Observation of cooperation and division of labor in colony formation of the tube-dwelling diatom *Frustulia vulgaris* in culture

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Abstract

The tube-dwelling freshwater diatom *Frustulia vulgaris* was cultivated for two years and its colony formation was observed under the optical microscope from a single cell to a fully developed colony. Cooperative behavior with division of labor allows the colony to expand rapidly. A group of diatoms at an end of the colony stretches the tube, transports it further and deposits mucilage. The volume of extracellular polymeric substances produced by this group was estimated. A second group of diatoms can usually be observed in the immediate vicinity, which widen and tighten the tube. The cooperative behavior of the diatoms and the division of labor can be characterized by symmetry properties of the spontaneous reversal of movement and the reversal behavior when reaching a barrier. The formation of the spatial structure of the colony is essentially a result of the movement of the colony ends during expansion. Random processes enable obstacles to be overcome. Even structures that are perpendicular to the previous direction of propagation can be colonized. Further observations concern the topology of the tubes. Branches can be formed by cooperation between the diatoms. When these branches are not being used, they become sealed. To investigate the elastic properties of the tubes, an external force was applied to a tube and a tensile stress-strain curve was created. An explanation of the phenomena requires mechanisms of self-organization. A hypothesis is presented for this. The ecological benefits of living in tubes are discussed in the context of the properties of the tubes.

Keywords: *Frustulia vulgaris*, colony formation, elongation process, stress-strain diagram, colony topology

1.1 Introduction

A number of pennate diatoms form tubes of extracellular polymeric substances (EPS) in which they live and move (for an introduction see Round et al., [1.28]) and are therefore called tube-dwelling diatoms. Manjumol et al. [1.26] mention *Navicula*, *Berkeleya*, *Nitzschia*, *Parlibellus*, *Haslea*, *Gyrosigma* and *Frustulia* as important genera of tube-dwelling diatoms. This life form is mainly found in marine species, but also in some freshwater species. There are also diatoms that colonize tubes that have been made by other diatoms. In the sense of the term, these cohabitants are also tube-dwelling diatoms. Extensive studies of tube-dwelling diatoms have been published by Cox (in particular [1.4], [1.5] and [1.6]).

The subject of this work is the pennate diatom *Frustulia vulgaris* (Thwaites) De Toni 1891, which is considered a synonym of *Schizonema vulgare* Thwaites 1848 (for details see [1.15], [1.20] and [1.19]). It is a cosmopolitan tube-forming diatom that occurs in freshwater, but also in weak brackish water [1.21] and is found particularly in running waters. G. H. K. Thwaites [1.31] describes the colonies in the original description as follows:

> “The species is most abundant in shallow streams, covering stones, &c. with a dark brown gelatinous coating, but in which a linear arrangement of the frustules may frequently be detected. When the plant occurs in deeper water, the ordinary *Schizonema* filaments make their appearance, which are much-branched when growing in rapid streams, but when occurring in still water, or where there is only a slight current, are simple or nearly so.”

As described by Krammer and Lange-Bertalot [1.21], they are also found aerially on wet rocks and as planktonic free-floating gelatinous tubes. Figure 1.1 shows the drawing from the description by Thwaites [1.31]. It shows a typical section of a tube with a branch and an already relatively dense population.

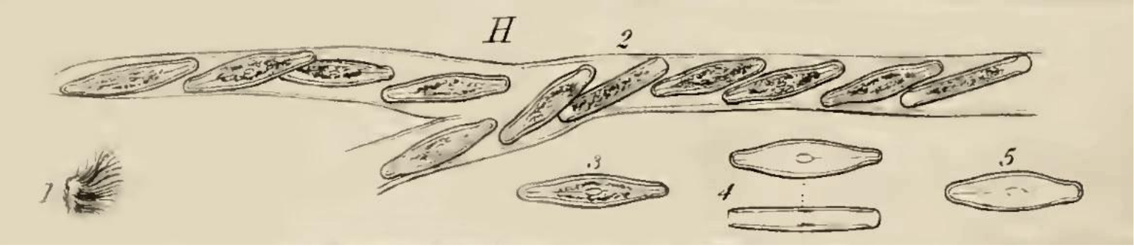

Figure 1.1 Historical drawing of an *F. vulgaris* colony. The branch joins the primary tube at a small angle. A whole colony is shown at the bottom left.

Aumeier and Menzel [1.2] point out that the tubes have evolved as a modification of a holdfast in the form of a hollow tube. However, little is known about the processes that enable the formation of the tubular structures. Houpt [1.16] [1.17] assumes an independent movement of the diatoms in the colony. In the context of

Navicula pseudocomoides (1987), it is spoken of the impression of complete independence. Lobban [1.25] states that the diatoms build the tubes at the tip. This does not contradict the statement that they function independently, but merely states that the tip is the site of extension. According to Houpt [1.16], in *N. pseudocomoides* a continuous extension is caused by the excretion of material by the cell at the front. Thus, only one cell involved is talked about. From these statements and observations, a coordinated activity and division of labor is not assumed. However, Lobban [1.25] goes beyond this and asks whether there is a division of labor between the diatoms and what the other cells do, by which he refers to the diatoms that are located away from the tip.

The formation of a colony of tube-forming diatoms has several interrelated aspects. These include the morphology of the colony and its topology, which often shows more or less pronounced branching [1.6]. As already mentioned for *F. vulgaris*, the density of branching can depend on the environmental conditions and the age of the colony. Unbranched colonies also occur, for example in *Berkeleya rutilans* [1.11]. Primary tubes can form thick secondary structures, such as in *B. rutilans* [1.25]. Complex structures also include tubes within tubes, which Cox [1.6] describes in *B. rutilans* and which may be colonized by other species. There are also spatial patterns that are difficult to interpret. Lobban [1.24] reported that in *Navicula bottnica* one or more strands of diatoms occur within a tube, each consisting of several strands of cells having a braided appearance. No tubes are visible around the twisted strands. All occurring structures must be considered in the context of the processes that lead from an initial colony to a complex system of tubes.

The topology of the network must furthermore be seen against the background of population dynamics. Houpt [1.17] regards branching and widening of the tubes as a possible reaction to rapid cell division. Even if a high growth rate is not the driving force behind branching, it allows the population to grow faster. This is one aspect of the question of how tube-dwelling diatoms cope with the limitations of an essentially one-dimensional habitat but does not answer the question of the benefit of this growth form compared to a benthic lifestyle without tubes.

The aim of this work is primarily to understand the processes of colony formation in *F. vulgaris* and to answer some of the questions raised by Lobban [1.25] with respect to the species under consideration. In particular, the cooperation of the diatoms in colony formation will be examined, which addresses cooperation and division of labor. This forms the link to the aspects of branching and the structure formation of the colony, including population dynamics. To better understand the processes, the elastic properties of the tubes are analyzed. Discussions on the control of the formation and breakdown of organizational structures lead to theoretical considerations on self-organization. Even if these are not the focus, the objective is a testable hypothesis. Finally, some aspects of the potential benefits of the life form, as a spin-off of the observations, are discussed.

1.2 Materials and Methods

1.2.1 Cultivation

Epilithic colonies of *F. vulgaris* were found and isolated in high density in the Neckar River (Germany 49°04'41.8 N 9°09'17.9 E) in October 2021. A clonal line was cultivated from this. At the time of sampling, the apical length was about 55 µm, in July 2023 the average length has been reduced to 48 µm. Colony formation was then irregular, and the colonies had a fuzzy appearance, so that observation could not be continued. Sexual reproduction did not occur in the cultures.

The f/2 medium according to Guillard [1.14] proved to be well suited. Except for a few cultures in which the spatial distribution in complex environments was investigated, the cultivation was done in polystyrene Petri dishes with a diameter of 55 mm. To study the external shape of colonies in a spatially irregular environment, cultivation was performed in a larger cuvette with an inserted wire frame. All cultures were exposed to an artificial day-night cycle with a light phase of 11 hours and a dark phase of 13 hours, whereby the intensity of the light exposure was 400 lx to 900 lx. The room temperature ranged from 19° C to 21° C. New batch cultures were inoculated with segments of colonies from mother cultures when these were two or three weeks old. Replenishing a culture with medium was only used in exceptional cases, because it is difficult to carry out systematic observations in a multilayer of tubes.

To observe the formation of a new colony from a single cell, it is necessary to inoculate with individual diatoms. It is possible to remove these from outside the tubes when a culture has reached a very high population density, typically after 4 weeks, and many layers of tubes are stacked on top of each other. Individual diatoms that have escaped from the colonies can be found below the colonies on the substrate.

In another sample taken in May 2023, *F. vulgaris* was not isolated but enriched with Guillard's nutrient solution. This allowed the observation of *F. vulgaris* in an environment similar to the natural environment in terms of biocenosis.

1.2.2 Recording technique

Long-term recordings were taken directly in the culture using an inverted microscope (Zeiss Axiovert A1), whereby time-lapse videos were generated from image sequences.

Details such as the wall thickness of the tubes or bacterial colonization were observed using an upright microscope (Zeiss Axioplan). Because colonies adhere so strongly to the substrate that they cannot be transferred to a microscope slide without damaging their structure, a colonized cover slip was used for such studies. For this purpose, a cover slip is placed in the Petri dish before inoculation so that it can be colonized on the upper side. After cutting off the tubes protruding over the edges of the coverslip, they can be placed on a slide with the colonized side down.

