diatoms. Therefore, video recordings are particularly important. Either a digital camera or a video camera is used. You can mount it on a trinokular tube or on one of the eyepiece tubes.

Personally, I prefere the video camera. The control is done completely on the PC, pictures and videos are immediately available and a separate power supply is not necessary if the video camera has a USB connection. A camera with USB 3 interface allows a sufficient framerate.

Further hardware

Depending on the observation, small auxiliary devices may still be needed. This will be discussed in the relevant sections.

Software

Here we refer only to the software, which is important in many recordings. In addition to drivers, capture software from the camera manufacturer and codec, this is:

- VirtualDub (www.virtualdub.org) is used to create a video from separate frames and to edit video sequences.
- Fiji (www.fiji.sc) is a universal tool for image processing and image analysis.

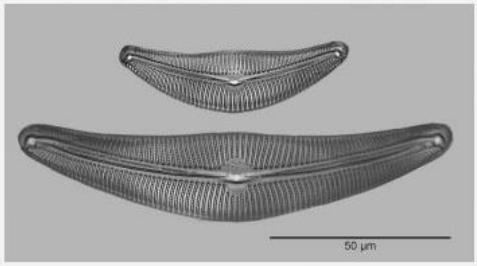
Specific software (such as programs to track the motion) is referred to in the appropriate section.

Cymbella culture with asexual and sexual reproduction
(2x time lapse)

Cymbella culture some time after the onset of sexual reproduction
(40x time lapse)

Sexual reproduction in *Cymbella* (allomixis)

Many of the observations presented on this website deal with the movement of diatoms and the formation of colonies. However, this and the following post are about the sexual reproduction of two species of the genus *Cymbella*.



At the end of June 2016 I gathered and cultivated a *Cymbella* species from a small artificial lake (Ebnisee, 48°55'25.5"N 9°36'32.8"E). From apex to apex the length was about 63 µm at that time. The picture of the valves can be seen in the upper half of the top picture of the image gallery (click to enlarge) on the left (100x-objective with oil immersion). I assume that this is *Cymbella cistula*.

In the middle of November large *Cymbella* appeared in one of the cultures. It turned out that they were the result of sexual reproduction. As many diatoms reached almost simultaneously the lower limit of the size, sexual reproduction occurs in many places in a culture. In the following weeks, this also happened in other cultures which were cultivated at about the same time or later.

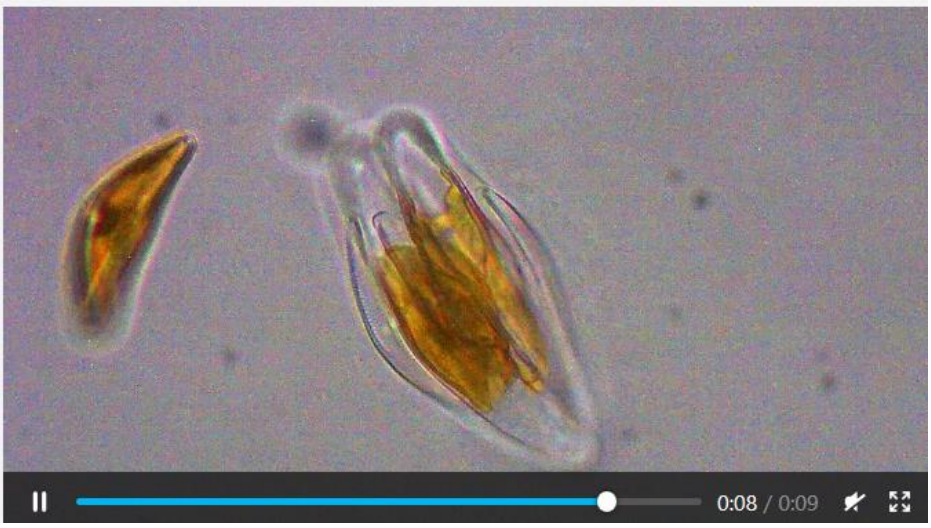
The length of the small diatoms at this time was typically 54 µm, while the large *Cymbella* (initial cells) measured 120 µm. The ratio of lengths is 2.2. The lower half of the first picture shows the valves a few generations after sexual reproduction. One can recognize the similarities and differences between valves of extreme lengths.

Sexual reproduction begins with the formation of haploid gametes (Geitler (1954), Geitler (1957), Geitler (1967), overview in the book of Round et al. (2007)). The second picture of the image gallery shows two gametes in each of the copulating diatoms. Then fusion takes place between adjacent gametes of the two gametangia, two auxospores develop, which mature into large vegetative cells. So there is a cross-fertilisation (allomixis). In the following picture, the fusion of the gametes seems to have begun. This image combines several images of different focus levels. For this stacking the program CombineZP (open-source software by Alan Hadley) was used.

A pair of copulating diatoms and the process of the gametes fusion were sketched by Lothar Geitler (1954). (*Not presented here for legal reasons.*)

The top left video shows the situation after almost completed development of auxospores. On the side to the auxospores, the empty valves of the small diatoms which have copulated are visible.

The following time lapse video shows the growth of two auxospores (9000x time lapse). It is my impression that gametes and auxospores do not tolerate high light intensities over a longer period of time. The video was therefore captured at very low light intensity. This leads to high image noise. The recording lasted for 22.7 hours.



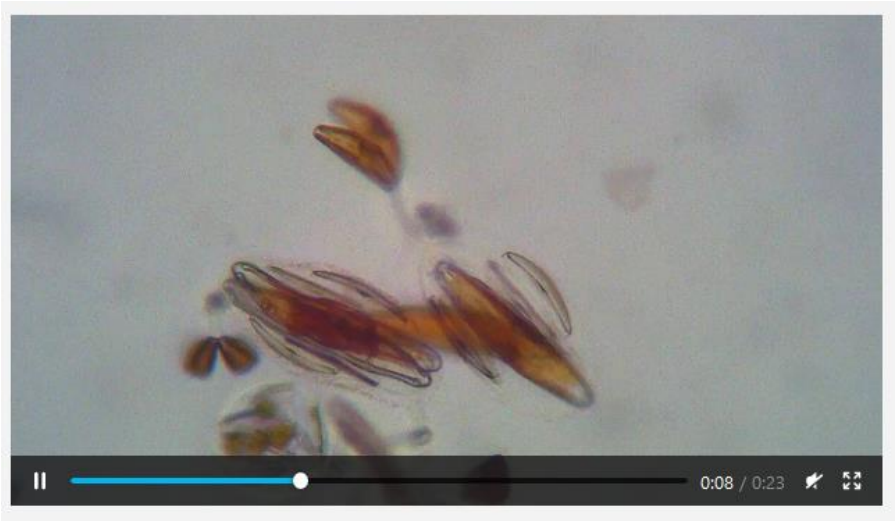
As the auxospores do not lie parallel to the bottom of the petri dish, a short image sequence is attached after the dark pause, in which the focus is moved through the sample. The structure becomes even clearer with higher resolution and by use of an image stack, as it is shown in the picture on the left. Here one also easily recognizes the enveloping copulation jelly. The new diatoms are evidently already well developed, because one can see the structure of the valves.

In the combined image only the small valves that are close to the objective remain visible. Behind this are the corresponding other halves. They can be recognized at a lower magnification and thus a higher depth of focus, but then its structure is not dissolved. Stacking with selected images provides a moderately good image. To illustrate the spatial structure, a video was prepared in which the focus level is moved forward and backward through the sample. The

upper and lower small valves are alternately visible. Like all other videos, this video can be viewed full-screen. Often the resolution of the microscopic images is insufficient for this, but in this case it is recommended.

It should be noted that an upright microscope was used for higher resolution images of living specimens. For this purpose, the objects from the petri dish were put on a microscope slide using a pipette and a 63x dry objective (Zeiss) was used for observation.

The finished cells of maximum size escape from this copulation envelope. This is probably the first benefit that the new cells get from their ability to move. The activity was recorded in a time lapse video with a speed factor of 75 (one image every 3 seconds, played with 25 fps). This is the resulting video:



The two diatoms slid out in a short time interval. In the second escaping diatom, one can see how the jelly yields elastically. The speed is considerable, but can only be estimated roughly by the frame sequence. Within three seconds (two consecutive frames) the first escaping diatom covers a distance of about half the cell length, which makes about 20 µm per second. The second diatom begins at first slowly and then arrives at a similarly high velocity, the elastic copulation envelope probably contributing to the driving force.

The number of small diatoms in culture is reduced by copulating and dying small diatoms. The number of large diatoms increases due to sexual and asexual reproduction, so that they quickly dominate. This situation can be seen in the video at the top right.

The lower half of the first picture of the image gallery shows the valves a few generations after sexual reproduction. One can recognize the similarities and differences between valves of extreme lengths.

It is worth mentioning that sexual reproduction is not always successful in cultures. It is no rare phenomenon that one sees dead auxospores. Whether this occurs in nature with similar frequency, we cannot judge. This may also be due to the high light sensitivity of gametes and auxospores.

The observation of the sexual reproduction of this *Cymbella* gives the possibility to determine the maximum and minimum length and to measure the age of the cell line in the aftermath in units of

length. When the generation rate is assumed to be constant, the average length of the diatoms in culture decreases linearly with time. This is, of course, only a rough assumption over the period of many cultures and is valid only as long as the smallest size is not reached again. In addition, the width of the size distribution (in the ideal case binomial distribution) can be tracked over the entire time and compared with the theory.

Note (January 24, 2018):

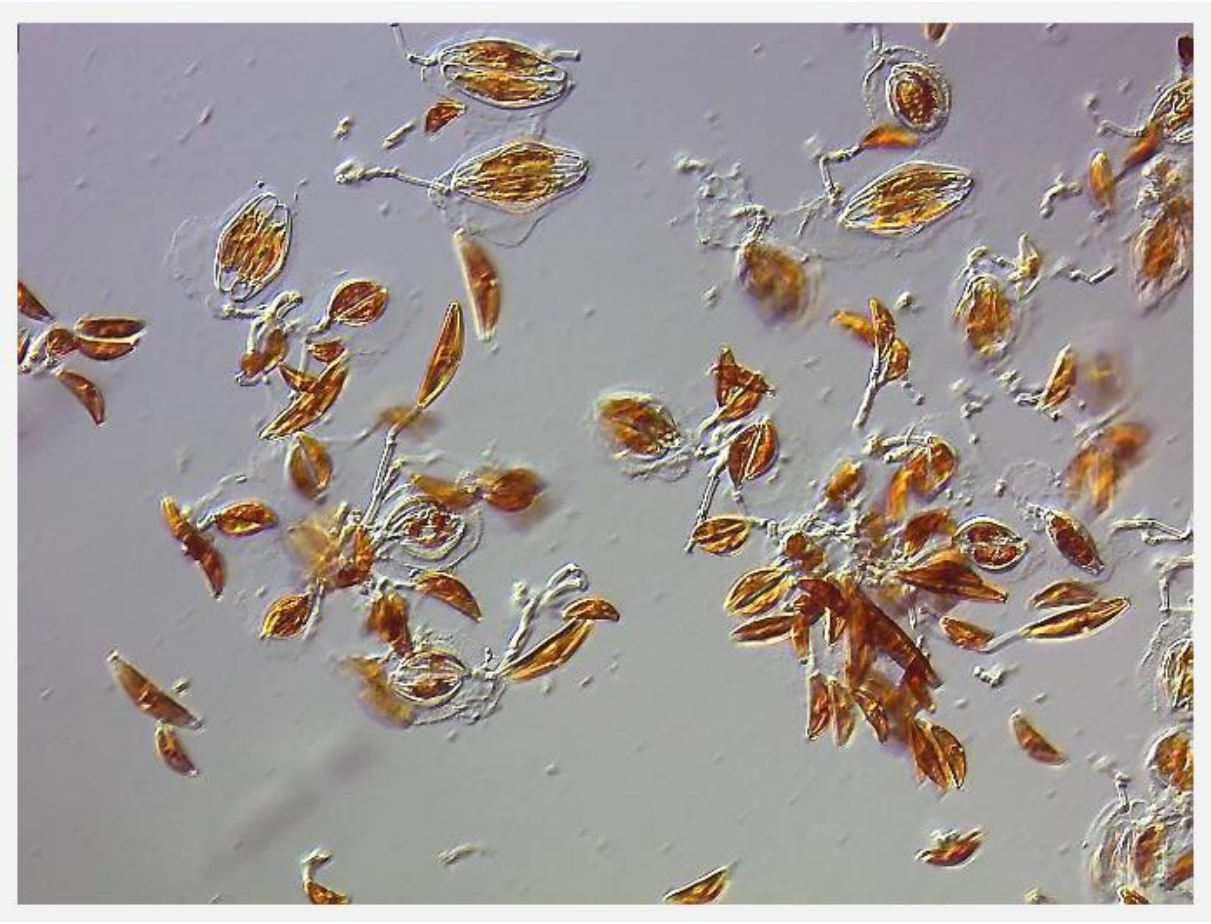
All cells of the original culture were derived from a single diatom, thus possessing the same genome. Cultivation showed that not all diatoms formed from auxospores had good viability. This could be a consequence of inbreeding.

The viable large diatoms could be further cultivated without any problems. Eleven months after the observations described above, the length of the diatoms again was so small that sexual reproduction began for a second time. The picture below shows a sight into a culture in a region with high local density of sexual reproduction (click to enlarge).

Note (April 02, 2019)

In a second strain of *Cymbella cistula* the third "round" of sexual reproduction took place in spring 2019. The following video shows a look (30x time-lapse; PlasDIC) into a culture in which many sexual

reproductions occur in parallel. The cells of smallest length, initial cells and the formation of pairs of auxospores are clearly visible.



Geitler, Lothar (1954) Lebendbeobachtung der Gametenfusion bei *Cymbella*. Oesterreichische Botanische Zeitschrift. Vol. 101(1/2). pg. 74-78.

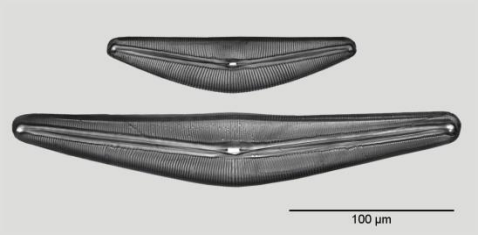
Geitler, Lothar (1957) Die sexuelle Fortpflanzung der pennaten Diatomeen. Biological Reviews. Vol. 32. pg. 261-295.

Geitler, Lothar (1967) Paarung und Auxosporenbildung bei *Cymbella*. Oesterreichische Botanische Zeitschrift 114(4):484-489 · August 1967

F. E. Round; R. M. Crawford; D. G. Mann (2007), Diatoms: Biology and Morphology of the Genera, Cambridge University Press; 1 edition (2007)

Automixis in *Cymbella aspera*

In July 2017 I isolated diatoms of the genus *Cymbella* found in a pond in Hohenheim (Stuttgart, 48°42'34.0"N 9°12'29.1"E). The size of the diatoms at the time of isolation was about 240 µm and was in the range of 150 to 180 µm after 5 months in culture.



The picture of a valve with a length of 151 µm can be seen in the upper half of the picture on the left. I assume it is *Cymbella aspera*.

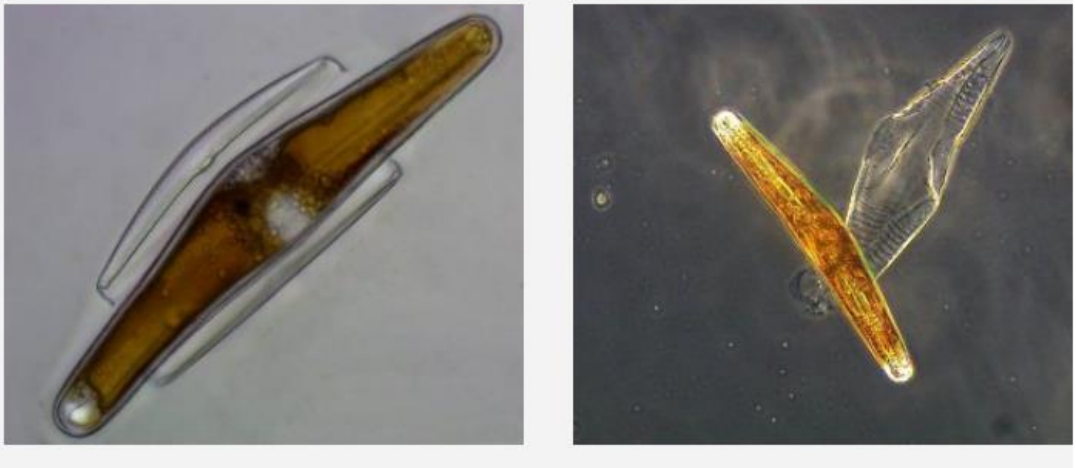
At several locations in a culture dish, sexual reproduction began at this diatom length. Auxospores with attached valves and initial cells were found.

The size of the initial cells was not uniform. They had a length between 270 µm and 302 µm, whereby a length of 280 µm was very common. These first cells were considerably longer than expected for this species. The ratio of length from the initial cell to a small cell showing sexual reproduction is approximately 1.8 to 2. In the picture on the top left below the

small diatom a large diatom (length 270 µm) is shown, which originates from the first culture, which has developed from an initial cell. So it is at most a few generations away from the initial cell.

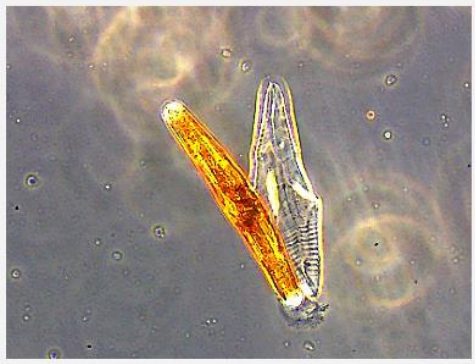
A typical image of a culture is shown in the image on the left (click to enlarge). Here you can see in several places evidence of sexual reproduction. A closer look reveals that each auxospore comes from only one cell. In a single diatom, two gametes are formed by meiosis, which then fuse to form a zygote (auxospore). It is a self-fertilization, or more precisely automixis. The valves of the diatoms open and an auxospore grows up. Automixis in *Cymbella aspera* was described by Lothar Geitler in "Automixis, Geschlechtsbestimmung und Pyknose von Gonenkernen bei *Cymbella aspera*" (Planta 1956, Vol. 47. pg. 359-373).

The auxospore is enclosed in a structured membrane, the so called perizonium. Inside the perizonium the auxospore grows and develops new frustules. The frustules of the original diatom stick to it from the outside. Finally, a finished diatom of maximum size leaves the perizonium. An almost finished initial cell can be seen subsequently on the left (click to enlarge). The cell has still not left the perizonium yet. In the picture to the right of it this already happened (click to enlarge).



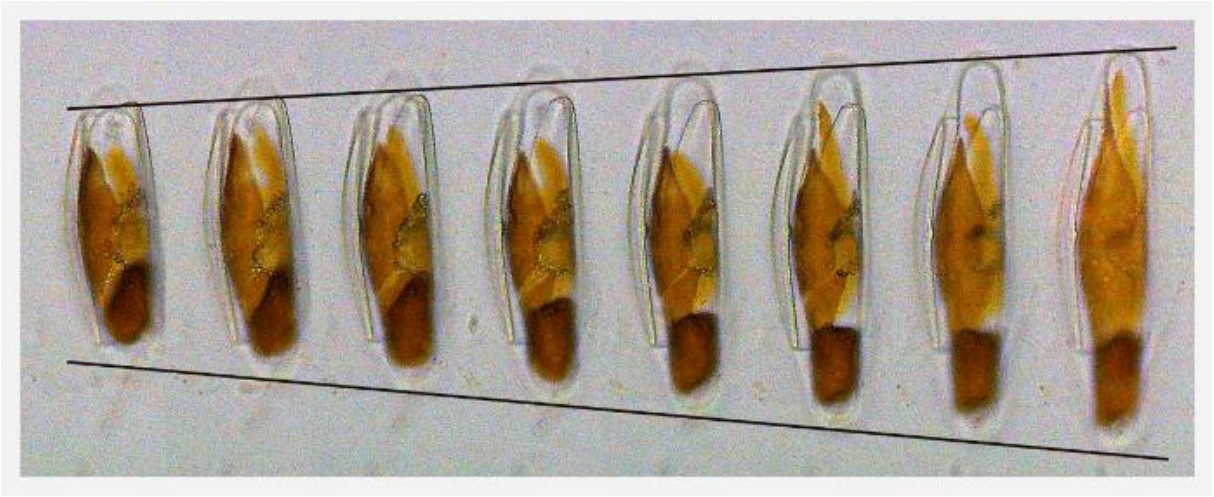
The structure of the perizonium is clearly visible in phase contrast, but not in the brightfield. The following picture shows the brightfield and phase contrast alternately (2 seconds and 5 seconds).

In culture it turned out that the gametes often died after their formation and there was no fusion of the gametes. This happened far more often than the successful formation of an initial cell. Some of the initial cells also proved to be not viable or not cultivatable.



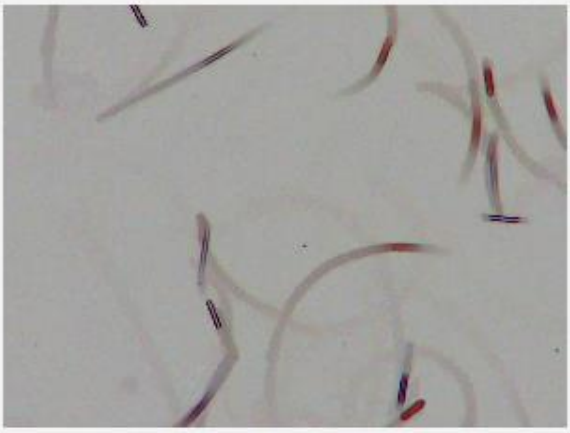
The continuous observation of the formation of an auxospore turned out to be difficult. In both *Cymbella cistula* and *Cymbella aspera*, it was striking that auxospores often died during several hours of observation. I suspect that auxospores have a very high sensitivity to high light intensities as they are typically used for microscopic observation.

A video of a growing auxospore can be seen on the left. The shown fragment is taken from a long-term observation and extends over a period of 84 hours. To protect the auxospore, the intensity of the light was kept extremely low, which leads to a strong image noise. In the beginning, the day-night rhythm was maintained as in cultivation. The nights are indicated by a short dark period in the video. After the second phase of darkness it became clear that the development had progressed in darkness. After that no dark phase was used. As the diatom and the auxospore do not lie parallel to the bottom of the petri dish, the lower part of the diatom is out of focus (objective with 20 x magnification). In the following picture, single images are placed next to each other with a time interval of about 4 hours (250 minutes):



The superimposed lines show that the auxospore grows linearly over time within the limits of the image resolution. The growth rate was about 3.4 µm per hour.

Cymatopleura solea
(60x time lapse)

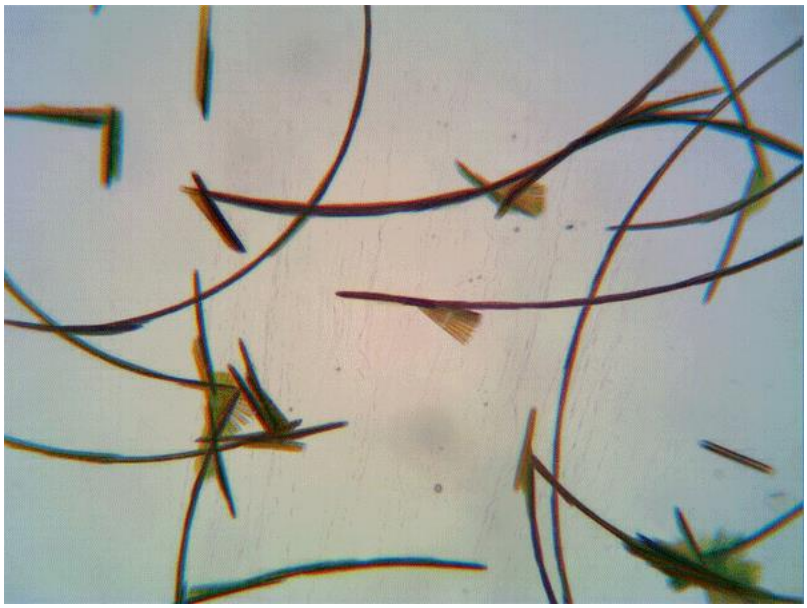


Cymatopleura solea "motion blur"-Filter
(60x time lapse)

Forms of the paths

When observing the movement of the diatoms with the view from above onto the substrate on which diatoms move, then one often notices almost circular, spiral or straight paths, which are interrupted again and again by reversals of direction. As mentioned earlier, a back-and-forth jerking or pivoting movement takes place around large angles, depending on the species. Particularly in small diatoms, in combination with the linear movement, a wagging occurs around the direction of movement.

An example of such paths is shown in the video above. On the left, the movement of some *Cymatopleura solea* (Length: approx. 180 µm) in a still sparsely populated petri dish can be seen in 60x time lapse. The video to the right was made by using VirtualDub, whereby the paths were highlighted by the 40-times application of the filter "motion blur".

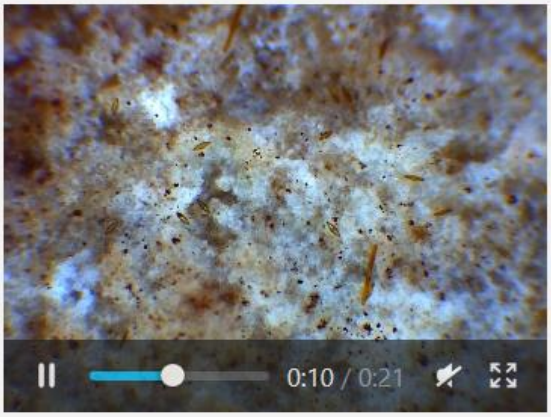


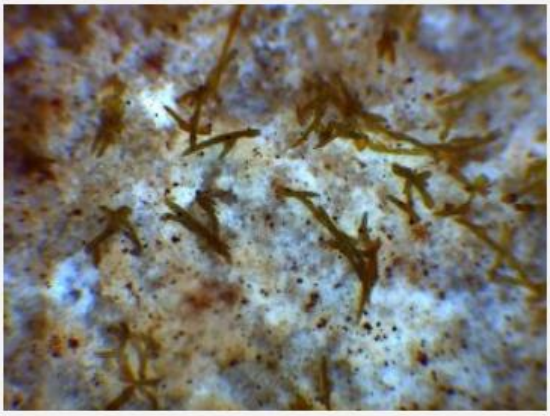
The animated picture on the left shows how perfect a circle is sometimes passed through. In the picture the paths of several *Nitzschia* of the same species are marked by red dashed circles. The circles have different radii. The largest has a radius of slightly less than a millimeter.

These regular paths are an artifact under laboratory conditions, because here the diatoms move on smooth surfaces. In nature the substrate consists, for example, of a rough surface of a stone or of rugged plant surface with a complex structure. Even before the irregularities in thickness of the substrate reach the order of the height of

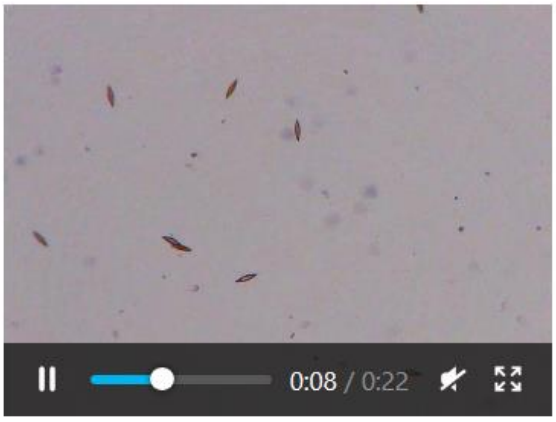
the diatom, the path is significantly disturbed. On precisely circular paths, reversals of direction alone would not make it possible to change the position beyond the circle. Nevertheless, the recordings of the motion on smooth substrates are of particular interest because they are almost free from disturbances and allow conclusions on the mechanisms of motion.

The picture on the left shows the paths of unknown diatoms. Here a small pebble from a brook was observed in reflected light. From a short video a typical image of a motion path in nature resulted from the minimum of all frames. One only recognizes rudimental circular paths. Nothing is known about the species and it is unclear how the corresponding motion would look on a smooth substrate. This situation can easily be simulated with a known species from a culture. For this purpose, a sterilized flat pebble was placed in a petri dish with nutrient solution. Afterwards, it was inoculated with a few clearly distinguishable species. After a few days the following video was recorded, in which one sees in particular all *Craticula cuspidata* (Length: approx. 120 µm) in motion. The recording time was 600 seconds. On the right, you can see the paths that were calculated from the video (click to enlarge).





For comparison, a very sparsely populated culture was filmed in a petri dish over the same period under the same light conditions. The video and the paths are shown below.



Although such experiments show strong variability, the influence of a rough substrate is unmistakable.

It should be noted that there are also cases where movement is restricted by colony formation. For example, *Bacillaria paxillifera* can only move relative to their adjacent diatoms, which leads to a movement of the entire colony (see video). The question of the path curvature of a diatom does not arise at all.

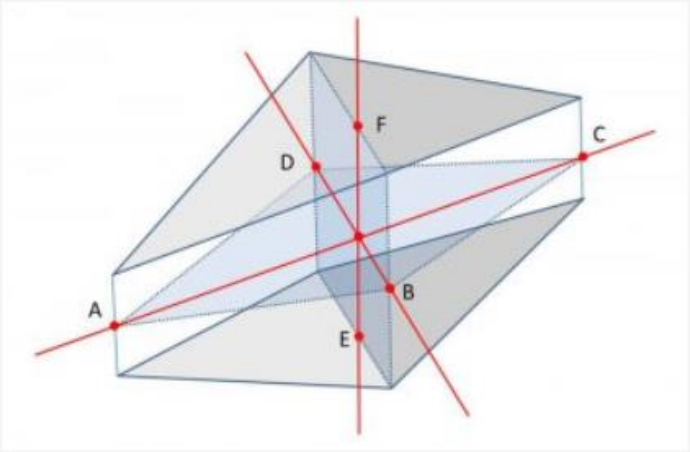
Many motile diatoms do not show any motion as long as they are bound in colonies, but they can move as individuals or in small groups (see *Eunotia* and *Cymbella*).

Another example are tube-dwelling diatoms as seen in a water sample from a small pond in the video on the left below (unfortunately, cultivation of these tube-dwelling *Cymbella* was not successful). The degrees of freedom of movement are restricted by the tube and the distance that can be covered is narrowed by the positions of the neighbouring diatoms. Tube-dwelling *Frustulia vulgaris* from the river Neckar (locality: Lauffen am Neckar) can be found in the video to the right. The high elasticity of the tube is striking.

Tube-dwelling *Cymbella*
(60x time lapse)

Tube-dwelling *Frustulia vulgaris*
(60x time lapse)

On the following pages the kinematics, i.e. the formal description of the motion paths, and the boundary conditions for the analysis will be discussed. Afterwards, the visualization and the tracking of the trajectories will be briefly presented. Analysis methods and results of the analysis follow in the last chapter (see menu on the left).



Axes and planes of a pennate diatom

- A-C apical axis
- B-D transapical axis
- E-F pervalvarous axis

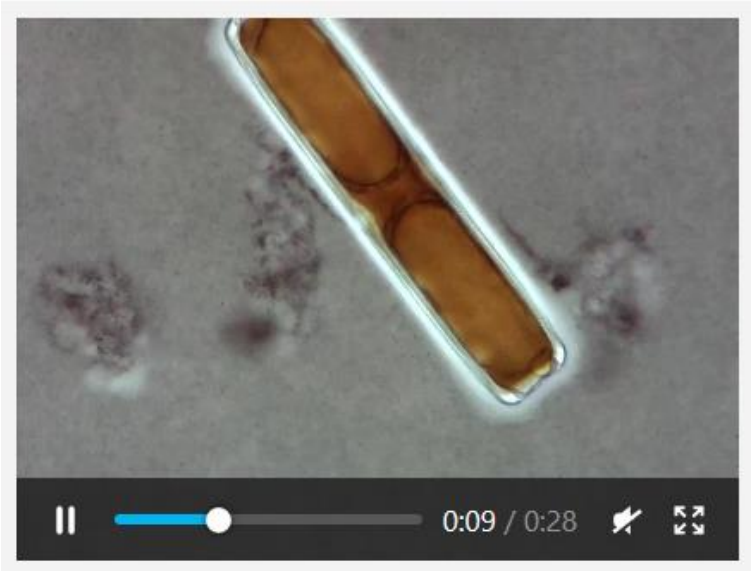
- ABCD valvar plane
- BFDE transapical plane
- AECF apical plane

Description of the trajectories

The use of video recordings for observing diatoms was already discussed in detail in Lesley A. Edgar (1979) and Ayumu Murasea et. al (2011). In both publications, diatoms are treated as point-shaped objects whose position can be described by the coordinates in the plane. In view of the sometimes complex movements of the diatoms, a complete kinematic description would have to capture the position of the diatoms in space. The basic axes and planes of a pennate diatom are schematically shown above (click to enlarge).

The ends of the diatoms (apices) lie at the points A and C. The valves were deliberately not represented as planes, in order to indicate that there is not necessarily only a single stable position in which the diatoms lie on a valve.

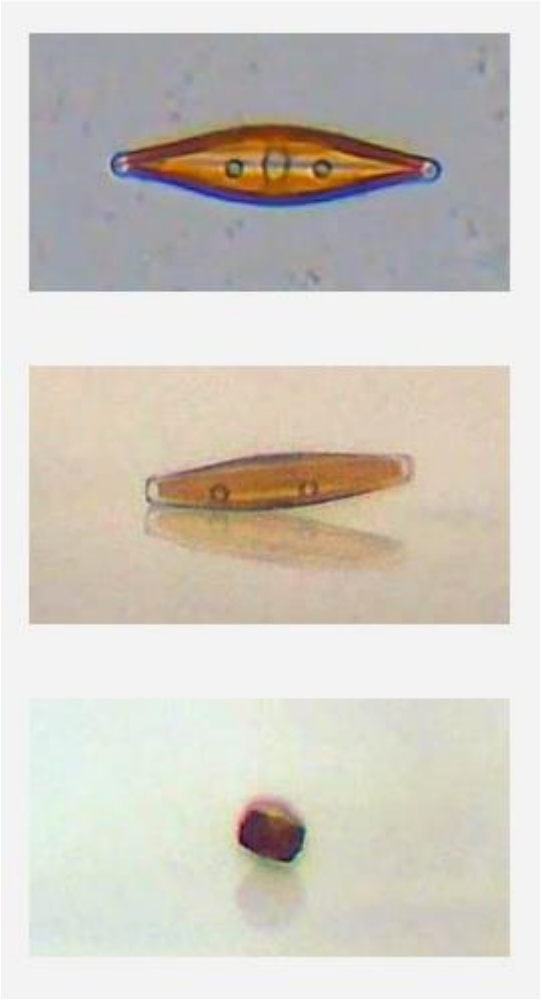
The next section is restricted to trajectories or sections of trajectories in which the diatoms do not erect, nor change between valve and girdle views. In the observation period, the valvar plane or the apical plane should lie in a good approximation parallel to the substrate.



Some diatoms (*Navicula*) are usually observed in valve view, some mostly in girdle view (*Nitschia sigmoidea*). Others often alternate between these positions, such as *Cymatopleura solea*. The ability to move can be significantly different in these two positions. Diatoms, which lie on a girdle face, can move freely provided their raphe is in mechanical contact with the substrate. If the raphe does not have this contact, as in the case of *Pinnularia*, only back and forth movements are possible in which a lump of extracellular polymeric substances (EPS) adheres to the substrate as well as to the raphe. The lump acts as an artificial substrate and couples the diatom to the substrate (MA Harper & JF Harper (1967)). In the 16x time-lapse video on the left you see a

diatom of the genus *Pinnularia* (Length: approx. 220 µm) in valve view, which moves by means of two lumps of mucilage. These were made visible with Indian ink. The marking with ink also clearly shows the activity of the raphes. This old method will be discussed elsewhere on this site.

The requirement that the valvar plane or the apical plane is parallel to the plane of the substrate is rarely met perfectly. The small pictures of *Craticula cuspidata* show an example of deviations from this condition.



The first picture on the left shows the typical valve view, which is taken up from the vertical direction onto the substrate, i.e. perpendicular to the valvare plane. When *Craticula cuspidata* moves with a uniform motion, it is raising as is seen in the second picture when looking perpendicular to the apical plane. You see the diatom from almost horizontal direction as it is moving to the right, quasi "from the side". As its mirror image can be clearly seen on the substrate, the twice angle of inclination against the horizontal can be easily measured. In this case, the inclination is about 7.5°, but even 10° is not unusual. The observed length in valve view then appears foreshortened by the factor of the cosine of the inclination angle. At this small angle this results in less than 1% shortening, which is not relevant in the valve view and meets the given boundary condition sufficiently. There is a separate contribution about observation of *Craticula cuspidata* from a horizontal perspective.
The third picture shows the diatom viewed perpendicularly to the transapical plane. It moves toward the observer. Here, in the video from which the picture was taken, different lateral inclination angles in sequence can be observed. If the diatom rotates 90 ° around the apical axis, it is lying on the girdle band. Such tilting may occur at the points of movement reversal during movement and as a result of collisions between diatoms. This is to be excluded within the analyzed section of a trajectory.

Formal description of the trajectory

For the mathematical description of the trajectory, the following coordinates are appropriate under the constraints described above (diatom lying on a valve with low inclination to the substrate):

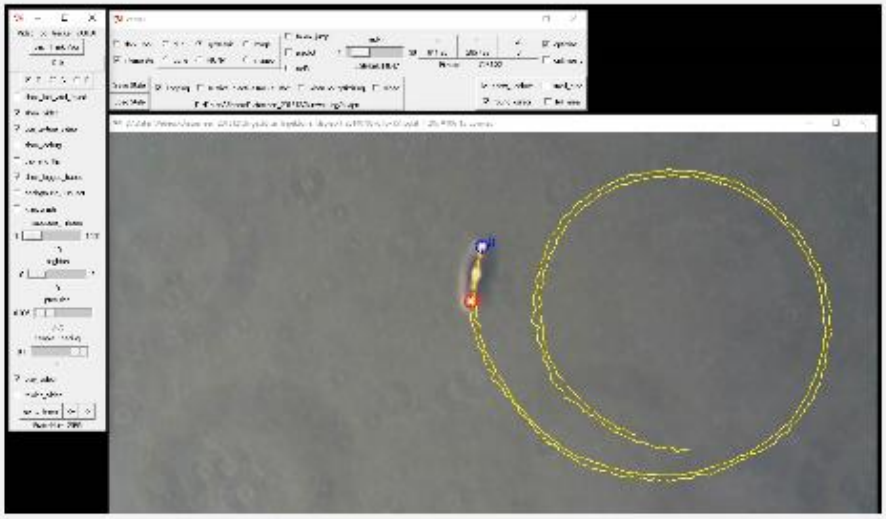
- coordinates of the center of the diatom and the direction of the apical axis
- coordinates of the two apices

The choice of the cartesian coordinate system results in practice from the video recording.

Ayumu Murasea, Yosuke Kubotaa, Shigeyuki Hirayamaa, Yoshikazu Kumashirob, Teruo Okanob, Shigeki Mayamac, Kazuo Umemura (2011) Two-dimensional trajectory analysis of the diatom *Navicula* sp. using a micro chamber, Journal of Microbiological Methods, Volume 87, Issue 3, Pages 316–319

Lesley A. Edgar (1979) Diatom locomotion: Computer assisted analysis of cine film, British Phycological Journal, 14:1, 83-101, DOI: 10.1080/00071617900650111

M.A. Harper & J.F. Harper (1967) Measurements of diatom adhesion and their relationship with movement, British Phycological Bulletin, 3:2, 195-207, DOI: 10.1080/00071616700650051



On the left you can see the "Video Spot Tracker" at work. The program is tracking a trajectory of *Navicula* sp. In this time-lapse, playback is performed at double speed.

