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Micrasterias – Little Stars

Part 2: Dictyosomes

The genus *Micrasterias* is very interesting for phycologists. That can be concluded from the fact that the section "Phycology" of the German Botany society proclaimed *Micrasterias* as "Alga of the Year 2008". To us as hobby biologists with the restriction on the light microscope, the genus *Micrasterias* offers interesting views into the cell. The first part of this report discussed, apart from the general taxonomy and morphology, the observations of mitochondria. The focus of the second part is particularly on one type of organelle, which can be observed extremely rarely in simple ways with the light microscope: the dictyosomes.

During photomicrography of *Micrasterias rotata* using high aperture objectives (Planapo 40/1.0 Oil, Planapo 63/1.4 Oil), I recognized a larger number of round particles just beneath

the cytoplasm layer situated under the central part of the cell wall in the vicinity of the nucleus. With DIC optics I was able to differentiate an internal structure in these parts, starting from an aperture of appr. 0.8. (Fig. 1). They showed concentric inner bodies with different refractive indices. Approximately 25 to 40 of such bodies per cell were visible with sizes between 2.5 µm and 5 µm.

Could these be spherosomes, e.g. oil droplets? It is far common with desmids to store oil as storage substances. However, their internal structure (interleaved spheres) did not fit this interpretation (Fig. 2a–c). Soon after, more particles were found in the cytoplasm that matched the appearance of oil droplets much better (Fig. 2a, lower part of the picture). It is also important to note that the oil droplets could be displaced as far as desired by the flow of cytoplasm, while the large round objects remained territorial and oscillated only slightly around a virtually central point. Which cell structure could fit these features? After talking to friends

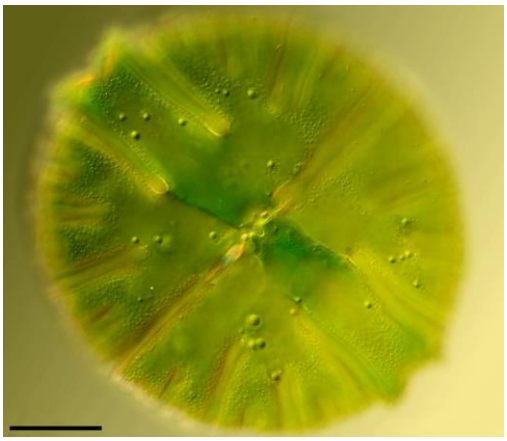


Fig. 1: Overview of *M. rotata*. The circular particles are dictyosomes. As the picture shows, the occurrence of dictyosomes is not limited to the vicinity of the nucleus. Scale bar indicates 50 µm.

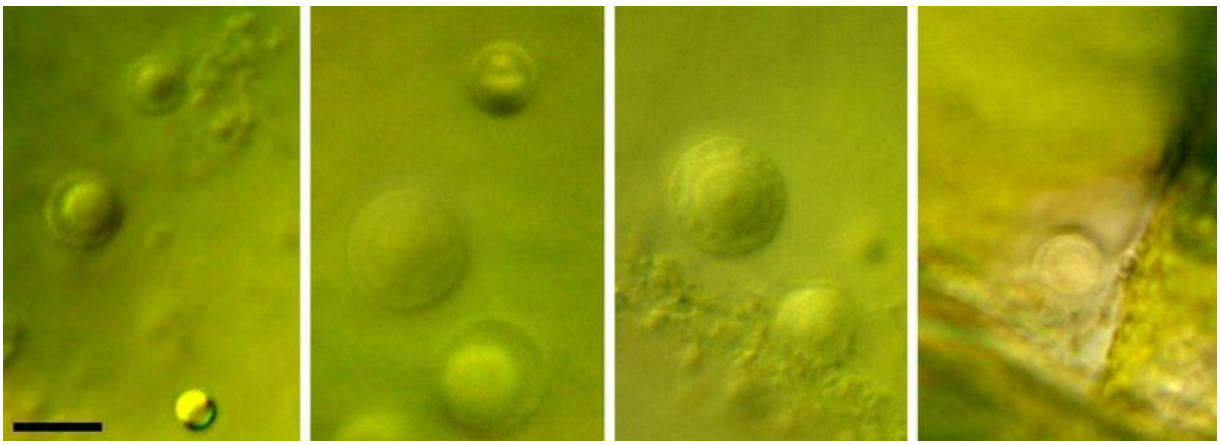


Fig. 2: Mucus producing dictyosomes of *M. rotata*. a: Frame also showing a oil droplet (bottom). b: Upper particle could be state of binary fission. c: Sometimes an internal vesicular structure was visible. d: Bright field shot. Scale bar indicates 5 µm.