Once the colonies have spread across the bottom of the entire Petri dish, it is not easily possible to observe the entire culture with such a high spatial resolution that the movement of the diatoms and all areas of colony growth can be recognized. For larger regions of interest, a stereomicroscope (Zeiss Stemi 305) was used.

1.2.3 Investigation of elastic properties

To investigate the elastic properties of the tubes, they were stretched, and the elongation and the force exerted were determined. The method of force measurement using bending of a glass fiber was used by M.A. Harper and J. F. Harper [1.18] to measure the adhesive and tractive forces of diatoms (Adhesive and tractive forces of diatoms have been measured by the bending of a glass fiber). This makes it possible to create a tensile stress-strain diagram.

Table 1.1 Symbols and Definitions

Symbol	**Definition**
$p_{lr}, p_{rl}.$	Transition probabilities per time for the spontaneous change of direction
p^{b}_{rr} , p^{b}_{rl} p^{b}_{ll} , p^{b}_{lr}	Probabilities for state transitions after the “barrier” event has occurred.
p^{s}_{rl} , p^{s}_{lr}	Transition probabilities per time for leaving the “Push to the right” and “Push to the left” states.
$v, \overline{v}$	Growth speed and average growth speed
Q	cross-sectional area of the tube
ΔV	EPS volume produced per time Δt
L	Length of a colony including branches
$N(t)$	Number of diatoms in the colony ($N_0 = N(0)$)
$n(t)$	Number of ends in a colony
λ	Growth rate in the case of exponential proliferation of diatoms
β	Proportion of time during which expansion occurs at one end
γ	Proportionality factor for the increase of branches
k	Spring constant
E	Modulus of elasticity
l, d	Length and diameter of the glass fiber
$\vec{F}, F$	Force and its amount
$\overrightarrow{F_1}, \overrightarrow{F_1}$	Tangential tensile forces
F_t	Amount of the tangential tensile forces
Δb	Deflection of the glass fiber
A, B	Points at which the tube is attached (see Figure 1.8)
α_0, b_0 c_0	Geometric quantities in the force-free case (see Figure 1.8)
α, b c	Geometric quantities when applying a force (see Figure 1.8)

1.3 Results

1.3.1 Colony forms

In the original sample, *F. vulgaris* tubes lay epilithic and flat on the substrate. The colonies formed in Petri dishes look similar. A system of branched tubes lies flat on the substrate, except for some tube ends, and the tubes are more or less frequently stacked on top of each other, depending on the stage of development. A smaller colony is shown in the overview images Figure 1.2a and Figure 1.2b. Small localized elevations (on the order of a millimeter in height) consisting of a tangle of tubes were common in well-developed cultures.

A region of a colony with crossing tubes can be seen in differential interference contrast (DIC) in Figure 1.2c and an even smaller section with diatoms during a passing process in Figure 1.2d.

In a cuvette (85x45x43 mm) containing an irregular frame of plastic-coated wire (diameter 0.85 mm) and a vertical cover glass, more complex structures appeared. The colony initially spread over a large area on the bottom and vertical cover glass. After 3 to 4 weeks, threads were formed that bridged open spaces. They continued to develop into reticular structures (see Figure 1.2e), with parts of the colonies detached from the substrate also contributing. Similar to the observations in Petri dishes, many cone-like structures with typical heights of 1 mm could be seen after about 5 weeks (see Figure 1.2f).

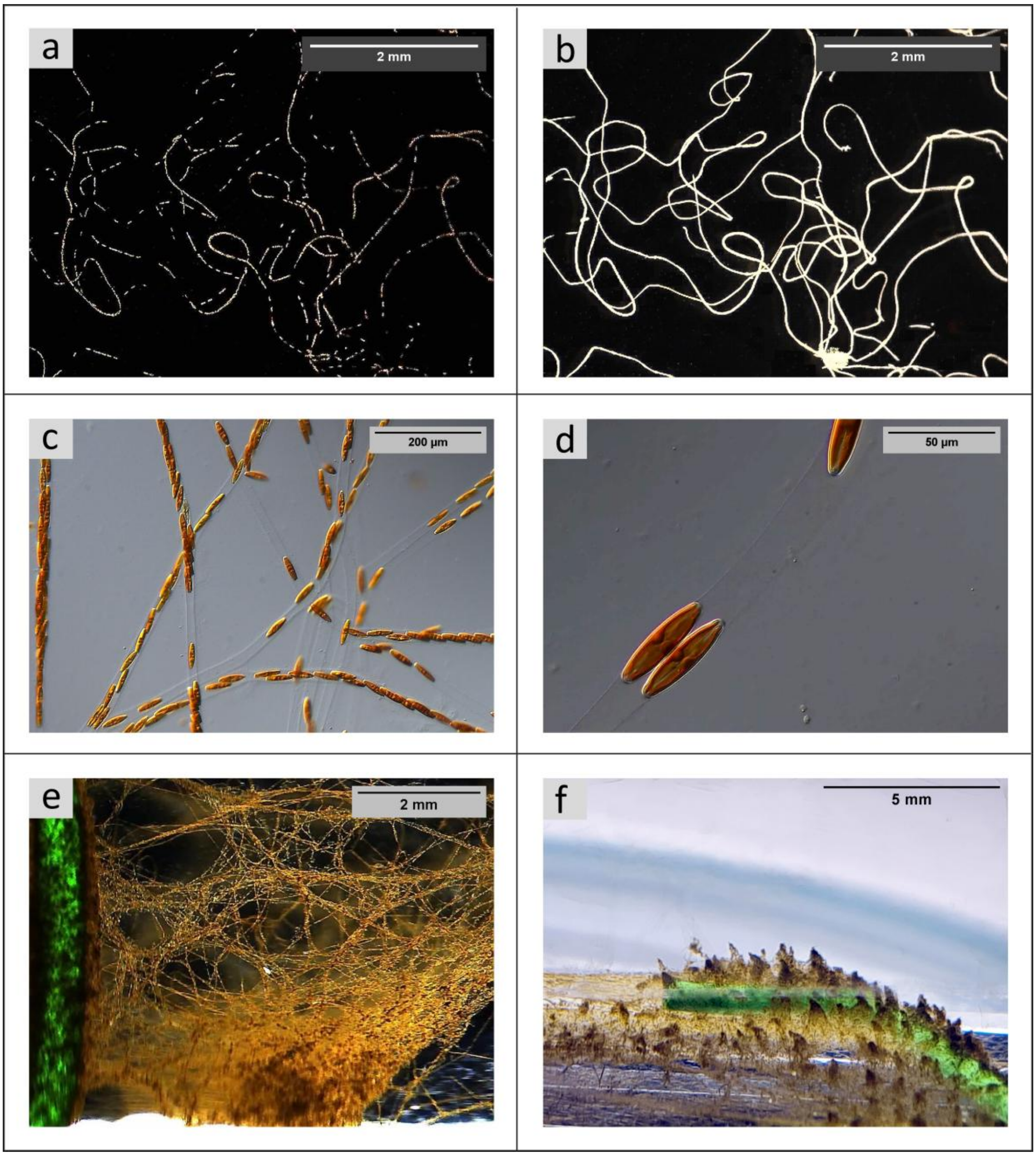

Figure 1.2 (a) Overview image of a colony in darkfield. (b) A composite of images of the colony shown in (a) over a period of one hour. The larger white spot at the lower edge is due to an end of the colony that is not attached to the substrate and exhibits mobility. (c) A colony grown on a coverslip in DIC. All optically crossing tubes are in different layers. (d) Close-up in DIC. On the lower left, one diatom is passing the other. Since the diameter of the tube is only about the transapical length of a diatom, elastic elongation of the tube occurs. The speed of the diatoms is very low in the position shown. (e) Formation of net-like structures on a frame made of plastic-coated wire. (b) Pyramidal structures in an older culture along a coated wire.

As expected, a frond typical of many tube-dwelling diatoms, as described by Thwaites [1.31] for the occurrence in deeper water and as shown in the first description (Figure 1.1), did not occur in culture. It would certainly be of interest to study cultures in deeper and in flowing water. It should also be noted that, except for very small colonies with a few diatoms, no unbranched colonies were observed. Metastructures consisting of many primary tubes were not found either in the samples or in the cultures. The different forms of colonies at the macroscopic level in cultures of *F. vulgaris* show that they are not suitable for characterizing the species.

1.3.2 Movement and collisions

1.3.2.1 Movement of diatoms inside the tubes

Tube-dwelling Diatoms move inside the tubes (see eg. [1.16] and [1.25]), which can be more or less densely populated. They often move in groups of diatoms lined up next to each other. Compared to the mobile diatoms, there are few resting diatoms in *F. vulgaris*, even at low light intensities. Often there is a spontaneous change of direction, which in the case of groups can be seen at the rear ends. Sometimes several diatoms will split off and continue in the opposite direction. This is probably caused by a reversal of movement of a single diatom within the group, which causes the following diatoms to change direction. The spontaneous change of direction results in a collision within the group.