(*Navicula* sp. was isolated by Mr. Oliver Skibbe and provided in form of a pure culture)

I like to thank Computer Integrated Systems for Microscopy and Manipulation (CISMM) at UNC Chapel Hill, supported by the NIH NIBIB (NIH 5-P41-RR02170) for free of charge use of Video Spot Tracker.

Motion Tracking of Diatoms

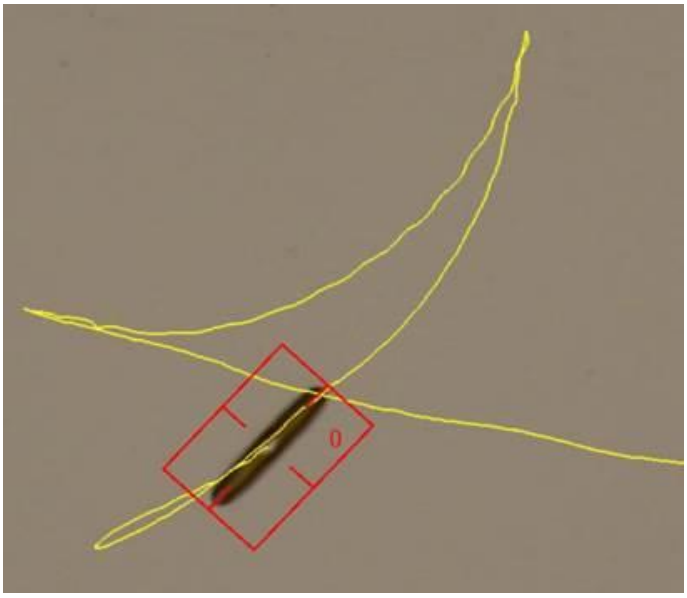
The visual tracking of movements gives only a rough impression of the pathways of the diatoms, especially when they are moving slowly. Some movements can only be recognized in time lapse.

It is helpful to combine the frames of a video recording or a picture sequence into one picture. If the diatoms are darker than their surroundings, one can achieve this by calculating the minimum. If they are brighter than their surroundings (dark field) the maximum is calculated. This can be done very easily and fast using the CISMM Video Optimizer. Information about the dynamics of the movement is thereby lost.

The possibility to get an impression of the sequences by using the filter "motion blur" in VirtualDub has already been pointed out. This is visually appealing but has hardly practical benefits.

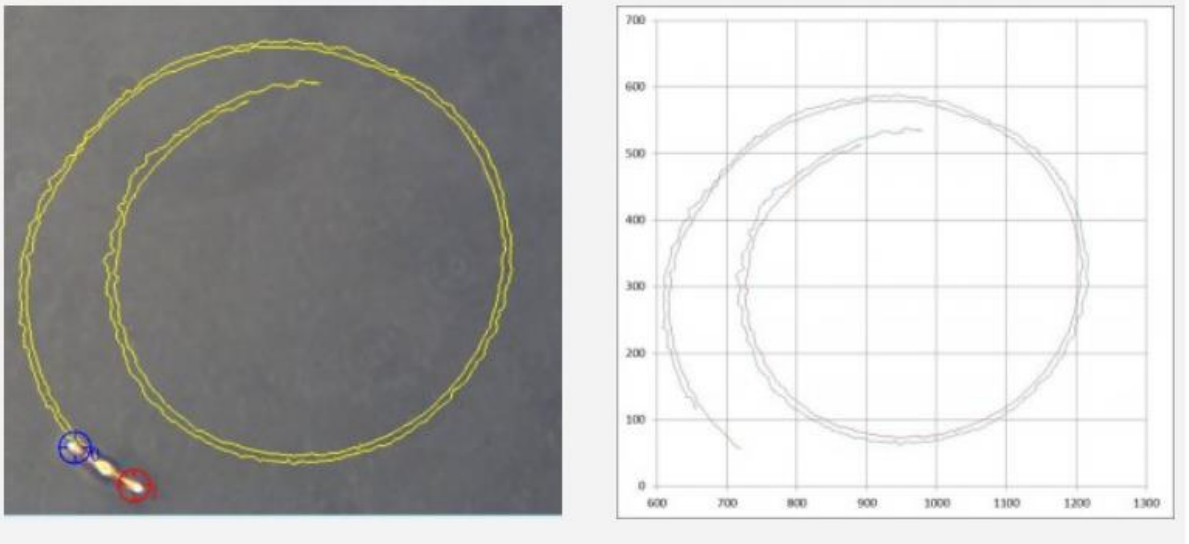
Motion tracking of Diatoms helps in tracking the movement on the basis of a video recording or a sequence of images. This is not primarily about the visualization of the trajectory but a quantitative determination of the position information, which allows numerical analysis.

For motion tracking the Video Spot Tracker was used. It provides circular and rectangular trackers (called rod).



If circular trackers do not adhere stably to the apices of the diatoms, even if the parameters are favorable, the change to a rectangular tracker often helps. For *Navicula* and others, it has proven useful to record videos in the phase contrast, because here the diatoms get a highlighted apex. The Video Spot Tracker displays the traces of the trackers in the video and saves the recorded coordinates to each frame in a csv file (comma-separated values). This can be imported and analyzed, for example, in MS Excel.

For a rectangular tracker, only the trace of its center is displayed (see picture on the left). However, because the orientation of the tracker is also outputed, the coordinates of the apices can also be determined by knowing the length of the diatom. The analysis can then be carried out in the same way.



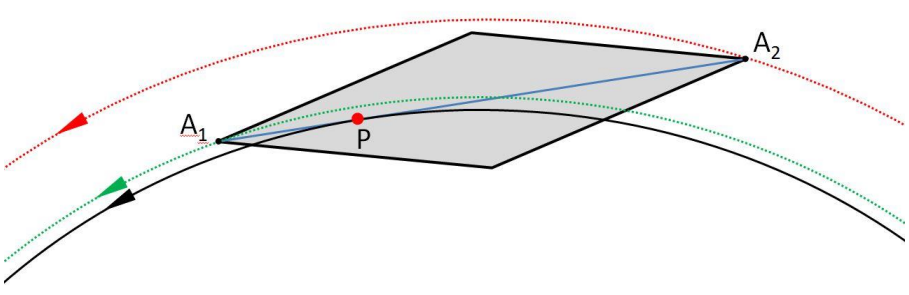
Analysis of trajectories I

On the previous page the tracking of a trajectory of a *Navicula* using the Video Spot Tracker was shown. The result as a screendump can be seen in top left image. If you import the coordinates into an Excel sheet, you can generate this as a diagram as shown in top right diagram (click to enlarge). As the Video Spot Tracker places the origin of the coordinates in the upper left corner of the video and the positive axes pointing to the right and down, the trajectories are mirrored.

The trajectory consists of a long spiral curve. According to Round et. Al. (2007) *Navicula* spp. move on a straight line, as their raphe is not curved. They never did it in my observations. The following characteristics of the trajectory I regard furthermore as remarkable:

- The leading apex describes a curve that is closer to the center of curvature than the trajectory of the trailing apex.
- The trajectories are not entirely smooth, with the inner curve having minor fluctuations. Admittedly, a non-well-positioned tracker leads to statistically fluctuating local coordinates, but these recorded fluctuations mainly describe the movement of the cell.

In diatoms such as *Navicula*, the raphe system is located in a narrow region around the connecting line between the apices. Except for the distal end, the raphe has such a small curvature that tangents to the raphe lie in a good approximation parallel to the connecting line through the apices. A hypothesis for the different paths of the apices is that there is a point P on the raphe, so that the diatom (more precisely, its apical axis) moves in a good approximation tangentially on the path of motion of this point. The following drawing shows this assumption:



The observed paths are shown in red and green doted lines. If the hypothesis is true, the path of the diatom (except for fluctuations) can be limited to the black trajectory, which the point P passes through. The use of a coordinate pair is sufficient then.

If the two radii of the curvature of the observed trajectories are determined from a short path segment, the distance from P to A_1 can be calculated with elementary geometry. In view of the inaccuracy of the determination of the radii on a short curve segment and the stochastic effect, this simple approach has not proven itself.

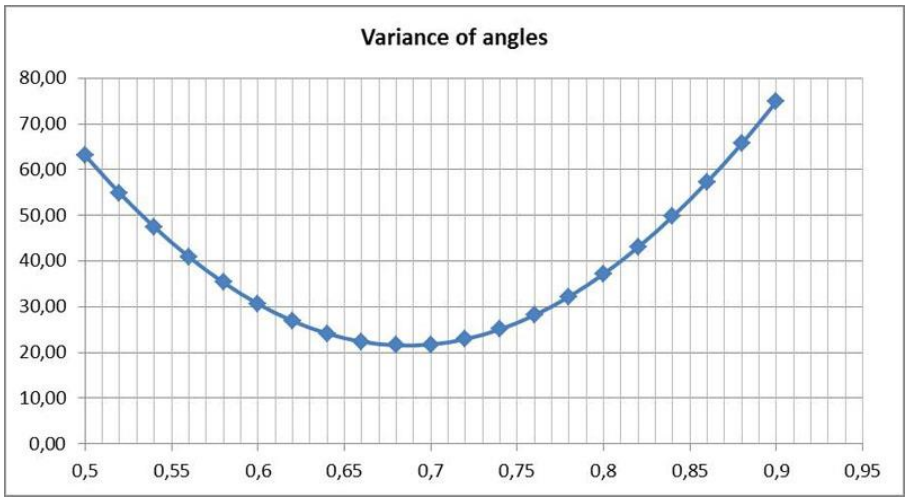
It is much more precise to determine P by making an assumption of its position, and then evaluating the angle between the tangent to the trajectory of the hypothetical P and the apical axis along the whole available trajectory. The sum of the squares of the angles between the tangent vector and the apical axis is very suitable for this purpose. The required P minimizes the mean squared deviation (variance) of these angles. By varying the position of the assumed point the minimum is quickly found.

Incidentally it is also possible to use the summed scalar products between tangent vector and apical axis as criterion.

The partly strong stochastic fluctuations prove to be cumbersome. Therefore the curve was smoothed tangentially and transversally by an FIR filter (low-pass).

By means of simulated, artificially disturbed curves, one can check whether the method provides valid results. It has been found that the analysis yields reliable results.

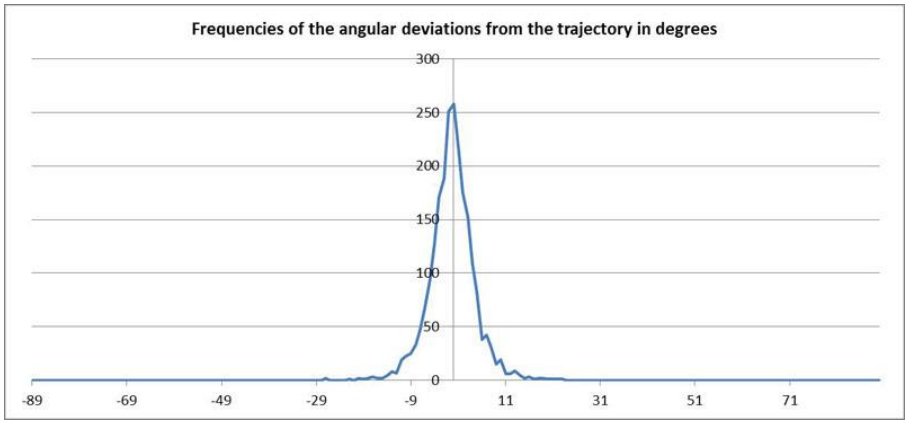
For the specific path curve presented here, the following graph shows the variance of the angles plotted against the position of the assumed point P:



The positions of the trackers are used as a reference for the position of the point P. The value 0 corresponds to the trailing tracker, the value 1 to the leading tracker. At 0.5 the point P lies exactly between the trackers. As the trackers do not sit exactly on the apices, but are slightly shifted inwards, a correction has to be done. On the normed segment A_1A_2 the maximum lies at about 0.64. The point P is located on the side of the apex in the direction of movement, as it is also shown exemplary in the above sketch. As a trajectory of the diatoms, I denote only the path of the point P.

The analysis of other trajectories of the same species often showed similar values. The values for 10 analyzes were between 0.58 and 0.77. Two other species of *Navicula* yielded somewhat lower values.

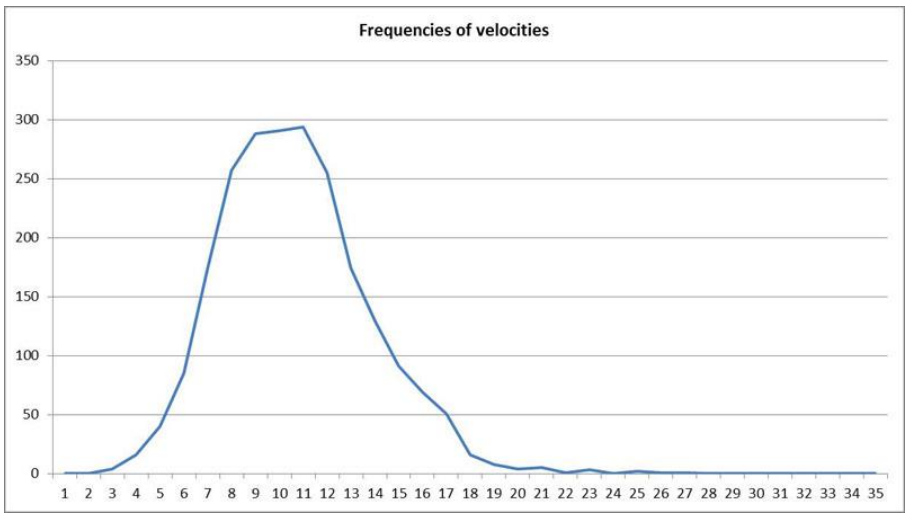
As the orientation of the diatoms varies around the direction of the tangent to the trajectory of P, the frequency of the angular deviation can be represented in form of a histogram:



The measured value for P was used here. If the position of P is incorrect, this frequency distribution is not symmetrical to the origin.

The existence of the point P with the described relationship between tangent to the trajectory and orientation of the apical axis is heuristic and should be viewed critically. On the other hand, it has proved its worth in the studied *Navicula* spp.

In the subsequent analysis the distribution of the velocity (measured µm/second) can be seen:

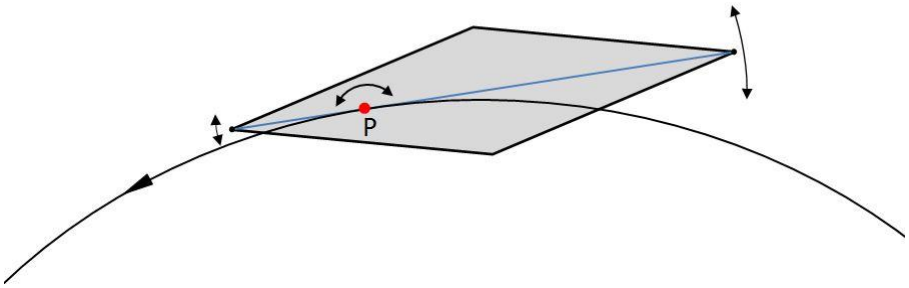


The mean value here is 10.16 µm/s and the standard deviation is 3.07 µm/s. It facilitates the imagination if we relate the velocity of diatoms to their size by measuring in the unit “bodies per second”. In this case the value is about 0.25 bodies per second. The speed can be very different even for the same species. For *Navicula* the observed range is from a few µm/s to almost 20 µm/s. It turns out that these values strongly depend on environmental influences such as the light field intensity. Some species become mobile only in daylight, others also move during the nighttime.

Analysis of trajectories II

In the previous chapter it was shown that the motion of a *Navicula* on a smooth substrate can be described by the trajectory of a point P to which the apical axis of the diatom is tangential. In addition a more or less strong “wagging” occurs around the direction of movement.

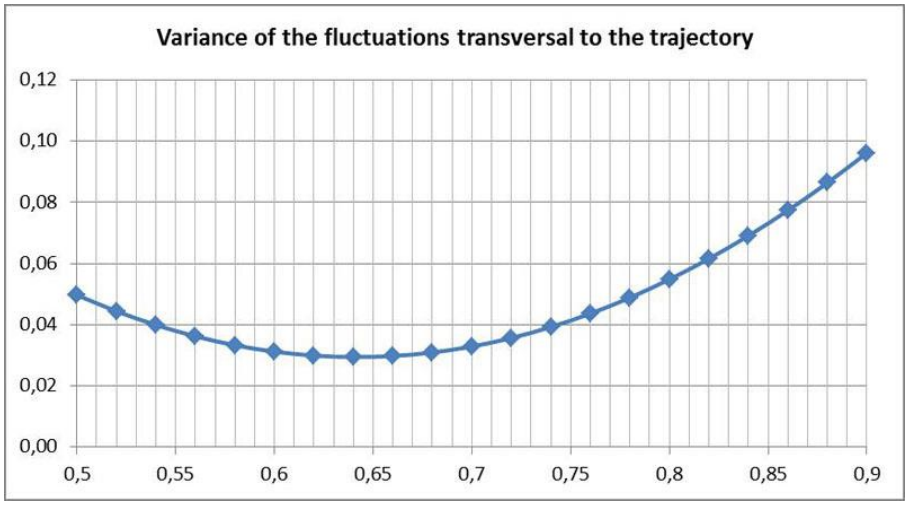
As mentioned at the outset, it is striking that in this example the path, which the leading apex describes, looks smoother than the outer path of the trailing apex. The following drawing shows that this is an immediate consequence of the position of P:



The closer the point P is to an apex the smoother its trajectory and the bumpier the trajectory of the opposite apex. If one follows the path of diatoms visually, one can often see with some experience which end shows the larger fluctuations and conclude on which side of the diatom the center of rotation P is located.

From the magnitude of the fluctuations the position of P can be determined in different ways. As the drawing shows, the amplitudes of the fluctuations relate as the distances of the apices to P. I didn't try to calculate P from the ratio of fluctuations of the movements of the apices, because I expected inaccurate results.

A more suitable alternative criterion for determining P is the magnitude of the fluctuations. As P is the point of rotation of the directional fluctuations, there should be no fluctuations in the case of uniform motion. Due to the fact that the velocity of the diatoms can change rapidly, it is convenient to consider only transverse fluctuations (perpendicular to the trajectory of P). It is therefore possible to use as a criterion that no or only slight transverse fluctuations should occur at the point P. In the following diagram the variance of the transverse fluctuations against the hypothetical values for P is plotted. This was obtained by comparing the observed motion to the smoothed curve (low-pass filtering):



Here, a somewhat smaller value is obtained. That means P is closer to the center. This is probably due to a lower accuracy of the method.

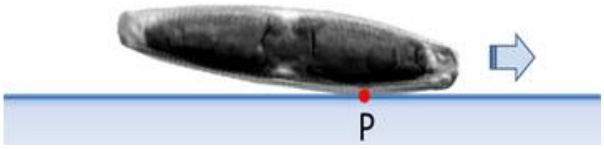
At this point an important remark is to be made. The definition of P using the requirement that the apical axis is tangent to the trajectory of P is fulfilled for straight paths for each point on the apical axis. It does not provide a criterion for the determination of P. However, a determination by consideration of the fluctuations remains usable. Basically a different definition of P is used here. Thereby, P is defined as the pivot point for the fluctuations, i.e., the deviations from a perfect tangential movement. The very restrictive conditions on the form of the raphe in the practical use of the earlier described tangent method do not occur. This method should therefore be preferred.

It turns out that some large diatoms have only a very small fluctuation around the direction of motion. If they move in a straight line, there is no specific point P. P is not needed to describe the trajectories of the apices, as their trajectories are identical and a pair of coordinates is sufficient to describe the path.

If the diatom moves only a short distance between two reversal points, this can prevent a determination of P. Here, an observation from a horizontal view helps.

Interpretation

Navicula and many other diatoms have no valve, which has a larger planar surface in the valvar plane. Such diatoms do not lie horizontally on a flat substrate. In case of Navicula there is a vaulted valve between the apices. Therefore, only a short section of the raphe is in contact with the substrate at a time. The point P lies in this contact area and represents the center of force of the drive.

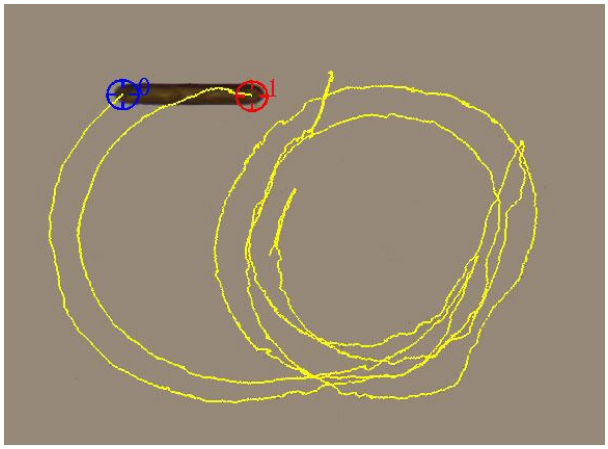


As already mentioned, this is proven by images which are made perpendicular to the apical plane (side view), while the diatom with the raphe touches the substrate and moves. The inclination angle of the axis of the apical axis to the substrate, and thus also the position of P, will typically be reached after a certain time from the last reversal point and fluctuate to a certain extent during the movement. The behavior in the vicinity of the reversal points and the observation in side view will be discussed elsewhere.

For all four observed species of Navicula, the point P was on the side of the leading apex. Accordingly, this apex was closer to the substrate and one can say that the diatom is pulled. In case of other diatoms the point P is closer to the trailing apex so that they are pushed. An example is the movement of Craticula cuspidata. Here, the position of P values was around 0.2.

In the general opinion the curvature of the trajectory is produced by the curvature of the raphe (Round et al., 2007). This sounds plausible, at least, for the diatoms, which rest at a short section of the valve. It is only necessary to consider the curvature of the raphe at point P. If the radius of the path is large compared to the length of the diatom, this means an almost inconspicuous curvature of the raphe. Images of Navicula show that there is a curvature in the correct order of magnitude at P. Even a raphe which is straight at first sight often has a sufficient curvature for macroscopic circular paths. I think it is also conceivable that spatial irregularities in extruded EPS influence the path. Even in the case of Craticula cuspidata one can occasionally observe curved paths.

The curvature of the raphe at the point P is presumably not always solely responsible for the size of the paths' radii. If the valve rests flat on the substrate, the raphe system contributes to the locomotion as a whole. In addition, the friction force of the valve on the substrate must not be neglected. P can then no longer be interpreted as the center of force.



An example of this is an observed species of Pinnularia. One recognizes that the diatom is drawn, the force coming from a region near the leading apex. The trajectories are not everywhere smooth, but an evaluable fluctuation around the direction of movement did not occur (see picture on the left). As Pinnularia has a fairly flat valve, the diatom rests level and a simple mechanical interpretation of P as the center of the driving force is not meaningful. Also the influence of the friction is probably essential. In addition, Pinnularia is often surrounded by a thick layer of mucilage, whose role is not clear with respect to motility. The Raphe has a complicated structure. It is almost closed over a long distance. I assume that for the movement of Pinnularia the tongue-shaped end of the raphe, the so-called helictoglossa, is of particular importance. It could be essential to the position of P near the apex.

For the clearly visible circular paths that e.g. Nitzschia, Cymatopleura and Rhopalodia species exhibit, friction could play an important role. When the raphe is in contact with the substrate, it is not positioned in the center. The friction forces and driving forces act at different points of the valve and could contribute to the formation of the circular paths.

It should not be concealed that the model presented is suitable above all when certain conditions prevail. Only in the case of well-trackable, sufficiently long and smooth trajectories a meaningful analysis is possible. It was pointed out that a simple interpretation of the observations requires a small contact area on the raphe. Some species show irregular patterns of motion that cannot be covered by these methods. One example is the Cymatopleura elliptica (see video on the page about creating cultures and care).

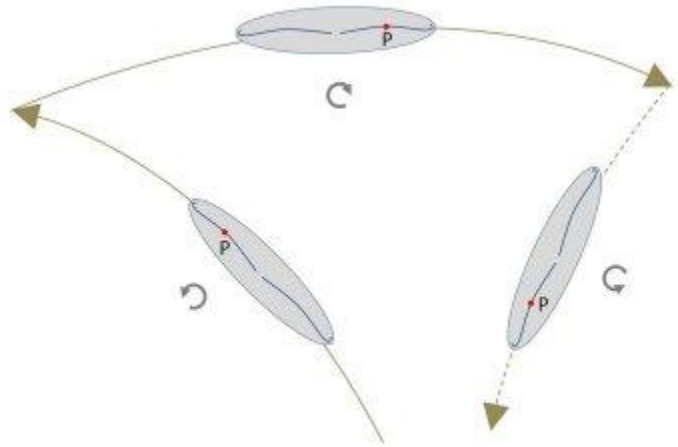
F. E. Round; R. M. Crawford; D. G. Mann (2007), Diatoms: Biology and Morphology of the Genera, Cambridge University Press; 1 edition (2007)

Curvature of the trajectories by the example of *Navicula*

On this page we will discuss how the curvature of a path changes when the movement reverses. It is not about the question of the absolute value of curvature, but the question of the direction of curvature, whether it is positive or negative. The curvature of the path of a diatom is positive when it bends to the left for someone who is moving with it. With respect to the center of curvature, the diatom then moves counterclockwise. However, if the curve bends to the right, the curvature is negative. Correspondingly, the diatom moves clockwise.

An important prerequisite of the following considerations is therefore that the trajectories exhibit clearly recognizable curved and smooth path segments. Diatoms like *Cymatopleura elliptica* do not meet this requirement (see video on the page about Creating cultures and care). In diatoms that cover only short distances or frequently collide with other diatoms, the curvature is often not observable. In addition, only paths where the raphe is in contact with the substrate should be considered. Movements such as at *Pinnularia* in girdle band view (see video on page Description of the trajectories) are excluded. Furthermore, only path sections with "uncomplicated" changes of the direction of the movement are considered. In particular, the diatoms should not rotate around the apical axis and should not straighten up. Many motile diatoms meet these requirements over a sufficiently long observation period.

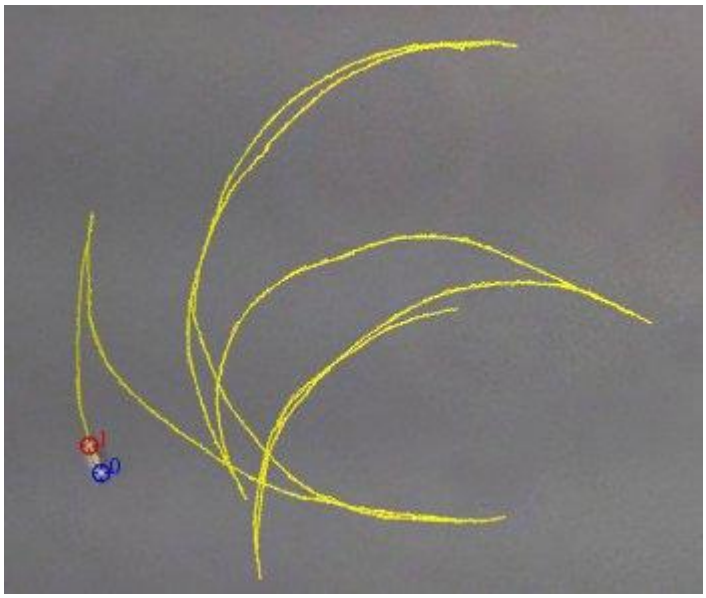
In the following, diatoms will be considered whose valves are symmetrical with respect to the transapical plane. This includes many genera such as *Navicula* or *Rhopalodia*. Sigmoid forms such as *Gyrosigma* are not treated here.



The drawing on the left (click to enlarge) shows the movement of a diatom where the valve is symmetrical with respect to the transapical plane. The partition into two raphe systems as shown in the figure and the changing position of the point P (see Analysis I) plays no significant role here. In addition, it is unimportant whether P is located on the leading or trailing apex side. However it is essential that the location of P changes only between points whose centers of curvature lie on the same side of the diatom. The curvature of the raphe is deliberately exaggerated in comparison with the curvature of the trajectory.

As already explained, it is assumed that the curvature of the path follows the curvature of the raphe. Consequently, the diatom moves in the first section of the path counterclockwise (positive curvature). From the viewpoint of a co-moving observer, the center of curvature is located to the left of the trajectory. Eventually, a reversal of direction occurs. At the same time the diatom usually rotates horizontally by a certain angle. In the second segment of the path, the diatom moves clockwise according to the curvature of the raphe (negative curvature). The center of curvature is now to the right of the trajectory from the viewpoint of the co-moving observer. After the next change of direction, the movement is counter-clockwise again. The rule is that the curvature changes its sign on every reversal. Clockwise and counter-clockwise rotations alternate:

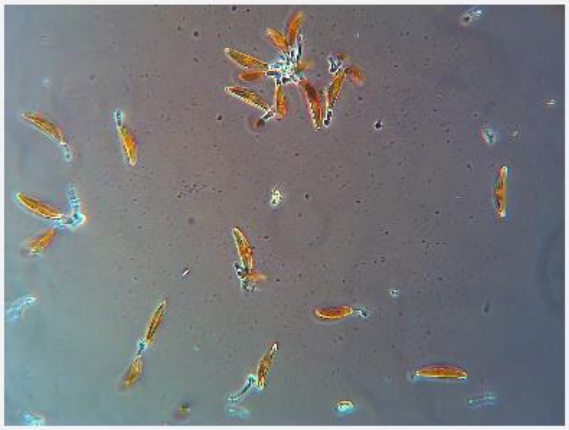
.... → + (counterclockwise) → - (clockwise) → + (counterclockwise) →....



If there were no horizontal pivoting movements at the reversal points, and if the radius of curvature remained constant, the diatoms simply behaved as a car, which changes the direction of travel when the steering wheel is fixed.

It turns out that these ideas often correspond to the observation. An example of accordance with the rule in case of *Navicula* is shown in the picture on the left (click to enlarge).

In four studied *Navicua* spp. (length range: 30-50 µm) and some species with similar regular movements the rule has proved its worth. These observations support the thesis that the curvature of the raphe in P determines the curvature of the path.

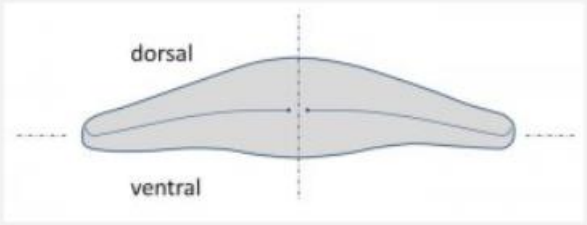


Paths of diatoms of the species *Cymbella cistula* (30x time lapse)

Visualization of the movement by calculating the maximum over all frames and removing the background (click to enlarge)

Curvature of the trajectories by the example of *Cymbella*

Diatoms of the genus *Cymbella* show symmetry with respect to the transapical plane, as assumed on the previous page. There is usually no symmetry of the valves with respect to the apical plane. One can distinguish a stronger arched dorsal and a weaker arched vetral side. The view on the valvar plane is sketched in the picture on the left. Incidentally, the two valves are not parallel. The typical *Cymbella* is thicker on the dorsal side than on the ventral (dorsiventral).

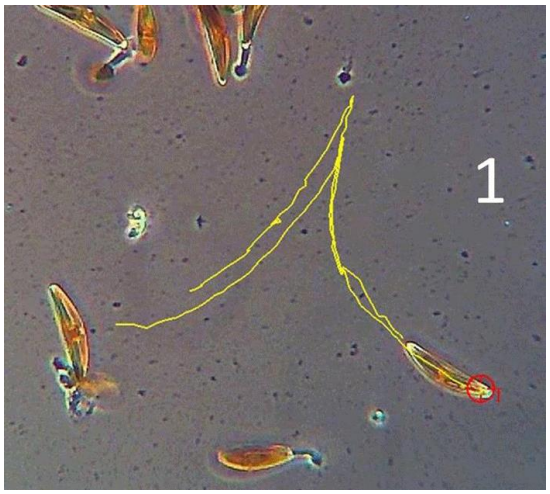


Most *Cymbella* spp. possess a distinct curvature of the raphe with a center of curvature on the ventral side, as shown in the sketch. A curved trajectory is therefore expected in which the centers of the curvature are also located on the ventral side. Actually, this is very often the case. A video of a cultivated species (Length: approx. 190 µm) can be seen on the left (30x time lapse). The video on the page for introduction into the movement and the associated visualization also show this curve direction of the path.

If the center of curvature of the path is always on the ventral side, the alternating sequence of clockwise and counterclockwise movements is given per se. If the path at the reversal points would not change the direction of curvature, the center of curvature would have to change to the dorsal side. The only difference to diatoms like *Navicula* is that one can recognize the curvature of the raphe already by the contour of the valve.

This would not be noteworthy if one did not also encounter a completely different movement. The video on the top left shows diatoms of the species *Cymbella cistula* (other posts provide information on size, sexual reproduction and formation of colonies) in which the center of curvature can be on the ventral as well as on the dorsal side. It often (but not always) keeps the sign of the curvature at reversal points (see diatom left). During movement in one direction a change in the curvature direction and thus a sigmoid path can occur in this species. In the video, the diatom on the left shows a half-turn around the apical axis at one location. Notable in this trajectory is the repeated change of the position of the center of curvature at the reversal points of the movement between the dorsal and ventral side. As a result, the diatom always moves with positive curvature. The movement was visualized in the image to the right of the video by calculating the maximum over the frames and and subsequent removal of the motionless background (using Fiji or ImageJ and manual correction). The uniform curve direction is marked by symbols.

It is obvious to carry out the analysis according to the methods of page analysis I in order to check whether there is a relation between the trajectory and the orientation of the diatoms. However it is important to remember that the assumption of a raphe near the connecting line between the apices is no longer exactly met. Therefore the behaviour will be illustrated on the basis of a few points of time first. In the animated picture on the left, several analysis steps are displayed at intervals of 5 seconds with periodic display. Only the first two segments of the trajectory from the video were used:



1. The trackers were placed close to the apices and the video was tracked over two segments. The first picture shows the result.

2. The images of diatoms at the same time intervals were copied over the trajectories.

3. In order to indicate the orientation of the diatom, line segments (in red) were drawn through the apices.

4. The images of the diatom were removed again so that the relation between the orientation of the diatom and the trajectories becomes better visible.

The first path segment is characterized by a center of curvature on the dorsal side of the *Cymbella*. It can be seen that the diatom orientation is in a good approximation tangential to the path of the leading apex (left ascending curve). Therefore, the model (see analysis I) is valid and the point P is located in the vicinity of the leading apex. So one can see that the diatom is drawn at a point near the leading apex and one may assume that here is a center of the force. The numerical analysis shows that this point is even a little outside the position of the tracker that means, almost at the apex. Looking closer at the diatom it is noticeable that the distal raphe ends are dorsally deflected. Some *Cymbella* sp. also shows hooked raphe endings. There is only a very small region of the raphe with a dorsal center of curvature. A picture of the valve of *Cymbella cistula* showing this curvature can be

seen in the section on sexual reproduction (left in the picture gallery). Similar to *Pinnularia*, this area of the raphe seems to be essential for the movement behavior.

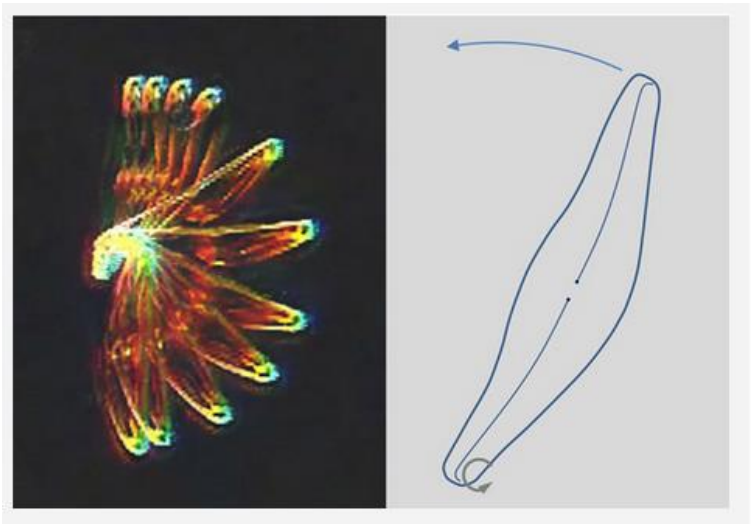
The direction of the activity of the raphe can typically change. Nevertheless, during the evaluation of the videos no movement could be found in which the *Cymbella cistula* is pushed from one point near the apex. Possibly the point P changes very rapidly its location when the direction of movement of the activity of the raphe is reversed.

In the second segment of the path a point P can only imprecisely be determined with the aid of the visualization. The proximate trajectories of the apices indicate a point P close to the proximal raphe endings. Because of the small distance between the proximal raphe endings it is not possible to assign it to any of the raphe systems. It could also be that a broad area of the raphe system is involved. With the presented methods this is not to be decided.

The rule of the alternating signs of curvature, which applies to *Navicula* and other genera, is not generally applicable because the point P can change between points on the raphe which have different directions of curvature.

Pivoting movements and direction of rotation at a dorsal center of curvature

In the video above, you see a *Cymbella cistula* on the right side which first moves on a small circular path with a center of curvature on the dorsal side. It then performs some pivoting movements in which the center of rotation is once at one end then at the other end of the diatom. Obviously, the radius of curvature of the movement at an apical center of force may be very different. This may be due to a more or less large contact area of the valve on the substrate, present mucilage or the activity of other raphe sections that are in contact with the substrate.



In an extreme case, the radius of curvature almost disappears and the diatom rotates horizontally about a pivot point near the apex by a certain angle. The raphe ending which is strongly curved or hooked towards the dorsal margin gives this species the ability to rotate on the spot. The picture on the left shows a pivoting movement by means of some superimposed images.

As a pushing movement from a point near the apex has not been observed, it is to be expected that the pivoting movement is always of such kind that the movable end of the *Cymbella cistula* moves in the ventral direction (see sketch). A diatome that moves along a small circle has the same direction of rotation. This direction of rotation is usually maintained, as one can recognise by viewing the pivotal movements in the video. After some observations however, it is obvious that there are also occasional reversals of the direction of rotation. But it does not change into a movement in which the diatom is pushed from the distal end of the raphe.

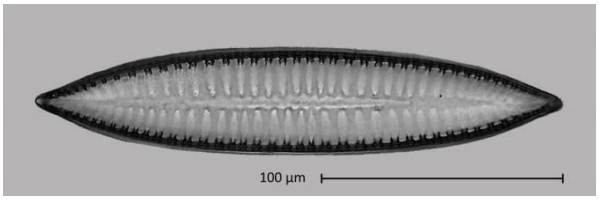
One obvious question is the influence of external conditions on the characteristics of the path. In the context of phototaxis it was shown that the times of the movement reversals depend on light conditions. It seems open to me whether there is also an influence on the change between dorsal and ventral center of curvature or the angle of rotation.

Surirella biseriata (600x and 700x time lapse)

Almost circular path (150x time lapse)

Curvature of the trajectories by the example of *Surirella biseriata*

In April 2017 I isolated a diatom of the genus *Surirella* in the creek Lein (48°53'04.6"N 9°38'29.2"E), the outflow from the Aichstruter reservoir.



The picture on the left shows the diatom in valve view. I assign them to the species *Surirella biseriata* (see http://cyclot.sakura.ne.jp/keisougazou/surirel/Surirell/sursei.html). The video above left shows an example of the movement of diatoms in a culture in 600x time-lapse. These diatoms usually move very slowly. In Petri dishes made of polystyrene, the fastest movements were around 2.8 µm per second. In order to cover their own length, which in my cultures was typically a bit over 210 µm, they needed more than one minute. Observations on vertical glass surfaces showed significantly higher velocities.