about these observations and researching the relevant literature, the assumption was confirmed that the observed particles were parts of the Golgi apparatus, the dictyosomes.
Electron-optical photographs show the fact that dictyosomes are surrounded by small membrane enclosed blisters, the Golgi vesicles (Fig. 3). In view of this fact, the smooth-edged objects in Figures 2a and 2b did not fit into the expected picture. But: Due to the limited resolution of the light microscope, the tiny Golgi vesicles are indistinguishable from the central body of the dictyosome. Sometimes, when these structures were close enough to the coverslip, I could even observe a hint of vesicle shapes (Fig. 2c). Even after switching to bright field, some dictyosomes remained visible if they were in favorable position (Fig. 2d). A single dictyosome showed a constriction in the central range (Fig. 2b). In the literature (Menge and Kiermayer, 1977; Noguchi, 1978) there were electron micrographs which showed that this picture could correspond to a phase of dictyosome's binary fission.

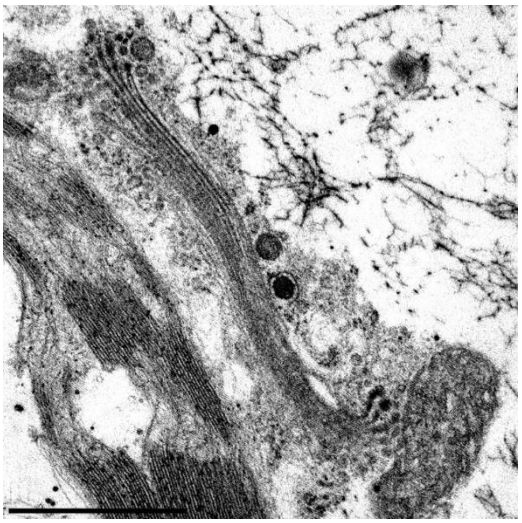


Abb. 3: Cross section of a dictyosome of *Micrasterias denticulata*. The image also shows a cross section through a mitochondrion (right down) as well as through a chloroplast (diagonally from top left to center to bottom). Preparation and micrograph by Dr. Detlef Kramer, TU Darmstadt, Germany. Scale bar indicates 1 µm.

The Golgi apparatus

The Golgi apparatus is an important cell organelle for synthesis. It produces and modifies proteins and polysaccharides in cooperation with the endoplasmic reticulum (ER) for many different purposes (e.g. enzymes, membrane proteins, basic substances for cell wall construction). After a substance is synthesized, Golgi vesicles are pinched off from the dictyosome. These are then moved to those places in the cell where they are needed by the cell's transport system, microtubules and motor proteins (Kleinig and Mayer, 1999; Plattner and Hentschel,

2006; Meindl et al., 1992). Dictyosomes also produce siliceous scales, for example those of the golden algae, the Euglyphida (Testacea) or the Heliozoa.

Dictyosomes are usually too small and delicate to be identified by an ordinary light microscope. In the *Micrasterias* species with large cells (diameter 200 µm and larger) the situation is different. Such large and high-contrast dictyosomes were already described in the 1960s. However, the micrographs in the articles cited were produced electron-optically. Without these confirmations, a reliable interpretation of the phenomena observed under light microscopy would be difficult. The same applies to mitochondria. The reliable confirmation for the interpretation of tiny cell structures is often provided by electron microscopy images alone (Drawert and Mix, 1961; Kiermayer, 1965, 1967, 1970; Staehelin and Kiermayer, 1970). However, differential interference contrast and phase contrast (in the case of thin specimens with little structure) allow observation under in-vivo conditions and, in the case of *M*. *rotata*, also a reliable interpretation of the organelles mentioned above.

Dictyosomes as producers of mucus

Desmids possess a special type of dictyosomes, which produce polysaccharide mucus for locomotion. Their diameter of about 5 µm is disproportionately large compared to other groups of algae and ordinary plant cells. Using this mucus, the desmid can move autonomously to match the direction of the light and even move towards the light source. When it gets dark, *Micrasterias* cells line up vertically (Fig. 4b). As a result, they have the largest possible "sensor surface" on the expected direction of incidence of the light at the beginning of the next day. At the beginning of twilight, they initially align themselves perpendicularly to the incidence of light. A movie of IWF (C1496, Wenderoth, 1985) shows this in an impressive way. When one sees the voluminous expulsion of mucus for locomotion purposes in this film, one involuntarily wonders where the cells get this wealth of material from. The process can only be understood if one assumes that the mucus, which is still compact in the Golgi vesicles, has a high swelling capacity. For example, it was measured on the root tips of corn that the mucus escaping there was 1,000 times more voluminous than in the mucus vesicles that supplied it (Steer, 1985).