A jerky back and forth movement in very rapid succession, as is seen in many motile diatoms, was not observed. When the ends of the colony were reached, the movement was usually reversed, particularly in the case of individual diatoms. These movements inevitably lead to encounters between diatoms or groups of diatoms moving in opposite directions. There are two different cases. Either the diatoms glide past each other, often forming adjacent rows with different directions of movement, or there is a collision in which diatoms change direction. Sliding past requires a sufficient diameter of the tube. If the diameter is sufficient but small, elastic stretching of the tube occurs as it passes (Figure 1.2d). The speed of the diatoms will initially decrease as energy is required to stretch the tube, and then increase again as the elastic energy of the tube accelerates the diatoms.

Since diatoms are usually found moving in the colonies, it can be assumed that the activities of the raphe system are synchronized, i.e. the raphes do not usually work against each other.

1.3.2.2 Collision processes

The following observations of collisions of individual diatoms and groups of diatoms explicitly do not refer to the behavior at the ends of the colony. There, unhindered movement in one direction is not possible and peculiarities arise that are related to the extension of the colony. The following processes can be recognized under this constraint:

1. The colliding diatoms do not come to a standstill, but all diatoms remain in motion after the collision.
2. There is usually no reflection of the two collision partners, with the colliding diatoms (or groups of diatoms) moving in opposite directions after the collision.
3. One direction of movement predominates for all diatoms involved, often, but not always, that of the originally larger group. This results in a bigger joined group.
4. When colliding with stationary diatoms, the direction of movement of the moving diatom (or group of diatoms) is dominant, but it is not clear whether the initially resting diatom is actively moving or being pushed.

Exceptions to (2) have been observed very rarely, whereby such an observation can also be explained by a collision according to (3) and an immediate spontaneous reversal of direction.

As the diatoms or groups of diatoms move at different speeds, there are also encounters between diatoms or groups of diatoms moving in the same direction relative to the tube. Either the faster groups will pass the slower groups without colliding or they will move together in a larger group at a uniform speed.

Individual events cannot always be clearly distinguished visually. The difficulties of observation are:

- Frequent spontaneous changes of direction, which can occur at any time, even when passing each other.
- High density, where processes such as gliding, passing, collisions and spontaneous reversals with internal collisions occur almost simultaneously in the group.
- Inside a thick tube and a dense population, the diatoms do not move in parallel rows. With this disordered movement, collisions can hardly be identified.

In contrast to collisions, sliding past does not change the group structure. Apart from a more or less pronounced slowing effect, the group cohesion remains. The rules for collisions (1) to (4) lead to the accumulation of diatoms into groups. This aggregation of diatoms was described by Houpt [1.16] [1.17]. However, in a longer tube without branches, not only one or two groups moving in opposite directions are observed, but a mixture of groups of different lengths and different directions of movement. The reason lies in the occurrence of spontaneous reversal of direction. It repeatedly reduces the size of the groups so that aggregation and separation are in a dynamic equilibrium. With the exception of the expansion of the tube system at its ends, which is still to be described, there is no discernible preferred direction of movement on average over time.

Jams are also common. Diatoms can become jammed in tube systems that are not yet densely populated, for example in narrow bends, at branches or when a larger group reaches one end. These jams clear after some time, which is achieved by the reversal at the fixed end and the spontaneous reversal of the direction of movement. This enables an even distribution of the cells across the limited living space.

1.3.2.3 Speed of movement of freely moving diatoms

The speed of free-moving diatoms and smaller groups that are able to move freely can vary considerably within a colony. The highest speed measured was 2.48 µm/s. Very often diatoms with a speed of slightly less than 2 µm/s were observed. In well-developed colonies under good growth conditions, all the tubes can be so densely packed that movement almost comes to a standstill.

1.3.3 Formation and growth of colonies

1.3.3.1 Formation of colonies initiated by a single cell

Wilson (1929) observed in *Navicula rhombica* Greg. that colony formation begins with a single diatom. When a single cell of *F. vulgaris* is placed on the substrate, a back-and-forth movement on the substrate begins. Sometimes the diatom rises vertically and then tilts back to a horizontal position. As with motile diatoms that do not form tubes, the raphe activity is coupled to the substrate so that movement of the diatom relative to the substrate can be seen. There is no limit to the radius of movement. During this movement, the diatom secretes a sticky EPS coat that gradually expands. Even before the first vegetative division occurs, a cocoon-like envelope can be seen around the diatom, adhering to the substrate. The diatom moves inside the tube and can no longer move freely on the substrate but is restricted in its movement by the tube. By moving back and forth, it reinforces the initial tube by excreting mucilage, as described by Houpt [1.16]. The length grows slowly. The elasticity of the short tube is evident because after a reversal of motion the tube shortens at the end, following the diatom.

Then the first vegetative division occurs. At this stage, the usable movement space is barely longer than the diatom itself. The generation time of diatoms is strongly dependent on environmental conditions such as light intensity and temperature (see e.g., Eppley [1.8]). Under the culture conditions described above, it was slightly more than one day. After division, the diatoms lie side by side and move back and forth relative to each other, reaching a total length greater than that of the cell, and thus expanding the surrounding cover in both length and diameter. However, at this stage of colony formation, length growth is so low that the colony's expansion after a few cell divisions would not keep pace with the rapidly growing population. Houpt [1.16] describes the colony formation of *N. pseudocomoides* similarly but speaks of a continuous extension at the open end. Such an open end is not observed in *F. vulgaris*.

1.3.3.2 Expansion of a small colony of a few diatoms

The further development of colonies is not uniform. Often there are subsequent divisions with successive expansion, in which the diatoms move towards each other in a disorganised manner. In a few colonies with only two diatoms, however, a form of coordinated expansion begins, in which the diatoms no longer act independently. The two diatoms lie one behind the other in the tube and together they exert force towards the end of the tube. Their forces add up. The result is a continuous process in which the tube is stretched by the two diatoms and strengthened by the secretion of mucilage. The resulting still very thin-walled tube adheres behind the two cells on a suitable substrate, such as the polystyrene surface of the Petri dish. During this extension of the tube, the diatoms repeatedly reverse by spontaneously changing direction, often together, probably initiated by the reversal of the leading diatom. To ensure that the diatoms quickly return to working together at the location of the expansion despite the tendency to spontaneously change direction, the probabilities of reversal in the direction away from the tip must be smaller than the probabilities of reversal in the direction towards the tip. While it is often observed that individual diatoms turn back immediately after reaching an end, this may not happen, or only with a low probability, as long as the colony is expanding. As mucilage is excreted during movement, this behavior should be useful for stabilizing and thus maintaining the expansion achieved. Over a period of expansion of several hours, the diatoms always return to the expansion at the same end of the colony. In one case an initial expansion of the first small tube was observed over a period of about 6 hours. This is followed by a period of no expansion at the same end of the colony and therefore no systematic movement towards that end.

The term ‘coordinated expansion’ refers to the temporal coincidence of the directed movement that enables rapid colony expansion. As this activity creates benefits for the diatoms in the colony, it is an elementary form of cooperative behavior.

The speed of propulsion of the tube with two cells in the cooperative phase, not counting occasional periods of reversal, is about 0.025 µm/s, two orders of magnitude below the speed of freely moving diatoms. Nevertheless, a phase of continuous expansion over 6 hours enables an extension of the tube at one end by 540 µm, which is significantly higher than the expansion speed of the initial tube without coordinated enlargement. The observation of the expansion of two diatoms was made under LED microscope illumination, the intensity of which was significantly higher than the intensity under culture conditions. Cultures that were only briefly exposed to the microscope light to measure the length of the tube showed lower increases. In addition, the rapid expansion of the colony due to the simultaneous action of two diatoms does not necessarily begin after the first cell division. An analysis of 98 3-day-old colonies showed that 12 colonies contained 2 diatoms, 6 colonies contained 3 diatoms (only one second generation diatom divided) and 78 colonies contained 4 diatoms. There was also one colony with only one diatom and another colony with 8 diatoms. No branching was observed at this stage of development. The mean length of the colonies with less than 4 diatoms was 104 µm, whereas the mean length of the colonies with 4 diatoms was 209 µm, i.e. twice as long. The standard deviation across all colonies was strikingly large at 85 µm. Among the colonies with 4 diatoms, there are small cocoons with 4 diatoms (minimum: 76 µm), but also longer tubes (maximum; 483 µm), depending on whether a coordinated expansion has already taken place.