As the video at the top left shows, those *Surirella biseriata* often only move a little bit back and forth. Even in moderately densely populated cultures, a noticeable EPS film is formed on the substrate. Diatoms glide on it at the slightest movement of the petri dishes and their active mobility is limited. To observe the movement, it is therefore advisable to pipette a sufficient number of diatoms into a fresh petri dish and observe them there. In my opinion, longer pathways are also covered mainly by diatoms, which have not yet stored many reserve materials. For longer sections of the path without reversal of direction, a high light intensity does not seem to be favourable.

If the diatoms do not travel very short distances between reversal points, it is obvious that the trajectory is curved. Between the reversal points there is clearly always one of the raphes in contact with the flat substrate. As the curvature of the raphe is uniform on each side of the diatom, the curvature of the trajectory can undoubtedly be assigned to one of the two raphe sections between the apices. At the top right you can see an example where the diatom moves approximately on a

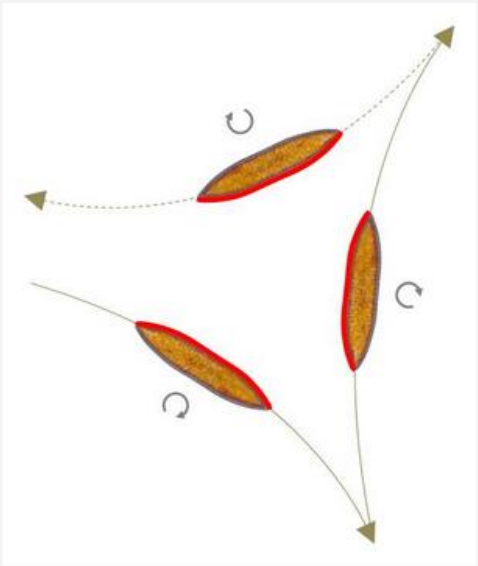
circular path. For purposes of clarity, a circle is shown. The video ends with a superposition of all frames. Apparently, the curvature of the path is determined by the exterior part of the raphe.

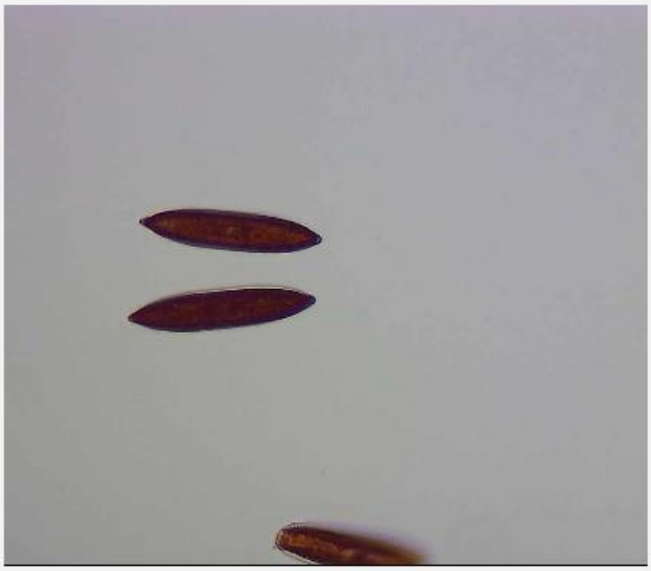
Surirella biseriata can be found in valve view (valve is uppermost) and girdle view (girdle is uppermost). When the diatoms rotate around the apical axis, which is often observed, they change frequently between these positions. Collisions between diatoms can also cause a change of position. Only the movement in valve view is to be considered, as here well observable and describable paths are run through. Particularly remarkable are the curvatures at the reversal points.
The prerequisites for an analysis of the tracks as presented in the post "Analysis of Trajectories I" are not given due to the strongly curved raphes on the outer edge. However, this geometry suggests a simple interpretation of movement patterns.

Reversal without changing the direction of curvature

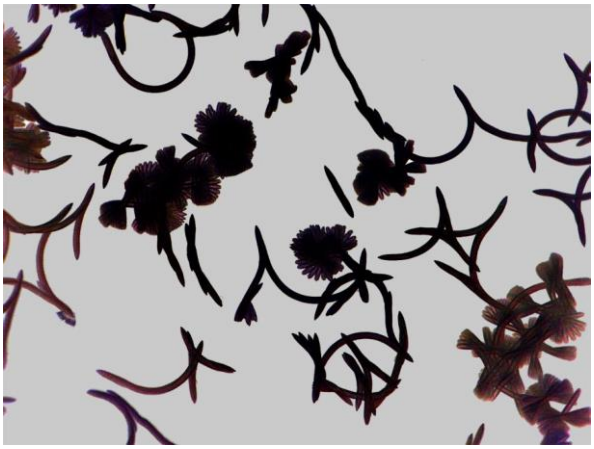
In this case, a diatom, which describes a path in a clockwise direction by viewing from above, continues to move in a clockwise direction after reversing. The same applies to the counterclockwise direction.

In the sketch below on the left side two such reversal points are shown in sequence. The raphe which is active in the sections is marked in red. It is in mechanical contact with the substrate.





From the trajectory nothing can be stated about the activity of the other section of the raphe-system. Obviously the side that is in contact with the substrate changes when reversing (tilting around the apical axis). In addition, the direction of the activity of the raphe sections is different, resulting in a reversal of movement. To the right of it is a 300x time-lapse video showing such a reversal.



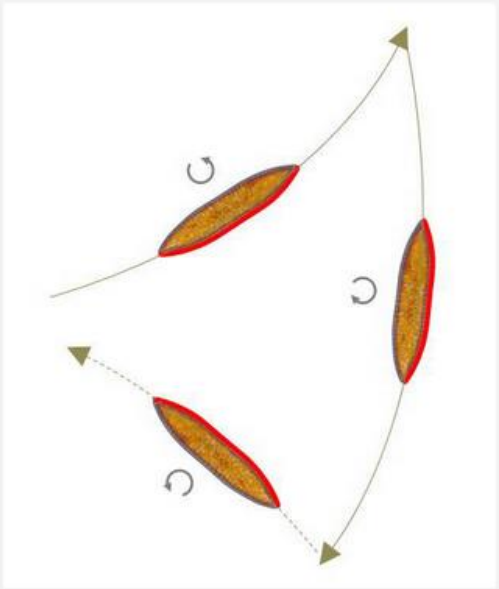
At the end of the short video, an image is displayed that shows the whole trajectory and that was created by superimposing all frames of the video.

This form of reversal with keeping the turning sense is very common. The picture on the left (click to enlarge) shows a superimposition of the frames of a video of the movement in a culture. In many places you can see the characteristic patterns of this behaviour.

It follows from this that very often the movement in the raphe halves is contrary. The activity of the entire raphe system is carried out in one direction of rotation, so to speak in a "circle".

Reversal with changing the direction of curvature

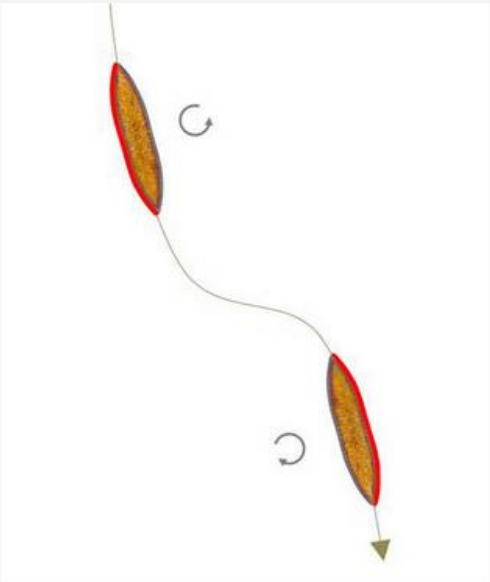
This form of reversal was presented as a typical form in *Navicula*. In *Surirella biseriata*, it is sufficient for the direction of the raphe's activity to reverse in case of continuous contact between the raphe and the substrate. The sketch on the left below illustrates the process.



In the video to the right (150x time lapse) you can see a reversal without changing the direction of rotation, as already introduced. Then the direction of the activity is changed. At the end of the video, the superimposition of frames is displayed again. I could observe this kind of change of direction only occasionally. This may also be due to the fact that such changes of direction occur in short succession, so that the curvature of the path between the reversal points is not recognizable.

Sigmoid trajectories

Round et. al (2007) are stating (p. 105): „Movement is directional, the path taken correspondingly fairly closely to the course of the raphe system (..) – curved where the raphe is curved (e.g. some *Nitzschia* species with eccentric raphe systems), straight where the raphe is straight (e.g. *Navicula*, *Pinnularia*), and even sigmoid where the raphe is sigmoid (e.g. *Pleurosigma angulatum*)." *Pleurosigma* and *Gyrosigma* have a raphe-system on each valve, which represents a sigmoid structure with the inflection point in the middle of the valve. A sigmoid path is run through when the centre of force changes from a section with a positive curvature to one with a negative curvature and the direction of travel is maintained. The same applies to the reverse change from negative to positive curvature. Apparently, it is not the position of the raphe-branches that is important but the existence of positive and negative curvatures. A sigmoid raphe-system is therefore not necessarily required. Therefore, the occurrence of sigmoid trajectories can be assumed in *Surirella biseriata*. A change of the mechanical contact to the substratum from one side to the other is sufficient, as long as the two raphe-branches work in the same direction as the sketch below left shows. The trajectory therefore has a inflexion point at the location of the change of contact.

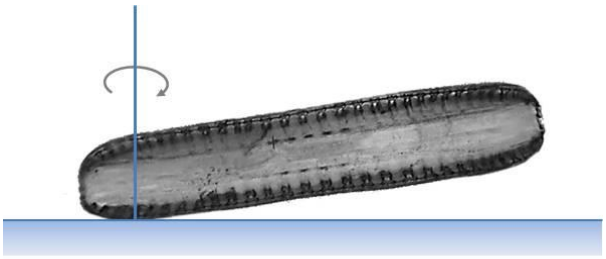


In the video next to it (900x time lapse) such a path followed by a superposition of all frames can be seen.

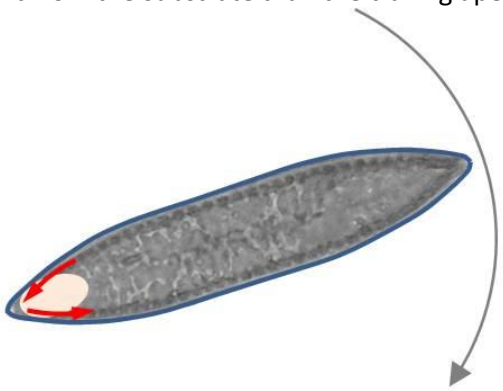
The reversal of the direction of movement described above without changing the direction of curvature of the trajectory (maintaining the sense of rotation) and the occurrence of inflection points are based on the change of the mechanical contact between the substrate from one half of the raphe to the other. Reversal with keeping the direction of rotation is much more frequent than the occurrence of inflection points. One can conclude from this that the activity of the raphe-segments on a valve after tilting is usually opposite and not parallel. By observing the trajectories, however, it is not possible to determine whether the activity of the raphe is uniform along the entire length between the apices and whether the direction of the activity already existed before tilting or whether it took the observed direction with tilting.

Pivoting movements

It is often observed that a diatom, which is lying on a valve and moves forward, suddenly stops and starts rotating horizontally around an apex. Sometimes it rotates around the apical axis and then lies on the girdle bands, so that the raphes of both valves determine the further movement. As mentioned above, only the motion sequences in which the diatoms lie permanently on their valves are to be considered here. As the rotation in this position typically takes place out of the movement, one can distinguish between a leading apex and a trailing apex. In my observations, the rotation always took place around the trailing apex. Since in my cultures there were frequent reversals of direction, so that the path curvature was often not noticeable, a correlation between the direction of curvature of the trajectory before the start of rotation and the direction of rotation of the diatom cannot be clearly demonstrated. In the clearly visible cases, the rotation took place in the same sense as the trajectory. Provided that the further movement lasted long enough and was not interrupted by stops, it typically took place in the same direction of rotation.



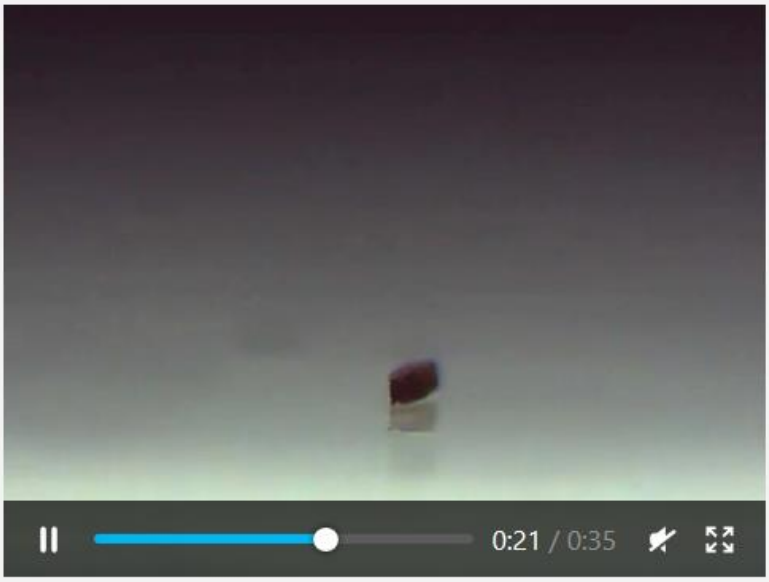
I suspect that rotation occurs when the centre of force is close to the trailing apex and the diatom on both rape branches near the apex comes into contact with the substrate. Due to the convex surface of the valves, the leading apex is more distant from the substrate than the trailing apex (see picture on the left). The apices cannot be focused at the same time in this situation.



As discussed, the activity of the raphe-branches is usually in the opposite direction, so that it comes to a rotation around the contact surface. The diatom rotates in the opposite direction to the EPS transport of the raphes. This is sketched in the picture on the left. The contact surface was indicated by the oval area. The blue short arrows indicate the direction of transport of the raphe.

Typical rotations can be seen in the following videos. In the video at the bottom left (150x time lapse), the diatom runs through a repeated sigmoid curve before it starts rotating. It probably touches the substrate alternately with the opposite raphe halves. Several rotations can be seen in the video to the right of it (1500x time lapse).

Side view observation



In the section on observation with horizontal viewing direction, a method is described that allows diatoms to be observed from a nearly horizontal viewpoint. One looks at them like a tiny observer standing on the substrate would see them. On a smooth substrate you can also see the mirror image of the diatom. The 30x time lapse video on the left shows such a recording of *Surirella biseriata*. The diatom moves partly in several S-curves. The lateral inclination of the diatom also changes. The connection between inclination and curvature of path is however not clearly recognizable from this perspective.

I hope to have succeeded in showing that the analytical methods presented allow a better understanding of the trajectories of diatoms. In particular, they allow investigating the interaction between the activity of the raphe and the pattern of movement.

"The Diatoms: Biology and Morphology of the Genera
F. E. Round; R. M. Crawford; D. G. Mann
Cambridge University Press; 1 edition (2007)

Observation with horizontal viewing direction

In connection with the detection of trajectories, an unusual camera angle on diatoms has already been mentioned, in which the viewing direction is inclined at a slight angle to the plane of the substrate. Here, one can recognize aspects in which the contact area between the diatom and the substrate is essential. The interaction between contact surface and trajectory has already been discussed.

After the explanation of the recording technique the following contributions will deal with the observation of certain genera and species. This method is also used elsewhere on this homepage, as in the contribution to the curvature of the trajectories of Surirella biseriata.

Vertical view

In the observation of living diatoms in the light microscope one sees them with a perpendicular view onto the substrate, ie in a vertical view. In the case of an upright microscope, you can either look at the top of the slide (bird's eye view) or with the inverse microscope from below into the culture vessel.

Horizontal view

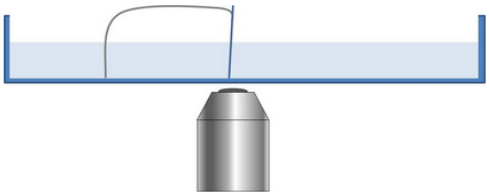
If one looks at the diatoms from the perspective of a fictional tiny observer standing on the substrate, one sees them in a horizontal view. The word "side view" does not describe the situation quite correctly, as the diatoms can take different positions to the substrate. It is useful to look at the substrate with a slight angle, because then one can recognize the mirror image of the diatoms. This helps with measurements, because usually the horizon line is not clearly recognizable.

Benefits of the horizontal view

Probably the most important aspect is that the contact with the substrate becomes visible.

If you want to measure the inclination of the apical axis to the plane of the substrate, you could in a vertical viewing direction use the focus setting of the microscope at high magnification to determine the distance between the apices. Alternatively, the foreshortening of the distance between the apices can be measured. The first possibility is not suitable for video recordings, the second one is very imprecise at small inclinations as the shortening is proportional to the cosine of the inclination angle. From a horizontal view, the inclination of the apical axis is immediately recognizable.

Setup



In a particularly simple method for recording with a horizontal view, a cover slip is placed almost vertically in a petri dish filled with water. A wire bow is attached to the cover glass and serves as a supporting leg. A sketch is shown on the left. If sufficient diatoms are brought into

the petri-dish, then after a while diatoms will have migrated to the cover slip. If necessary, one can place the cover slip flat on the bottom of the petri dish and then carefully tilt it into the vertical position.

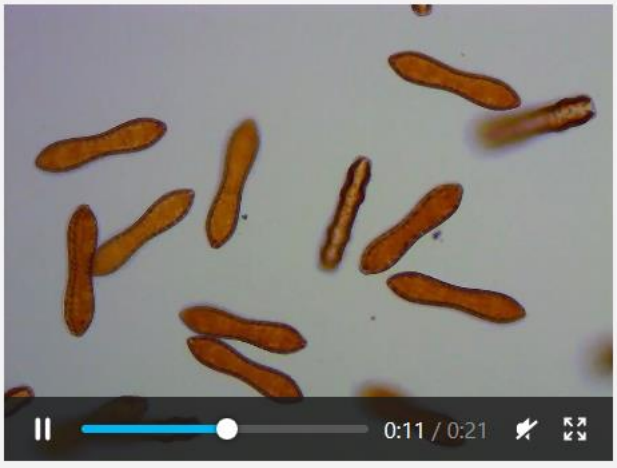
A more favorable setup for such observations would be an inverted microscope, which is tilted by about 90°, so that the optical path through the sample is almost horizontal. The observation takes place in a cuvette in which a transparent, very thin substrate (film) is arranged horizontally above the bottom so that the illumination beam path is only slightly influenced. The diatoms can then simply be put on the substrate and observed. So far I have avoided the effort to build this.

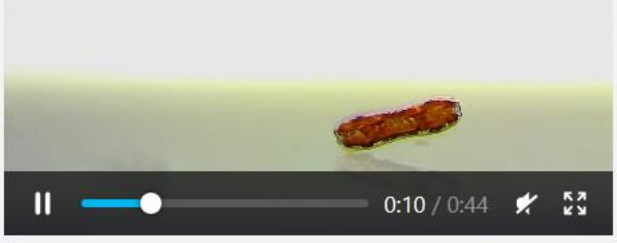
Difficulties

It is not surprising that the horizontal view is not widespread in light microscopy because one has to struggle with some difficulties:

- The distance between the bottom of the petri dish and the diatom can be very large. If this distance becomes too big, you can no longer focus the diatom. Even before reaching the maximum working distance, there is a water layer between the bottom of the petri dish and the diatoms, for which larger magnifying objectives are not corrected.
- The distance of the diatoms from the substrate can change considerably during the course of an observation. This requires frequent focusing.
- The illumination beam path is disturbed by the inclined cover slip.

An example



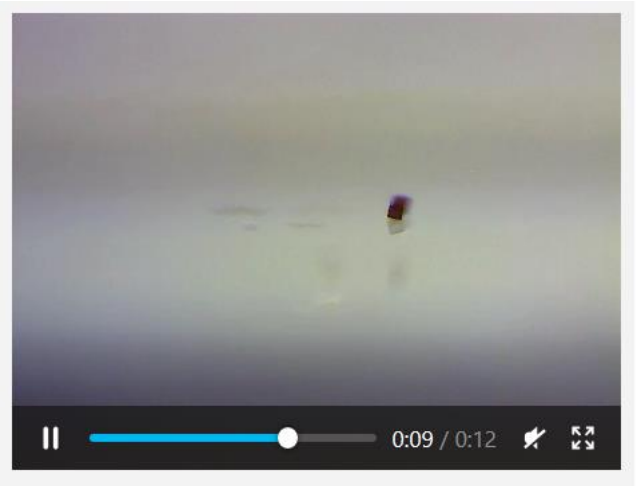


Diatoms of the species *Cymatopleura solea* (length approx. 80 µm) are considered as examples.

The video (60x time lapse) on the left shows a culture under the inverse microscope at different magnification levels. The optical axis is perpendicular to the substrate. It can be seen that the diatoms lie partly on the valves and partly on the girdle bands. There are changes between the positions, spontaneously or triggered by collisions.

A video recording (5x time lapse) from a horizontal perspective with respect to the substrate can be seen on the left below. The frame format has been changed by removing the top and bottom edges of the image. The inhomogeneous illumination therefore does

not disturb the visual impression. The video shows a diatom which moves almost perpendicular to the viewer and changes its position to the substrate twice. Because the optical axis is almost perpendicular to the apical axis and the diatom has a small depth at this sight, the picture was taken successfully with a 10x objective. The focusing had to be adjusted only slightly, for example after the diatom tilted around its apical axis. The diatom moved at a short distance from the bottom of the petri dish.

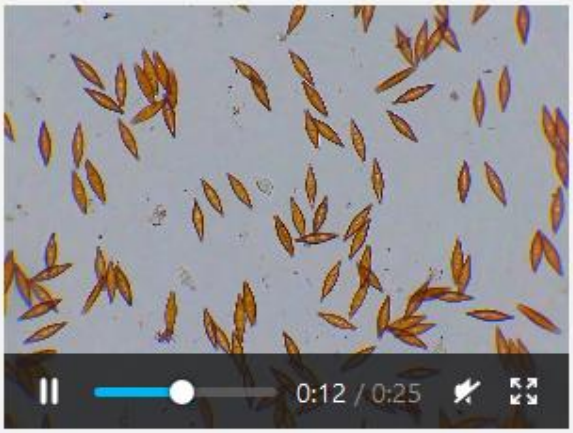


Pictures from this perspective are informative when they show the *Cymatopleura solea* with a view parallel to the apical axis, i.e. perpendicular to the transapical plane. The third video on the left (20x time lapse) shows a diatom from this point of view (full screen mode is recommended). It shows that the diatom does not lie flat on its valve, but is tilted significantly around the apical axis. Therefore, the raphe can only be in contact with the substrate on one side of the apical axis. This inclination could be a consequence of the convex shape of the valve. A simple relationship between the direction of curvature of the trajectory and the inclination does not seem to exist in *Cymatopleura solea*.

Observation on natural substrate

The described method shows the movement of diatoms on a nearly vertical smooth glass surface. Observations in a natural environment tend to be made by chance.

In the 4x time lapse video on the left is a sample of a pond with a fiber on which a diatom moves. The contact between diatom and fiber is visible. Such images of natural samples can unfortunately hardly be used without an assignment to the species. In addition, the desired observation conditions are met only for a short time. In particular the direction from which the diatom is viewed changes frequently. Other objects can block the view onto the object of observation.

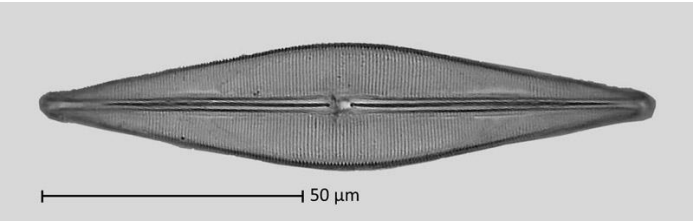


Craticula cuspidata observed from a perpendicular direction of view with respect to the substrate (5x time-lapse)

Craticula cuspidata observed from a horizontal direction of view with respect to the substrate (8x time-lapse)

Craticula cuspidata from a horizontal view

The video above left shows the view of a culture of *Craticula cuspidata* (found in Ebnisee 48°55'25.5"N 9°36'32.8"E, Length approx. 120 µm), as you are used to from an inverse microscope. The viewing direction is perpendicular to the substrate. A diatom of the species *Craticula cuspidata* can be seen to the right of it in a horizontal view with respect to the substrate. The diatom changes the direction of the movement several times. With longer movement in one direction, the diatom is elevated against the horizontal, with the trailing apex closer to the substrate. Therefore, the diatom is pushed. The point P determined by means of the described method from the trajectory is in accordance with this direct observation.



More important is the behavior of the diatom at the points of motion reversal. As *Craticula cuspidata* has two raphes on each valve (see picture on the left; click to enlarge), one might assume that the driving raphe loses contact with the substrate and the other raphe comes into contact with the substrate by tilting the diatom. In case of the opposite direction of transport of mucilage, the direction of movement would be reversed.

This recording and several other videos show that the sequence is different. In the first step, the driving raphe changes its direction of transport so that the diatom moves in the opposite direction. Thereafter, the inclination angle of the diatom to the substrate gradually changes until finally the trailing apex is close to the substrate. Three points seem to be essential:

- The diatom is not generally pushed, but is pulled in a short time frame after the movement reversal. A statistical evaluation of the trajectory as described earlier can hardly reveal this because it requires a sufficiently long distance and directional fluctuations in the vicinity of the reversal.

- The diatom is always tilted to the other raphe after the change of direction, without a second reversal of the direction being observed. Obviously the two raphes operate synchronously in the same direction. Strictly speaking, this observation can make a statement about the synchronicity only for the time frame shortly after the change of direction.
- The diatom systematically changes its angle of inclination after the change of direction. It is not recognizable which mechanism causes this effect. Possibly the water flow (small Reynolds numbers) could play a role. It would be interesting to know whether there is a correlation between the velocity of the diatom and the angle of inclination. I consider it more likely that the drive mechanism in the local environment of the contact results in a displacement of the contact point.

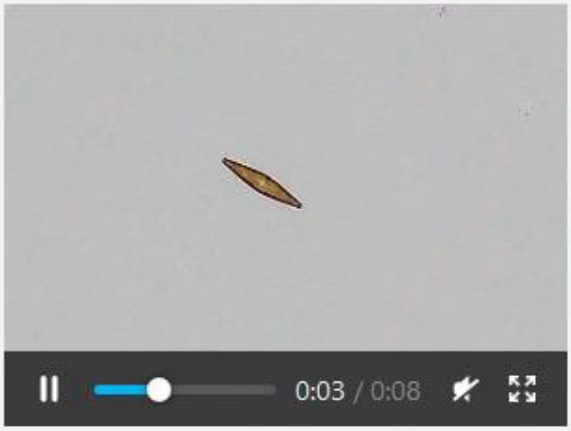
In some diatoms spp. one observes that often after long unidirectional trajectories, a rapid back and forth jerking occurs until a longer movement section follows. A possible explanation is that in these cases the raphes work against each other. If the activity of the other raphe was opposed after a change of direction, a new change of direction would occur as soon as the diatom reached the horizontal position. Then the diatom would jerk back and forth until a uniform direction of the activity of the raphe takes place. So far I have not been able to observe this situation in horizontal view.

Note on requirements for a theory of the locomotion

A complete theory of the movement must not only be able to explain how a change of direction takes place at a molecular level. It also has to explain why some diatoms are pulled and others are pushed, how the synchronization between the raphe systems is accomplished and how the shift of the contact point occurs when the direction of movement changes.

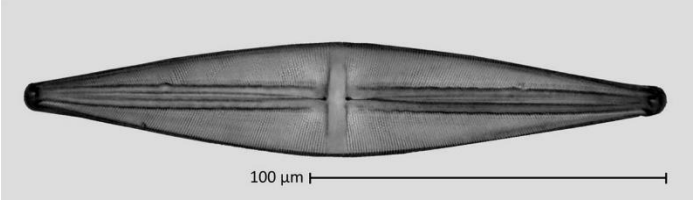
It would be important to see whether the sequence for *Navicula* is analogous to that of *Craticula cuspidata*. Here, however, it would always have to be changed into a pulling position after reversal of the direction in which the leading apex is closer to the substrate.
Video recordings of a species of the genus *Rhopalodia* show a tilting of the diatoms on the opposite apex without a direction change. *Craticula cuspidata* does not show such a tipping.

Stauroneis sp. observed from a perpendicular direction of view with respect to the substrate (time-lapse)



Stauroneis sp. , short section of a trajectory (150x time-lapse)

Stauroneis sp. from a horizontal view



From a pond near Hohenheim (48°42'32.2"N 9°12'40.3"E) a diatom of the genus *Stauroneis* was isolated, which was about 180 µm long. It is likely to be *Stauroneis phoenicenteron*. The picture of its valve can be seen on the left (click to enlarge). The video above left shows the movement in different magnification levels (5x to 40x objective) and time-lapse factors (5x: 450x time-lapse; 10x first scene: 300x time-lapse: 10x second scene: 600x time-lapse; 20x: 300x time-lapse; 40x: 150x time-lapse).

The speed of the diatoms can be very different. In the first video sequence a fast-moving diatom appears at the bottom of the screen.

In a short section of this video you can see diatoms that separate after asexual reproduction (10x objective, 600x time-lapse). Here, the outline is clearly visible in girdle view (girdle is uppermost). Obviously, the diatom in a valve view (a valve is uppermost) cannot lie level on a flat substrate but has only a small contact surface.

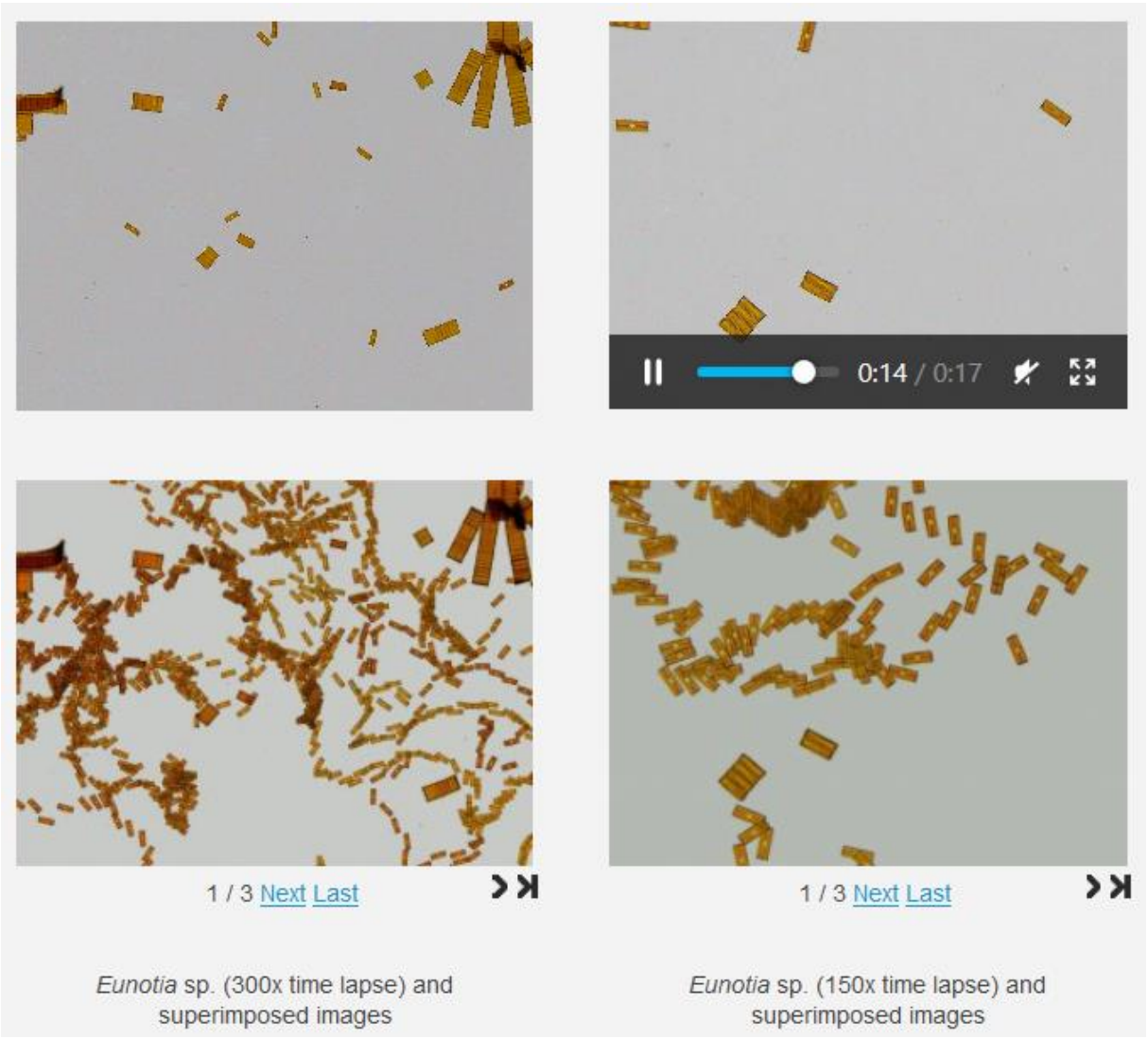
Due to the shape of the raphe only slightly curved trajectories usually occur. A detail enlargement from a video showing a typical path can be seen at the top right. At the end of the video a superposition of all frames is shown. Occasionally, stronger curved paths are observed. I suppose adherent EPS lumps are the cause. In such cultures diatoms in girdle position also show an astonishing activity of movement.

Therefore, a calculation of the position of the center of force using path curvature (see contribution to the analysis of trajectories) is not reliable. Also the evaluation of the not very large directional fluctuations around the direction of movement did not provide a clear information for short paths between reversal points. In the case of longer sections between reversal points, the diatom seems to

be pushed from a point near the trailing apex. This analysis also corresponds to the visual impression when viewing the fluctuations.

For reliable evaluation, observation from a horizontal perspective in relation to the substrate is appropriate. A typical video is shown on the left side (50x time-lapse). There is a strong similarity to the movement of *Craticula cuspidata*. In case of longer movements the subsequent apex is closer to the substrate. This position is taken shortly after reversing the direction. It is not very stable as occasional fluctuations can be recognized. Sometimes the diatom moves for a short time with its apical axis parallel to the substrate. When changing direction quickly, no constant inclination of the apical axis to the horizontal is established.

As with *Craticula cuspidata*, the reversal of direction in the video does not occur as a result of tilting to the other raphe with an opposite direction of its activity. A change in direction is caused by a change in the direction of the transport of the raphe. When in the course of the movement the inclination of the apical axis changes in the way described, the other raphe comes into contact with the substrate. If the direction of transport of the raphes is different, the movement must stop at horizontal position of the diatom. It would then have to reverse itself if the diatom remained in contact with the substrate. A fast jerking back and forth would be the result if the diatom remained approximately in a horizontal position. During the observation period of a few hours there were a few cases where the direction of movement was reversed while the diatom was in an almost horizontal position. It is unclear whether this reversal was caused by the opposite transport direction of the raphes or by a simultaneous change of an identical transport direction. I assume that a reversal of direction as a result of opposite raphe activity rarely or never occurs and that the transport direction of both raphes of a valve is usually the same.



Eunotia sp. (300x time lapse) and superimposed images

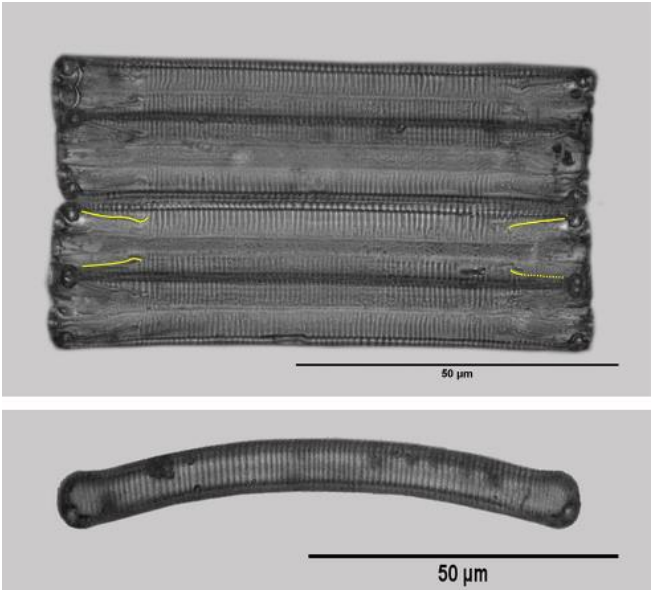
Eunotia sp. (150x time lapse) and superimposed images

The movement of *Eunotia* sp.

As the previous examples have shown, different properties of the paths of diatoms on smooth substrates can be understood on the background of the shape of their raphes. These involve path curvature, position of the center of force and the characteristics of curvature at reversal points. However, trajectories are not always easy to interpret. The movement in *Cymatopleura elliptica* shows (see video) that certain forms of valves in connection with the shape of the raphe only allow a random movement. *Eunotia* will be discussed here. The movement of diatoms of this genus is unusual even in the plane, i.e. without erecting.

Species and Raphe

In March 2018 a *Eunotia* sp. was isolated in a pond near Stuttgart/Hohenheim (48°42'34.0"N 9°12'29.1"E) and kept in clonal culture. I assume that this is *Eunotia glacialis* Meister 1912. In view of the existing images, it could also be *Eunotia glacialifalsa*, *Eunotia glacialispinosa*, *Eunotia belgica* or *Eunotia valida*, according to indications by Dr. Nélida Abarca (Botanic Garden and Botanical Museum Berlin, Free University of Berlin). Corresponding to the documented ranges of the mentioned species in Baden-Württemberg (Mattern et al. 2019) the assignment to *Eunotia glacialis* seems probable. Since a residual doubt remains, it is referred to as *Eunotia* sp. here.



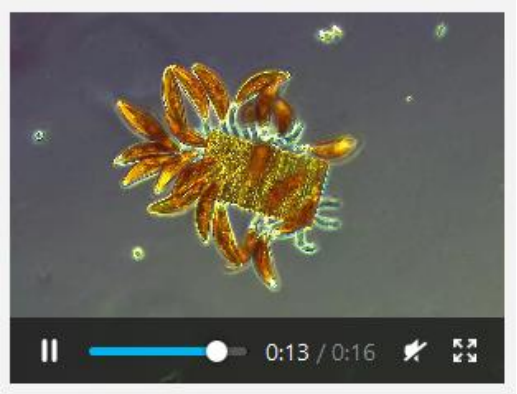
The pictures on the left show the girdle and valve view. The diatoms have just developed from cell division and therefore their width (expansion in the direction of the pervalvarous axis) is small.

In the girdle view, the short raphe is periodically displayed as a yellow line, as far as it is clearly recognizable. The picture of the valve view suggests that the raphe at the poles still run a bit on the upper side of the valve. Such raphe branches are typical for *Eunotia*.

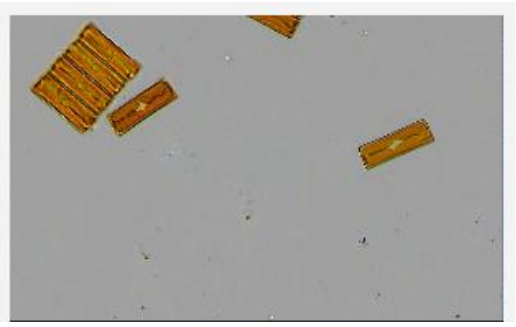
Usually these diatoms lie on the girdle bands on the substrate. Apparently, at least in culture, the concave side usually faces the substrate. In this position, up to four raphe branches can have contact with substrate and contribute to the propulsion. The criteria for the analysis procedures already described are apparently not fulfilled here.

Motility

In the following, only the movement in the plane in girdle position is considered. The movement patterns of *Eunotia pectinalis* described by Bertrand (1993) impressively show that the raphe systems of this species also enable various movements in which the diatom stands upright. I could not observe such movements. In the observed species in culture, tilting into the valve position occurred only as a result of collisions between diatoms.