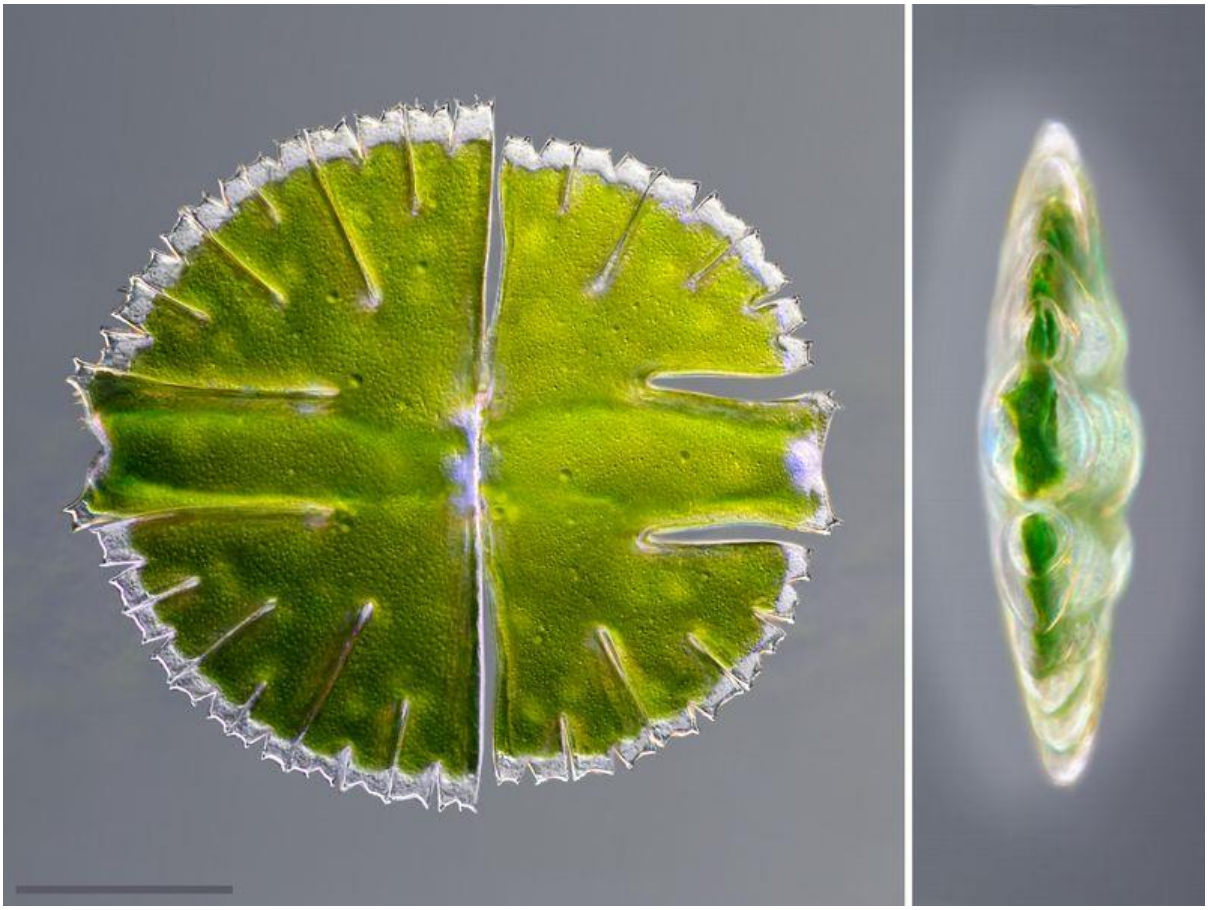


Fig. 4: *Micrasterias rotata*. a: Synoptic picture of shape, surface texture and mucus producing dictyosomes. This picture is manually stacked using 40 frames.
b: *M. rotata*, taken up with an inverse microscope, standing more or less perpendicularly on the coverslip. Manually stacked using 27 frames. Scale bar indicates 50 µm.