1.3.3.3 Expansion in a more advanced state of the colony

Through continuous vegetative cell division, the population of a colony in culture initially grows exponentially. As the colony grows, more diatoms can participate in the expansion of the tube. Three spatially separated sections can be distinguished, in which the diatoms perform specific tasks. Although the allocation of diatoms to these functions changes dynamically at the boundaries of the sections, they can be assigned visually. Figure 1.3a shows one end of a tube where diatoms are actively contributing to the expansion. In this section, which will be referred to as Section I, there are diatoms, which stretch the tube and strengthen it by secreting mucilage. This is not fundamentally different from expansion by two diatoms. Due to the forces and the elasticity of the tube, the diatoms are not positioned apex to apex, but always offset laterally. Apart from the spontaneous reversals of direction described above, the diatoms exert a force towards the tip of the tube. The addition of these forces results in faster propulsion than with two diatoms. This is shown in an idealized illustration in Figure 1.3b. Instead of the typical 'scrum', the diatoms are lined up apex to apex in the idealization. A higher number of diatoms in Section I also implies a higher release of EPS to the tube per time. The typical number of diatoms involved in propulsion was 5 to 10 diatoms, although the number is not constant over time. A large number of pushing diatoms bears the risk that the force at the tip ruptures the thin newly formed membrane. Sliding past in Section I is infrequent and is probably prevented by the tight, tensile membrane. Occasionally two parallel rows of diatoms can be seen in this section. The tube expansion phase lasts for a limited period of time, usually a few hours. The diatoms then withdraw from the tip. The statements made about the asymmetry of spontaneous directional changes and the reflection behavior when reaching the tip also apply to expansion by more than two participating diatoms.

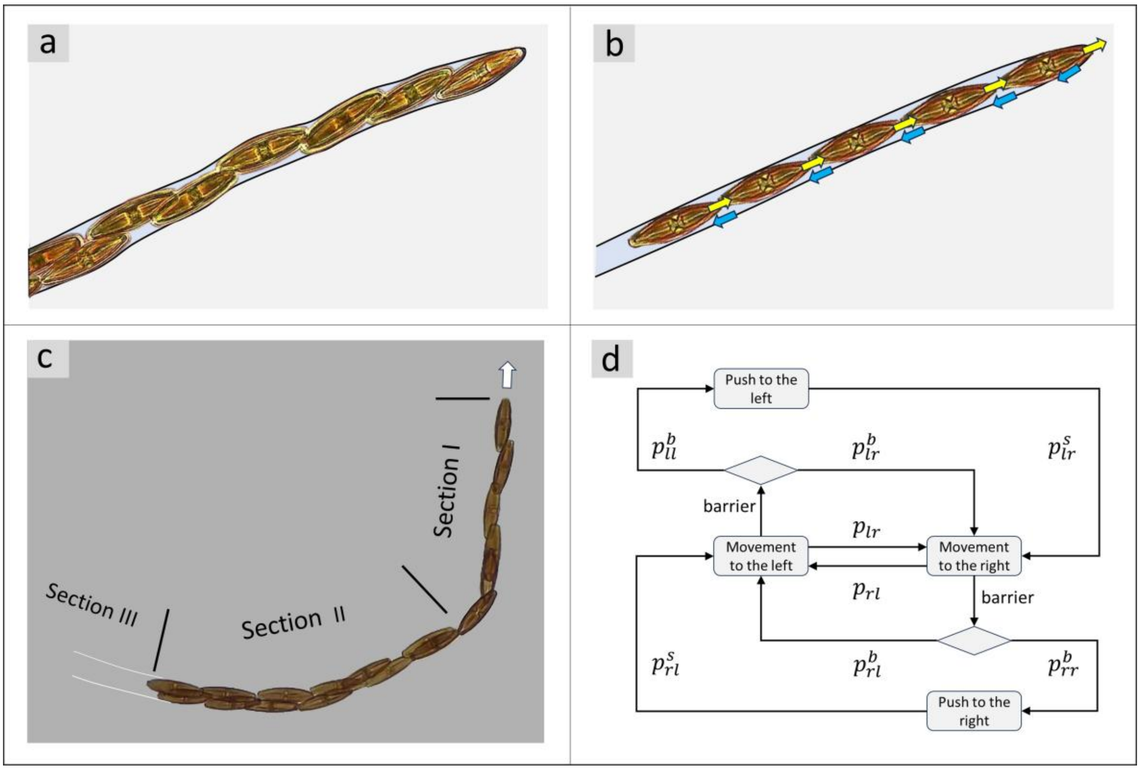

Figure 1.3 Illustrations of the cooperative expansion of the tube system. (a) A group of diatoms in Section I, near the end of a tip that is in the process of expansion. The background has been removed from a phase contrast image and the EPS envelope added manually. (b) Idealized arrangement of diatoms to illustrate the forces at work. The yellow arrows represent the pushing force of the corresponding diatom. The leading diatom also exerts such a force. The blue arrows represent the opposing forces acting in the EPS film. They were drawn as an example at one location, but act around the entire circumference of the tube. (c) Accumulation of diatoms extracted from a bright field image. The tip progresses in the direction of the white arrow. Opposing movements must be possible in Section II, whereas in Section I they only occur occasionally. In this example, there are no diatoms in the indicated Section III. (d) Modeling the reversal behavior of the diatoms in the form of a state diagram. It does not take into account the collision of diatoms in motion, but only the spontaneous reversal and the reaching of an impassable barrier, such as the end of a tube or other diatoms in Section I.

With about 7 diatoms, the typical speed of extension was 0.15 µm/s. The highest speed measured amounted to 0.31 µm/s, with 10 diatoms involved. These figures should be regarded as rough reference values, as different speeds were observed. They vary and are certainly dependent on environmental conditions such as light intensity and temperature. Such dependencies are subject to further investigation. It is important to note that the achievable speed is about one order of magnitude higher than the speed of expansion by two participating diatoms. The speed of freely moving diatoms or groups of such diatoms is again one order of magnitude higher than the observed maximal speed of expansion.

1.3.3.4 Stabilization and widening of the tubes

In many cases, Section I is followed by a section in which the tube is reinforced and radially widened (see Figure 1.3c), which can be recognized by light microscopy. This is referred to as Section II. An empty zone between Section I and II is occasionally observed, at least temporarily. The tube in Section II has sufficient diameter to allow diatoms to easily pass each other. They exhibit the typical movement patterns described, in particular sliding past diatoms moving in the opposite direction, passing diatoms with the same direction of movement, collisions and spontaneous reversal of direction. The individual processes can hardly be differentiated in the dense movement in both directions in this section. The diatoms in Section II do not exert any systematic tangential stretching forces on the tube.

To ensure that in particular the section adjacent to Section I is strengthened, and the group forms a closed unit, the reversal behavior must be asymmetrical as in the diatoms at the tip, so that there is a tendency to remain close to the section of extension. Nevertheless, the behavior differs from that of the diatoms in Section I, because they do not increase the force in the direction of the tip but generally reverse when Section I is reached. An asymmetry regarding the collisions, which favors a movement towards the tip, would benefit the stability of the arrangement, but is not easily recognizable in the dense teeming. Occasionally, a diatom from Section II changes its role and joins the diatoms in Section I. The role change also occurs in the opposite direction. A need for simultaneous reinforcement of the tubes becomes apparent when diatoms leave Sections I and II. It can occasionally be observed that the tube at the end shrinks by a few diatom lengths in the axial direction. As will be explained later, there can be no strong adhesion to the substrate in Section I.

In Section III (Figure 1.3c), which is not directly involved in the construction of the tube, the diatoms move individually or in groups without a preferred direction. Symmetrical reversal behavior ensures that all usable parts of the tube system are reached. The tubes are thus constantly reinforced and protected. A clear distinction between Section II and Section III is only recognizable if the colony is not densely populated, which is no longer the case after a few weeks of high growth rates in culture. A distinction between Section II and Section III is then no longer meaningful.

1.3.3.5 Division of labor and characteristics of the diatoms involved

The various functions that diatoms perform in the colony can be interpreted as a division of labor in a higher-level process. There are functions for formation, for stabilization of the newly formed tube and for maintenance of the entire tube system.

Each function is associated with a spatial allocation, which has been designated as Sections I, II and III. Each of the three functions is characterized by a behavior with respect to a spontaneous change of direction and in the event of a collision with an impassable obstacle. The completeness of these features requires further detailed investigation. The behavior in collisions (symmetry) or the forces generated by the cells could be further function-dependent characteristics.

For a better understanding of the processes, a state diagram of a continuous Markov process is shown in Figure 1.3d (for introduction see Garfinkel et al. [1.12]). Since the reversal of direction can happen at any time, this is a continuous Markov process. The designations refer to a chosen horizontal orientation of the tube. If a diatom moves from left to right, it is in the state “Movement to the right”, if it moves in the opposite direction, it is in the state “Movement to the left”. There is always the possibility that the diatom will reverse its direction of movement. The transition probabilities per time are described by p_{lr} and p_{rl}. An asymmetrical behavior that favors one direction of movement over a longer period of time is present for $p_{lr} \neq p_{rl}$, i.e. in Sections I and II. If the diatom encounters a barrier during a movement to the right, such as the end of the tube, the “barrier” event occurs. There is the possibility of reversing direction (probability p_{rl}^{b}) or continuing the movement in the direction of the obstacle (probability p_{rr}^{b} with $p_{rr}^{b} + p_{rl}^{b} = 1$). For diatoms in Section III, the reversal of direction on reaching the end is the normal case. Therefore, $p_{rr}^{b} \ll 1$ holds. If the diatom does not reverse, the state “Push to the right” is taken. Although a movement to the right also takes place here, albeit only a slow one corresponding to the stretching, this was modeled as a separate state. The “barrier” event cannot occur in this state. A spontaneous change of direction is possible in this state, which occurs with the probability per time p_{rl}^{s} and leads to the state “Movement to the left”. Whether $p_{rl}^{s} = p_{rl}$ applies requires further investigation. After such a spontaneous change of direction, it again depends on the asymmetry of the changes of direction whether there is a tendency to return to the “Push to the right” state. With a barrier on the left-hand side, the mirrored case shown in the diagram applies. In this simple modeling, collisions between diatoms are not considered. A list of functions and associated behaviors can be found in Table 1.2. A combination of symmetrical reversal behavior and maintaining the direction of movement in the event of a barrier was not observed. It would not be suitable for expanding the colony.