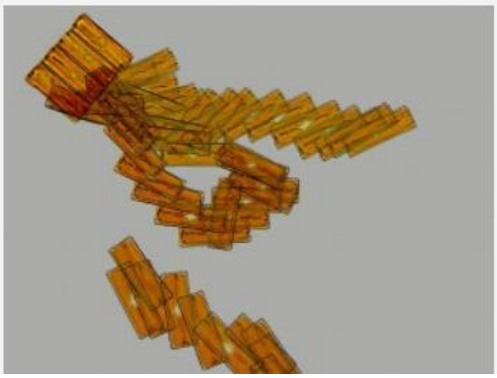


The cultivated *Eunotia* forms chain-like colonies, which can become very long in vitro. Some pictures can be found on the pages on the size sequence and synchronicityronicity of chain-like colonies. The individual diatoms that have separated from colonies are mobile. Only rarely one observes several connected diatoms in motion. This is why they can



probably also be colonised epiphytically. In the first raw culture, besides *Eunotia*, *Cymbella* was also present, which colonized *Eunotia* colonies. The video on the left (4x time lapse) shows the situation.

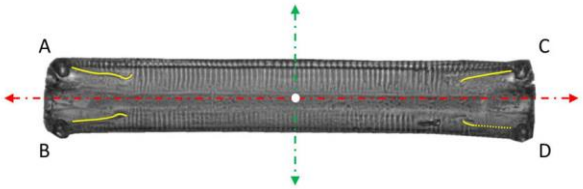
In the two videos at the top of this page (300x and 150x time lapse) you can see some diatoms in motion. Below each of them there is an image gallery with superimposed frames in different time intervals. The second video on the left shows the movement in higher magnification and 20 x time lapse. Below it are again different superimpositions.



In time-lapse, the movement appears erratic. The direction of movement and orientation of the cell change frequently. Therefore, the movement of the center of the diatoms was used to measure the velocity. The speed is relatively low. In the measurements carried out, it was between 1.1 µm/s and 2.3 µm/s. With a length of about 70 µ from apex to apex, the diatom typically takes between half a minute and one minute to cover its own cell length.

Forms of movement

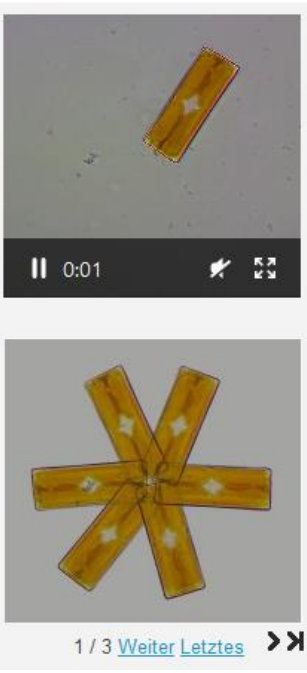
The forms of movement are marked by the raphes and their activity. In *Eunotia* in girdle position, they are versatile, as four independent raphe branches can be in contact with the substrate at the same time. As the videos of movement and the superimposed images show, the apical axis (dashed, red in the picture on the left) and the trajectory of the center (marked white) of the diatom usually form a clearly observable angle, i.e. the alignment of the diatom is rarely tangential to the movement of the center. Besides the velocity component in the direction of the apical axis, there is thus usually an essential component in the direction of the pervalvarous axis. Often the motion occurs roughly in the direction of the diagonals of the rectangular contour line (in direction of theline segments AD or BC). This suggests that opposite raphe branches (e.g. the raphe branches at A and D) contribute mainly to the propulsion and are active in the same direction. Occasionally the component in the apical direction becomes very small or disappears and the diatom moves in the direction of the pervalvarous axis (dashed, green). This can be explained by an activity of the raphe branches in the same direction, for example at A and C.



Large components of movement in the direction of the pervalvarous axis give the movement of the diatoms in time-lapse photography something floating. They seem to drift, although they have a strong cohesion to the substrate.

Rotation

Rotary movements can also be observed. The following video shows a rotation around a full circle. Below the video you can see superimposed images with the same time interval.



In addition to the high regularity, it is noticeable that the pivot point is slightly outside the diatom. An evident explanation of the rotational movement is that only the two raphe branches A and B (or C and D) are in contact with the substrate and their activity proceeds in opposite directions. One raphe works in apical direction, the other in distal direction. This is sketched in the following picture.

In the specific case, a rotation around 2π took almost exactly 60 seconds, which means an angular velocity of ω = 0.105 radians per second. As the width of the diatom is 24.8 µm, the radius of the circle is approximately r = 12.4 µm. With the relation v=r*ω the velocity at the raphe is 1.26 µm/s. This corresponds to the typical velocity of this species.

Motion sequences

The given description is roughly simplified, because the directions of movement change continuously. Rotations and diagonal linear movements often overlap, so that fan-like structures appear on superposed images. On the other hand, the orientation of the apical axis does not necessarily change in case the trajectory of the center changes direction.

In many motile diatoms one can observe that longer paths are traversed and suddenly a reversal of direction takes place. Here, a raphe branch is usually in contact with the substrate, whose direction of activity changes. In this clear form this cannot be seen in the observed *Eunotia*. Only a jerking back and forth occurs, during which a change of direction is difficult to recognize. A possible explanation is that in *Eunotia* up to four raphe branches can contribute to movement and it is very likely that more than one raphe branch contributes to movement. Then the reversal of the direction of movement of a raphe branch leads to a change of direction, but not to a reversal of the diatom.

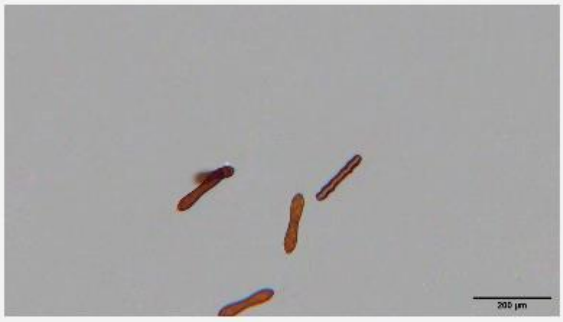
Notice

On the subject of the movement of diatoms of the genus Eunotia Ehrenberg, Paula Furey has published the following paper, which also refers to this page:

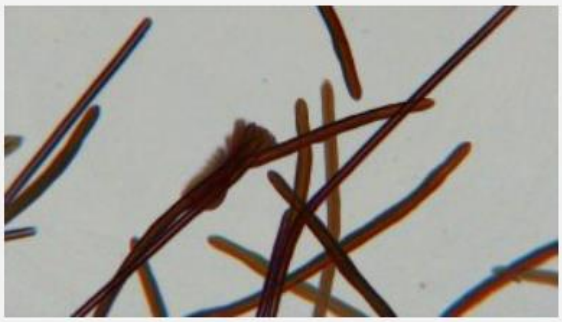
Furey, Paula C. "Motility in the Diatom Genus Eunotia Ehrenb." *Diatom Gliding Motility* (2021): 185-209.

Bertrand, J. (1993) Mouvements des diatomées. III. Le pivotement polaire vertical de *Eunotia pectinalis* (Kütz) Rab. Essai de quantification des forces. *Cryptogam. Algol.* **14**(4), 157-172.

Mattern, H., Stutz, S., van de Vijver, B. and King, L. (2019). Klasse Bacillariophyceae. In: Beiträge zu den Algen Baden - Württembergs. Band 2: Spezieller Teil: Euglenozoa und Heterkontobionta (Ed. by S. Stutz & H. Mattern), pp. 94–371. Verlag Manfred Hennecke, Remshalden.



Cymatopleura solea at the beginning of cultivation (30x time lapse)



Superposition image from all frames of the video (click to enlarge)

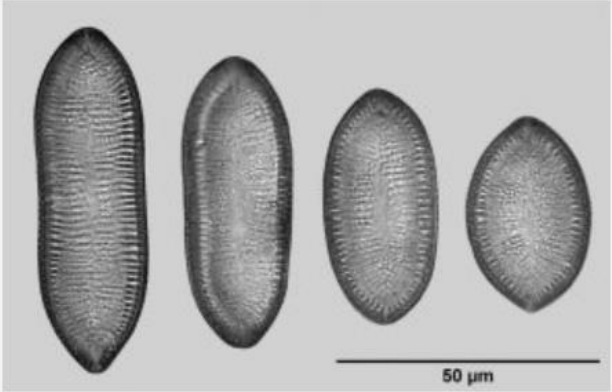
Change in the trajectories of *Cymatopleura solea* as a result of cell size reduction

As described in the introduction, a vegetative division produces an equally large and a smaller diatom (MacDonald-Pfitzer rule). If one begins the cultivation with a single cell and generates a sequence of batch cultures by taking a larger number of diatoms from a mother culture and inoculating the following culture, then one observes a gradual reduction of the cell sizes. Each culture is started with a sample from the mother culture. As a result of the vegetative divisions, the mean value of the size decreases in each culture.

With this reduction of the cells, particularly the distance between the apices decreases. The transapical size changes comparatively little. Therefore, the cells of different sizes are not similar to each other, but have different proportions.

In the previous contributions, the relationship between the paths of diatoms and the shape of raphes was discussed. As the proportions of the diatoms and thus the radii of curvature of the raphes change with successive reduction, it is to be expected that the properties of the trajectories will also change. This will be shown below with the example of Cymatopleura solea.

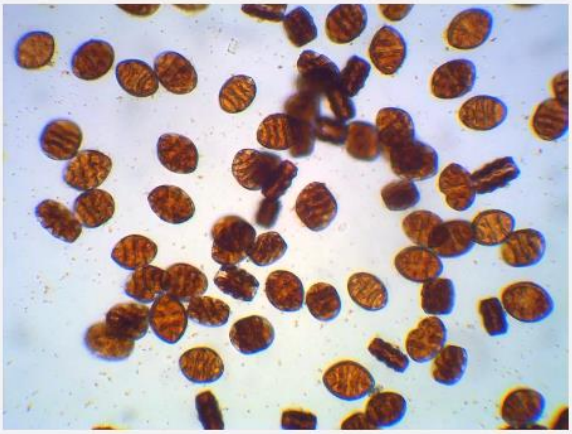
Diatoms of the species Cymatopleura solea were found in a sample of Lake Ebnisee (48°55'25.5"N 9°36'32.8"E) on 25 March 2017, isolated and kept in clonal culture. New cultures were inoculated approximately every two weeks, with a larger number of cells (in the order of 100) being transferred into a new nutrient solution every time. The video above shows a 30-times time-lapse view of such a culture on 16 May 2017. At that time, the diatoms still had an almost uniform length of about 160 µm. To the right is a superposition image of all frames. Apparently, the paths between the reversal points and collision locations are straight or slightly curved.



In February 2019, almost two years after the beginning of cultivation, the shape of diatoms had changed considerably. The characteristic violin-like shape in valve view could only be found among the longest diatoms. Below a certain length (approx. 50 µm) the diatom is convex in the valve view. The diatoms with

the strongest shortening exhibit a striking similarity to Cymatopleura elliptica. In the picture on the left (click to enlarge) four valves with decreasing length (63 µm, 54 µm, 43 µm, 36 µm) are shown. Because of the used superposition of focal planes, the undulated valve face hardly becomes noticeable. It is probably less pronounced than in Cymatopleura elliptica.

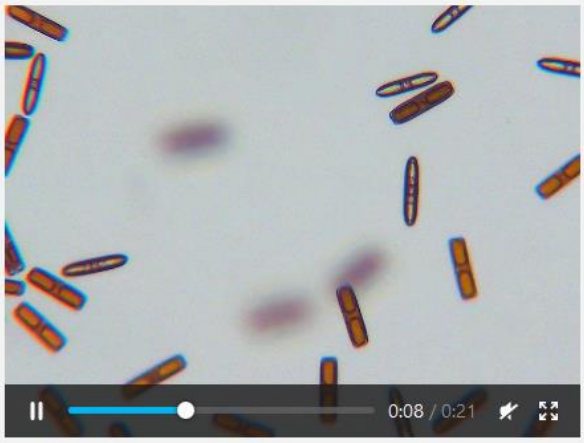
The movement of the diatoms can be seen in the following video on the left in time lapse (20x at low magnification, 10x at all higher magnification levels).



The first sequence was recorded in a culture with a strong EPS deposition that influenced the movement. Subsequent sequences were obtained with diatoms previously removed from a culture. In comparison to the original culture, one recognizes stronger curved paths. This is probably a consequence of the more curved raphes. In the case of the shortest diatoms, rotation is frequent. This movement is not accidentally reminiscent of the movement of Cymatopleura elliptica, as can be observed in the video on the right.

In conclusion, it can be said that the trajectories of motile diatoms also depend on the number of reduction steps passed through. This is certainly not only true for the species *Cymatopleura solea* considered here.

Biofilm of *Craticula cuspidata* (30x time lapse)



Pinnularia viridiformis within the biofilm and on its surface (25x timelapse)

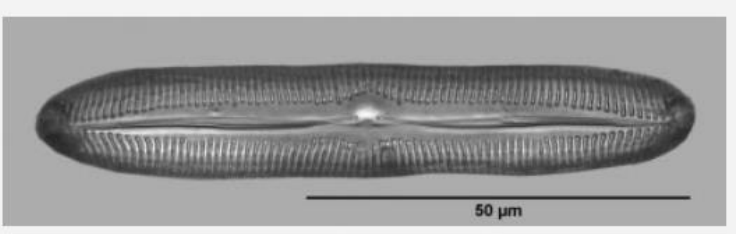
Movement of diatoms in and on biofilms

According to the explanations for the active motion of pennate diatoms one expects that a direct or indirect contact between raphe and a substrate is necessary. The requirements on the hardness of the substrate are often not high, because movements on agar are possible (Iwasa, K., Shimizu, A., 1972).

Motile pennate diatoms that form biofilms, like many diatoms of the genus *Pinnularia*, *Rhopalodia* or *Craticula* can move well in their biofilm. A biofilm that has detached itself from the substrate is shown on the page about nutrient solution. In the top left video such a detached biofilm of *Craticula cuspidata* (Length: approx. 120 µm) can be seen under the stereomicroscope in dark field illumination. One can recognize a strong movement of the diatoms in the transparent biofilm.

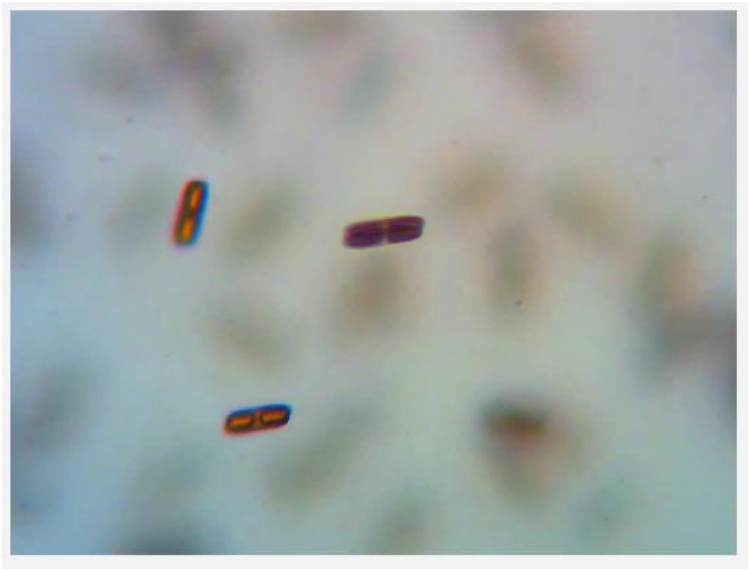
Biofilm in *Pinnularia viridiformis* cultures

Diatoms of genus *Pinnularia* excrete excretes extracellular polymeric substances (EPS) during the activity of the raphes, which by and by can form a soft deposit in the cultures above the bottom of the petri dish (overview of EPS in diatoms in Aumeier, C. and D. Menzel, 2012). On the left a very small *Pinnularia* (length approx. 87 µm) is to be seen, which very quickly builds up a thick biofilm (click to enlarge). It is a *Pinnularia viridiformis* morphotype 3 sensu Krammer 2000.



At the beginning the video on the upper right shows three diatoms of this species which are staying at the bottom of a petri dish. On top of that a layer of EPS is deposited, on which most diatoms are located. Afterwards these diatoms are brought into focus. Movements can be observed in both layers with movement on the biofilm predominantly occurring in pivoting motion. At the bottom of the biofilm the *Pinnularia viridiformis* often straighten up vertically, which they do not do in pure nutrient solution.

Under the stereomicroscope diatoms are easily found between the bottom of the petri dish and the surface of the biofilm. The diatoms between the bottom of the Petri dish and the surface of the film, which are rare compared to the population on the surface, often rotate around changing axes. The video on the left shows *Pinnularia viridiformis* within the biofilm. The time-lapse factor is 12 times as large as the one in the video at the top right. In the background, the faster moving diatoms on top of the biofilm can be seen out of focus. I assume that a migration of the diatoms from the bottom to the top of the film is possible. To the opposite direction such a change is presumably rare or impossible. This is suggested by the found far greater number of diatoms on top of the biofilm.



Properties of the biofilm

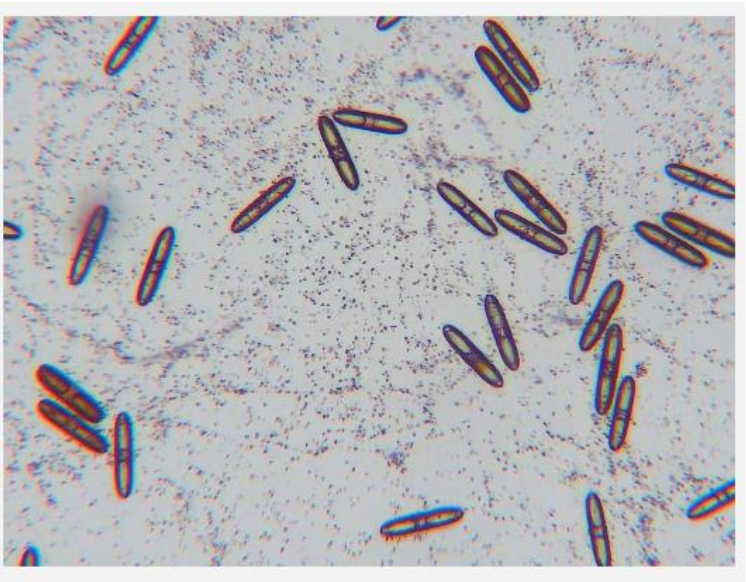
The layer of EPS shows viscoelasticity, so it is neither purely viscous nor purely elastic. The viscous property of the gel allows the movement of the diatoms at the bottom of the petri dish and within the biofilm. Because of the elasticity the diatoms are able to stay on the surface without sinking to the bottom of the petri dish.

As the biofilm is colorless and transparent, its surface cannot be recognized under the microscope. If small particles are placed from above, these remain on the gel and mark the surface of the biofilm to the water.

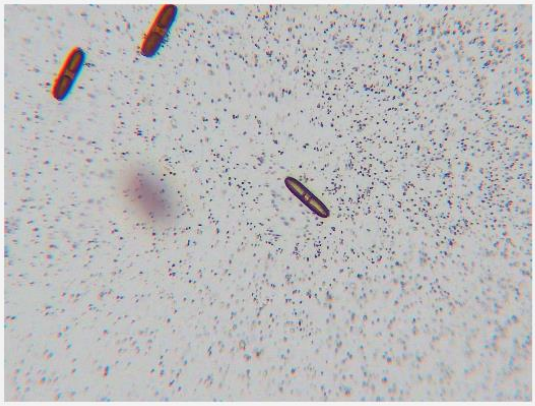
In the observed culture (petri dish with diameter of 5.5 cm) the thickness of the biofilm was between about 150 µm and 380 µm. For comparison, the length of this Pinularia is at about 90 µm. At the time of recording the culture was 30 days old. Locally the layer is flat enough to focus the diatoms in the field of view at the same time.

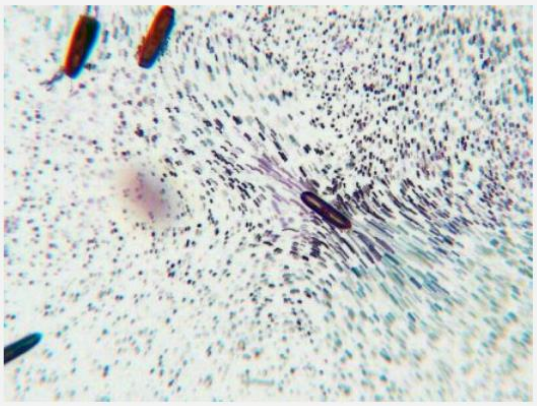
Use of the biofilm to study the activity of the raphes of *Pinnularia*

The elastic properties in connection with the possibility of marking the boundary surface between the gel layer and the water can be used to observe the activity of the raphes. The modulus of elasticity proves to be so small that elastic deformations which are easily observable occur during the movement of the diatoms. In the experiment mineral particles with a linear expansion of typically less than 3 µm were distributed on the gel layer.



In the video on the left (30x time lapse) one can see how the surface of the gel layer is elastically deformed by the movement of the *Pinnularia viridiformis*. It is striking that there is a slow build-up of tensions and a rapid, jerky drop of the tensions. The cause for this becomes understandable if one considers the process at a location with a low population density. In case of steady movement of the diatoms no conspicuous deformations occur. Frequently however, the movement of the diatoms stops without the raphes ending their activity. Through the transparent valves one can see that both raphes are now working towards their proximal ends. The gel in the vicinity of the diatoms is thereby elastically deformed due to the adhesion of the raphe to the substrate. It is compressed (negative strain) at the proximal ends and in the neighborhood of the diatoms in the direction of the transapical axis. A short video of such a process together with a superimposition of the frames during the standstill of the diatom can be seen below (click to enlarge):





The deformation has a visible effect at a distance of several diatom lengths.

After a while the raphes no longer work against each other, and the movement is continued in the same or opposite direction. The EPS layer returns to its equilibrium position. The gel expands in a short time, which is reflected in the movement of the gel surface in some distance from the diatom.

Pinnularia viridiformis lie on a planar, solid substrate flat on their valves, as can be seen from the silhouette of the valves in the belt view. It can be assumed that raphe systems that work against each other also in this case cause the movement to stop.

In this type of motion the diatoms consumes energy. It would be interesting to know whether this energy consumption is useful, or whether there is a lack in the coordination of the raphes. This question also arises for another reason. It is easy to show in diatoms of the genus *Pinnularia* as well

as in many other diatoms that often raphes are active which are not coupled to the substrate and thus do not contribute to the movement.

A simultaneous activity directed to the distal ends of the raphe is not observed in this and several other videos. It would lead to an elastic expansion of the gel. With this observation technique it cannot be determined whether both raphe systems are involved in uniform motion of the diatom.

Apparently particles can also be displaced during the opposing activity of the raphe. In the upper video on the left, the initially evenly dispersed particles already show striking clusters.

The method provides in principle the possibility of quantitatively determining the forces exerted on the substrate by the raphe. For this purpose the elasticity module and the displacement of the particles have to be determined.

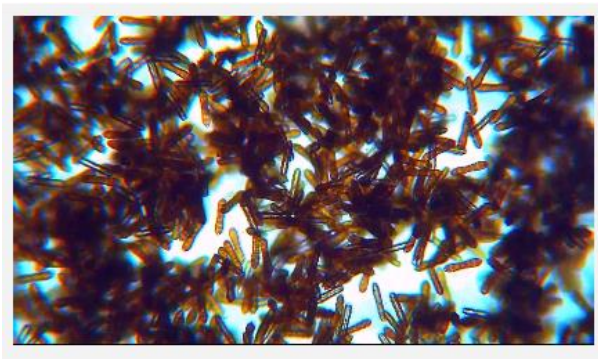
It is also a good idea to use this method to investigate the activity of the raphes even in species which do not produce such a biofilm. For this purpose a gel (agar or gelatin) could be superimposed with nutrient solution and the top of the gel layer could be marked with particles. The difficulty consists in the production of an elastic layer with a very small modulus of elasticity. If this fails, diatoms could be placed on the biofilm produced by *Pinnularia*.

Aumeier, C. and D. Menzel, Secretion in the Diatoms, in Secretions and Exudates in Biological Systems, J.M. Vivanco and F. Baluška, Editors. 2012, Springer Berlin Heidelberg. p. 221-250.

Iwasa, K., Shimizu, A., 1972. Motility of the diatom, *Phaeodactylum tricornutum*. Exp. Cell Res. 74, 552–558.

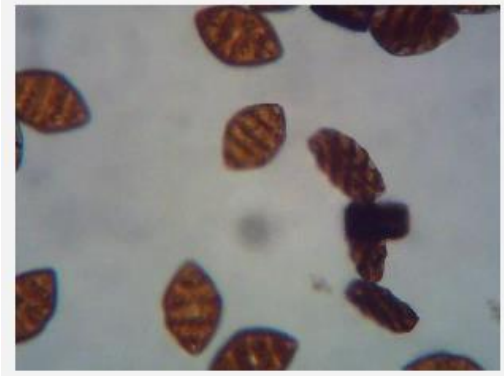
Notes on flake formation and movement in flakes

The observations on *Pinnularia* in a biofilm have shown, among other things, that benthic diatoms move in a suitable environment in a three-dimensional space. However, the diatoms are often located on the surface of the substrate or of the biofilm. Motile diatoms in cultures necessarily no longer move in one plane but within a more or less thick layer when the population density becomes so high that they cannot all be in contact with the substrate at the same time. The video on the left shows *Cymatopleura solea* in such a culture (4-x time-lapse). The focus of the objective lies in the plane of the bottom of the Petri dish. The diatoms move in mutual contact with each other, but do not form a homogeneous spatial pattern. Hydrated extracellular polymeric substances (EPS) are probably the reason why the diatoms lump together locally.



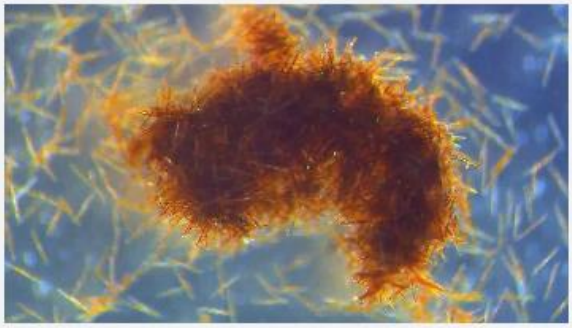
In some genera the diatoms form flakes even at lower population densities. The diatoms are bound in a more or less pronounced matrix of mucilage. In cultures of a single species and low bacterial density, this matrix is formed by these diatom species alone.

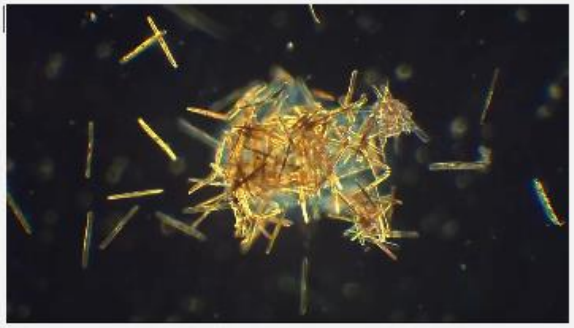
At first we will consider *Cymatopleura elliptica*. The video below left shows a culture in 80x time-lapse in which many diatoms move independently. However, it can be recognized that the diatoms adhere to each other locally and temporarily in certain areas. To the right of it is a video with flakes of this species in different sizes (300-x time-lapse).



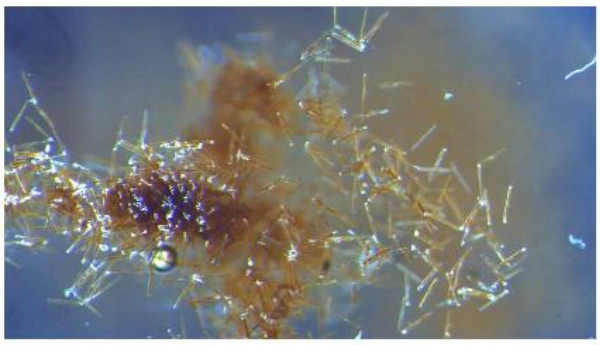
The diatoms apparently stick together. However, an EPS film between the diatoms is so thin at the chosen magnifications that it is not visible. The possibilities of movement are strongly limited by the connection to neighbouring diatoms. Often one recognizes rotary movements around the contact points. The mechanical coupling of several diatoms leads to complex motion sequences.

Nitzschia sigmoidea also forms flakes, as shown in the video below left (dark field, 40x time-lapse). A small flake at higher magnification can be seen to the right (dark field, 4x time lapse).





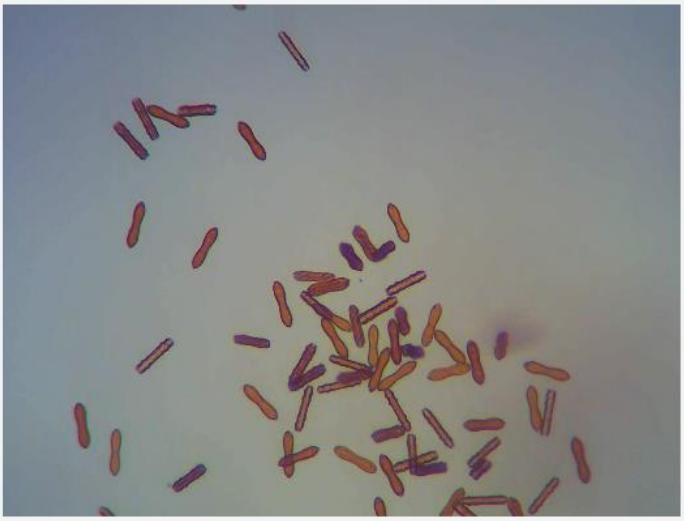
In dark field, the connecting mucilage is clearly visible at this magnification. It completely fills the space between the diatoms. Therefore, the movement of the diatoms in an EPS environment without direct contact between the diatoms plays an important role.



Oxygen produced by photosynthesis can sometimes accumulate within the EPS matrix into macroscopic bubbles. Their buoyancy is able to transport the flake to the water surface, as can be seen in the video on the left (see also post on biofilms). Such flakes represent a biofilm that is not or not generally connected to a substrate.

The tendency to the formation of such flakes increased in both species with the age of the culture strains. Often the diatoms do not move far apart after a vegetative division, but remain more or less loosely connected by EPS.

***Cymatopleura solea* on the water surface**



On the water surface of densely populated cultures occasionally float diatoms, which are in mutual mechanical contact and show a relative movement. Surprisingly, there also is the case that diatoms can move on or near the water surface without having such a contact. In the 4x time-lapse video on the left you see *Cymatopleura solea* with a length of approximately 180 µm drifting with the water which moves relative to the observer. In addition to this drifting movement of the water, locomotion of the diatoms relative to the surrounding water can be recognized. This also applies to diatoms which are not in mechanical contact with adjacent diatoms. These questions are particularly relevant:

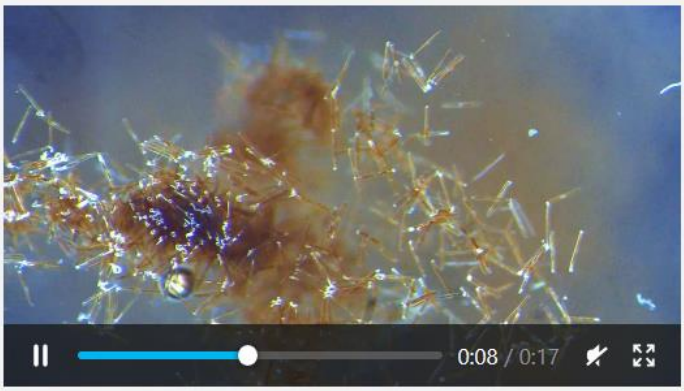
- How do the diatoms come to the water surface?
- Why do the diatoms not immediately sink again, but remain for a long time on the water surface?
- Can diatoms survive on the water surface for a long time?
- Why can they move near and on the water surface?
- What is the difference between the motion on the water surface and the motion on the substrate?
- Do diatoms have an advantage from the ability to float and to move on the water surface?

This post about *Cymatopleura solea* on the water surface is very speculative, because I cannot answer most of these questions. I will make some assumptions for discussion. In the subsequent contribution to *Nitzschia sigmoidea* the situation is clearer.

How do the diatoms come to the water surface?

Diatoms have a higher density than water. They can therefore not reach the water surface by buoyancy. An active lifting from the substrate and directed swimming through the water body is not possible.

If a diatom is not attached to the substrate with strong adhesive forces, a slight water flow is sufficient for some species (e.g., *Nitzschia sigmoidea*) to detach them from the ground. Then they remain in the water body (tychoplankton) for a period of time and can also be transported to the surface. Obviously, it is sometimes sufficient to carry the petri dish to the microscope. One can check this by swirling the water in the culture with a rod. After the flow has decreased additional floating diatoms often occur. In *Cymatopleura solea*, however, the success of the procedure was not convincing. Only occasionally some diatoms were found on the surface.



In densely populated cultures groups or flakes of diatoms adhering to each other by extracellular polymeric substances (EPS) can often be observed. Occasionally gas bubbles are included, which may be composed of oxygen. Such flakes may have sufficient buoyancy to bring the flake to the water surface. This situation can be seen in the video on the left (4x time lapse), but with the example of *Nitzschia* spec. In the case of the video of the *Cymatopleura solea* neither EPS nor gas bubbles can be recognized.

The question therefore arises whether these two approaches are sufficient.

Why do the diatoms not immediately sink again, but remain for a long time on the water surface?

Staying on the water surface can take a very different time. Some diatoms of the species *Cymatopleura solea* sink relative quickly, others can be found on the surface even after a few days. In the case of floating *Nitzschia sigmoidea* a permanent stay on the water surface is possible.

As diatoms have a greater specific gravity than water, a longer presence on the surface requires an explanation. Extracellular polymeric substances with included gas have already been mentioned as an option for transportation to the surface. This would also explain whereabouts. In the case of the video of *Cymatopleura solea* this possibility is obviously to be excluded.

It is striking that diatoms on the surface of the water often accumulate. If this is caused by hydrophobic properties, it would explain swimming. This can be proved for *Nitzschia sigmoidea*.

Can diatoms survive on the water surface for a long time?

Even Diatoms of the species *Cymatopleura solea* that float flatly on the water surface seem to be surrounded by water to survive for several days. After a few days, however, the proportion of dead diatoms is significantly higher than any of the diatoms on substrate. The water surface is not a habitat of *Cymatopleura solea*.

Why can diatoms move near and on the water surface?

As our cultures are not axenic one observes on the water surface bacteria when using DIC or phase contrast. Mostly, they are scattered or form small contiguous colonies. With their low mass compared to the diatoms and their small size, they have no noticeable influence on the movement behavior. However, if they form a closed layer, they could form a kind of substrate on the water surface. In principle, I think this is quite possible, but not in the case of clean cultures like the culture shown in the video of *Cymatopleura solea*.

A movement within a floating flake of EPS is possible, as one can see in the above video of the *Nitzschia* spec. This corresponds to the discussed movement within a biofilm. In the video *Cymatopleura solea* seems to be mobile without this EPS.

It would also be conceivable that the individual diatoms carry lumps of EPS with a sufficiently large mass and volume on their valves and transport them along their raphes. Then one would expect, however that the diatoms cannot touch easily. In the video there are actively moving diatoms, which are colliding with other diatoms. There is no evidence of such lumps. Therefore, this does not seem to be a convincing explanation for the movement.

Finally, one should consider whether Diatoms of the species *Cymatopleura solea* have a sufficient interaction with water even without a substrate. A model which could explain this is not known to me. It may be important that *Cymatopleura solea* has a canal raphe.

What is the difference between the motion on the water surface and the motion on the substrate?

In the case of movement on the bottom, a substrate exists which is fixed from the observer's viewpoint. Except for back and forth movements caused by lumps of EPS, there is a contact between the raphe and the substrate. This gives the diatom a mechanical boundary condition and thus a reduction of the degrees of freedom. In addition, movement usually follows the orientation of the raphe.

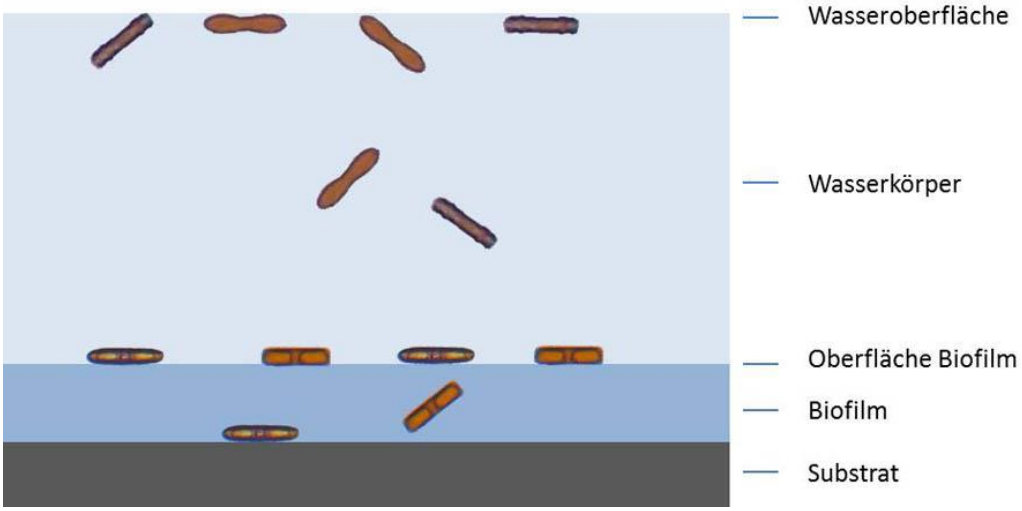
These external forces, which limit the movement, do not exist in the situation of single floating diatoms. They show rotational movements in all spatial directions and short back and forth moves. Longer straight paths apparently do not occur. This speaks is in principle for the model of a transport of transparent EPS lumps along the raphe.

Do diatoms have an advantage from the ability to float and to move on the water surface?

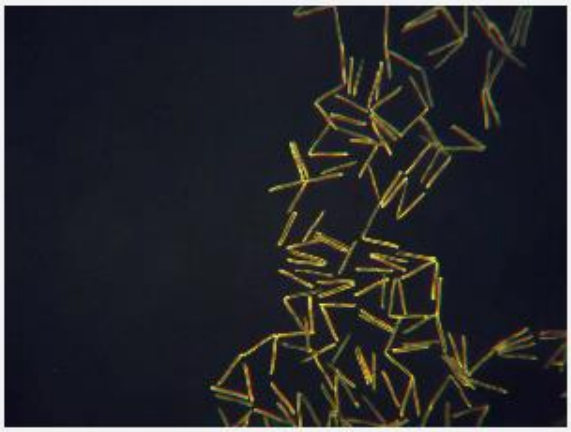
I doubt this and consider drifting on the surface of the water as an artifact. At best, it would be helpful to spread the species by drifting (hydrochory). Without indications to the occurrence of the phenomenon in nature this is pure speculation.

Locations of diatoms in cultures

In the context of the previous page I would like to summarize the places where diatoms live in our cultures.



Diatoms in the body of water can in small culture vessels only be observed for a short time after the water has been whirled up. At the small height of the petri dishes, sedimentation occurs rapidly. Occasionally diatoms that have been swirled up remain attached to the water surface for a long time. If diatoms form sufficient thick biofilms, a distinction between diatoms within or on the biofilm can be made.



Nitzschia sigmoidea floating on the water surface
(4x time lapse)

Nitzschia sigmoidea moving on a substrate
(10x time lapse)

***Nitzschia sigmoidea* on the water surface**

In 2015 we cultivated *Nitzschia sigmoidea* from the Aichstruter Stausee (reservoir) and the creek Lein, its outflow. In autumn 2016 I was able to cultivate very large specimens (360 µm) from a pond near Stuttgart-Hohenheim.

It turned out that densely populated cultures had many diatoms that floated on the water surface. The movement patterns of these diatoms and the observable properties appeared so remarkable that they are the real reason for the post about floating diatoms.

The floating *Nitzschia sigmoidea* are visible to the naked eye due to their size. The observation of the movement can therefore be performed well with a stereomicroscope. For details an inverse microscope with phase contrast or DIC is appropriate. Observation is often hindered by convection in the petri dish which is caused by the illumination. It is very helpful to insert a cylindrical ring to reduce the convection. On the top left is a time-lapse video recording of floating diatoms from the Aichstruter Stausee which was recorded in dark field with a stereomicroscope. For comparison the movement on substrate is shown in the top right. For readers with a closer interest in the movement sequences, some more videos are compiled on a separate page.

In a newly prepared culture the phenomenon of floating diatoms is evident after about two to three weeks, the first floating isolated diatoms often being found after about a week. In a fully developed culture, contiguous structures can be formed that cover a large part of the water surface. There are cultures in which the number of diatoms on the water surface is several times as high as that on the substrate.

While in the case of a vertical view onto the substrate this species occasionally can be seen in valvar view, it is never found on the surface of the water in this position, but only in the belt view. This is for a floating *Nitzschia sigmoidea* the position of equilibrium. On the surface of the water a possibility to rotate around the apical axis does not seem to exist.

The same questions arise as have already been discussed in the context of the floating *Cymatopleura solea*.

How do the diatoms come to the water surface?

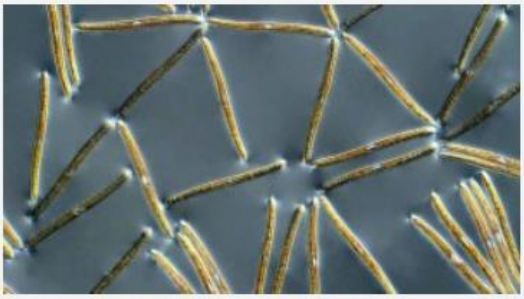
Apparently, even small water currents lead to the detachment of *Nitzschia sigmoidea* from the substrate. There is some evidence that carrying the culture to the microscope is essential for the transport of the diatoms to the surface. As the diatoms obviously do not sink again, they accumulate on the surface of the water. In order to check whether the phenomenon is sufficiently explained, one would have to make experiments with stationary cultures.

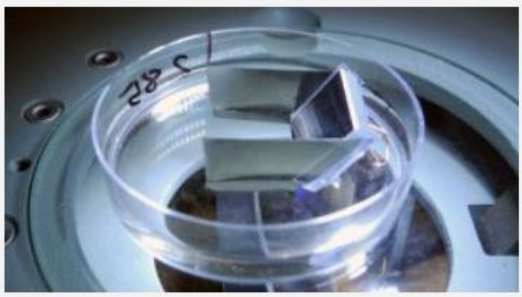
Can diatoms survive on the water surface for a long time?

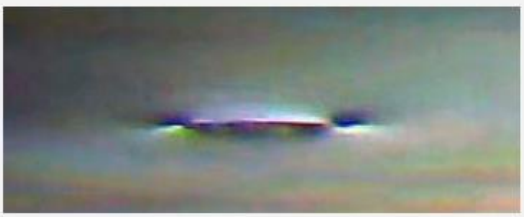
Nitzschia sigmoidea can apparently survive many days on the surface of the water and even reproduce asexually. The sometimes small number of benthic diatoms would not explain the rapid increase of diatoms on the surface of the water. In addition, a large proportion of diatoms in the process of cell division can be seen on some video recordings. I would not like to claim that the surface of the water is actually a habitat in nature.

Why does diatoms of species *Nitzschia sigmoidea* do not sink to the bottom?

In the phase contrast pronounced brightening is visible on most apices.







Significant changes in brightness also occur in differential interference contrast (DIC) which can be seen in the picture on the left (click to enlarge). This indicates phase objects which are based on different optical path lengths. Under the stereomicroscope one can see with an oblique view of the water surface that the surface is arched around the apices. There is a more or less pronounced convex meniscus which explains the appearance in phase contrast and DIC. The diatom lies deep in the water. This is not easy to be photographed. With some effort pictures are taken with the help of a mirror, which is inserted into the petri dish of the culture. Its inclination is adjusted so that the water surface is observed at a small angle. The equipment is shown on the left. Below you can see a picture which has been taken with the equipment.

In the following figure a diatom from a horizontal view is sketched (valvar plane). The ends of the diatoms obviously have hydrophobic properties which lead to the deformation of the water surface and give it a buoyancy, as is known from the water strider.

Wang Y et. al. (2012) have found that valves of *Coscinodiscus* sp. float on water surfaces. However, the ability of living diatoms to float is not reported. Here too, hydrophobicity is the cause. After examination of cleaned valves the authors conclude that the hydrophobicity is based on the convex form and 40 nm sieve pores. I consider it an open question whether this explanation applies to

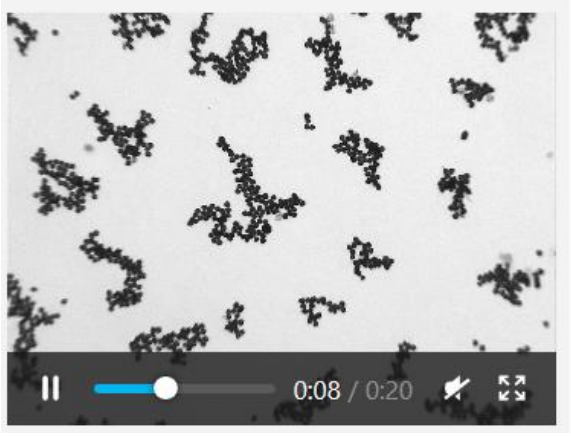
floating living *Nitzschia sigmoidea*. The structures of the valves differ. Moreover, living diatoms have an EPS cell coating which could prevent the hydrophobic effect of sieve pores. I think it is quite possible that EPS, which is secreted at the apical pores, has hydrophobic properties. I cannot answer the question of the nature of the hydrophobia with the tools available to me. I would appreciate if this question would be taken up by visitors of the site.

In this context it should be noted that after a few months the cultures of *Nitzschia sigmoidea* lost the ability to float. At first the typical patterns of connected diatoms on the water surface became less regular and finally disappeared completely. The floating diatoms did not always lie side by side but more and more frequently crossed, and occasionally they hung with only one end on the surface of the water. Later, the proportion of diatoms on the surface of the water diminished. At the moment I can only speculate about the cause.

I am just watching this weakening hydrophobia again. The video on the left (4x time lapse) shows a culture in which the phenomenon is already clearly visible.

Attractive forces between diatoms on the water surface

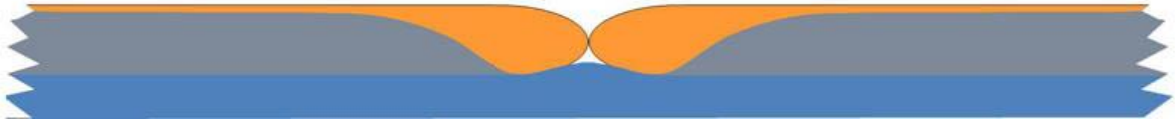
When looking at the patterns that form floating *Nitzschia sigmoidea*, one can see that the apices of the diatoms very often lie next to each other. This leads often to parallel diatoms, but also to star-shaped, linearly concatenated and polygonal patterns. Even dead diatoms can be part of these structures. Also Wang Y et al. (2012) report on the formation of regular structures in Coscinodiscus. This is a consequence of the hydrophobic properties. If floating hydrophobic bodies accumulate, energy is released to the environment. The system prefers the state of minimal energy. This phenomenon of spreading has been known for a long time. Wang Y et al. (2012) call this effect ‘self-assembly’.



One can observe such a self-assembling well by scattering *Lycopodium* powder on a surface of water. You can see this in the video on the left (4x time lapse). It is remarkable that sometimes existing contacts between particles are broken. In total, energy is reduced in the multi-particle system, for example by establishment of other connections at different locations. To explain the processes one must consider the many-particle system.

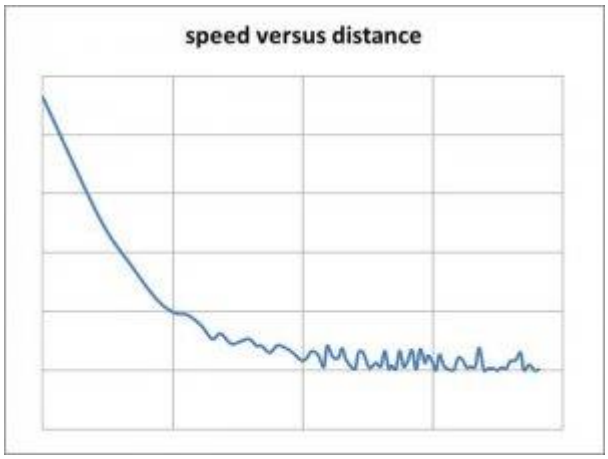
Between hydrophobic bodies there is an attractive interaction at the water surface. Specifically, in this case there is an attractive force between the ends of the diatoms. A similar behavior would result if the ends of the diatoms were hydrophilic, because there is also attraction between hydrophilic floating structures. However, there is a repulsive force between hydrophobic and

hydrophilic bodies. The following drawing shows two diatoms in the valvar plane with the resulting water surface.



The water surface has a lower energy than in the case of two separate diatoms.

Regarding the form of the diatoms an analytical representation of the law of force can certainly not be given. A simplified modeling consists in the replacement of the diatom by two rotationally symmetric hydrophobic particles, which are connected by a rod having the length of the diatom. Unfortunately, even in this approximation no analytical description of force is known to me.



For the experimental determination of the attractive interaction one can examine the movement of diatoms, which move toward each other or the movement of a hydrophobic particle like spores of *Lycopodium* in the vicinity of a diatom. Under the plausible assumption that the inertia force can be neglected in the equation of motion, and Stokes' law holds, the velocity is proportional to the force. I have not systematically carried this out. In the picture on the left the velocity of a diatom which is proportional to the force is plotted against the distance of two approaching diatoms. This is an exemplary result in which it is not ensured that the water surface is sufficiently calm. In addition, the temporal resolution in the vicinity of the collision is not sufficient. The picture is only intended to illustrate the principle of the procedure.

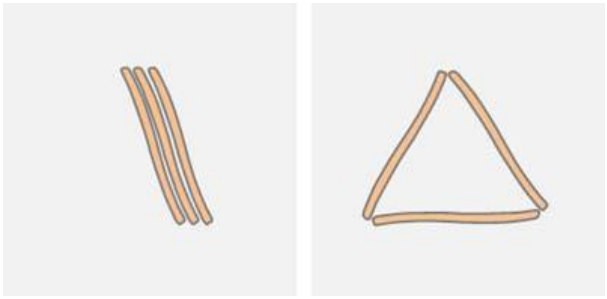
As mentioned at the beginning, not all diatoms have a pronounced hydrophobic apex. It is enough to stay on the water surface, but the attractive interaction is barely recognizable in the movement. There are also systematic differences between the strength of the hydrophobicity of the diatoms from different localities. This affects the accuracy of the patterns of movement.

Wang Y, Pan J, Cai J, Zhang D (2012), Floating assembly of diatom *Coscinodiscus* sp. microshells. Biochem Biophys Res Commun 420(1):1–5

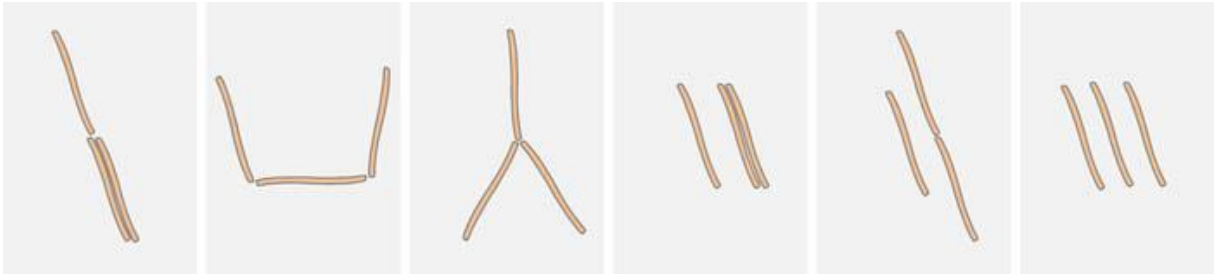
Movement patterns of floating *Nitzschia sigmoidea*

As the videos of the movement illustrate, the motion sequences are fundamentally different from movement on a substrate. On the water surface there is no interaction with a solid substrate, which determines the paths of benthic diatoms. Here, the attractive interaction of the hydrophobic apices also occurs. If there were only these attractive interactions, a distribution on the surface would be similar to that of *Lycopodium* powder, whereby the movement would come to rest when a local energy minimum was reached. "Local" means that the energy cannot be reduced by a small change. An absolute minimum is not reached because of the stability of the local minima and the active movement of the diatoms. Lattice structures as in crystals probably do not occur in such clusters of living motile diatoms.

The pattern of the diatoms can be described by undirected graphs. The apices form the nodes. Parallel diatoms represent multiple edges (multigraph). If a diatom touches a second diatom at a point other than its apex, there is no bond. According to the simple model of two hydrophobic particles in the distance of the apices one expects structures with few unconnected nodes (i.e. unbound apices). For example, if three diatoms are within the range of the surface forces, the configurations where all ends have bonds to other diatoms should prevail:

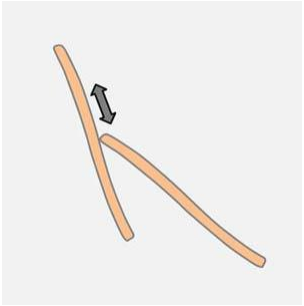


Energetically unfavorable are patterns with free ends, as are shown below:

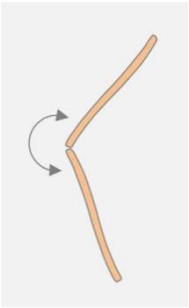


A chain of three diatoms should close to a triangle because of the attractive interaction of the free ends. The diatoms however, are not passive floating objects, but show active movement. The diatoms release energy and thus temporarily produce patterns with a higher energy.

There are elementary sequences which are observable locally in the entire structure pattern:

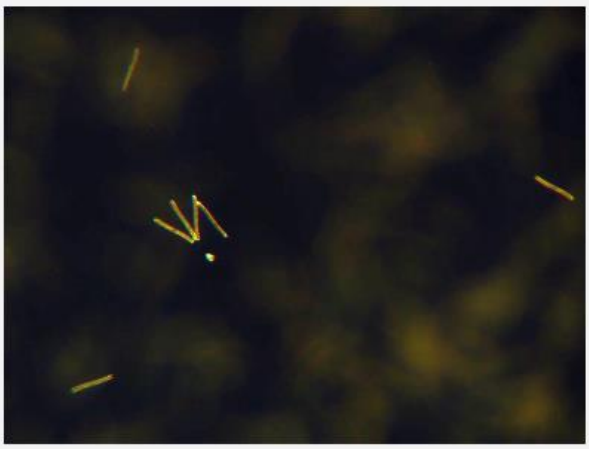


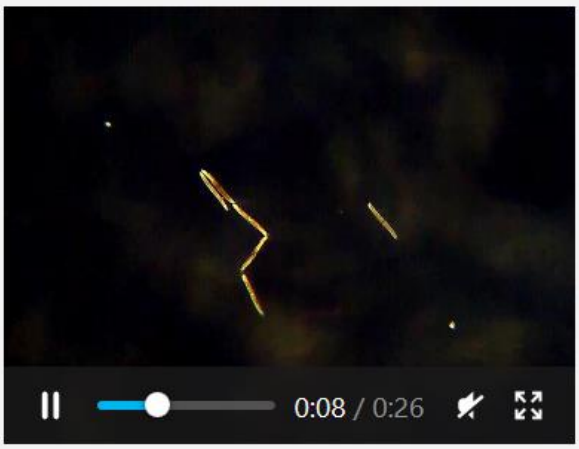
The end of a diatom can be moved by another diatom along its raphe (see drawing on the left). An existing connection at an apex of the moved diatom can be broken.



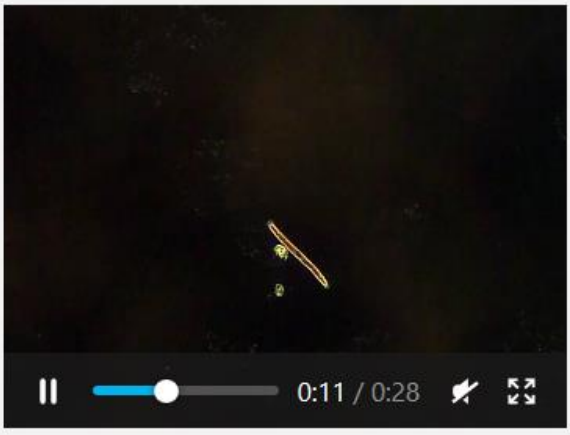
Very frequent are angular changes at connected apices (see drawing on the right). Diatoms that adhere with the apex on a substrate are capable of performing pivoting around the cell pole. It is probably the same mechanism which is observed on the surface of the water.

The static attractive interaction of the apices, the active movement along the raphe and the active change in the angle between two connected diatoms can produce a huge amount of patterns and sequences in a system of several diatoms. In the time lapse video (time lapse factor 30) at the bottom left we see a cluster of only four diatoms which, by means of the described types of motion, take different forms successively, the number of free ends varying between 2 and 5. To the right of it, another example of motion processes (time lapse factor 32) is presented, whereby some particles floating on the surface illustrate the activity of the raphes.

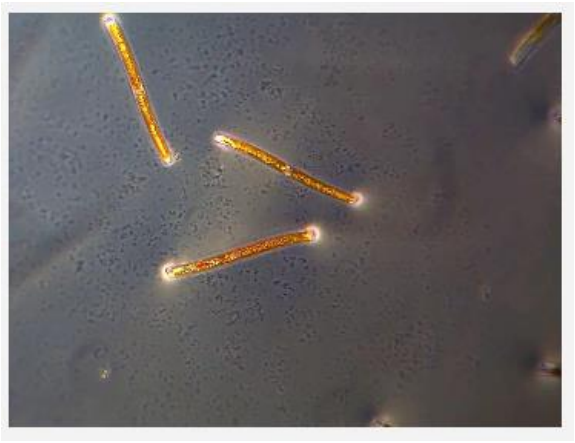




Older cultures are often contaminated with EPS. This can also drift on the surface and be transported by the raphes. In the following video on the left (32x time lapse) there are a diatom and two particles. Due to the extruded EPS new patterns of movement on the surface of the water are caused. On the right side, a video with a large time lapse factor (64) shows two diatoms, which have coordinated sequences in their movement.



Although no EPS is recognizable, I suspect EPS as the cause. The two diatoms could be in contact with the lump and transport it along their raphes, which lead to mutual interaction. I would not assume that there is a long-distance effect which is caused e.g. by water vortices.



More difficult is the interpretation in another case. In the video on the left (80x time lapse) you see in phase contrast many bacteria on the surface, which make the water movement visible. The raphe appears to generate a water flow, which is causally connected with the movement. EPS on the raphe and between the diatoms is not recognizable. However, I do not want to rule out this possibility. In this context, it should be mentioned that even individual diatoms are sometimes capable of small movements on the surface of the water. I cannot judge whether lumps of EPS at the Raphe cause this movement.

Light intensity also plays an important role. Thus, in a culture which is exposed to a low intensity of light, parallel diatoms are frequently found. Under the more powerful microscope illumination the activity increases at the raphes and the structure changes into an irregular structure.

Diatoms of diverse localities differ in terms of the movement sequences and the accuracy of the connections at the apices. I suspect a differently strong hydrophobic interaction as reason. Against this background it is worth watching the watching the compilation of the videos.

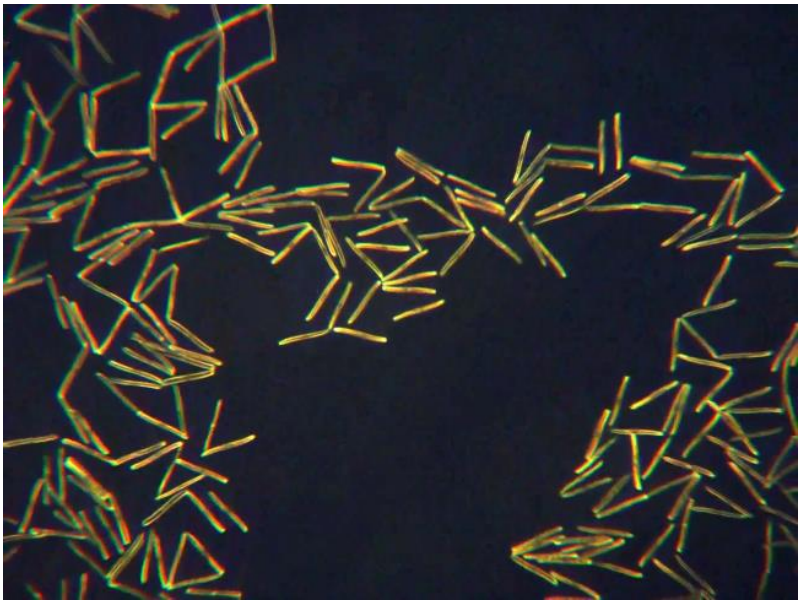
Due to the mechanical coupling of many diatoms, water flow, activity of the diatoms at their pole and raphe a complex pattern is generated that change in time. I believe that the given presentation is only for discussion. It should not give the impression that all processes of the movement can be explained with the illustrated approaches. Some aspects require further investigation.

Examples of floating *Nitzschia sigmoidea*

The lengths of the observed *Nitzschia sigmoidea* ranged from 180 µm to 380 µm.

Locality: Aichstruter Stausee (48°54'06.6"N 9°38'25.2"E)
Found in April 2015

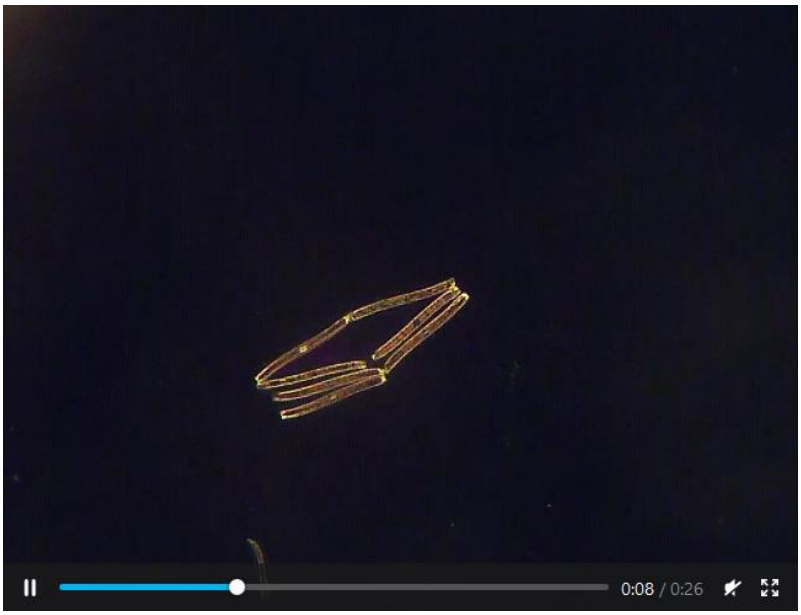
Time-lapse factor: 4 (i.e. four times faster than real time), dark field



Locality: creek Lein (48°53'04.6"N 9°38'29.2"E)
Found in August 2015

Time-lapse factor: 20, dark field

Time-lapse factor: 10, dark field



Locality: pond in Hohenheim (Stuttgart) (48°42'34.0"N 9°12'29.1"E)

Found in November 2016

Time-lapse factor: 24, bright field

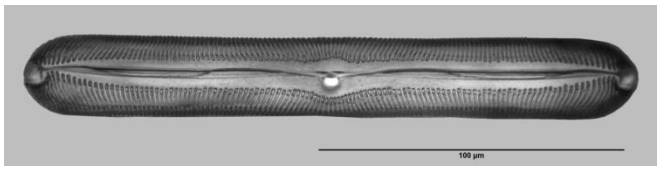
Time-lapse factor: 10, bright field

Time-lapse factor: 4, PlasDIC

Pinnularia sp. on the water surface and on a substrate (150x time lapse)

Pinnularia sp. on the water surface

When *Pinnularia* cultures are prepared, a fast sedimentation of inserted diatoms is usually observed. An exception among my cultures was *Pinnularia gentilis* (Donkin) Cleve from a small pond in Hohenheim (Stuttgart, 48°42'32.2"N 9°12'40.3"E). At the time of observation, the diatoms were already in culture for 6 months and had a typical length of 200 µm. Such a *Pinnularia* is shown on the left (click to enlarge).



In the case of diatoms from these cultures, it was striking that many diatoms were swirled up and sedimented relatively slowly on the substrate when the petri dish was carried to the microscope. If one observes the surface of the water immediately after swirling up the diatoms, one regularly finds *Pinnularia*, which float on the surface of the water. In a densely populated petri dish with a diameter of 55 mm, the water surface contains a few to several - tens of diatoms. Diatoms often float in groups on the water.

The video (time lapse factor 150) top left shows *Pinnularia* on the water surface and afterwards diatoms moving at the bottom of the petri dish. By chance, all diatoms are located on the surface of the water in a valve view (valve is uppermost). A video, in which the girdle view (girdle is uppermost) can be found on the water surface, can be seen at the top right. Some observations will be described below.

The sinking of the diatoms

Already in the first minutes many of the diatoms sink to the ground. Others remain on the surface for hours and only a few for days. The videos shown here were taken immediately after the swirling up of the diatoms. Therefore, the sinking of diatoms to the bottom can often be observed.

In all observed cases, the diatom starts to sink with the diatom taking a position perpendicular to the water surface. Often it remains on the water surface in this orientation for a while. The diatom often sinks to the ground in this orientation. However, it is not unusual for the diatom to rotate around the transapical axis or pervalvarous axis as it sinks. It is not possible to identify which axis it is because the automatic image series focused on the diatoms on the water surface. It is also not clear whether the direction of rotation changes.

In the two videos above, the sinking with and without rotation is recorded. For better visibility, a spatial and temporal fragment of the video in the upper left corner (90x time lapse) is shown on the left-hand side below. In this video you can watch twice rotating diatoms while they sink. Please note that there are other special details in the video. The diatom in the upper right-hand corner, which later sinks down under rotational movements, is temporarily in a vertical position, but returns to the surface in a horizontal orientation "without external help". This cannot be explained without active movement in the resting water body, because the potential energy of the diatom in the floating state with horizontal alignment is higher. For a longer period of time another diatom hangs vertically on the water surface. Later it is transported to a horizontal orientation after collision and floats on the water surface. Apparently, it is not alive anymore.

To second video shows two cases of sinking diatoms (30x time-lapse) with no rotation. The sequences are separated by a short dark pause.

Movement patterns

The two videos shown above already give a good impression of the typical movement patterns. As on substrate, *Pinnularia* in valve view have a high mobility and cover longer distances, while back and forth movements are carried out in girdle view position. Such long distances are not covered by *Nitzschia sigmoidea* at the water surface. The question arises which way this is accomplished with a raphe that is below the surface of the water. To my regret, I cannot give a satisfactory explanation.

Furthermore, the top right video shows a *Pinnularia*, which rotates around its apical axis and thus reaches the valve view from the girdle view. So it can cover a wide curve before sinking. Here, as in other sequences, these approximately circular paths are typical. The trajectory is distorted by a more or less pronounced drift movement. As in the case of the movement on substrate, I suspect that the EPS transport in the area of the helictoglossa is the cause of rotation about the apical axis and the curvature of the path.

The video on the left shows three movement patterns. They are detail enlargements from a larger image format. The first *Pinnularia* describes a typical roughly circular orbit, which is distorted by additional drifting motion, the second one a rotation around the pervalvarous axis almost without forward motion and the third one finally a wagging motion, thus a change of the direction of rotation in quick succession. After each video sequence an image of the superposition of the frames is inserted.

Interaction between diatoms

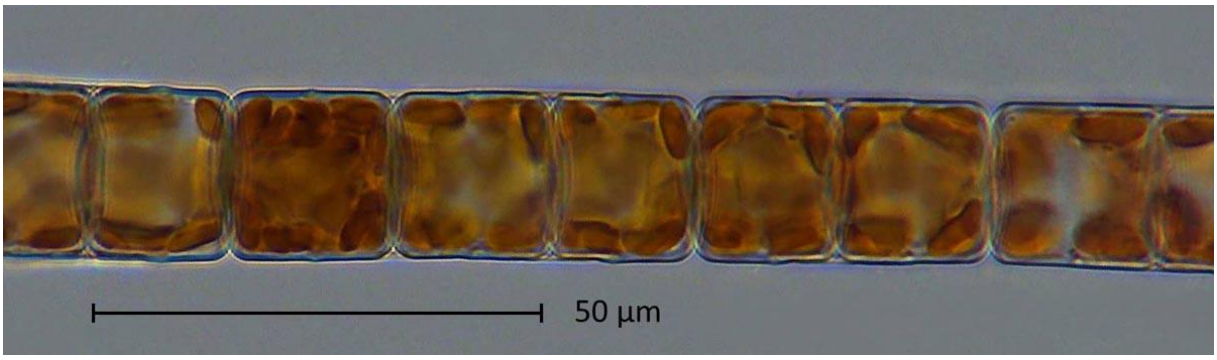
Collisions of diatoms on the water surface are frequently observed. As the videos shown so far show, they are mostly unspectacular and differ little from collisions on the substrate. Within a few hours of observation I could observe and record one exception. The video on the left shows the "dance" of two *Pinnularia* in 60-x time-lapse. There is an obvious long-distance interaction. The cause is not recognizable. Since this interaction is apparently only rarely observable, it seems plausible to assume an interaction via non-visible adhesive EPS flakes. Hydrophobic or hydrophilic areas at the diatoms are also plausible, analogous to the hydrophobic properties of *Nitzschia sigmoidea*. Water motion could also play a role.

In the absence of a sufficiently strong, systematic and attractive interaction, pattern formation as in *Nitzschia sigmoidea* does not occur.

Summary

While some aspects of the movement of *Pinnularia* on a water surface can be explained, many aspects seem to require further explanation. It should be noted that there were only small concentrations of bacteria on the water surface. They never formed a continuous film at any time.

At least in cultures, diatoms on the water surface are not uncommon. I was also able to observe floating diatoms of the genera *Cymbella* and *Rhopalodia*. The latter moved like *Pinnularia* in large circles. Therefore, anyone who cultivates diatoms in petri dishes is recommended to have an occasional look at the water surface.

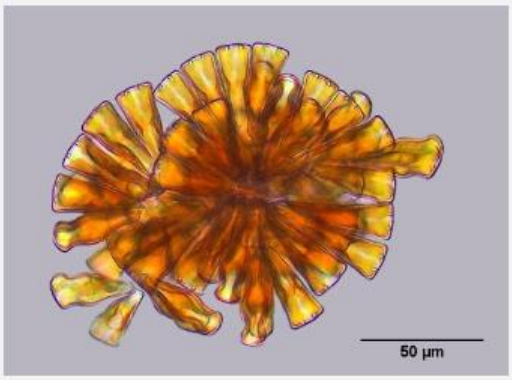


Introductory words and pictures of chain-forming diatoms

Some diatoms do not separate after an asexual reproduction, but adhere together and form chain-like colonies. These colonies can take the form of filaments, ribbons, stars or fans. The picture above shows a section of a colony of *Melosira varians* (click to enlarge). In cultures one can often observe such colonies consisting of thousands of connected diatoms.

All following images are taken from freshwater cultures. Click on the image to enlarge it. By using the arrow keys or by clicking on the small thumbnails at the bottom, you can view more images.

In the subsequent image gallery on the left you can see colonies of *Gomphonema capitatum* forming overlapping fans. *Gomphonema capitatum* is a motile diatom. It can detach from colonies and form a new colony elsewhere. Especially in newly created batch cultures, this *Gomphonema* can be seen moving between the still young colonies. This movement between such colonies is shown in the video on the left in 8-fold time-lapse.
Meridion circulare also forms fan-shaped colonies, which can be seen to the right.

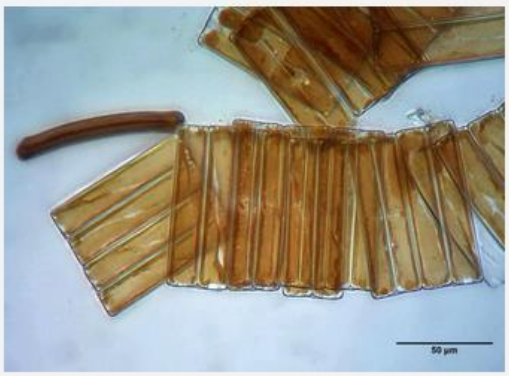


Gomphonema capitatum

Meridion circulare

In the following series of pictures on the left there are pictures of an *Eunotia* culture in which long ribbon-shaped chains are formed. *Eunotia* is also motile. At another section there is a contribution on their complex movement patterns.

To the right you find pictures of *Melosira varians*. First you see a small beaker containing nutrient solution, into which a few fragments of a young Melosira colony have been given. Already after a few days in suitable lighting, a fine web of long colonies appeared. This web was taken with the stereo microscope at two magnifications. In order to achieve sufficient contrast, the photos were taken with dark field illumination. The original colony has already been broken into several fragments.

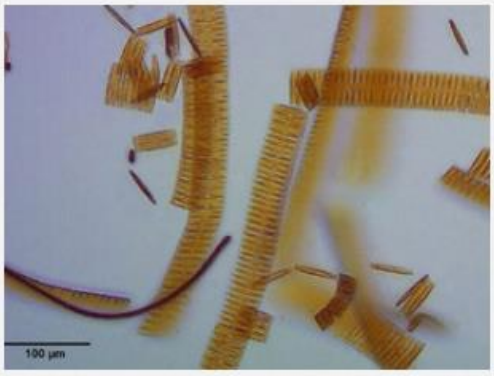


Eunotia sp.

Melosira varians

Images of a *Fragilaria* culture (probably *Fragilaria capucina* not completely excluded is *Fragilariforma virescens*) are shown in the image gallery below left.

To the right pictures of *Diatoma vulgaris* in culture follow. The diatoms do not lie parallel to each other, because they usually do not separate completely, so that zigzag forms develop. In the DIC images, the ESP pads connecting the diatoms are clearly visible.



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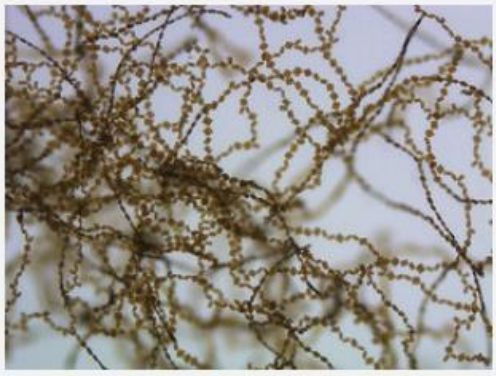
Fragilaria sp.



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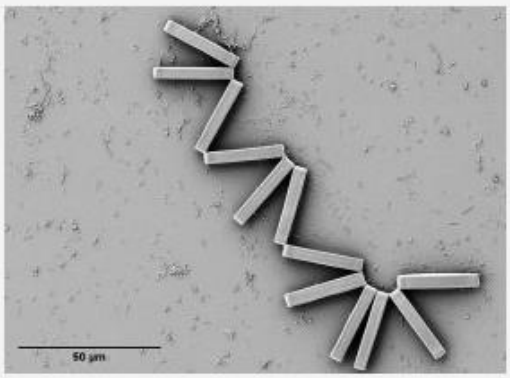
Diatoma vulgaris

An image gallery of a *Diatoma* culture of unclear species is shown below left. This diatom also quickly develops a ball of connected diatoms in a beaker with nutrient solution. SEM images have been taken for this *Diatoma* cell line. Before inoculating a new culture, small round coverslips were placed into the Petri dish, which were then colonized by the diatoms. The diatoms on the cover glass were fixed with glutaraldehyde, rinsed with distilled water and dehydrated in baths with increasing isopropanol concentration. The SEM images were kindly produced by Dr. Wilfried Nisch, NMI Reutlingen, https://www.nmi.de/en/. On the images (picture gallery below right) you can see well the extracellular polymeric substances (EPS), which connect the diatoms to a chain near the apices.

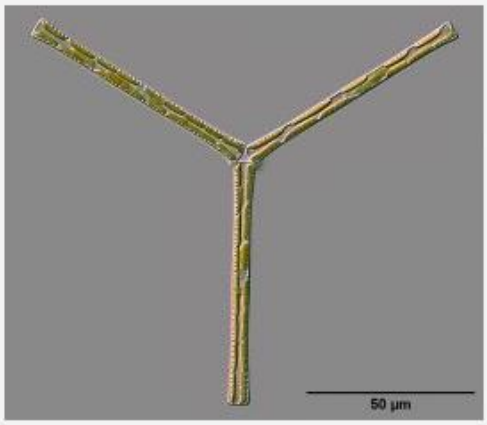
Diatoma sp.



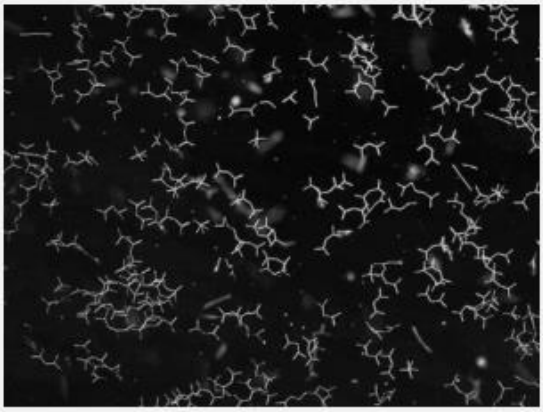
Diatoma sp. SEM images

In the following image gallery, some images of the cultures of *Diatoma tenuis* are shown. Longer chain-like structures, as well as short planktonic colony forms, are observed in this species. In culture, structures of one star formed by three diatoms and structures of up to four such connected stars occurred. In addition, there are transitional forms. The video on the right shows the sedimentation of *Diatoma tenuis* colonies in a Petri dish in 200-fold time lapse. The focus was on the bottom of the dish.

As a final example of chain-shaped colonies, a image gallery and a video of *Bacillaria paxillifera* (*Bacillaria paradoxa*) is shown below.



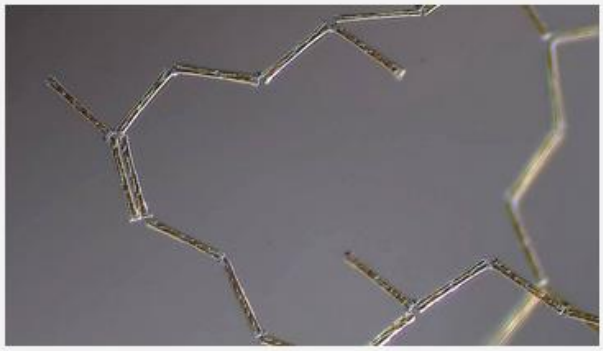
Diatoma tenuis (40x-Objektiv, DIC)



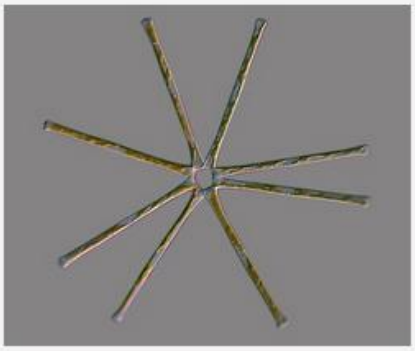
Diatoma tenuis, 3600-facher Zeitraffer

With *Diatoma ehrenbergii* another species of the genus Diatoma could be kept in culture. As shown in the images of the image gallery on the lower left, chain-like colonies, which adhere to the substrate, occur in coexistence with planktonic, mostly stellate colonies. Thereby, the short planktonic colonies are often formed by separation from long sessile colonies.

To the right of these images are presented from cultures of *Asterionella formosa*. All cultivated planktonic species tended to multiply rapidly and reached high densities.