Table 1.2 Overview of the observed functions and behaviors

Section	Function	Spontaneous reversal of direction	Reversing at a barrier
I	Stretching and stabilizing the tube	asymmetric	no
II	Strengthening the new section	asymmetric	yes
III	Maintenance of the tube system	symmetric	yes

1.3.3.6 Estimation of the EPS volume produced during expansion

The extracellular polymeric substances excreted by the diatoms take up water quickly and are highly hydrated [1.9] [1.10] [1.13]. A volume of hydrated mucilage is produced in Section I, which can be estimated. To accomplish this, the states in the section of the advance at time Δt and $t + \Delta t$ are compared. The colony is extended by $v\Delta t$ in the time Δt, where v is the speed of the advance.

If the number of diatoms in Section I does not change, the shape of the tip in this Section is approximately unchanged and thus also the amount of EPS in it. However, a tube of length $v\Delta t$ was formed in Section II (see Figure 1.4). Its volume is $\Delta V = Qv\Delta t$, where Q is the cross-sectional area of the tube when it leaves Section I. It can be determined by measuring the outer diameter and inner diameter. The EPS volume produced per time is therefore given by $\Delta V/_{\Delta t} = vQ$.

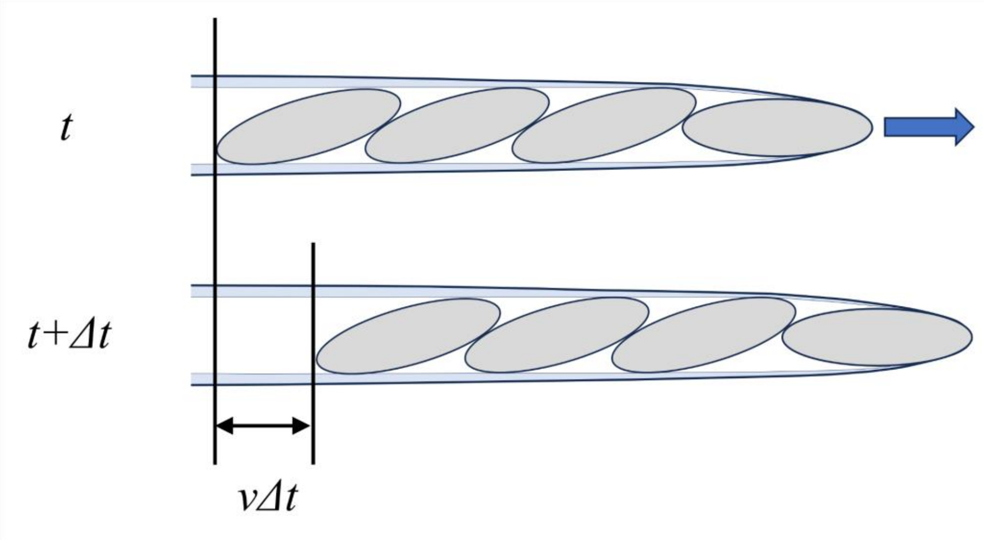

Figure 1.4 This represents Section I at the specified time points, *t* and *t* + *Δt*. The tube formed within the time interval *Δt* enables the amount of hydrated EPS produced to be determined.

A measurement carried out yielded 0.98 µm^3/s. (There were 5 diatoms in Section I, the outer diameter was 10.1 µm, the wall thickness 0.2 µm and the speed of advancement 0.15 µm/s). As the measured values are subject to natural statistical fluctuations and environmental conditions, this should be regarded as an exemplary value. This value could only be determined imprecisely due to the difficulty of determining the wall thickness by optical microscopy. This is where the potential for more accurate measurements is greatest.

1.3.3.7 Movement of the tip and the search for ways forward

Even on a flat substrate such as the bottom of a Petri dish, growth at a tip does not follow a straight line, but moves forward in more or less pronounced curves. It can often be seen that the tube in Section I does not yet adhere to the substrate and has its own mobility. Even with a flat substrate, the end protrudes into the body of water from time to time and performs irregular movements. In particular, when there is no contact with a substrate due to the shape of the bottom, this moving part can reach into Section III. Figure 1.5 shows overlays of image series of a colony in dark field, in which the freely moving ends can be seen as tuft-like structures.

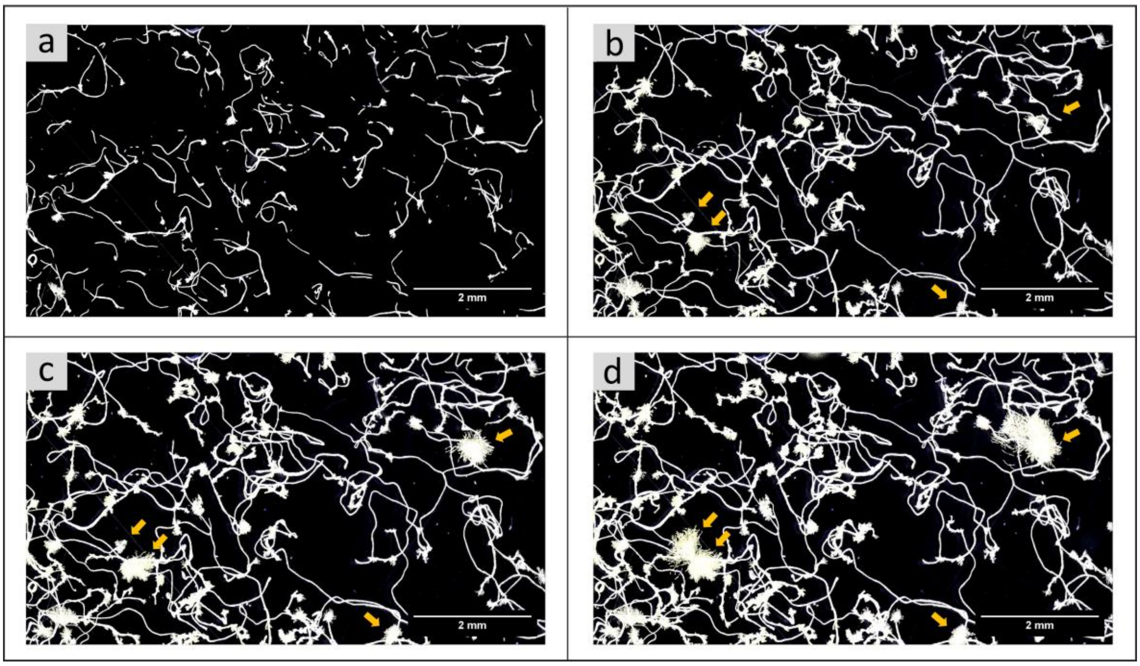

Figure 1.5 Dark field image of a growing colony. An image was taken every 20 seconds. The image series was then superimposed up to a fixed point in time so that the movements of the ends with propulsion are visible as white tufts. Between images a, b, c and d there are 3.75 hours. The yellow arrows point to some moving ends.

The phenomenon is particularly noticeable when the tip comes into contact with an obstacle, such as an existing tube of the colony. Then the interaction of the tip with the obstacle usually causes the tip to straighten up. The tip is probably deflected mechanically. The trajectory taken by the growing end of the culture depends on the geometry of the obstacle. A tube of the colony is typically traversed, i.e. the tube descends behind the obstacle and sticks to it in Section II or Section III. Sometimes it is also deflected horizontally in the plane of the substrate, so that the growth takes a tight bend but remains predominantly horizontal. If a cover slip (thickness 0.17 mm) is placed in the Petri dish, the colony continues to grow on the top of it. The colony can climb up vertical obstacles, such as a vertical glass fiber. Apparently, a random contact of the colony serves to decide in which direction further growth will take place. A targeted reaction to a touch stimulus, as known from tendril plants, cannot be seen. Additionally, growth movements in which the shoot axes of bindweed or climbing plants circulate around a support structure are not observed. Tactile stimuli applied to the tip do not lead to a recognizable reaction. In this sense, the search movement is random and not systematic.

The increase in obstacles as a colony spreads out on a flat substrate leads to an increase in changes of direction due to collisions and thus tight windings and tubes stacking on top of each other. At first, flat structures are colonized, whereby the free movement of the ends also enables angles and gaps to be bridged. Tubes that have already been formed subsequently serve as support points, resulting in mesh-like structures as shown in Figure 1.2e

Two aspects are important for understanding the active movement of the tip of a tube that is currently being extended. According to the described continuous generation of a new tube in Section I, a stretching of the material between the contact points of the diatoms takes place. Therefore, the tube cannot adhere statically to the substrate in this section. The observed movement is due to the fact that the diatoms are not completely static in the tip. In addition to the spontaneous reversal behavior, small displacements of the diatoms towards each other occur continuously, possibly due to fluctuations in the forces. Apparently, the cooperating diatoms, with their force directed towards the tip, are necessary to enable this macroscopic movement.

1.3.4 Branching

1.3.4.1 Formation of branches

In *F. vulgaris*, the trigger for branching is observed to be diatoms that are not oriented parallel to the tube due to the pressure of other diatoms. Narrow bends are often places where this happens, but branches in less curved tubes also occur. A bulge is created by the pressure of the following diatoms. Such a small structure represents a new end that leads to elongation according to the described cooperative behavior. These observations largely coincide with the description given by Houpt [1.17].

Figure 1.6a shows a section of a tube in which a branch (white arrow) has already formed at a point with a slight curvature. The newly formed branch is in a growth phase. Figure 1.6b shows (yellow arrow) a cross-lying diatom that does not align itself parallel to the tube again but becomes the root of a new branch. The resulting branch grows and is not lying flat on the substrate at the time of the image (Figure 1.6c) but protrudes into the water body at a small angle. The tip is therefore out of focus. A more advanced stage is shown in Figure 1.6d at a lower magnification. Both side branches are in a growth phase. It is astonishing that in this case, the advance develops very quickly with the division of labor described above. In the older part of the tube, from which the two branches originate, no diatoms move during this process, as they are all involved in the formation of the branches.

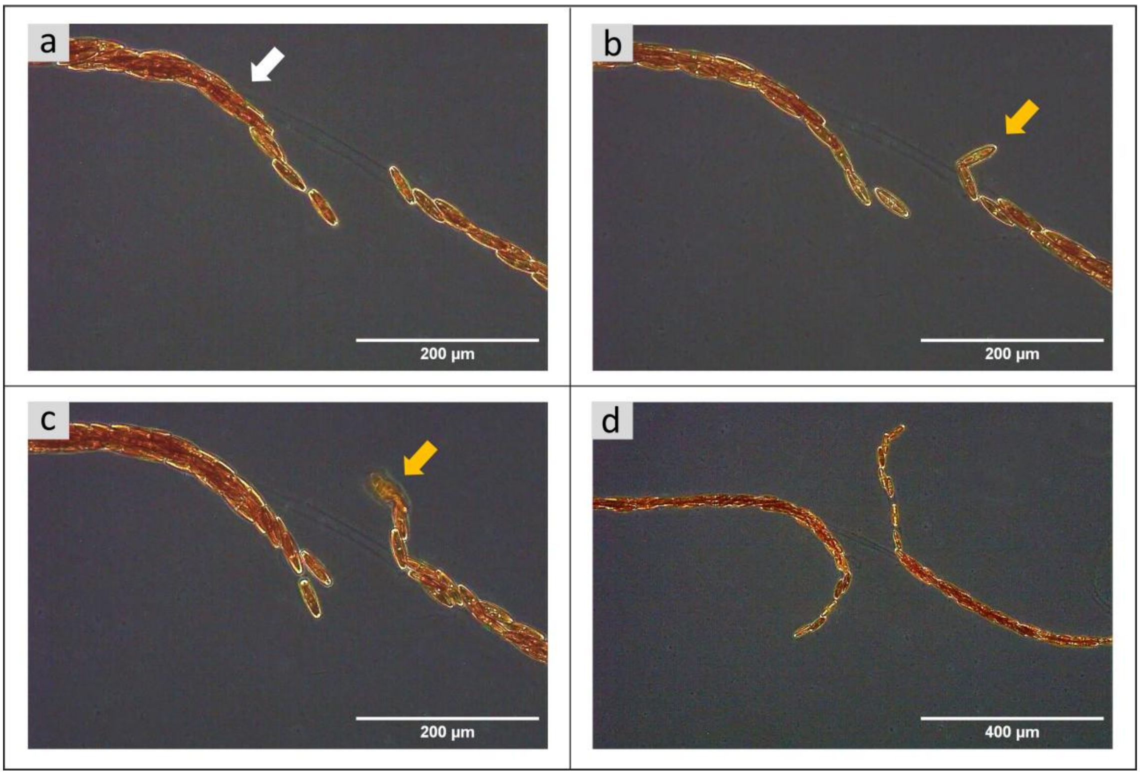

Figure 1.6 Formation of a branch in phase contrast. (a) In the section between the two groups of diatoms, the main tube can be seen, from which the group of diatoms on the left has branched off. The right group of diatoms moves along the tube to the left. (b) Presumably due to strong pressure, one diatom has become transverse. (c) The small

bulge gives the following diatoms the opportunity to follow an alternative route. (d) The result are two branches close to each other.

1.3.4.2 Topology and distances between branches

A *F. vulgaris* colony is a three-dimensional structure of tubes, even when flat. Since the movement of the diatoms is essentially limited to one dimension, it seems reasonable to focus on the topology, i.e. to restrict oneself to the graph consisting of nodes and edges. Starting from an unbranched colony, lateral branches are formed which grow and in which branching can also occur. This simple structure is the rule. An exception to this are highly developed, very densely colonized colonies. In these, loops can be found in which diatoms move in closed paths. It can be assumed that an advancing tip has perforated a tube from the outside due to a lack of alternative paths. This process could not be observed. It could enable cohabitation with other species. In the samples, only *F. vulgaris* of the same size could be found in the tubes, but the sample material was not very extensive. As mentioned above, secondary structures consisting of ordered primary tubes were not found, nor were nested tubes.

The distances between the branches can only be easily determined by light microscopy in small colonies, as the structures quickly become complex due to the superposition of the tubes. In two image analyses carried out on young colonies with several branches, the distances were between 3.4 mm and 15 mm. These values are only intended to illustrate that the distances between branches are large in relation to the apical length of the diatoms. No statements can be made about the distances in more advanced colonies.

1.3.4.3 Flow of movement and sealing of branches

The movement currents at junctions depend on the angle that a branch forms with the tube from which it has branched off. At a junction with a small angle, almost all diatoms move in such a way that they do not undergo any major change in direction, as illustrated with arrows in Figure 1.7a.

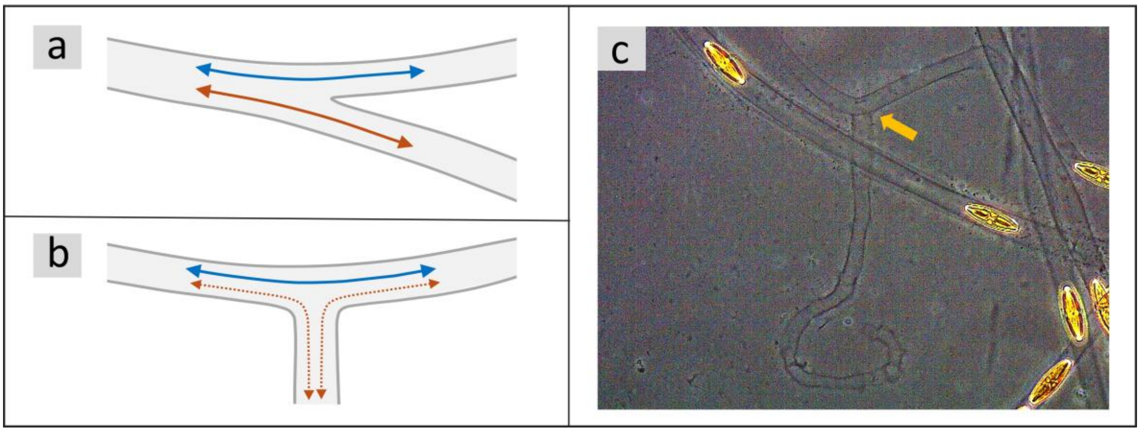

Figure 1.7 (a) The arrows show the main paths used by diatoms at a junction. A trajectory that leads around the acute angle only occurs in rare cases, for example, when diatoms are crowded at the junction as a result of a confluence of diatoms. (b) The dotted curves indicate rarely used paths. (c) The yellow arrow points to the sealing of a branch. The original tube runs from top left to top right. A branch was formed (vertical orientation in the image), which was obviously hardly used, so that the passing diatoms have sealed it.

The greater the change in direction required to enter or exit a side branch, the less the path will be used. At a right angle, most of the diatoms move past the branch (Figure 1.7b). As the diatoms that pass the branch secrete mucilage, unused branches can become sealed (Figure 1.7c).