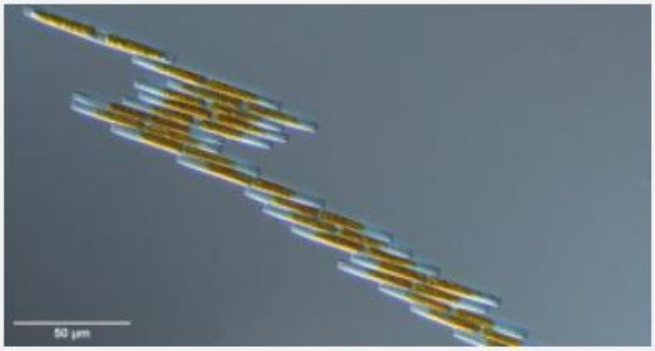


Diatoma ehrenbergii



Asterionella formosa

As a final example of chain-shaped colonies, a image gallery and a video of *Bacillaria paxillifera* (*Bacillaria paradoxa*) is shown below.



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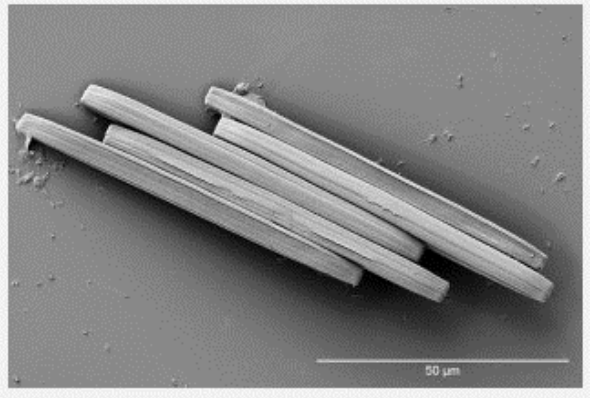
Bacillaria paxillifera (40x lens, DIC)

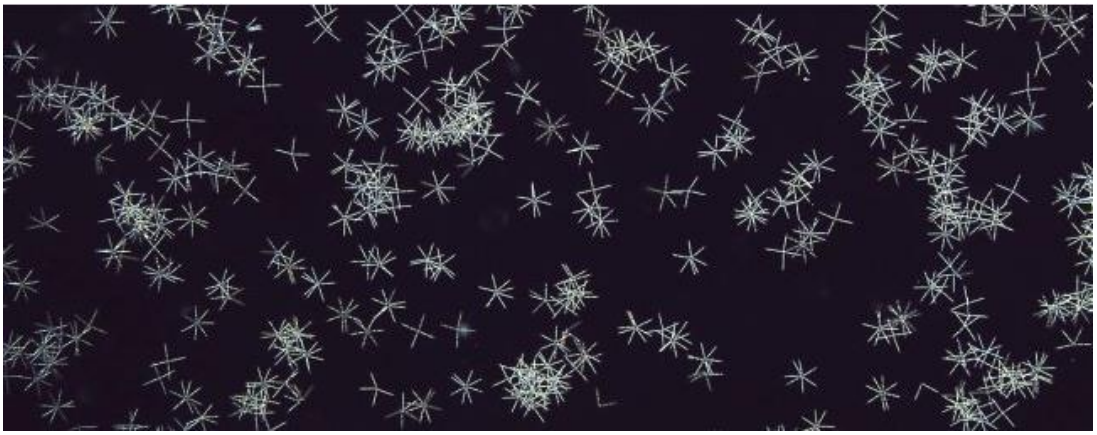
Bacillaria paxillifera (20x lens, 4x time lapse)

The diatoms have a raphe and exhibit a remarkable mobility, which is apparent in parallel displacement of the diatoms relative to neighbouring diatoms.



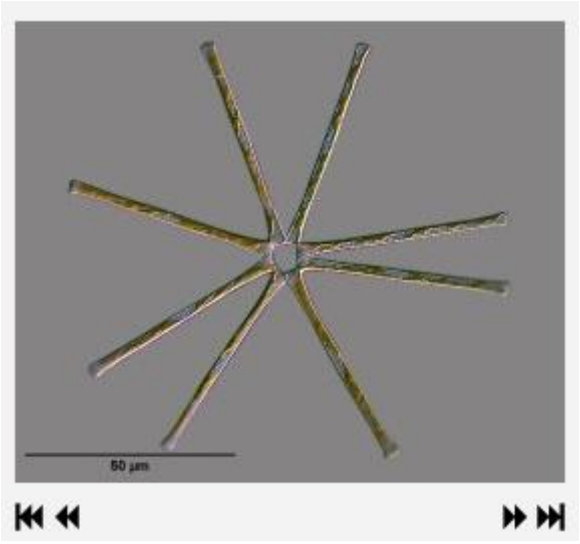
Dr. Nisch has also taken SEM images of *Bacillaria paxillifera*, which can be seen in an image gallery on the left. One can easily recognize the EPS, which serve the relative displacement. The preparation corresponds to that of the Diatoma species. Since thereby the samples were dried in the air after dehydration, artifacts are to be expected.

In nature, large colonies are easily broken into fragments by external influences such as a turbulent flow, so that only small colonies usually are found. Fragments can easily be drifted and may help spread.



Colony formation in *Asterionella formosa*

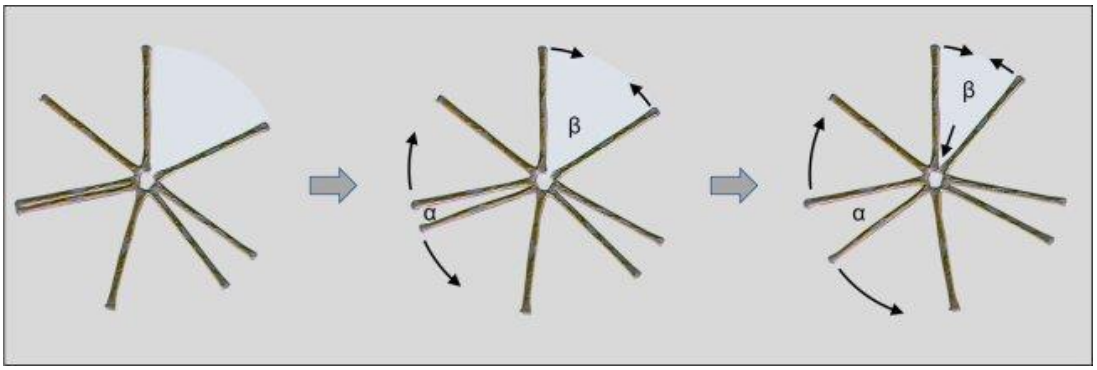
Diatoms of the planktonic species *Asterionella formosa* were found in the river Neckar (Germany 49°04'41.8 N 9°09'17.9 E). Since planktonic organisms typically do not occur in flowing waters, they probably originated by upstream barrages.As the name *Asterionella* indicates, they form star-shaped colonies. On the left side the picture gallery from the introduction is shown again.



For phytoplankton, it is usually important to have a sinking speed as low as possible. Because of the small size of organisms and colonies, the friction force is well described by Stoke's law (small Reynolds numbers). Thus, the sinking speed is proportional to the density difference of phytoplankton and surrounding water and reciprocally proportional to the form resistance, which depends on the geometry of the sinking body (Ostwald 1912). Star-shaped structures have a high form resistance compared to a sphere of the same volume. Judit Padisák et al. (*) have experimentally shown by experiments with differently shaped models that the shape resistance of *Asterionella* increases with the number of diatoms in the star up to 6 diatoms and remains approximately constant thereafter. Deviations from symmetry, i.e. unequal angles, decrease the shape resistance.

Stellate and zig-zag shaped colonies of different species may differ with respect to the concatenation of adjacent diatoms (connecting points), the angles between adjacent diatoms, and the lengths of the chains. The rules leading to pattern formation or morphogenesis at the colony level are correspondingly different. *Asterionella formosa* probably exhibits the simplest possible formation processes. This is due to the fact that the connection points of neighboring diatoms are always located at the same apex. Therefore, there can also be only one possibility of separation of neighboring diatoms. Star-shaped colonies always transform into star-shaped colonies when they divide. A synchronicity of the divisions is not necessary for this (no transitional forms). The following composite photograph illustrates this exemplarily. In the star-shaped colony on the left, a pair of diatoms is about to split into a V-shaped structure. If no external forces act, as in the case presented,

this opening occurs in a short time compared to the generation time. In the middle image, the splitting has occurred (angle $\alpha > 0$) and thus the angle β between the two diatoms at the ends of the chain decreases. In the following time, the angle α increases and β diminishes correspondingly.



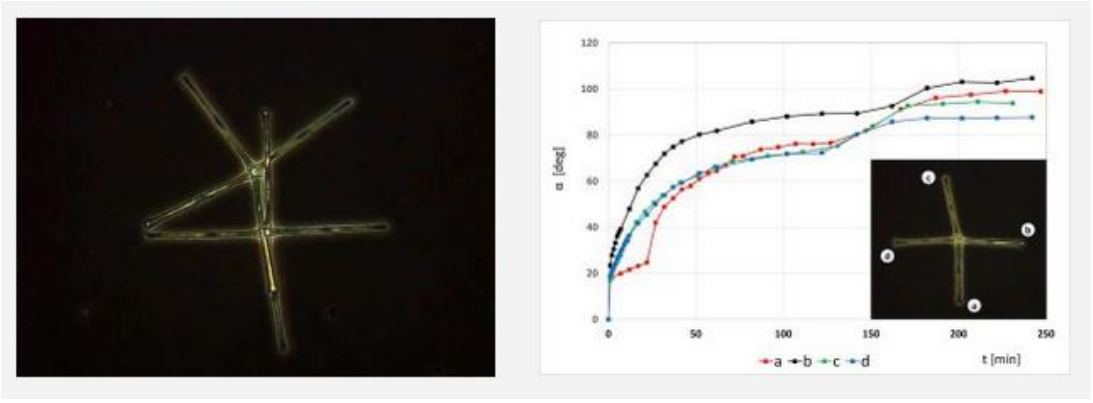
For simplicity, it is assumed that no further division occurs during the process shown and that the other angles remain unchanged. The two halves then rotate against each other as a rigid entity. In the case shown, contact of the diatoms at the ends of the chain occurs at the apices in the center of the star (marked by short arrow). Unless the ends of the chain slide over each other, which is often observed, each further increase of α and each further division leads to mechanical stresses in the chain. This can cause the angles in the colony to change and eventually break the chain, creating two new colonies. Even if the ends slide over each other, further divisions will eventually cause diatoms to become hooked and the chain to break. Rotations of the partial chains in space can also be observed. Thus, the colonies do not always lie in the plane of the substrate. The size of the colonies is limited by the fractures that occur sooner or later. The breakage of colonies seems to be the result of external tensions as a rule, but spontaneous breakage at older joints cannot be excluded either.

From a considered diatom, the doubling of the number of diatoms per generation quickly results in longer sequences that break into pieces. Since the splittings are approximately synchronous over some generations, one often observes that after a longer phase without splittings (about one generation time) the splittings happen in rapid succession.

The asymptotically reached opening angles vary strongly, so that also smaller regular colonies with similar angles, as well as formations with more than 8 diatoms are found. Star-shaped colonies with 8 diatoms are frequently observed and are often shown in the literature.

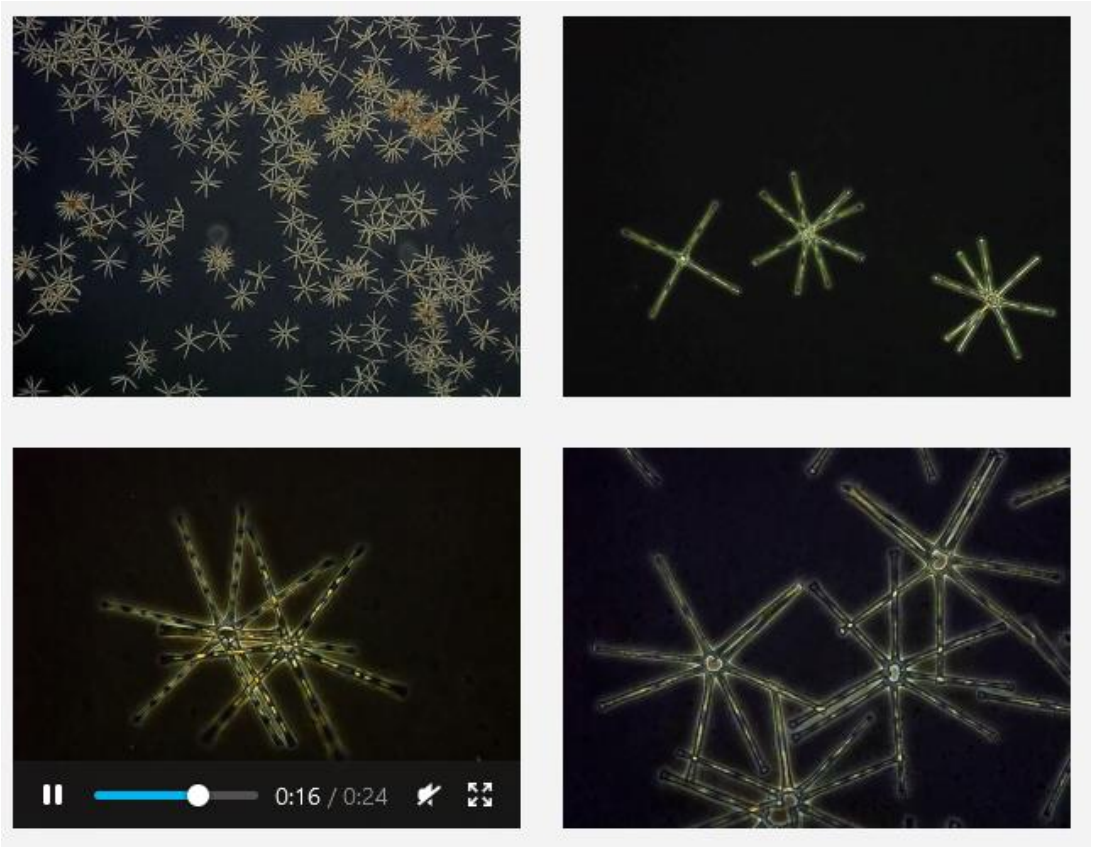
Each split begins with a sudden opening of the neighboring diatoms to a V-shaped structure. Apparently, a mechanical tension built up in the EPS pads at the hinges, which exceeds the adhesion forces between newly formed diatoms. Thereafter, the increase in opening angle is uniform, with a decrease in angular velocity.

Without collision between parts of the same chain or with other objects, the final angle is reached asymptotically. Such behavior was also seen in all observed *Diatoma* species (*D*. *tenuis*, *D*. *eherenbergii*, *D*. *vulgaris*). In the video below, on the left, 1500-fold time-lapse views of the splitting of a chain initially consisting of four diatoms are shown. To the right, the opening angles of the four diatoms are shown as a function of time. The moment of splitting was set to the origin of the time axis in each case. There are clearly recognizable deviations between the functions.



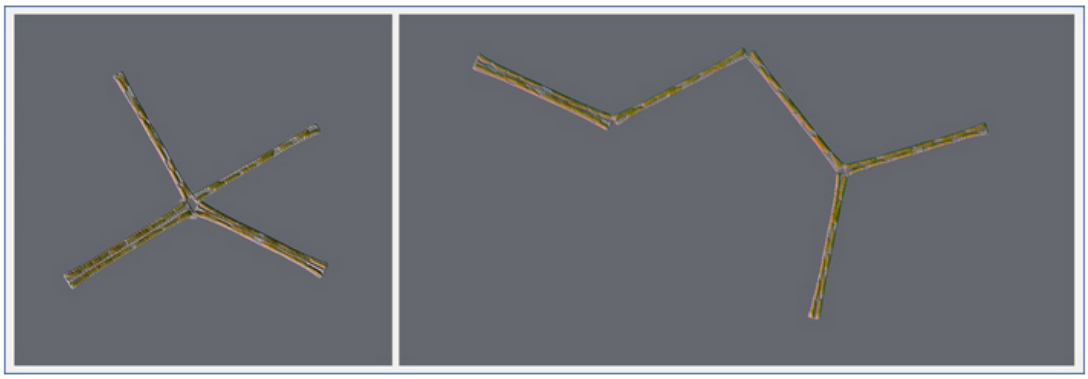
The angles taken at sudden opening are 17°, 23°, 17° and 19° in the order given.

A series of other observations of divisions can be seen in the following time-lapse videos (time lapse factors 1500 and 1600). The upper video on the left shows a larger part of a culture that is not yet very dense. The individual separation processes are better visible at a higher magnification.



In summary, it should be noted that in *A*. *formosa* the formation of the star-shaped structure, which is advantageous for a low sinking speed, is achieved by a simple splitting process and a fracture mechanism due to mechanical stresses. From this point of view, *A*. *formosa* has a model character and is a good starting point for studies on other star-shaped and zigzag shaped colonies.

(*) Padisák, J., Soróczki-Pintér, É., and Rezner, Z. (2003) Sinking properties of some phytoplankton shapes and the relation of form resistance to morphological diversity of plankton—an experimental study. *Hydrobiologia* 500(1), 243-257.

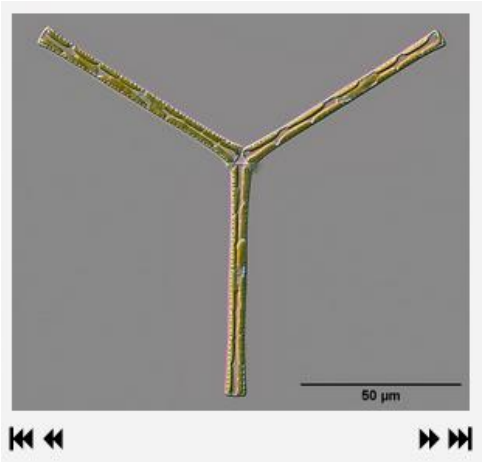


Notes on colony formation in Diatoma tenuis and Diatoma vulgaris

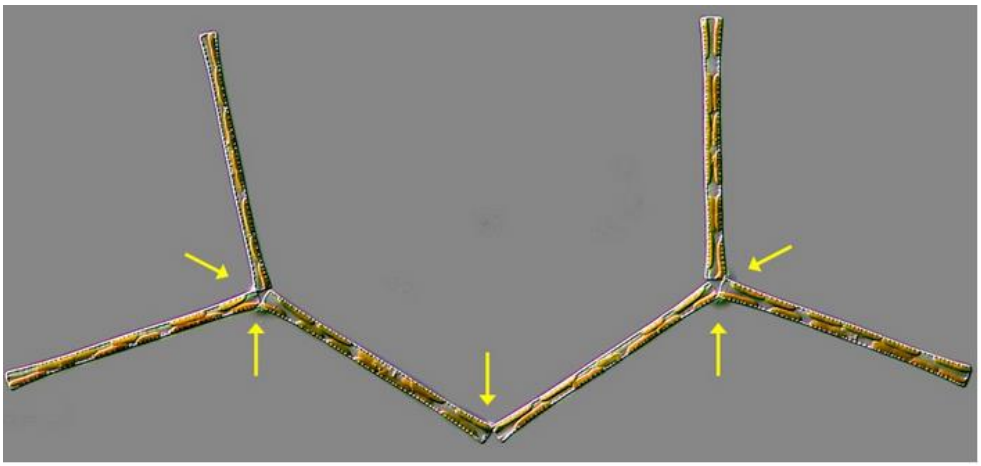
Colony formation in Diatoma tenuis and Diatoma vulgaris represent extensive topics and will only be briefly discussed here. Publications have been submitted on this subject.

Diatoma tenuis

Diatoma tenuis can occur in the form of longer chains and star-shaped structures, with up to four such star-shaped structures connected. Transitional forms also appear. In culture, only star-shaped and linked star-shaped colonies were observed. On the left again images from the cultures are shown.

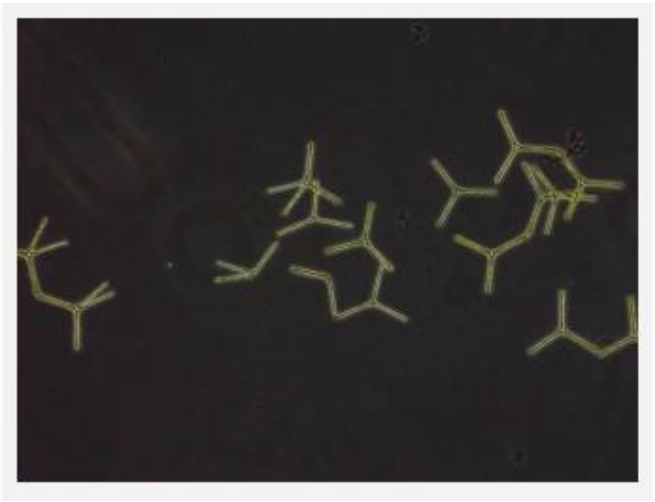


A simple star-shaped colony of three diatoms does not seem to differ from *Asterionella formosa* with respect to the connection points at first sight, but with two connected stars it becomes clear that the connection points of the diatoms in the chains are not generally located at the same apex (see image below). The two diatoms that connect the two star-shaped structures each have their connections at diagonally opposite locations. The rules of formation must take this into account and allow the typical forms of simple and chained stars to reproduce themselves repeatedly.



For the preservation of the structures over the generations, the breaking of the chains must also take place appropriately. It almost always occurs spontaneously without external forces and exclusively at the connection points of the star-shaped substructures, so that star-shaped fragments are always formed.

Transitional forms arise between completed concatenated star-like structures. At the top of the page two examples of such forms are shown. In the example on the left, two separations are required for the almost fully developed diatoms to form a colony of two star-shaped structures. In the example on the right, there is just one. In the phase of exponential growth, the proportion of transitional forms is low, which can be explained by sufficient synchronicity of the divisions.



The video on the left shows the vegetative reproduction in a low-density culture in 3600 time-lapse.

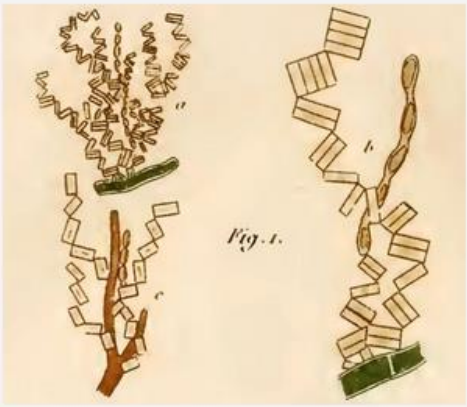
The asymptotically reached angles between adjacent diatoms range around 120° and do not scatter by far as much as in *Asterionella formosa*. This high symmetry gives them a low sinking speed despite the small number of diatoms in the star. The influence of the asymmetry on the sinking speed can be roughly estimated. Furthermore, it is essential that the opening of the angles happens quickly compared to the generation time, so that favorable conditions exist for a longer time.

A study of pattern formation in Diatoma tenuis, including the timing of colony formation, was submitted under the title " Colony and Pattern Formation in *Diatoma tenuis* " for the following book:

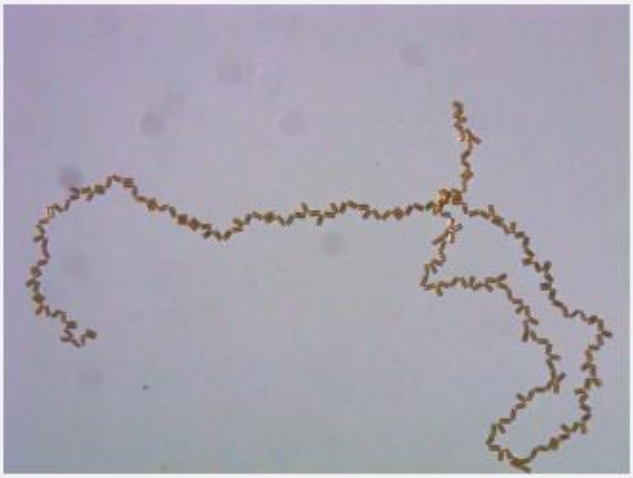
Chain Diatoms [DCHN, Volume in the series: Diatoms: Biology & Applications, series editors: Richard Gordon & Joseph Seckbach, in preparation]" Tiffany, M. and Ghobara, M. (eds.) (2024).

Diatoma vulgaris

The image gallery of *Diatoma vulgaris* shown again below left exhibits long zig-zag shaped colonies. The connection points are located at changing positions of the diatoms. The discoverer of the species, Bory de Saint-Vincent (see his drawing right below) wrote that he could not recognize an order structure in it.



Nevertheless, it is possible to find rules for the structure formation, which, however, also have a stochastic part. Agreement with a deterministic approximation is found only over lengths in the order of 20 to 30 diatoms. Further limiting the observability of the deterministic approximation are deviations from the synchronicity of the cell divisions.

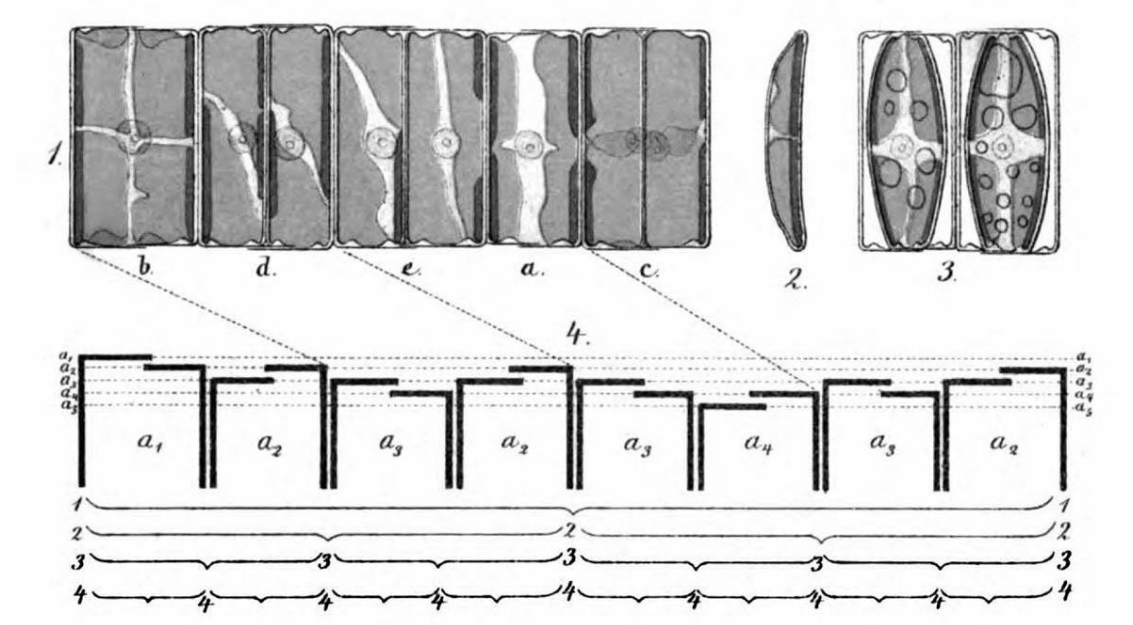


The opening of diatoms after division into a V-shaped structure begins with a sudden opening, as in all observed species that form zig-zag or star-shaped chains. An example of a chain in dividing is shown on the left in 4500x time-lapse.

If you are interested in these investigations, I would like to refer you to the following publication:
"Pattern Formation in *Diatoma vulgaris* Colonies: Observations and Description by a Lindenmayer-System",
submitted for:

The Mathematical Biology of Diatoms [DMTH, Volume in the series: Diatoms: Biology& Applications, series editors: Richard Gordon & Joseph Seckbach, in press]. J.L. Pappas and R. Gordon, (eds.) Wiley-Scrivener, Beverly, Massachusetts, USA. (2023)

If you have any questions about these topics, please contact me.



Size of the diatoms in a chain-like colony I

In this article we will discuss the sequence of diatoms sizes in such a culture and if one can observe them.

Note: A more detailed analysis of the size sequence of diatoms in chain-shaped colonies is given in this publication:

Harbich, T. (2021), On the Size Sequence of Diatoms in Clonal Chains. In *Diatom Morphogenesis* (Diatoms: Biology and Applications) Vadim V. Annenkov (Editor), Richard Gordon (Editor), Joseph Seckbach (Editor), Wiley-Scrivener; First published: 29 October 2021, https://doi.org/10.1002/9781119488170.ch3

The mathematical aspects are treated more formally in the paper than here. As examples for measured sequences, *Eunotia* sp. is used. Furthermore, a rule of thumb for the loss of synchronicity is derived.

As early as 1871, Pfitzer illustrated the successive reduction in size on the example of a short chain of diatoms of the genus *Eunotia* (see figure above). Specifically, attempts are being made to determine possible positions of a fragment of such a colony in the theoretical sequence. For this purpose, the sequence of sizes and orientations of the diatoms in a clonal chain must be known first. Ussing et al. (2005) have argued that the generation rule for this sequence can be described by a one-dimensional Lindenmayer model (see Lindenmeyer, A. (1968)). This model has already been successfully used for various species (e.g. cyanobacterium *Anabaena catenula*).

Both the measurement of the size of diatoms as well as the assignment to the theoretical sequence prove to be no easy task. A closer look at the Lindenmayer system for chains of diatoms makes it possible to successfully analyze an example from nature. This is probably not the case for all species. Furthermore, one cannot assume that a found assignment is unique. In the following, the mathematical basics are explained and the practical challenges described. Then the mathematical method for analysis is presented and demonstrated by an example. So if you want to know more about it, you will find the details below.

Description of the sequence of diatom sizes

Preliminary remark on asexual reproduction

The asexual reproduction of diatoms has already been briefly described in the introduction. Each cell division produces a diatom of the same size and a smaller one. In the following, the possible sizes of the larger valve are indexed by consecutive numbers k, where k = 0 corresponds to the largest possible diatom and k_{max} to the smallest. Starting from a cell of maximum size (generation 0) there exist in the generation n

$$\binom{n}{k} = \frac{n!}{k! * (n-k)!}$$

diatoms of the size k (Animation in the introduction shows Pascal's triangle). The applicability of the formula presumes the synchronicity of the divisions and applies only until the smallest possible cell is reached. If the results for a generation n are normalized to 1, the probability function for the binomial distribution is obtained (probability p = ½). In such an exemplary culture however, there is no probability distribution of the diatoms, but the number of diatoms of a certain size is deterministic. If samples are taken they are following a binomial distribution.

Lindenmayer system

Now the modeling of the colony by a Lindenmayer system is shown. A Lindenmayer system is a triple D = (A, P, ω) consisting of an alphabet A, replacement rules P (also called productions) and an axiom (start word). The elements of the alphabet are intended to describe the orientation and size of the diatoms in a colony. A colony or a fragment thereof is characterized by a string of characters over the alphabet. The replacement rules specify how this string changes from generation to generation. A starting condition, the so called axiom, determines with which string to start the calculation.

Alphabet A:

Let us imagine the colony as a sequence of characters (a string) written horizontally. The subsequently used notation for the characters of the string has been adopted from Ussing et al. (2005). A diatom whose left valve is larger than its right valve and whose larger valve is given by the size index k is called L^k.

Graphical representation of L^k: 

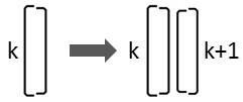
Correspondingly, a diatom whose right valve is larger than its left valve and whose larger valve is given by the size index k is called R^k.

Graphical representation R^k: 

L^k and R^k are mirror-symmetrical. The alphabet consists of the union of sets { L^k | k = 0, 1, 2, …. k_{max} } and
{ R^k | k = 0, 1, 2, …. k_{max} }.

Production rules:

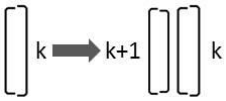
If a diatom that is characterized by the character L^k divides, two diatoms are generated in this arrangement:



Consequently the following replacement must be carried out:

$L^k \rightarrow L^k R^{k+1}$

If the larger valve of the diatom is on its right side, it is only oriented differently to the observer:



This replacement rule results from mirroring:

$R^k \rightarrow L^{k+1} R^k$

It is assumed that the cell divisions are synchronous. In the transition from one generation to the next generation, all elements must be replaced according to these rules so that the number of diatoms is doubled. The strings generated correspond to snapshots between the divisions.

With these replacement or production rules the Lindenmayer system is deterministic and context-free and is called a D0L-system.

Axiom:

As an axiom (starting point) I choose a single cell of maximum size corresponding to the status after sexual reproduction. As the orientation of the cell is arbitrary, the axiom can be selected as $\omega = L^0$. Ussing et al. (2005) use $\omega = R^0$, which leads to mirrored, thus reversed chains. For practical reasons, which will be explained later, I prefer L^0. In connection with the properties of the chains produced, their dependence on the axiom will also be discussed.

Beginning with the axiom, the productions are applied iteratively to all elements of the string in parallel. This results in a sequence of strings G_i which describes the colony after the i-th iteration, which is nothing but the i-th generation.

$G_0 = \omega = L^0$

$G_1 = L^0 R^1$

$G_2 = L^0 R^1 L^2 R^1$

$G_3 = L^0 R^1 L^2 R^1 L^2 R^3 L^2 R^1$

etc.

In the following, I will term the string that is created after n iterations as "n-th generation".

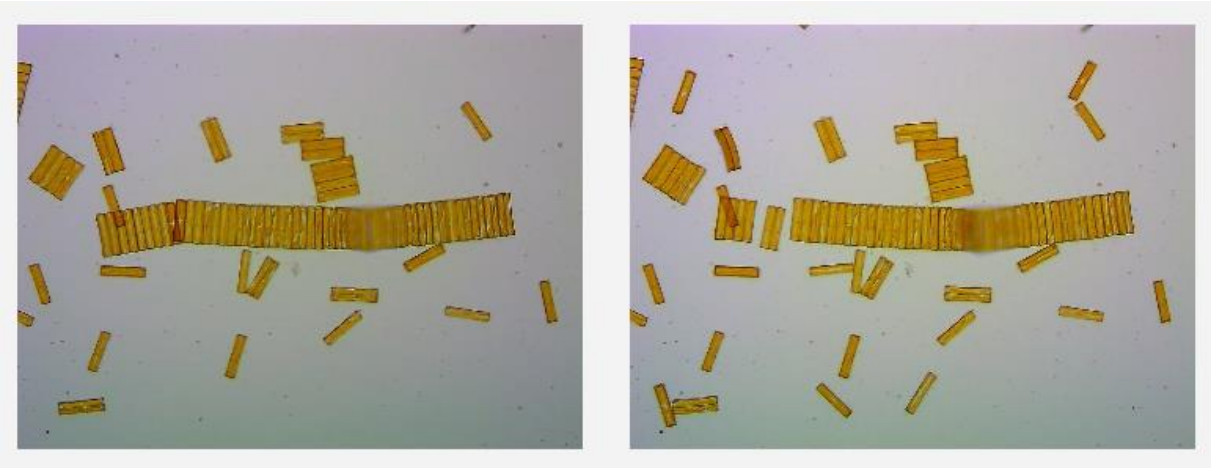
Observation and challenges

As the MacDonald-Pfitzer rule has often been proved and the description of chain-like colonies is based solely on this rule, a proof of the sequence of sizes should not be difficult at first sight. Nevertheless, I had to realize that this is by no means the case. The following difficulties arise:

- Missing assignment of sizes to size index: When measuring a valve size, there is usually no way of assigning this value to the size index introduced above. In particular, the length of the largest possible valve is not known.
- Too short chains: Even if a fragment consisting of only a few diatoms can be measured well, a match with a theoretical sequence of sizes is only of limited value. It could be due to chance.
- Dead cells in the fragment.
- Small differences in the size of the valves of a diatom: The conception of valves which lie clearly inside one another, may not apply to many diatoms that form chains. The valve sizes appear to differ only by a fraction of their thickness. Natural fluctuations in the size of the valves could also play a role. A sufficiently accurate evaluation of the sequence of sizes of the *Melosira* colony which has been shown above was not possible to me.

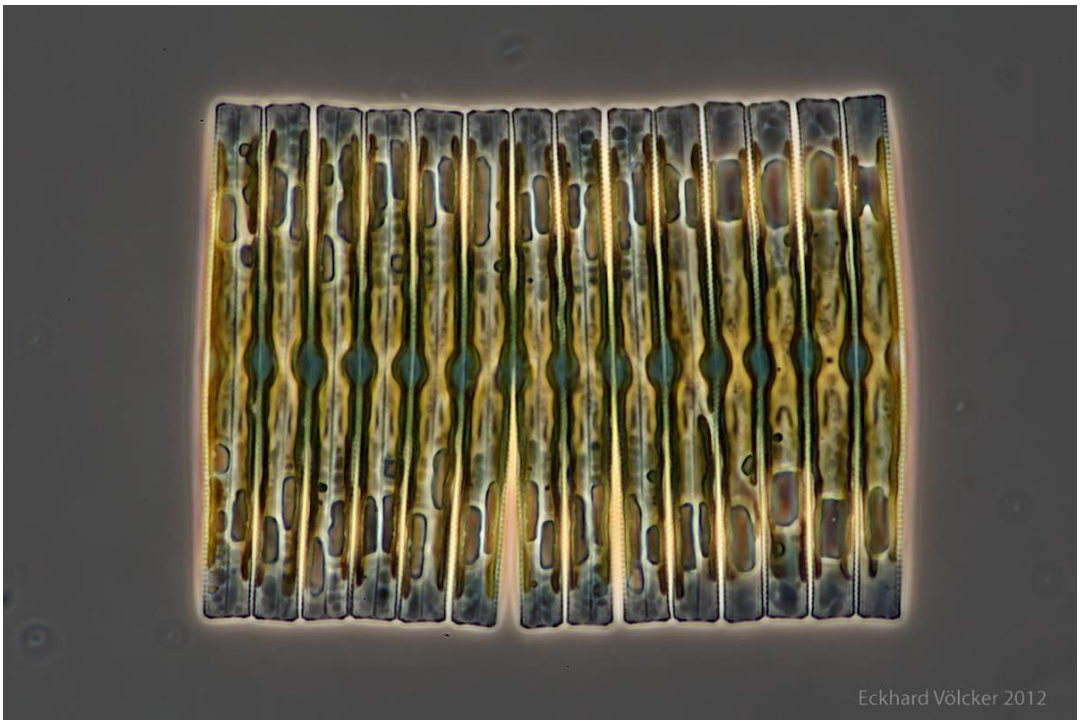
The latter difficulty could possibly be overcome by the use of scanning electron microscopy. Even if all these challenges are mastered, the question remains whether the cell divisions that led to the whole fragment were really synchronized.

In the investigation of the lengths of *Eunotia* sp. the motility of the diatoms led to further problems. Fragments of several diatoms can separate from the colony. Individual diatoms often migrate away from the ends of the chain and can also detach and move away from the inside of the colony. Such a gap sometimes closes again as a result of expansion through cell division, so that the change remains undetected. The video below left shows several such events in 1500-fold time-lapse. Scenes further apart in time are interrupted by a dark pause. Surprisingly, it even happens that a diatom connects to the end of a chain, as can be seen in the video (1500x time lapse) at the bottom right (near the left edge of the frame). Individual free diatoms settle after some time and form a colony by dividing. It should be mentioned that these observations were made on cultures using the inverted microscope. The illumination (LED) of the microscope replaces daylight. In darkness the movement of the *Eunotia* comes to rest.



Ussing et al. (2005) discuss the sequence of size on the background of studies on *Bacillaria paradoxa*, but there is no indication as to whether this principle was observed and whether this was successful.

By chance I found on the internet at http://www.wunderkanone.de/ an excellent picture of a *Fragilaria* colony, which offers itself at first glance as an examination object. The next picture is shown by courtesy of Eckhard Völcker (see http://www.penard.de/):



Each of the diatoms shows significant differences in size between their valves, which is evident in the irregular upper and lower edges. The fragment contains 14 diatoms. A quite advanced breaking point is visible between the 6th and 7th diatoms from the left.

An easy possibility to assign the sizes of the diatoms to a size index is not given. In order to be able to verify the agreement with the theory, however, it is useful to study the properties of this Lindenmayer system in more detail. Therefore, I return to the theory.