1.3.4.4 Relationship to population dynamics

An unbranched colony can have a maximum length increase per time of $2v$, where v is the highest speed of extension at one end. This linear growth in length leads to a problem if the increase in diatoms is faster than linear. Branching allows the living space to be enlarged, in particular if the number of branches formed per time increases with the density of colonization of the colony. Let us consider the length growth of a colony with n ends. Since the advance does not always occur at all ends and an average speed $\bar{v}$ must be assumed, the average increase in length L is given by

$$\frac{dL}{dt} = \beta n(t)\bar{v} \tag{1.1}$$

where β is the proportion of time during which expansion occurs at one end. Observing the ends of colonies, it is noticeable that there is often no expansion for hours. It is therefore to be expected that β is significantly smaller than 1. The size β also depends on the number $N(t)$ of diatoms in the colony, because optimal expansion requires that sufficient diatoms are present to drive expansion in the colony. To do this, $N(t)$ must grow sufficiently fast with $n(t)$. With a constant number of diatoms, as is the case in the stationary phase of logistic growth, the maximum increase in length is limited by the number of possible ends at which expansion can occur simultaneously. Even if further side branches are created, the total length only grows linearly with time, because in the optimal case the diatoms can only extend the tubes at a maximum number of ends. In the following, it is assumed that there are enough diatoms for an optimal expansion, so that β takes on a maximum. If we consider a simple model in which the probability of branching increases proportionally to the number $N(t)$ of diatoms in the colony, i.e.,

$$\frac{dn}{dt} = \gamma N(t) \tag{1.2}$$

then it follows from equations (1.1) and (1.2) for the total length

$$\frac{d^2L}{dt^2} = \gamma\beta\bar{v}N(t)\ . \tag{1.3}$$

If we consider the period of exponential growth, i.e. $N(t) = N_0e^{\lambda t}$, the integration equation (1.3) shows that the total length also increases exponentially for times that are large compared to the generation time, and that the increase in living space corresponds to the increase in the population. The spatial density, i.e. the number of diatoms per length, tends under this assumption towards

$$\frac{N}{L} = \frac{\lambda^2}{\gamma\beta\bar{v}}\ . \tag{1.4}$$

High values of λ are achieved at optimal light and nutrient supply and optimal temperatures [1.8]. At high spatial density, many diatoms lie next to each other and the required diameter of the tubes increases. In cultures of *F. vulgaris*, it can be observed that tubes can burst or rupture and release diatoms. Occasionally one can see open ends with diatoms protruding from them. Whether such growth also occurs under natural conditions is unclear. A high density strongly reduces the mobility, which in turn prevents the colonies from expanding. In the given modeling, this reduces the parameter β.

Exponential growth is limited in time in nature as well as in the batch cultures used. In a logistic model (see e.g., [1.32]), the population reaches a steady state. Although the mechanism of branching can reduce the disadvantages of spatial dispersal in the case of life in a 1-dimensional structure, it does not create any benefits in comparison to freely moving diatoms. A dense coating of diatoms on the substrate worsens the growth conditions for individual diatoms as well as for colonies in tubes.

In an analogous calculation, the length per branch *L*/*n* can be determined in the case of exponential growth. For sufficiently long times, in which the linear and constant terms can be neglected, the following holds:

$$\frac{L}{n} = \frac{\beta \bar{v}}{\lambda} \tag{1.5}$$

With a generation time $\ln(2)/\lambda$ of 24 hours and an average speed of expansion at one end of 1.5 µm/s, values of β in the range of 0.02 to 0.1 are required to get to the order of magnitude of the observed distances. This consideration is merely intended to demonstrate that branching is indeed a significant adaptation, particularly in a limited period of exponential growth.

1.3.5 Mechanical properties of the tubes

1.3.5.1 Elongation at break

To understand the processes involved in the formation of the tubes, it is helpful to know their mechanical and, in particular, elastic properties. The elongation at break is a measure of the elongation of a material at which a break occurs, whereby elongation is understood as the relative change in length in relation to the initial length. It was roughly determined by stretching a part of a tube under observation with a stereomicroscope until it broke. The ratio of the increase in length until breakage to the initial length typically took on values of 40%. There is a relevant natural range of variation. The tube exhibited elastic properties. When the tension was removed again, the tube returned to its original length according to visual observation.

The elongation at break sets a limit to the elongation that must not be exceeded during extension to prevent the tube from breaking. The number of diatoms in Section I is therefore limited. However, even before a break occurs, the problem arises that the diatom at the tip is pressed through the EPS membrane by the pushing diatoms and cannot return into the tube. The EPS layer at the tip immediately closes again so tightly that advancement is still possible afterwards. SEM images of *N. pseudocomoides* show that the tubes consist of a network of thin mucilaginous threads [1.16] (see also [1.17]).

This is also true for *Navicula hamulifera* [1.27]. If *F. vulgaris* should have such a microstructure, this could explain these observations.

1.3.5.2 Stress-strain diagram

A stress-strain diagram describes how a sample stretches when a tensile stress is applied. For an introduction to stress-strain curves, see for example Roylance [1.29]. The tensile stress is defined as the acting force per cross-sectional area and the strain as the relative change in length compared to the tube that is not under tension.

To measure stress and strain, a section of the tube adhering to the substrate was carefully detached using a fine needle. It continued to adhere next to the detached section. A vertical glass fiber was placed in the middle of this detached section in such a way that a force is applied between the glass fiber and the tube when the sample is moved. The glass fiber is attached to a micromanipulator at the upper end. Figure 1.8a illustrates the experimental set-up.

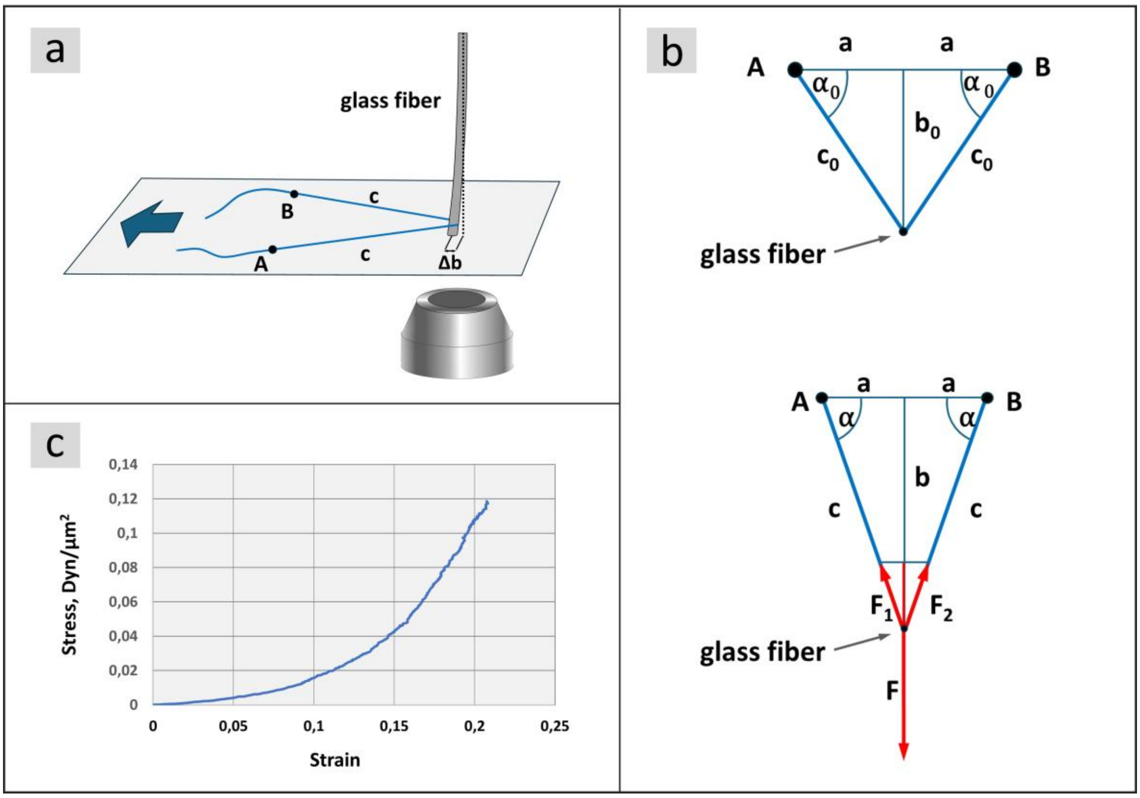

Figure 1.8 (a) Device for measuring the stress-strain relationship. In the illustration, a force is exerted on the fiber so that it is deflected by *Δb* from its rest position. The tube is detached from the substrate up to points A and B. (b) The setup at the beginning of the measurement is shown in the upper half. No force is applied to the fiber and the tube is not stretched. In the lower half, a force is acting on the glass fiber and correspondingly on the tube. (c) The stress-strain curve shown is obtained by measuring *Δb* as a function of Petri dish displacement.

With increasing displacement of the sample in the direction of the blue arrow, the counterforce of the glass fiber increases. The amount F of this force $\vec{F}$ is given in linear approximation by Hooke's law [1.22]:

$$F = \frac{3\pi E d^4}{64\, l^3}\, \Delta b = k\Delta b \tag{1.6}$$

Here, Δb is the deflection, E is the modulus of elasticity, d is the diameter of the fiber and l is its length. The fraction denotes the spring constant k. The values used in the measurement were $E = 64.7$ Gpa (kN/mm2), $d = 121$ µm, $l = 47$ mm and therefore a spring constant of approximately $1.97 \cdot 10^{-8}$ N/µm

Figure 1.8b shows the geometry and the forces when looking vertically at the substrate. Points A and B form an isosceles triangle with the glass fiber. At the top, the triangle can be seen in the non-deflected rest position $\vec{F} = 0$. The corresponding lengths are $b = b_0$ and $c = c_0$. The base angle is $\alpha = \alpha_0$. Displacement of the substrate results in elongation of the tube and the formation of the counterforce F, which is proportional to $\Delta b = b - b_0$. The force $\vec{F}$ generates the tangential tension forces $\overrightarrow{F_1}$ and $\overrightarrow{F_2}$ in the tubes, with $\vec{F} = \vec{F}_1 + \vec{F}_2$. The amount of these forces $F_t = |\vec{F}_1| = |\vec{F}_2|$ can be seen from the drawing (similar triangles):

$$F_t = \frac{F/2}{\sin\alpha} = \frac{Fc}{2b} \tag{1.7}$$

With equation (1.6) we get

$$F_t = \frac{1}{2} ck \frac{\Delta b}{b} = \frac{1}{2} k\sqrt{a^2 + b^2}\,\frac{\Delta b}{b} \tag{1.8}$$

The quantities a, Δb and b can be measured with a microscope. Dividing F_t by the cross-sectional area of the tube yields the stress, whereby in the diagram the cross-sectional area at rest is always used. Figure 1.8c shows a stress-strain curve. Here $a = 952$ µm, $b_0 = 1913$ µm. The cross-sectional area could only be determined very imprecisely from the outer and inner diameters of the tube in the rest position at 44 µm^2, whereby the outer diameter was approx. 15 µm. The curve could only be plotted up to a strain of just over 0.2, because the tube then detached from the substrate at points A and B. It can be seen from the measurement of the elongation at break that only about half of the strain up to the break of the tube has been recorded.

In agreement with the observation for elongation at break, the tube was elastic in the investigated range of elongation, as it returned to its original length when the tension was released. There was no hysteresis. No statements can be made about the subsequent range up to the break. As the tube was at least a few days old, it cannot be ruled out that a newly formed tube would behave differently.

Regarding the elongation process described above, the non-linear increase in tensile stress is of importance. If there are m diatoms in the Section I and each exerts the same force F_0 on the diatom in front of it in the direction of movement, then the force mF_0 will act at the tip. According to the stress-strain curve, the additional strain decreases with each further diatom. The strain as a function of the stress grows more slowly than m, so that the efficiency of additional diatoms in Section I becomes increasingly lower. However, it should be noted that the thickness of the EPS membrane decreases towards the tip of the tube, so that the absolute change in length does not necessarily grow slower than m. An upper limit for the number of diatoms is reached at the elongation at break, which has never been observed. The mentioned escape of individual diatoms at the tip does not indicate a break of the tube, as no open end is generated.

With a small number of pushing diatoms, the force exerted is low. Due to the low slope of the curve at low force, it is still possible to achieve sufficient advancement.

Since the number of diatoms in Section I fluctuates, one advantage of the non-linear increase could be a stabilizing effect, also with regard to the evenness of the thickness of the wall.

1.3.6 Hypothesis on self-organization, cooperation and division of labor

The cooperation of the diatoms in Section I is that they collectively exert pressure towards the end, thereby stretching the tube, transporting it further and applying EPS. An organizational mechanism must exist that ensures that the diatoms involved exhibit the required behavior, i.e. act accordingly when encountering obstacles and when spontaneously reversing direction. The same applies to Section II, where the diatoms must remain in the required region and thus exhibit an asymmetrical behavior regarding the spontaneous change in the direction of movement.

This organizational structure and division of labor also require a formation process and a process for terminating the activity, because an expansion of the colony at one end is limited in time. It usually builds up slowly, typically persists for a few hours and then disappears. During the formation process, diatoms stay increasingly in the region of one end, gradually become denser, with strong fluctuations in density, and finally begin to extend the tube. This process can take several hours. The end of the growth is indicated by the migration of diatoms into Section III, until finally no more noticeable activity can be seen. Since there is no central control, mechanisms of self-organization must underlie this behavior. This mechanism cannot be inferred from visual observations. There is one observation that gives an indication of chemical signaling and chemotaxis. Diatoms that are pushed through the tube at the tip initially move slightly away from the colony. If they encounter the tube again, they remain in its immediate vicinity and move only slightly. This could be an indication of chemotaxis, whereby the tube must be permeable to the assumed signaling substance.

As an aggregation process with the section structure only is meaningful in the vicinity of an end, it is reasonable to assume that the organization begins when a diatom hits an end. Such a diatom usually reverses without visibly affecting other diatoms. For no discernible reason, it can remain in the vicinity of the tip, whereby the usual spontaneous reversal of direction is not restricted. The hypothesis is that it changes its own behavior and produces a signal substance that causes other diatoms to adapt their own behavior and release this substance in turn. Due to the frequent spontaneous changes in direction, this is not immediately recognizable. It is even possible that the region behind the tip empties completely again for a short time. Nevertheless, aggregation tends to occur at one end of the tube and the tube begins to expand. Since the direction in which the tip is located is an essential information, the gradient of the signal substance should enable directional sensing.

To ensure that the division between Section I and Section II occurs and the number of diatoms in Section I remains limited, the measurement of the concentration of the signaling substance could serve as a criterion. A concentration above a certain threshold value could prevent further aggregation.

This explanatory model fulfills essential requirements but is incomplete because it cannot give any criteria for when an order structure arises and when it disappears again. It is minimalistic in the sense that it is limited to a single signaling substance.

Additional substances could facilitate differentiation of the cells and separation of the sections.

1.3.7 Ecological aspects

The construction of tubular colonies requires additional energy resources and forces them to restrict themselves to a one-dimensional habitat. The disadvantages must be compensated by benefits that outweigh them overall. Drum [1.7] and Lobban [1.25] provide indications of possible advantages, such as protection against strong changes in salinity or diseases. Only a few aspects that became apparent from the observations will be addressed here.

In samples enriched with nutrient solution, amoebae of different sizes were found which could not be determined and which enclosed diatoms in their foraging. In the case of diatoms, which were small compared to the amoeba, successful grazing can be assumed despite the protection provided by the frustules (see Schaeffer [1.30]). The number of amoebae increased significantly within a few days. *F. vulgaris* colonies were surrounded by amoebae, but only partially due to size and adhesion to the bottom. In all observed cases, phagocytosis was aborted, suggesting effective protection against amoebae. The formation of a larger system of tubes and the adhesion of planar colonies may also provide advantages over individual diatoms in other grazers.

Another aspect could be access to light and nutrients in colony forms that rise above the substrate. Tufted growth as well as the observed free-hanging tubes or nets provide this advantage. On the other hand, this structure weakens the protection against grazing, as there is no adhesion to a substrate.

Since *F. vulgaris* also thrives aerially on wet stones, it would be advantageous to provide protection in the event of short-term drought. Lobban (1989) mentions protection against desiccation as a possible benefit of the tubes. However, experiments in which the water was tipped out of a colonized Petri dish and drying was observed under the microscope showed that the tubes of *F. vulgaris* immediately collapse and the diatoms die as soon as drying begins. *F. vulgaris* does not resist desiccation for a relevant duration.

A more or less thick layer of bacteria is often observed on the outer surface of colonies of tube-forming diatoms. Houpt [1.17] found no bacteria in the tubes of young, still actively growing colonies, but did find bacteria in the tubes of older colonies. In the observations of young *F. vulgaris* colonies, no bacteria were found inside. The occasional escape of diatoms during colony expansion does not appear to lead to the entry of bacteria. The described formation process begins with the mechanical separation of a diatom from the self-created EPS cover. The initial short tube, which later develops into a structure with branches, has no macroscopic openings until a possible breakage at high population density. Such an *F. vulgaris* colony could represent a natural axenic clonal population. As the tubes are subject to ageing processes, it is to be expected that bacteria can migrate into old colonies. The observed bursting of tubes due to strong cell growth is also likely to result in colonization of the inside of the tubes with bacteria. This is probably atypical for colonies in natural environments.

1.4 Discussion

The observations presented for *F. vulgaris* demonstrate a complex behavior for eukaryotic unicellular organisms and a comparatively highly developed organization. Long-term observations are expected to reveal further details. However, other methods are needed to investigate the underlying mechanisms. In particular, successful detection of the hypothetical signaling substance would be crucial. It would be the key to studying chemotaxis, gradient sensing and self-organization.

More formal modelling and simulation could be used to test whether the assumed mechanisms in terms of self-organization, asymmetry, response to barriers and collisions reproduce the observed behavior. Similar to the simulation of pedestrian behavior, a cellular automaton could be the basis (see [1.3] for an exemplary application to pedestrians). Agent-based modelling (an example of pedestrian modelling can be found in [1.23]) might provide a more accurate reproduction of the observations.

Even though the shape of *F. vulgaris* colonies can vary according to environmental conditions, the processes of colony expansion can be assumed to be the same. This raises the question of what processes occur in other tube-forming diatoms. Experience with *F. vulgaris* suggests that cultivation in suitable culture vessels that allow long-term observation is recommended for this purpose.

Although the mechanisms of coordination, division of labor and self-organization described above suggest a high degree of organization, the term ‘non-neural intelligence’ has not been used. The reason for this is the lack of evidence for a specific response to environmental stimuli or even the ability to learn. A discussion of this term in the context of the coordinated movement of *Bacillaria paradoxa* can be found in [1.1]. The term 'swarm behavior' has also been avoided, as cooperation is clearly evident, but not reaction to the behavior of other diatoms, in particular neighboring diatoms. The behavior in collisions with aggregation and separation of groups can only be interpreted to a very limited extent as swarm behavior.

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