Properties of the Lindenmayer system

Two simple rules of this D0L-system prove to be particularly important:

Symmetry:

A chain of diatoms is characterized by a string with characters of the alphabet L^k and R^k with K = 0 .. k_{max}. If you look at it mirrored, so that the right and left are interchanged, the corresponding string must be reversed. In addition, each L must be replaced by an R and each R by an L so that the orientation of each diatom is also changed. The operator of the reflection is denoted by S. The production rules (operator P) are invariant under reflection by construction, so that $P \circ S = S \circ P$ holds. It is irrelevant whether you first perform a mirroring and then an iteration or vice versa. This simply means that the growth of a chain-like colony does not depend on the direction from which it is viewed.

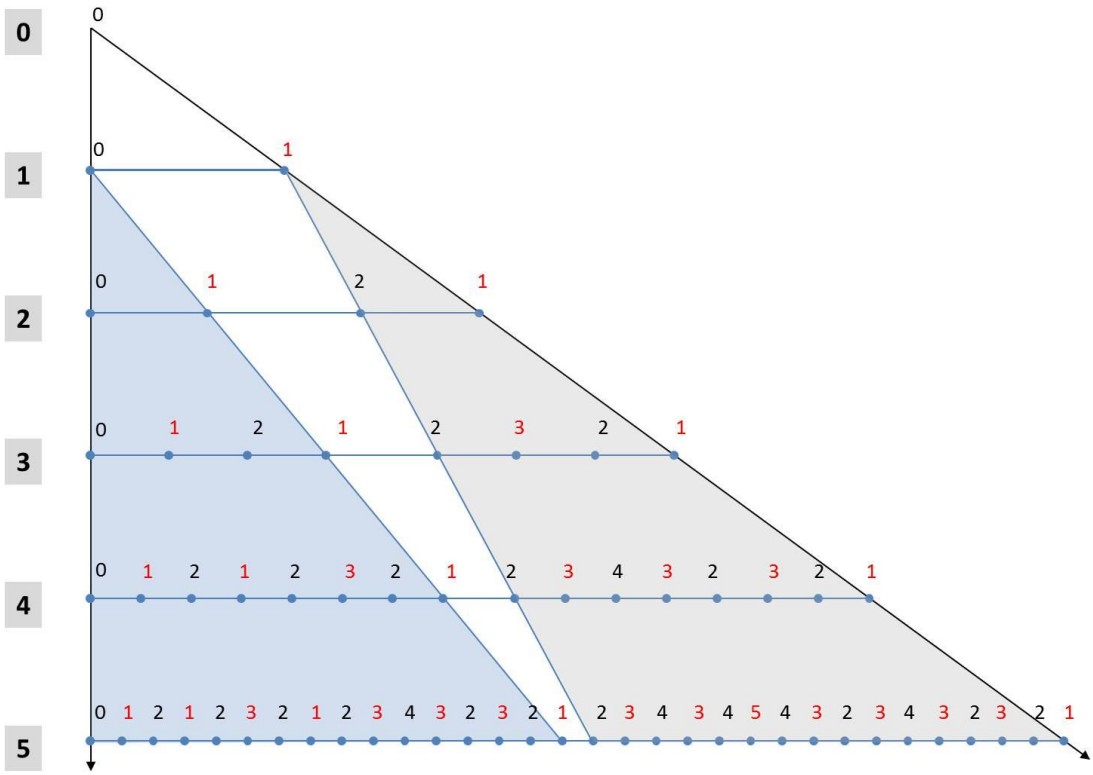
Change of size index:

It is helpful to consider the strings generated by iterations of the production rules of the described D0L-system as a function of the starting point. According to the production rules, a diatom of size k produces in the case of asexual reproduction a diatom of the same size k and a smaller one with size index k + 1. If one starts with the axiom L^k instead of L^0 where k> 0, then in the first generation according to the Production rules, all size indices are increased by k. This is also true in the second and subsequent generations.

If therefore the development of two colonies is observed where each colony starts with a single diatom of different size, similar size patterns are produced, but the size indices in one chain is shifted by a constant value relative to the other chain. If the differences of the size indices of successive diatoms are calculated, these differences are identical for both chains. This is the basic idea when answering the question of how far a fragment of a colony can be assigned to a theoretical sequence without knowledge of the mapping of size indices to absolute lengths.

Alternative formulation

These two rules make it possible to derive an alternative formulation for the calculation of generations. In the following figure, the first 5 generations are arranged one below the other starting from the axiom. The numbers denote the size indices. In order to simplify the notation, the orientation of the diatoms is marked by font colors. Black characters stand for "L" and red for "R".



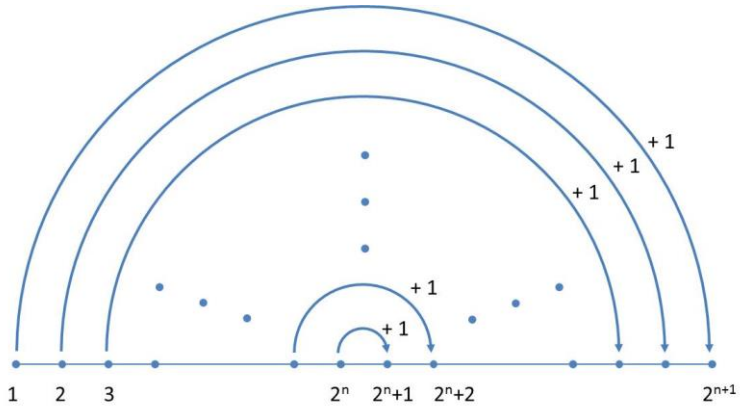
The fifth generation to Axioms L^0 can be seen with its 2^5 elements in the last row. The light blue triangle shows that its first 2^4 elements are equal to the 4th generation with respect to the same axiom L^0. The same applies obviously for all other iterations beginning with the first iteration. In each case, the first half of the n-th generation is identical to the (n-1)-th generation to the same initial value L^0.

A little more complicated is the rule for the 2^{n-1} elements of the second half of the generation. From the gray triangle it can be seen that the second half of the n-th generation is the (n-1)-th generation with respect to the initial state R^1. The two above-mentioned rules can be used to determine the associated characters. All elements of the n-1-th generation belonging to the initial value R^1 are higher by the value 1 compared to a start with the hypothetical value R^0. In addition, they are mirrored in comparison to a generation which originates from the starting element L^1. The values of the second half of the n-th generation can thus be obtained from the (n-1)-th generation belonging to the axiom L^0 by mirroring (exchanging "L" and "R" and reversing the order) and incrementing all size indices. The (n-1)-th generation is identical to the first half of the n-th generation.

This representation provides a simple scheme to determine generations. If a generation is available, the next generation can be written down by the following scheme without explicitly using the replacement rules:

- The present generation is the first half of the next generation.
- The second half of the next generation is obtained by mirroring (exchange of "L" and "R" and reversing the order) of the present generation with simultaneous increase of all size indices by the value 1.

In each generation the orientations (a note to the proof is given below) are alternating, so that one can limit yourself to the size indices. The simplified scheme is illustrated below:



Now it becomes clear why, in contrast to Ussing et al. (2005), a mirrored axiom is used. As we write from the left to the right, the next generation can always be completed at the end.

Denoting the size indices of the n-th generation with a_i^n, where i takes the values 1 … 2^n, the generation rule reads as follows:

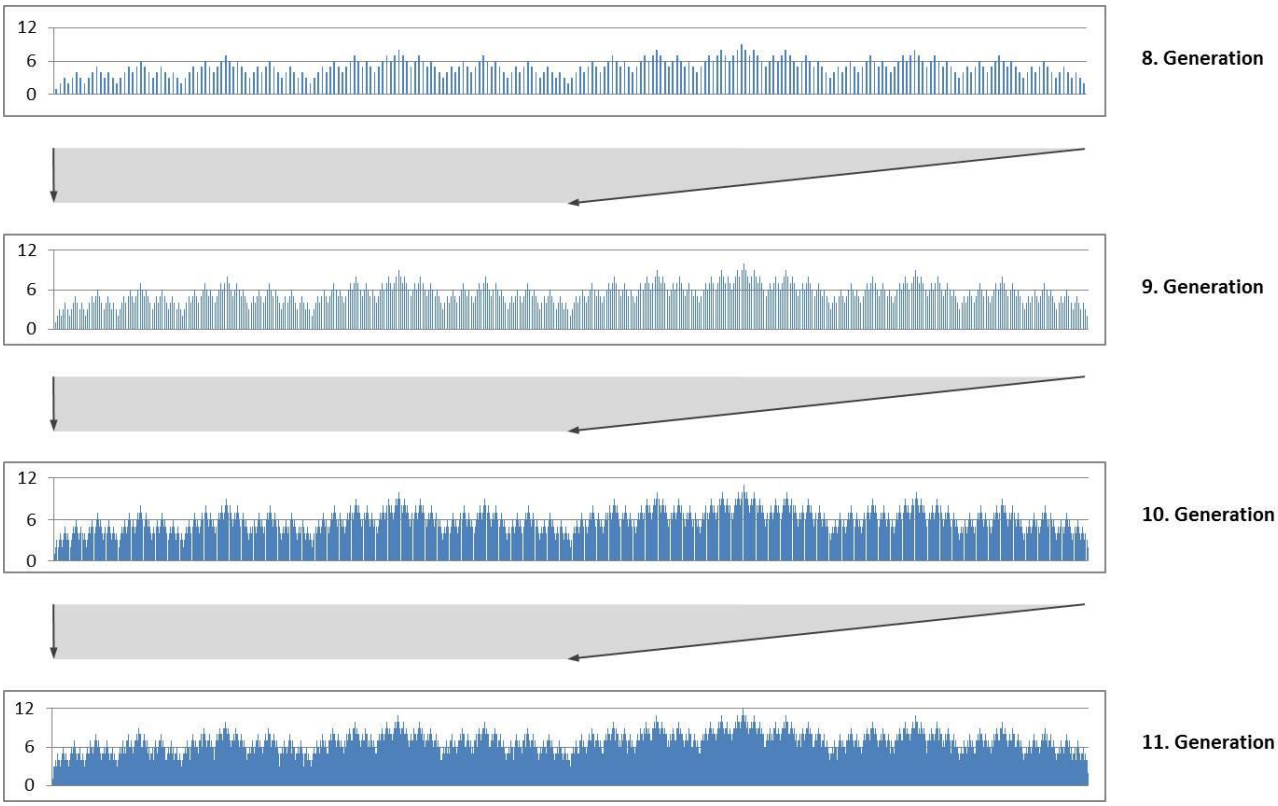
$$a_i^{n+1} = \begin{cases} a_i^n & \text{für } i = 1,2,\dots,2^n \\ a^n_{2^{n+1}-i+1} + 1 & \text{für } i = 2^n+1,\dots,2^{n+1} \end{cases}$$

The initial value corresponding to the selected axiom is $a_1^0 = 0$. A formal mathematical proof is given in the publication cited above, "On the size sequence of diatoms in clonal chains".

If one knows the absolute indices of the size of a found fragment of a colony, one can search for the generation in which this or the mirrored pattern appears for the first time. All subsequent generations contain the same pattern, so that there can be no unique assignment to one generation.

This formulation not only allows the rapid manual calculation of the generations, but also provides some mathematical insights immediately. For example there is no periodicity because of incrementing.

The repeated duplication with mirroring produces a self-similar fractal structure. The following diagrams show the size indices for the 8th to 11th generation. Each diagram forms the first half of the subsequent diagram..



Self-similarity and fractal structure are not surprising because they are typical for Lindenmayer systems.

When one increments the next maximum size index results from the previous maximum size index. In accordance with Pascal's triangle, the maximum value in the nth generation is n. For its position in the string it is easy to formulate a relationship. In the limit $n \to \infty$ the position converges to 2/3 of the length of the string.

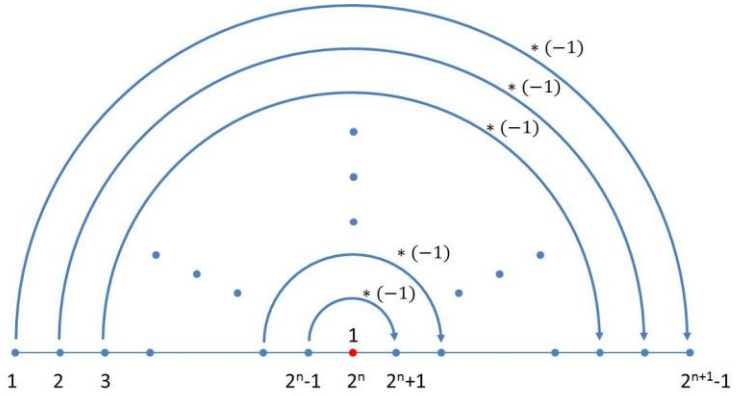
More important for our purposes are the following statements, which apply from the 1st generation for each further generation:

1. The orientations of the diatoms alternate.
2. The differences of successive size indices (size index of an element - size index of its predecessor) can only take the values +1 or -1.

Both assertions can be proved in a few lines by complete induction. The first statement can alternatively be derived immediately from the replacement rules. As base case the first generation is used. For the inductive step the upper half of the n + 1-th generation and the position where the lower and upper halves border each other have to be considered. The first half of the n-th generation need not to be considered more closely because it is identical to the (n-1) generation (induction hypothesis). The second statement turns out to be useful for the characterization of a fragment.

The difference sequence of neighbouring elements in the nth generation contains n-1 elements. They are independent of the size index used in the axiom. A simple schema can also be given for the generation of the sequence of differences which follows immediately from the schema for the size

indices. In the transition to the next generation, one firstly appends the number 1 to the existing generation and then reflects the previous generation whereby all values have to be inverted:



If the differences between the size indices of the n-th generation are denoted by d_i^n, where i takes the values 1 … $2^n - 1$, the iteration formula reads:

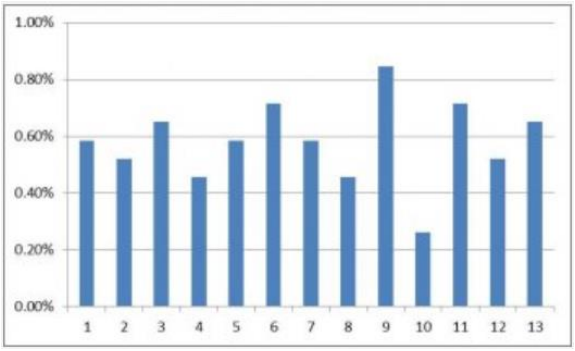
$$d_i^{n+1} = \begin{cases} d_i^n & \text{für } i = 1,2,\dots,2^n - 1 \\ 1 & \text{für } i = 2^n \\ -d_{2^{n+1}-i}^n & \text{für } i = 2^n + 1,\dots,2^{n+1} - 1 \end{cases}$$

As the iteration requires at least one difference we start with the initial value $d_1^1 = 1$.

Analysis of the lengths of the fragment

It is time to return to the analysis of the above shown fragment of a *Fragilaria* colony. First of all, one can see that the orientation of the diatoms is alternating in accordance with the theory.

In the picture on the left you see side by side narrow stripes which were cut out of the overall image showing the longer valve of each diatom (click to enlarge). Although this is an example where the sizes of diatoms can be differentiated their differences are relatively small. On average, length differences between adjacent diatoms are about 0.6% of their mean length. The absolute values of the differences in length between neighboring diatoms are not constant because of the limited resolution and presumably also of natural fluctuations. Small adjustments of the marks have a very strong effect due to these very small differences.



The bar chart below shows the absolute values of the differences in length (in percent of the mean cell length) of adjacent diatoms is therefore to be considered critically.

The biggest problem is the 10th value, which deviates extremely from the other values. Otherwise, there are no differences in length, which differ from the others by a factor of 2 or 3. If one considers the D0L-system in spite of this strong deviating value for plausible, the sizes of the diatoms can be analyzed except for an additive constant. One considers only the feature of whether a diatom is longer or shorter than its predecessor. If it is longer, the difference is -1, otherwise 1. The 10th value (-1) is to be regarded as uncertain because of the anomaly mentioned. These differences are shown in the figure above. Since, as mentioned several times, an assignment to the absolute magnitude index is missing, I consider the sequence -1 1 1 -1 -1 -1 1 1 -1 -1 1 -1 -1 as a "fingerprint" of the fragment of the colony.

Now one can check whether the fingerprint can be found in a sufficiently long sequence of differences. With a length of the fragment of 14 diatoms, i.e. 13 length differences, it can occur at the earliest in the 4th generation. It is found mirrored and inverted in this generation (1 1 -1 1 1 -1 -1 1 1 1 -1 -1 1). Here the sequence of differences of the 4th generation can be seen, whereby the mirrored inverted "fingerprint" of the fragment is highlighted by red color:

1 1 -1 1 1 -1 -1 1 1 1 -1 -1 1 -1 -1

In the sequence of sizes belonging to the axiom L^0 the fragment can be placed accordingly:

0 1 2 1 2 3 2 1 2 3 4 3 2 3 2 1

Here the size index of the first diatom from the left is 0, the largest occurring index is 4. The correspondence between observed and calculated patterns is convincing and proves again the MacDonald-Pfitzer rule. Above all, it demonstrates the practical usability of the D0L-system.

Number of pattern matches

As a result of self-similarity, the fingerprint of the diatom fragment can be found additionally in the next generation mirrored and inverted. For the fragment there are two places with matching patterns. To fit in the second location, it must be mirrored. With each generation the sites with matching patterns are doubled. If the pattern of the fingerprint appears for the first time in the generation m, then it is found in the generation j with $j \geq m$ exactly 2^{j-m} times. The unknown absolute assignment of the length to a size index thus leads to further possibilities of placing a fragment into the theoretical size sequence.

LINDENMAYER, A. (1968a). Mathematical models for cellular interactions, in development
I. Filaments with one-sided inputs. Journal of Theoretical Biology, 18, 290-299

Pfitzer, E. (1871) Untersuchungen über Bau und Entwicklung der Bacillariaceen (Diatomeen). *Botanische Abhandlungen* 2, 1–189.

USSING, A.P., GORDON, R., ECTOR, L., BUCZKO´ , K., DESNITSKIY, A.G. & VANLANDINGHAM, S.L. (2005). The colonial diatom ‘‘*Bacillaria paradoxa*’’: chaotic gliding motility, Lindemeyer Model of colonial morphogenesis, and bibliography, with translation of O.F. Müller (1783), “About a peculiar being in the beach-water”. Diatom Monographs, Vol. 5. Koeltz, Koenigstein, Germany.

Size of the diatoms in a chain-like colony II

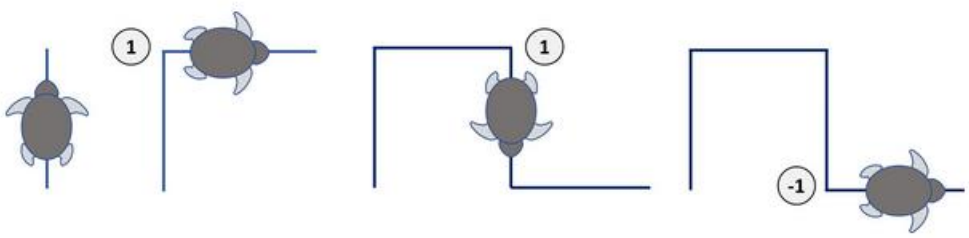
A surprising relationship between size sequence and dragon curve

On the previous page it was shown that the sequence of differences of length indices of neighboring diatoms in a chain-shaped colony can be calculated according to a simple scheme. For the practical observability of this sequence, synchronicity of the divisions must be assumed. A graphical interpretation of this sequence will be presented here. More details can be found in the publication (3).
As a reminder: If one assumes that the development of the size sequences is started with the axiom L^0, the sequences of the size indices result from the described L-system to (see also (4)):
$g_0 = 0$
$g_1 = 0\ 1$
$g_2 = 0\ 1\ 2\ 1$
$g_3 = 0\ 1\ 2\ 1\ 2\ 3\ 2\ 1$
$g_4 = 0\ 1\ 2\ 1\ 2\ 3\ 2\ 1\ 2\ 3\ 4\ 3\ 2\ 3\ 2\ 1$
….
The corresponding difference sequences are:
$d_1 = 1$
$d_2 = 1\ 1\ -1$
$d_3 = 1\ 1\ -1\ 1\ 1\ -1\ -1$
$d_4 = 1\ 1\ -1\ 1\ 1\ -1\ -1\ 1\ 1\ 1\ -1\ -1\ 1\ -1\ -1$
….

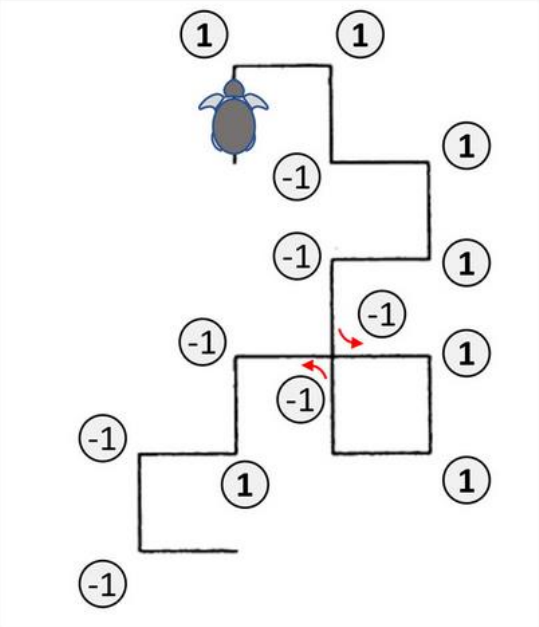
If one would start with j >0 instead of the size index 0, then each index of the size sequence would have to be increased by the value j. The sequences of differences remain unchanged. In (3) it is shown that one can also give a Lindenmayer system (L-system) for the sequences of differences.

For L-systems, the generated sequences are often visualized by a turtle graphics. Here, the generated sequence (for example) is run through from left to right, and depending on the letter in the sequence, a certain command is executed. Thus, depending on the letter, one can execute a rotation by an angle, raise or lower the pen, change the color, etc. These rules are to be used:

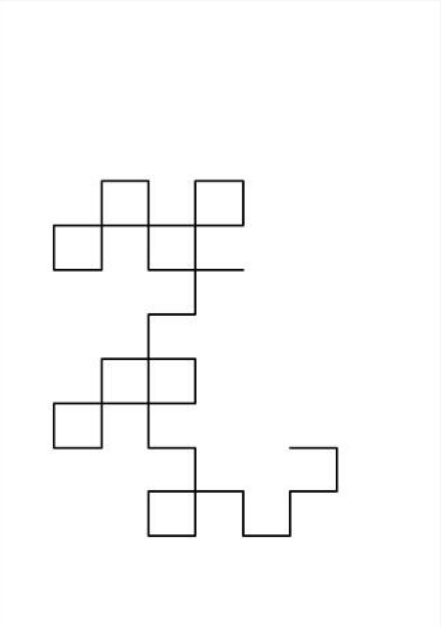
1. Go forward by one length unit.
2. Stop the movement if the end of the sequence is reached.
   Otherwise: Read the next letter, then turn right by 90° for value 1, turn left by 90° for value -1.
3. Go to 1 (jump instruction).



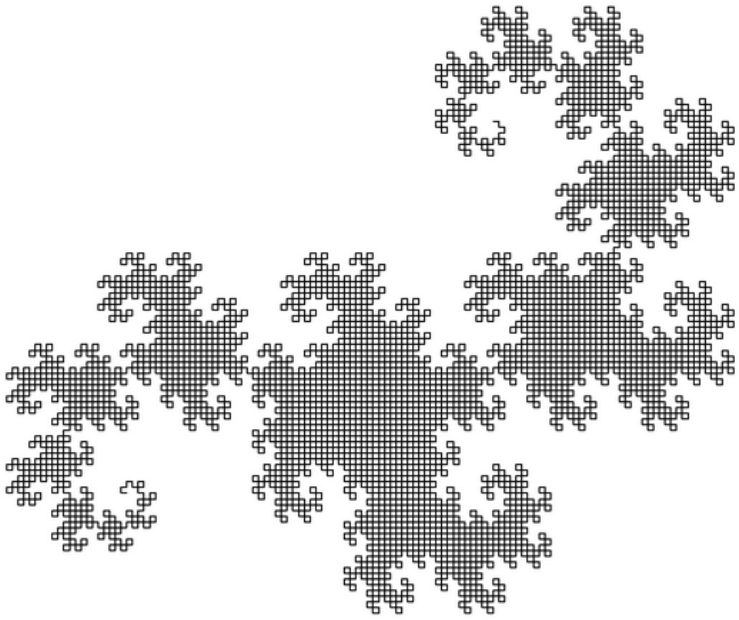
For d_4 = 1 1 -1 1 1 -1 -1 1 1 1 -1 -1 1 -1 -1 this figure is obtained:



This video shows the creation of the 6th generation.



These sequences and their visualization are well known. The curves are called (Heighway) dragon curves (Wikipedia). Dragon curves occur in the diatom chains in the sense in which Pascal's triangle occurs, not as a geometric figure but as a mathematical structure.

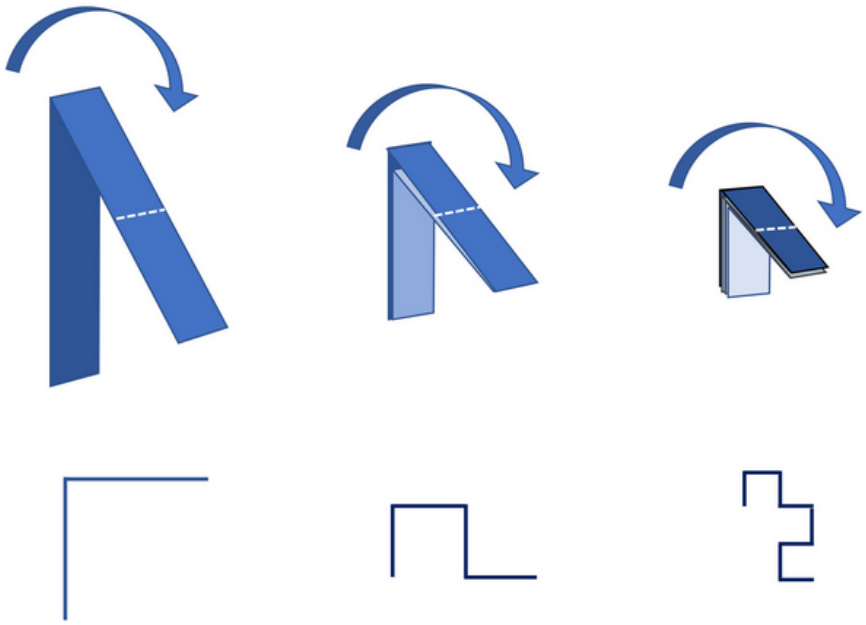


Dragon curve of the 13th generation. It contains $2^{13} - 1 = 8192$ angles.

If a fragment of a diatom colony matches the theoretical sequence, one can generate part of a dragon curve by checking diatom by diatom for differences in length and generating a turtle graphics in the way described. If the considered diatom is larger than the previous one, one performs a right turn (difference = 1), if it is smaller, one performs a left turn (difference = -1). An example of a correct sequence in a *Eunotia* chain comprising 25 diatoms can be found in (3).

The properties of dragon curves are well studied. Statements about them can be directly applied to diatom colonies. Dragon curves are exact self-similar fractals. It is plane-filling in the limiting case $n \to \infty$ (Hausdorff dimension = 2).

The sequences can be made very easily by folding a strip of paper (Wikipedia). This is folded several times in half to the right. Then you unfold the strip and form the bends to right angles.



1. Prusinkiewicz, P. and Lindenmayer, A. (1990) *The Algorithmic Beauty of Plants*. Springer, New York.

2. Benoit B. Mandelbrot *The Fractal Geometry of Nature ,* Freeman, San Francisco, 1982

3. Harbich, T. (2021), *On the Size Sequence of Diatoms in Clonal Chains* in Diatom Morphogenesis (Diatoms: Biology and Applications) Vadim V. Annenkov (Editor), Richard Gordon (Editor), Joseph Seckbach (Editor), Wiley-Scrivener; 1. Edition (9. Dezember 2021)

4. USSING, A.P., GORDON, R., ECTOR, L., BUCZKO´ , K., DESNITSKIY, A.G. & VANLANDINGHAM, S.L. (2005). The colonial diatom ‘‘*Bacillaria paradoxa*’’: chaotic gliding motility, Lindemeyer Model of colonial morphogenesis, and bibliography, with translation of O.F. Müller (1783), “About a peculiar being in the beach-water”. Diatom Monographs, Vol. 5. Koeltz, Koenigstein, Germany.

View into a *Eunotia* culture (click to enlarge)

Size of the diatoms in a chain-like colony III

Synchronicity

The essential prerequisite for the correctness of the description by a D0L-system is the synchronous division of the diatoms, which is not of permanent nature. It was stated that longer chain-like colonies are found primarily in cultures. An investigation of a longer chain that has grown in a culture is likely to be very time-consuming and not very promising. If only the consistency of short fragments with the theoretical sequence is examined, the requirements for synchronicity may be lower. The first occurrence of a fingerprint in a generation m means that m generations are required to produce the observed pattern for the first time from a single cell. Also the coincidences in later generations need precisely m generations to develop from a single cell. To match with the theoretical sequence it is therefore sufficient if synchronicity is retained over m generations. In the example the fingerprint appears in the 5^{th} generation for the first time, so that the relative deviations in the generation times must be less than 20% in order to preserve the pattern of the fragment.

If, for a fragment consisting of L diatoms, the number m is such that $2^{m-1} < L \leq 2^m$, then the pattern appears at the earliest in the generation m. Unfortunately you cannot rely on it. Let us look at the tree of generations once again. From the first cell two daughter cells are generated simultaneously in the first generation. In the nth generation, 2n-1 diatoms have emerged from each of these two cells. They correspond to two strings of length 2n-1, which are adjacent to one another in the middle of the n-th generation. A fragment containing diatoms from both halves in the n-th generation has different ancestors in the first generation, i.e. n-1 generations earlier. A synchronous development must then be given over n-1 generations in order to guarantee a consistency of the sequence of sizes with the theory. If one considers the development of sequences that arise from neighboring diatoms in other generations (> 2), one finds in the same way that fragments in the generation n can also have different ancestors n-2, n-3, .., 1 generations before.

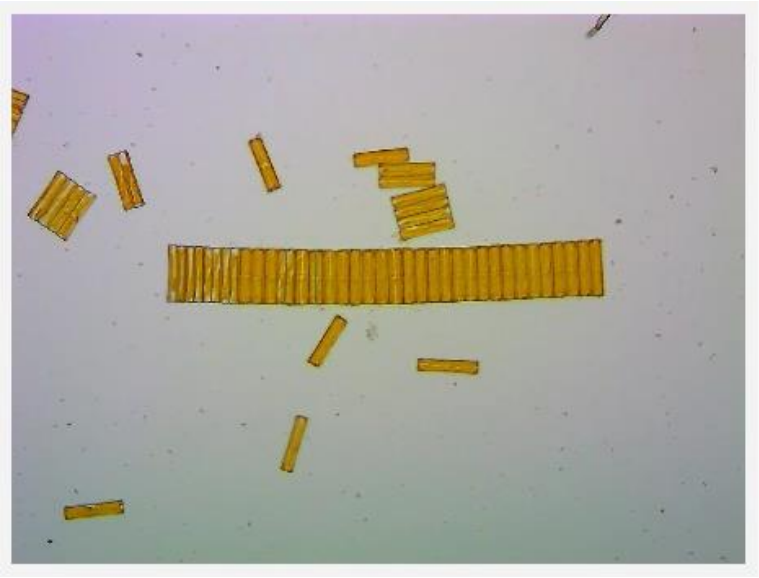
So far, the chains of diatoms have always been treated as if all the diatoms are located between two divisions. If the divisions are no longer synchronous, one must expect to find fragments in which the diatoms are in different stages of division. In this case it can be seen at first sight that the modeling as a Lindenmayer system is beyond its limits.

Example of synchronicity over a few generations

If a diatom culture is started with a single cell and the diatoms are counted at periodic intervals, one can often observe that deviations from the power law 2^n occur after three or four generations. The same will be expected for chain-like colonies. The picture on the left shows a sample from a small brook (click to enlarge). One can see a very young *Melosira* colony, which has developed after auxospore formation. This finding makes it possible to determine the maximum size of the diatoms, but the differences in size between the cells of adjacent diatoms are also in this case very small. The colony is, however, well suited for observing the synchronicity of cell divisions. It was transferred into a culture vessel containing nutrient solution, where it developed well. After three days it looked like this (click to enlarge):

The diatoms exhibit different stages of division with a small-scale order. The development seems to be synchronized over only two to three generations. This is even more striking in a time-lapse recording. For this purpose, the culture was observed for several days near a lamp at about 200 lux without additional illumination. A simple macroscope was used, as shown on the page about equipment for observation. The objective was a Zeiss Luminar with a 25 mm focal length.

On the left the resulting time-lapse video can be viewed (3600 times faster than real time; full screen mode is recommended). The brightness fluctuates due to changing additional daylight incidence. The dark sections in the video correspond to the dark phase of the lighting (daytime cycle with about 12 hours light per day). They were extremely shortened in the video. In darkness the division processes come to a standstill. It is noticeable that asexual reproduction occurs in changing more or less large sections. Here the chain expands. Between the cell divisions there are larger periods of time in which no progress can be observed from the outside at this low resolution.

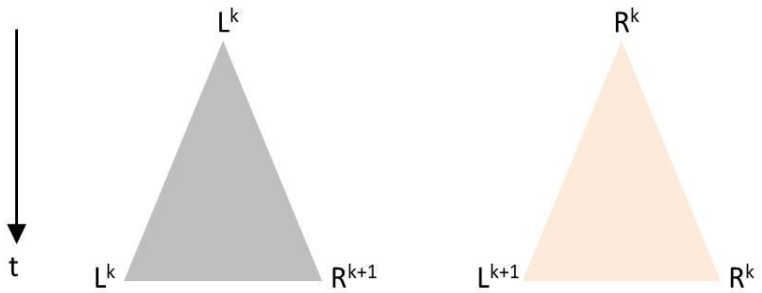


A similar observation was made on a cultivated *Eunotia* sp. On the left you can see a video in 7200-fold time-lapse. Here the culture was kept permanently under the microscope's LED illumination for a period of 70 hours. Since *Eunotia* is motile, diatoms can move away from the ends of the chain and even from positions in between. As the chain expands, the colony detaches and bulges from the substrate. As in the example of Melosira, one recognizes locally different stages of division and accordingly different temporal lateral expansion of the colony. In cultures, however, very long chains with a uniform structure often occur. It is unclear whether the day-night rhythm has a synchronizing effect here.

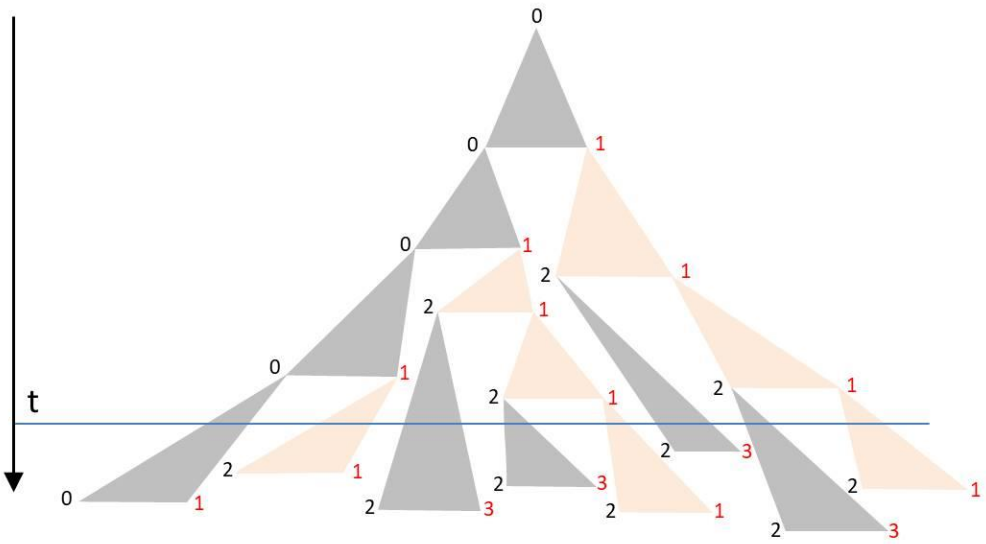
Beyond the D0L-system

Deviations of the generation time are likely to be of a statistical nature. The longer a colony develops, the more deviations from the deterministic model are to be expected. If the synchronicity is given over a maximum of k generations, there will always be sections with up to 2^k diatoms that match the D0L-system. The applicability of the model is limited to k generations.

Numerically one can model systems in which the time between successive divisions has a random component, but the predictive power of such a model with a stochastic component is limited. Presumably the general behavior, as it can be seen in the video of the Melosira culture, can be reproduced. An approach is briefly outlined. As soon as a diatom is generated by division, an individual time segment begins until two diatoms arise in the same initial state. As with Pascal's triangle or the sequence of the generation in the presented visualization, the time axis is oriented vertically from top to bottom. Each division is to be visualized by a triangle. The tip of the triangle represents a diatom before its division and the opposing vertices stand for the daughter cells after completed fission. The height of the triangle is chosen proportional to the duration of the division process. Depending on the orientation of the diatoms before division, there are these possibilities which correspond to the production rules:



The angles in the triangle are chosen in a way that the triangles in the drawing are not overlapping. Starting again with L^0, such a sequence for example could arise:



The orientation of the diatoms was again visualized by the font colors. The corners of the triangle form a binary tree. The duration of the subsequent cell division is assigned to each node. If one chooses them identically, the described D0L model is reproduced. The more these time intervals fluctuate around the mean generation time, the lower the synchronicity. For a realistic model one would have to determine the probability density of periods of cell divisions by observations. It is also possible to take into account dependencies and correlations. Laney et al. (2012) have shown in their paper 'Diatoms favor their younger daughters' that the daughter cells of the marine centric diatom *Ditylum brightwellii* have different periods of division. The diatom which inherits the smaller hypotheca exhibits a more rapid division than her sister, that got the larger epitheka. If this also applies to the chain-like colonies considered here, the synchronicity would already be lost due to this effect.
At a certain time t the colony is found in a state characterized by the intersection line along the horizontal. The blue line in the diagram gives an example. Due to the strongly fluctuating division periods there is no alternating orientation of the diatoms. As mentioned, the spatial separation of the diatoms becomes apparent only in the lower part of the triangle.

Flexibility of chains and breaking points

Finally, I would like to discuss a text passage from the publication by Ussing et al. (2005). It states:

"The flexibility of chains, which affects their entanglement (Karp-Boss et al. 1998) might depend on the nonuniformity in size predicted by this model. Smaller cells may also represent weak points in the chain at which it could fragment in a turbulent environment or during predation. So this simple mathematical model may have some life cycle and ecological consequences."

In many chain-forming species the relative size differences between successive valves are small. Even in the well analyzable example of the *Fragilaria* colony, these were only about 0.6%. The differences in the size of the valves for instance in the cultivated Melosira are so small that they are hardly perceptible even in big colonies. In a fragment which appears for the first time in generation m, the

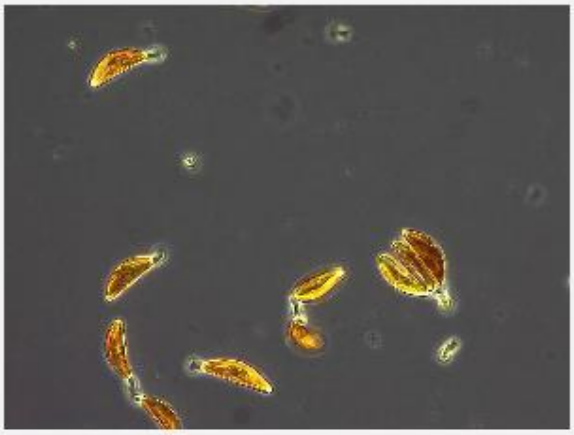
largest and the smallest size index differ by m at most in the case of synchronous division. This is not much in the case of small fragments which are usually found. In our example we have m = 5 but the difference between the size index of the largest and smallest diatom is only 3. As has been shown connected diatoms always have a difference in size of one step, at least in the case of synchronous divisions. You can hardly speak of a "predetermined breaking point ". If the cell divisions of diatoms are not synchronized, deviations from these rules may occur. As the duration of division process does not differ by orders of magnitude, this cannot drastically change the situation. I would not like to deny the influence of unequal sizes of diatoms but also not to overestimate it.

Karp-Boss, L. & Jumars, P. A. (1998). Motion of diatom chains in steady shear flow. Limnol. Oceanogr., 43, 1767-1773

S R Laney, R J Olson and H M Sosik, Diatoms favor their younger daughters, Limnology and Oceanography, Vol.57, No.5, pp.1572–1578, 2012.

USSING, A.P., GORDON, R., ECTOR, L., BUCZKO´ , K., DESNITSKIY, A.G. & VANLANDINGHAM, S.L. (2005). The colonial diatom ‘‘*Bacillaria paradoxa*’’: chaotic gliding motility, Lindemeyer Model of colonial morphogenesis, and bibliography, with translation of O.F. Müller (1783), “About a peculiar being in the beach-water”. Diatom Monographs, Vol. 5. Koeltz, Koenigstein, Germany.

Cymbella culture with colonies (660x time lapse)



Cymbella colonies (1800x time lapse)

Cymbella colonies on jelly stalks

In the post on sexual reproduction, a *Cymbella* species (probably *Cymbella cistula*) was presented that dwell on mucilage stalks. Their lengths ranged from 54 µm to 120 µm. In the observations described below, their length was approximately 110 µm. These diatoms adhere to the substrate, form a stalk and then divide. In nature, these colonies are attached to aquatic plants or stones. This gives them an advantage in streaming waters. They can also be found in ponds and lakes.

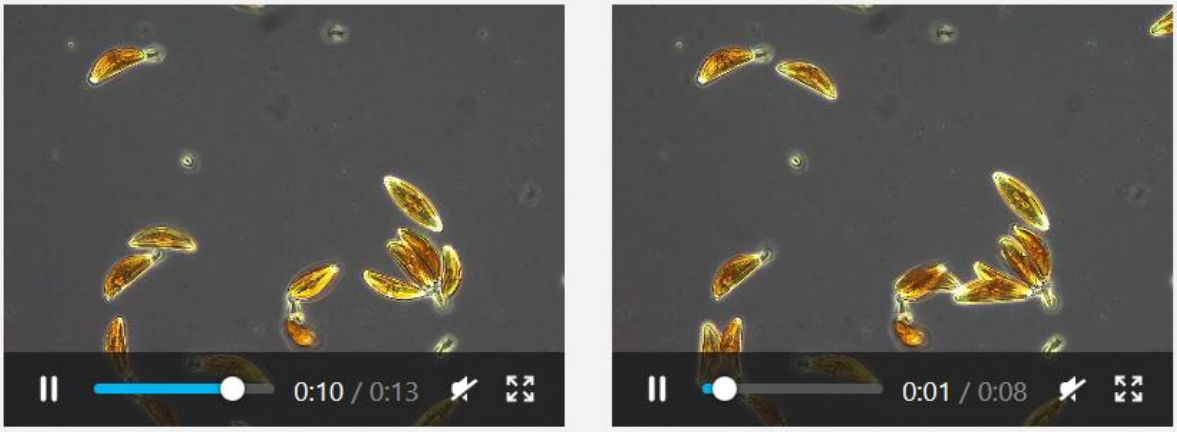
Colony formation

The time-lapse video recorded with PlasDIC at the top left shows a two-week-old culture. You can easily recognize the mucilage stalks, many of which were abandoned by the diatoms. The free diatoms move between the mucilage formations until they settle and generate their own stalk.

In the video in the upper right corner you can see a young culture in phase contrast. It is recorded with a time-lapse factor of 1800. The observation time was 13.6 hours. On the right side you can see two diatoms on a stalk, both of which have almost divided and are now close to separation. These cell divisions take place simultaneously. Subsequently, diatoms become detached from the mucilage stalk. Towards the end of the video, another division occurs on the same stalk, which can be recognized because of the transparency of the diatoms.

In the lower part of the picture you can see a diatom that settles, excretes a pad of EPS and within about three hours develops a stalk on this basis. Such a stalk grows only for a few hours. The diatom probably uses its energy after the formation of the stalk primarily for division.

If the diatoms lie on the substrate, it is very easy to detach from the stalk. An active raphe can exert force on contact with the mucilage stalk or other diatoms may knock against them. In the video you can see how diatoms break off from the mucilage stalk shortly after separation. Here are two scenes from the video above, but with a time lapse factor of 125:



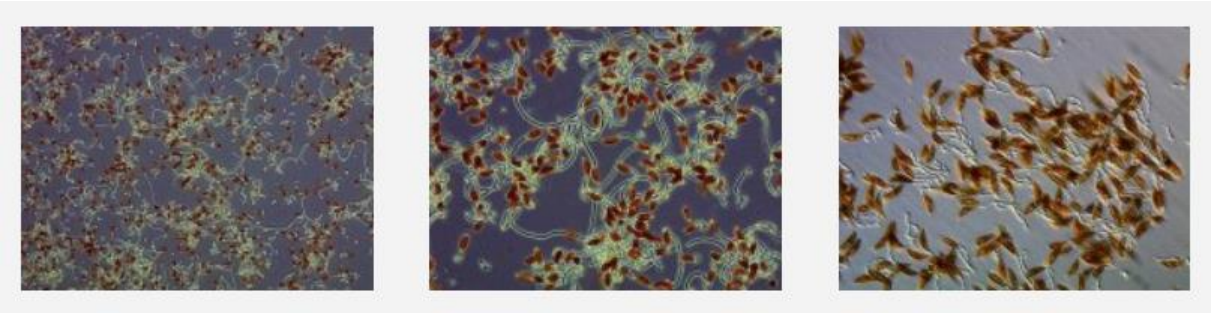
In one case a diatom that lies on its girdle bands at the dorsal side has helped somehow. In the other case the diatom may have already been loosened by the other diatoms. It is therefore to be expected that older cultures with a high population density will be easily destroyed by freely moving diatoms.

In some cultures I have observed that after a cell division, one diatom often falls off the stalk while the other remains for a long time. I suspect that the larger cell through whose apical pores the EPS was secreted for the mucilage stalk is more firmly connected to it.

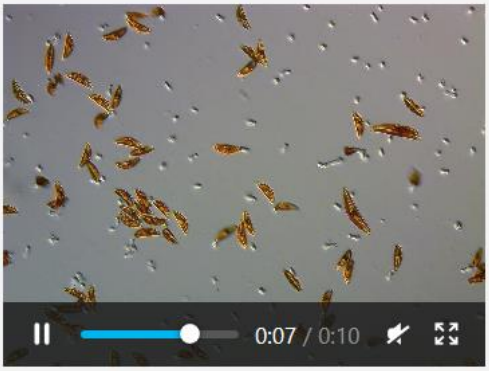
Form of colonies and spread

Cultures can develop very differently for reasons that I do not know. As you can see from the examples, there are cultures in which a diatom breaks off quickly after the division and moves away. Branching colonies do not develop. In other cultures, the diatoms remain on the stalks for a longer period of time on average, form their own stalks and divide. This creates a tree-like structure. Below you can see an example of such colonies in phase contrast (focus stacking) on the left. In the middle is an example of a colony in the DIC (again created from a stack of images), which has no branches. On the right you can see such a colony as it was drawn in the famous book "A History of Infusoria, including the Desmidiaceae and Diatomaceae" by Andrew Pritchards from 1861.

(All pictures can be enlarged by clicking on them.) Sometimes long stalks form, at the end of which there is often only one diatom, as can be seen in the lower left and lower center (both phase contrast). Quite often one comes across irregular branched structures, which are shown in PlasDIC at the bottom right.



(All pictures can be enlarged by clicking on them.) Below left a video of a culture of small diatoms and some initial cells is shown (60-x time-lapse, PlasDIC), in which no colonies have formed, because the diatoms apparently separated quickly from their stalks.

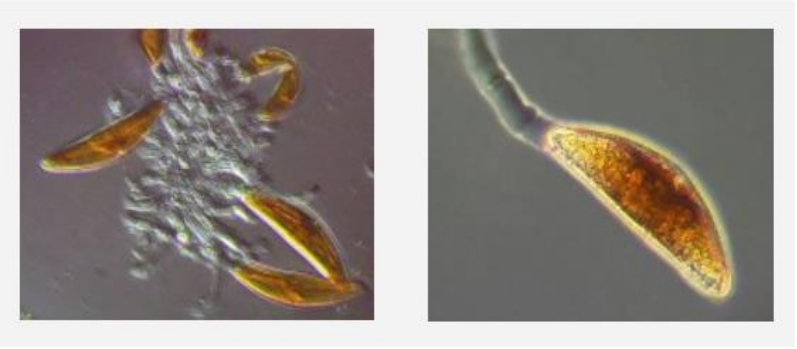


Different environmental conditions can be considered as a reason for the different forms of development in nature. In our cultures, however, the nutrient solution, temperature etc. are almost identical. There are differences in light intensity. It can be assumed that this plays a role in the stability of the tree-like structures, as it has a significant influence on the activity of the raphe. Incidentally, the largest structures were formed in my cultures in the first generations after sexual reproduction. The fast leaving of the jelly stalks could serve the search for a sexual partner in diatoms that are close to their minimal size.

As diatoms repeatedly break off from the tree, these diatoms are at least temporarily free living. At least in cultures the diatoms do not move far from their place of origin before they settle. On the smooth substrate the diatoms describe almost circular paths. This artifact makes it difficult to traverse long distances in cultures. Nevertheless, free moving diatoms in cultures enable a spatial spread in principle.

Structure of the mucilage stalks

During the formation of a stalk a thick gelatinous substance (EPS) is secreted through apical pores in the valves. If you look at the mucilage stalks with PlasDIC, you will find surface structures that run parallel to the direction of the stalks. The image at the bottom left was created by superimposing several images (focus stacking) and subsequently sharpening the picture (click to enlarge). In the phase contrast this can be recognized in some pictures, such as the branched small colony shown above.



This thick string is certainly not pressed through a single pore, but rather through an apical pore field (HUFFORD, T. L., COLLINS, G. B., 1972). The diameter of the pores and the distance between adjacent pores are far below the resolution of a light microscope. The structures could characterize the boundary of the pore field that contributes to EPS production. Similar structures in the stalk can be found, for example, in the genus *Gomphonema*.

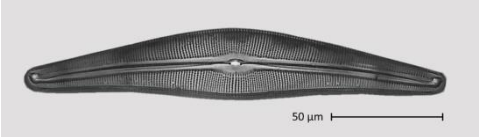
Also remarkable are nodular thickenings which appear frequently in longer jelly stalks. They are obviously formed when the creation of a new section begins after a pause and a new pad is formed. This can be seen in the picture (phase contrast) on the right (click to enlarge).

Pritchard, A. (1861) *A history of Infusoria*, *including the Desmidiaceae and Diatomaceae, Biritsh and foreign*. Whittaker and Co., London, 968 pp.

HUFFORD, T. L., COLLINS, G. B., 1972: The stalk of the diatom *Cymbella cistula*: SEM observations. J. Phycol. 8, 208-210.

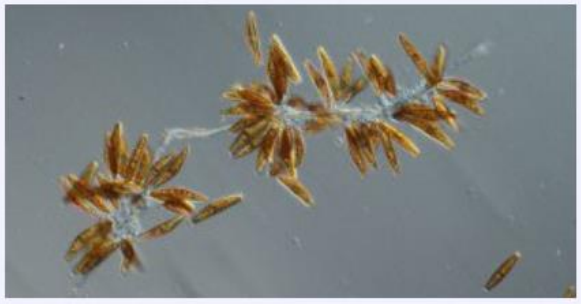
Flat colonies and loosely bound colonies

In the previous post colony-forming C*ymbella* were described which dwell on jelly stalks. Other C*ymbella* species do not form stalks. The diatoms are attached to the substrate at one end with the aid of a thick gelatinous substance. EPS excreted at apical pores causes a high adhesion. As shown on this page, this leads to the formation of local accumulations, which have a limited stability and are somewhat simplified referred to as colonies.



First of all it is reported on *Cymbella lanceolata* (Ehr.), which is about 190 µm long. Its valve is shown on the left (click to enlarge). A short video with the movement was presented in a post about the curvature of the trajectories (center of curvature is always on the ventral side).

The adhesive EPS excretions are clearly visible in phase contrast or DIC. Below you can see two pictures (click to enlarge) from the bottom of the Petri dish taken with PlasDIC. In the right picture the diatoms adhere to a fiber present in nutrient solution. Apparently, there is a preference for attachment to such fine structures.



Among my *Cymbella* cultures there were different species that formed such irregular EPS excretions.

Processes

If you observe such accumulations for a longer period of time, you can recognize these processes:

1. Diatoms detach from a colony. This is typically done at the edge region of the colony.
2. Diatoms move in the space between the colonies.
3. Diatoms meet an existing colony and remain in this cluster.
4. Diatoms stop their movement and attach themselves to the substrate.
5. Diatoms reproduce asexually inside and outside colonies.

The collision between individual diatoms and contact with colonies without a long stay are not listed here, as they are transient and not of great importance for the structure formation.

Diatoms that attach to colonies usually remain on the edge of the colony. As they themselves secrete EPS, the area formed by EPS deposits is continuously increasing.

If individual diatoms come to a standstill, the cause may also be existing EPS from previous attachments. This effect, which is not immediately apparent, will be discussed later.

Events 1,2 and 3 do not allow the formation of new colonies. A colony can develop from individual adherent diatoms according to 4 by following cell divisions and attachment of diatoms.

In the time-lapse video on the left (20x time lapse) you can see examples of the detachment and connection of diatoms to a colony.

This change between colonies was rarely observed in *Cymbella* on gelatinous stalks. Here too a diatom may collide with a colony and come to rest there, but it always remains outside of existing mucilage trees.

Influence of light intensity

The movement activity of diatoms requires sufficient light intensity. With increasing intensity, the raphes become active in the observed *Cymbella*, regardless of whether they move freely or are in a colony. This leads to the fact that more and more diatoms are detached from the colony in bright light.

The driving force then exceeds the adhesion to the substrate produced by EPS. In the video on the left (600x time lapse), a culture was irradiated with several thousand lux using microscope illumination. One recognizes a considerable reduction of two small clusters.

The removal of diatoms from a colony and migration requires sufficient light intensity. On the other hand, the activity of the movement decreases when the intensity becomes low. If diatoms encounter existing colonies or deposits of EPS on the substrate at low brightness, they adhere to the colonies because they cannot overcome the adhesion of the polysaccharides (process 3).

At very low light intensity or darkness, the free movement between the colonies of the diatoms comes to rest. The diatoms then excrete an EPS pad, which they use to adhere to the substrate. As

will be explained later, EPS deposits abandoned by diatoms can also lead to the attachment of freely moving diatoms.

Day-night cycle

In the last video it was demonstrated how very intense lighting can lead to the disappearance of existing *Cymbella* colonies. At lower light intensities, as we use them for cultivation, the effect is not so dramatic, but it is clearly visible. You can expect this to happen in a normal day-night rhythm in a body of water.

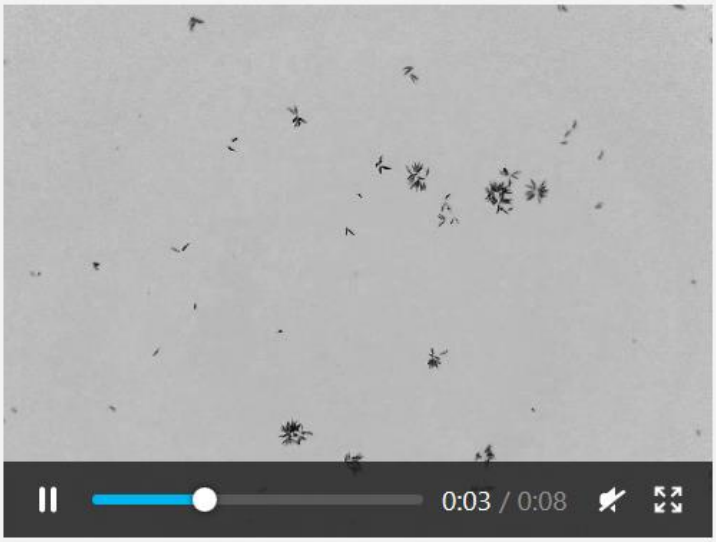
An exemplary time-lapse video, which was recorded over a day-night cycle with a time-lapse factor of 6000, can be seen on the left.

The image was taken with a macroscope (objective with 50 mm focal length). The observed culture is located between other cultures without additional lighting. To enable pictures to be taken in the dark phase, the culture was illuminated with low intensity from below by a diffusing screen with a white LED. In the light phase there was about 200 lux (incident light), in the dark phase (transmitted light) 15 lux. Probably because of this remaining brightness the movement never comes to rest completely.

Development of a culture

Various long-term observations were carried out with different *Cymbella* species. Subsequently, the longest observation on a very similar looking species will be described in more detail, which however was only slightly longer than 100 µm. The observation lasted for 24 days, with one image being taken every 10 seconds. For most analyses such a short time interval is not necessary. However, it is useful in connection with the evaluation of the activity of diatoms moving between colonies.

Also in this observation there was about 200 lux in the light phase and 15 lux in the dark phase. In addition, there was a window with fluctuating incidence of light. The light phase lasted 12.5 hours. The area visible in the pictures is 8.27 mm x 6.21 mm. As the culture was in a 50 mm diameter petri dish, only about 2.6% of the cultivated area was observed.



Particularly in the first few days after inoculation, the relative number of diatoms in the observation area fluctuates strongly, as the leaving and entering of individual diatoms has a significant effect. With the increasing number of moving diatoms and the formation of colonies, which started after about 5 days, the relative fluctuations decreased.

The video on the left shows a sequence of images of the culture in which it was recorded at a daily interval from the third day onwards. The status at the end of the observation is intentionally displayed longer. The images were converted into greyscale images and the differences in brightness due to varying incidence of light were corrected. The pictures were taken at 8 p. m., shortly before the dark phase. With this species and a light intensity of only 200 lux in the light phase, the activity of the diatoms between the colonies already decreased considerably before the beginning of the dark phase. Therefore, only a few diatoms are found outside the colonies.

A video with good temporal resolution and low compression cannot be played here because of its size. The following video was recorded with a time lapse factor of 40,000 and high compression.

Colony formation

A new colony can develop when a single diatom attaches itself to the substrate. It creates an adhesive area where other diatoms can get stuck. In addition, a colony typically grows by asexual reproduction. As has already been mentioned, diatoms attached to the substrate leave behind hardly soluble EPS, which can bind diatoms again. This can be seen by following the development of a colony, starting with observation before a stable colony is established. There is usually a small area in which diatoms are repeatedly attaching and releasing.

The video on the left shows in detail the formation of a colony. Pay particular attention to what is happening inside the white circle. Repeatedly diatoms attach themselves, divide or leave the area in light phase. Diatoms which get onto the already once populated areas adhere there preferably. After a few days, the population is so large that a permanent colony is created.

Not every abandoned EPS deposit is used for buildup again. If no diatom meets the stain for several days, its adhesive power decreases. I do not know whether it dissolves or is degraded by bacteria. In some cases EPS can be seen as a grey spot in the dark phase.

Natural environment

The described observations were carried out in a petri dish. Here, the diatoms inevitably form their colonies in a plane. Under favourable conditions, they can probably also develop flat colonies in a natural habitat such as a leaf surface or a rock. In a three-dimensional fibrous netting, on the other hand, heaps or spherical accumulations would be more likely to form. Diatoms escaping from dense populations must then move along thin filaments. The question arises as to whether the movement and exchange of diatoms between colonies is possible in such epiphytic situation.

In a raw culture with all kinds of fibres, such an accumulation developed by chance. As it was in the middle of a deeper mesh, the recording was difficult. The disturbance of the water surface also made it more difficult to take pictures. On the left you can see the colony with some diatoms in the near surroundings, which move more or less ‘skilfully’. There were several colonies in the braid, but at such a distance from each other that an exchange of diatoms seemed unlikely.

Quantitative analysis of directly attached *Cymbella* colonies

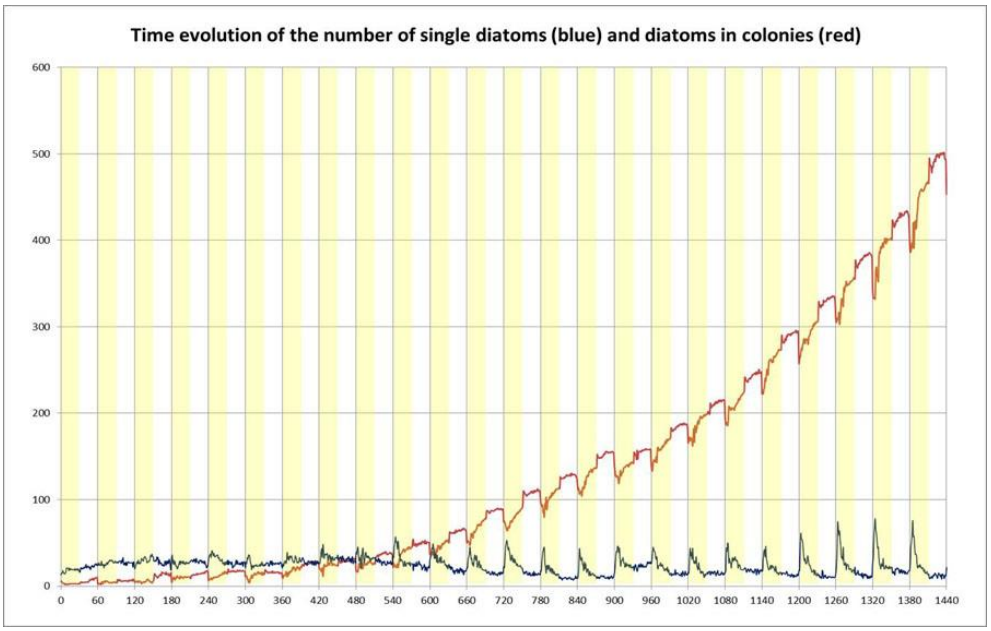
Growth of culture

In order to quantify the growth of the culture and colonies, the area of the diatoms in the visible region was determined. At first, the number of images was reduced to 60 images per day. This selection of images is analyzed as a stack of images using ImageJ (Fiji). After conversion to grey tones and setting a threshold value, a binary representation is obtained in which the diatoms appear black on white. The Particle Analysis ('Analyze Particles') now calculates the size of the contiguous image parts in number of pixels for each image, which can be output in the form of a csv-table. Therefore, Excel is the appropriate tool for further evaluation and graphical presentation.

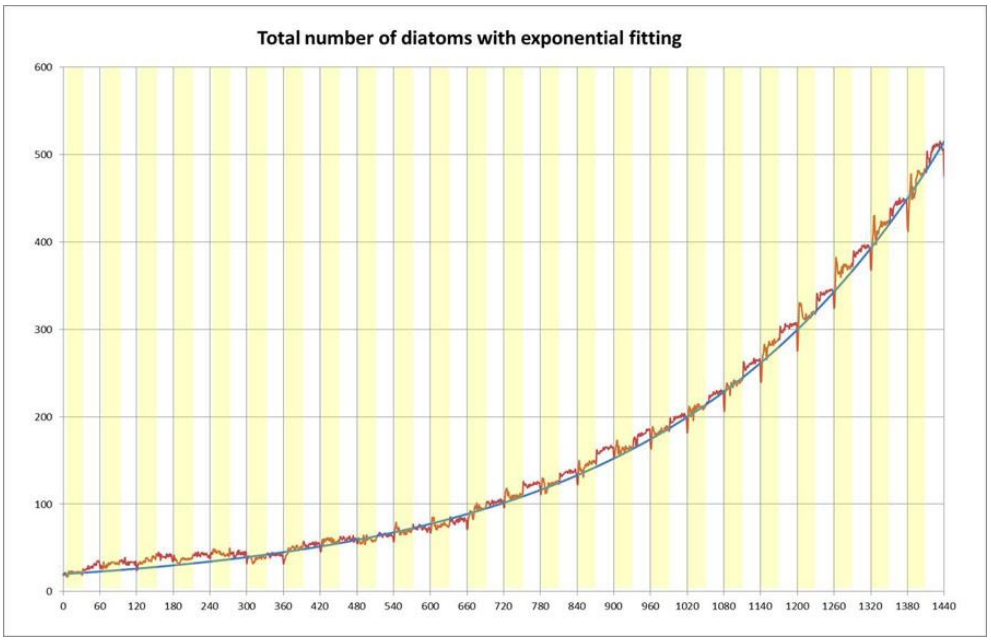
At first one would assume that the total area occupied by diatoms increases steadily with the development. The diatoms, which are released from the colonies at brightness, reduce the area of the colonies accordingly. In return, the diatoms between the colonies increase the total area. However, there are various effects that lead to fluctuations which are correlated with the light-dark cycle. Diatoms in colonies can overlap in the image. Furthermore, the area of a diatom in the image depends on its inclination towards the substrate. This inclination varies on average depending on the position in the colony. The number of diatoms in a colony is therefore only roughly proportional to the area taken. Apparently, the surfaces of the individual diatoms located outside the colonies are surprisingly small, as can be seen from the explicit comparison of images in darkness and subsequent brightness. This may be due to errors caused by the low resolution or effects of threshold value calculation. Therefore, the surfaces were divided into colonies and individual diatoms in an image. The classification has been validated by exemplary counting:

- Areas smaller than a lower threshold of a few pixels (about 1 to 4 pixels) are sorted out. They are caused by small particles on the substrate and irregular boundaries
- All objects larger than an upper threshold (in this evaluation 70 pixels) are considered colonies.
- All objects in between are mainly characterized by individual diatoms. It is possible, for example, that two diatoms overlap in the image and, due to their projection, occupy a small area so that they are counted as one cell, although they represent either two diatoms or a very small colony. However, this is rarely the case.

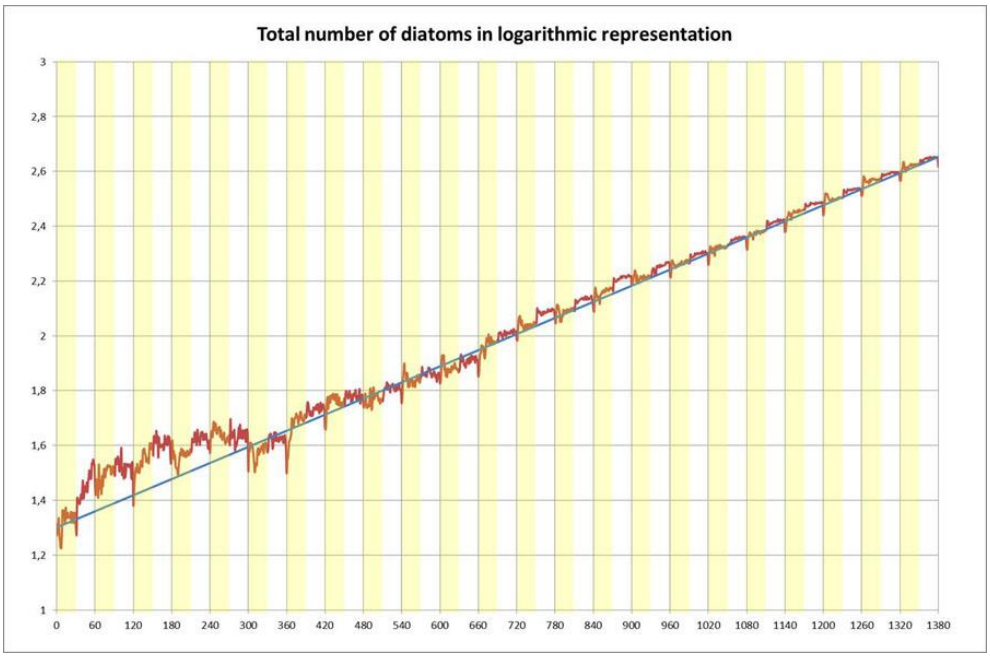
In rough approximation, the number of diatoms in the colonies can be estimated by dividing the total area taken by them by an average area of diatoms in a colony. This conversion factor must be determined appropriately.
The following figure shows the number of individual diatoms (blue) and the number of diatoms in colonies (red) over time. The phases of bright light are marked with a yellow bar.



With the start of the light phase the number of individual diatoms increases rapidly. It falls off again in the course of the light phase. The area of the colonies decreases in mirror image. The conversion factor was selected in such a way that the sum of the two curves shows the smallest possible fluctuations with the light-dark cycle. This is not a precise quantitative count of the number of diatoms in the colonies. The following figure below shows the total number of diatoms resulting from the sum of the two curves discussed. An exponential function (red line) can be easily adjusted:



The corresponding logarithmic representation shows strong fluctuations at the beginning of cultivation, as there are only a few diatoms in the observed range (see note above):

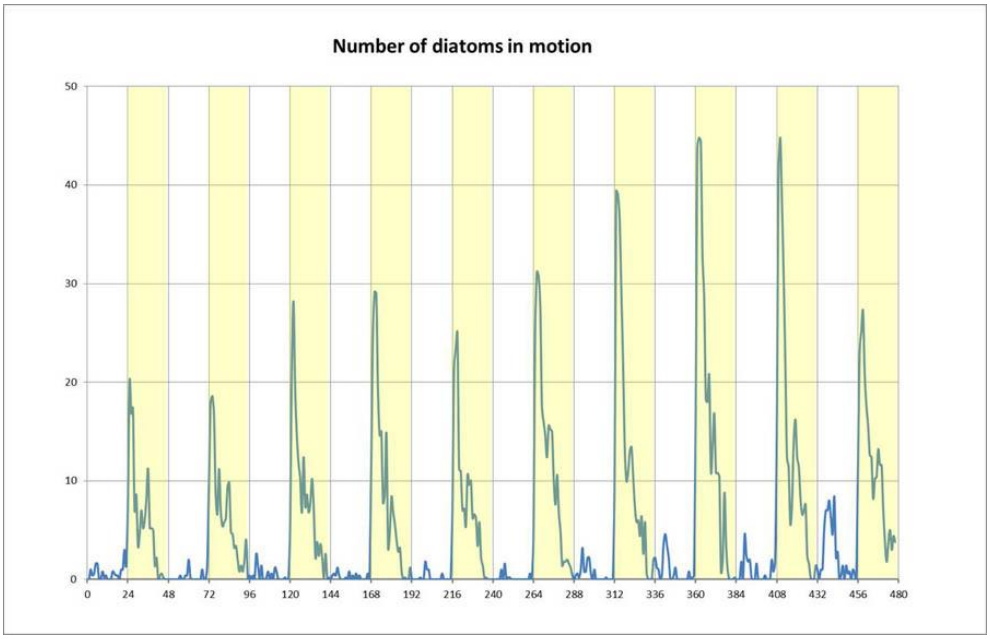


Movement activity

The number of diatoms between the colonies can be determined by classifying the sizes of the components of an image. However, this does not correspond to the number of diatoms that move, because some diatoms remain in place, especially at low brightness. In order to estimate the number of moving diatoms, the particle analysis plugin can be used well again. Diatoms of the genus *Cymbella* have a characteristic speed in their movement. If one superimposes two images whose recording times differ so much that moving diatoms have covered a distance of at least their own length, then they can be seen twice in this picture.

For superimposition one uses the calculation of the minimum offered by the Image Calculator. In the next step, the number of individual diatoms in the superposition image and in the single image is determined by classifying the size. The difference between the two numbers results in the number of diatoms shown twice, i.e. the number of moving diatoms. In the image on the left, even 6 binary images were superimposed, each of which was taken at intervals of one minute. The animated picture shows such superimpositions in succession during a light phase. In order to determine the number of moving diatoms, the difference between the number of individual diatoms in the superimposed image and the number of individual diatoms in the non-superimposed image is divided by 5 since the moving diatoms additionally appear 5 times. Overall, a period of 5 minutes is used to evaluate the

movement activity, during which the size of the colonies does not change significantly. The following picture shows the movement activity of the diatoms over the last 10 days of the observation time:



Apparently, the movement activity falls off quickly after the light phase has set in. However, this is just one example. *Cymbella* species can be observed that show high activity throughout the light phase. The already shown video on the activity in light and dark phase demonstrates this well.

It can be assumed that there is a strong dependence on light intensity. It is sufficient to bring the culture under the strong illumination of the inverse microscope to activate the diatoms at any time. As has already been shown, the colonies can be dissolved to a considerable extent at high light intensities.

The described procedure for determining the activity is reasonably easy to carry out, but it is also error-prone. Sometimes diatoms stop or resume their movement during the period of superposition. Diatoms can enter or leave the observed area. The colonies also change their shape to a certain extent through the movement of diatoms. Especially at high densities, images of diatoms may overlap with images of other diatoms that were there at another time. In the present case of small diatoms and a large observed region, the difficulty lies mainly in the quality of the binary images. This is expressed in imprecise classification. Nevertheless, I believe that the procedure is a simple and good approach, especially in comparison with the tedious manual counting process.

July 6, 2024	Complement	References: Further articles with reference to the homepage and publications by the author on the subject of diatoms.
February 07,2023	New post	Observation: Colonies: Notes on colony formation in *Diatoma tenuis* and *Diatoma vulgaris*
February 06,2023	New post	Observation: Colonies: Colony formation in *Asterionella formosa*
January 25, 2023	Complement	Observation: Colonies: Introductory words and pictures of chain-forming diatoms: Image galleries of *Diatoma tenuis*, *Diatoma ehrenbergii* and *Asterionella formosa* added.
October 28, 2021	Complement	Observation: Trajectories: Form of the paths: Video on tube-dwelling *Frustulia vulgaris* added
October 27, 2021	Complement	Observation: Colonies: Introductory words and pictures of chain-forming diatoms: Image gallery of Diatoma vulgaris added.
October 22, 2021	New post	Observation: Colonies: Size of the diatoms in a chain-like colony II - A surprising relationship between size sequence and dragon curve
May 15, 2020	Complement	Observation: Colonies: Introductory words and pictures of chain-forming diatoms: SEM images of *Diatoma* and *Bacillaria* added
March 9, 2020	Complements	References: Articles relating to the homepage Observation: Colonies: 'Size of the diatoms in a chain-like colony I' Note
March 6, 2020	Complement	Observation: Colonies: Introductory words and pictures of chain-forming diatoms: Example *Bacillaria*
February 13, 2020	Complements	Culturing: Purpose of cultivation: Video on the benefits of cultivation (*Bacillaria paxillifera*) Observation: Trajectories: Form of the paths: Constraints for colonies
February 7, 2020	Complements	Culturing: Challenges: Image on the sexual reproduction of Stauroneis sp. Observation: Devices for observation: upright microscope
February 5, 2020	Complement	Culturing: Nutrient solution: Nutrient solution for marine diatoms
July 27, 2019	Complement	Observation: Colonies: 'Size of the diatoms in a chain-like colony I' is divided into two parts. The extended part 'Introductory words and pictures of chain-forming diatoms' is placed in the beginning.
July 26, 2019	Complement	Observation: Another example of contamination with amoebae (*Meridion circulare*)
June 10, 2019	Complement	Observation: Trajectories: The movement of *Eunotia* sp.: Note on the observed *Eunotia* species
April 5, 2019	New post	Observation: Movement in EPS matrix: Notes on flake formation and movement in flakes (The menu item "Movement in EPS matrix" has been added.
April 2, 2019	Complement	Observation: Sexual reproduction: Further note

February 15, 2019	New post	Observation: Trajectories: Change in the trajectories of *Cymatopleura solea* as a result of cell size reduction
February 12, 2019	Complement	Culturing: Challenges: Malformations in *Cymatopleura solea*
August 21, 2018	New post	Observation: Trajectories: The movement of *Eunotia* sp.
August 11, 2018	Complement	Observation: Colonies: Size of the diatoms in a chain-like colony I: Images of further colonial diatoms
August 9, 2018	Complement	Observation: Colonies: Size of the diatoms in a chain-like colony I: two videos and text about difficulties in determining the size sequence of *Eunotia* sp.
August 1, 2018	Complement	Observation: Colonies: Size of the diatoms in a chain-like colony III: additional video and text on the development of an *Eunotia* colony
July 31, 2018	Complement	Observation: Colonies: *Cymbella* on jelly stalks: additional pictures and a video to the form of colonies
May 19, 2018	Complement	Observation: Movement of diatoms in and on biofilms: Video and text on movement within the biofilm
April 25, 2018	Complement	Observation: Sexual reproduction: Image of gametes fusion and of Automixis
April 4, 2018	New posts	Privacy statement and further legal notices
March 9, 2018	Complement	Observation: Sexual reproduction in *Cymbella* (allomixis): Video on the growth of auxospores
March 3, 2018	New post	Observation: Sexual reproduction: Automixis in *Cymbella*
February 24, 2018	New post	Observation: Movement on the water surface: *Pinnularia* sp. on the water surface
February 17, 2018	Complement	Culturing: Identification of genus and species: Annotation and pictures of *Cymatopleura elliptica* after annealing
January 24, 2018	Complement	Observation: Sexual reproduction: Note
January 8, 2018	Complement	Culturing: Challenges: Examples of malformations in *Surirella* und *Nitzschia*
December 10, 2017	Complement	Culturing: Creating cultures and care: Examples of contamination with epiphytic diatoms and amoebae
November 25, 2017	Complement	Observation: Horizontal view: Method of observation (Extension of the example)
November 8, 2017	New post	Observation: Horizontal view: *Stauroneis* sp. from a horizontal view

October 15, 2017	New post	Observation: Trajectories: Curvature of the trajectories by the example of *Surirella biseriata*
October 4, 2017	New posts	Observation: Colonies: ▪ *Cymbella* on jelly stalks ▪ directly attached *Cymbella* I ▪ directly attached *Cymbella* II
September 26, 2017	Complement	Culturing: Auxiliaries for cultivation (Notes on the microscope) Observation: Devices for observation (Notes on the microscope)
July 7, 2017	New post	Culturing: Example of a difficult cultivation (*Gyrosigma*)
June 29, 2017	Complement	Observation: Colonies: Size of the diatoms in a chain-like colony II The previous post about chain-like colonies has been extended and split into two pages.
June 10, 2017	New post	Observation: Colonies: Size of the diatoms in a chain-like colony
March 3, 2017	New posts	Observation: Movement on the water surface: ▪ *Cymatopleura solea* on the water surface ▪ *Nitzschia sigmoidea* on the water surface ▪ Movement patterns of floating *Nitzschia sigmoidea* ▪ Examples of floating *Nitzschia sigmoidea*
March 1, 2017	New post	Observation: Movement of diatoms in and on biofilms
January 18, 2017	New posts	Observation: Trajectories: ▪ Curvature of the trajectories by the example of *Navicula* ▪ Curvature of the trajectories by the example of *Cymbella*

December 31, 2016	First release	Welcome
		
		Diatoms:
		▪ What are Diatoms?
		▪ Introduction to motility
		▪ References
		
		Culturing:
		▪ Purpose of cultivation
		▪ Forms of cultures
		▪ Creating cultures and care
		▪ Nutrient solution
		▪ Light and Lighting
		▪ Auxiliaries for cultivation
		▪ Identification of genus and species
		▪ Challenges
		
		Observation:
		▪ Devices for observation
		▪ Sexual reproduction in *Cymbella* (allomixis)
		▪ Trajectories
		  ▪ Form of the paths
		  ▪ Description of the trajectories
		  ▪ Motion Tracking of Diatoms
		  ▪ Analysis of trajectories I
		  ▪ Analysis of trajectories II
		▪ Horizontal view (originally in a single post):
		  ▪ Method of observation
		  ▪ *Craticula cuspidata* from a horizontal view

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