Vesicle detachment in *Micrasterias rotata*

Further observations on the focus level of the dictyosomes showed sometimes the separation of huge vesicles on their equatorial plane (Fig. 5). Here is a brief description of the complete observation: First the edge of the outline was bulging (look at Fig. 5, first frame). Then the bulge was expanded into a bubble and further reshaped into a dumbbell-shaped structure with unequal spherical diameters. Gradually the connecting strand was stretched out and thinned out. After separation the vesicle disappeared in the current of the cytoplasm. Such processes each lasted about five minutes. Noguchi (1978) examined mucus producing dictyosomes of *Micrasterias americana* by transmission electron microscope (TEM), she called them curved dictyosomes. They have an elliptical cross section; the membrane stacks are bent like a trough and giant vesicles with diameters of 1.2–1.8 µm are formed.

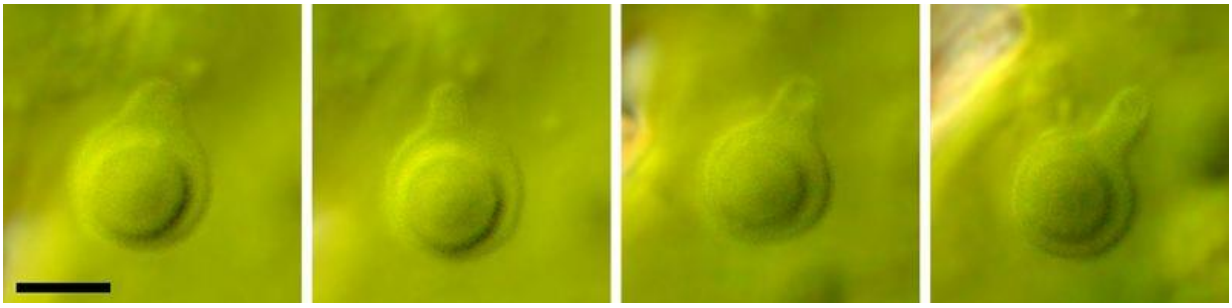


Fig. 5: *M. rotata*. Debonding of a huge vesicle from a dictyosome. Scale bar indicates 5 µm.

The comparison to *Micrasterias apiculata*

M. apiculata bears two kinds of particles with similar size and refractivity as the Golgi stacks seen in *M. rotata*. Figure 6a shows the first type, situated mostly at the periphery of the cells. They have a high optical density and a good visibility in bright field observation. They don't exhibit the manner of oscillation which features dictyosomes. However, their brightness is lower than that of most spherosomes (oil droplets). It would be necessary to use TEM to determine. But: In the vicinity of the nucleus there were structures known from *M. rotata*, and these should be dictyosomes, too. The Figures 6b and 6c illustrate the two types of particles situated in the same area. Arrow heads point to dictyosomes whereas arrows indicate the other type of spherosomes.

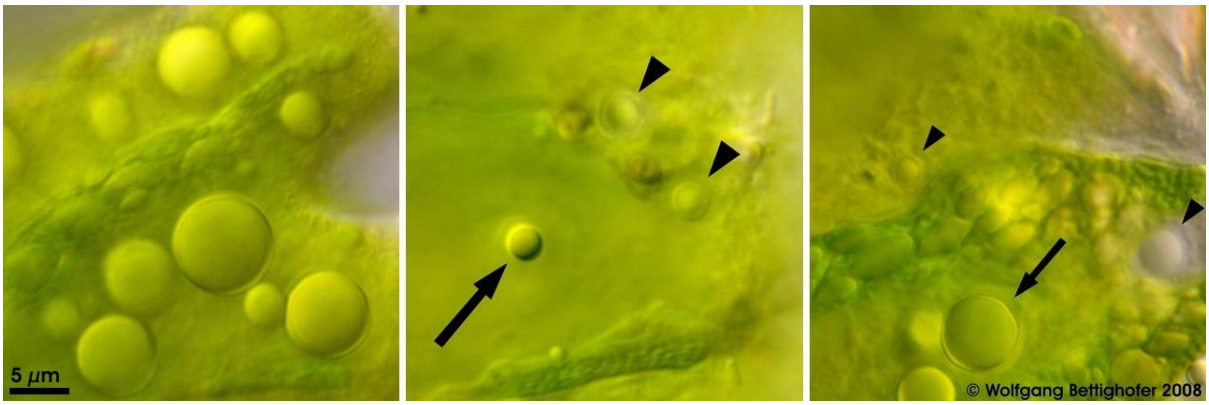


Fig. 6: *Micrasterias apiculata*. a: Particles of uncertain classification. b and c: Dictyosomes (arrowhead) and parts of the other type of spherosomes (arrow) close together in the vicinity of the nucleus. Scale bar indicates 5 µm.

The ordinary type of dictyosomes

With *Micrasterias denticulata* und *M. thomasiana* var. *notata* (Figs. 7 and 8) as objects, I was able to find another type of round body with a diameter of 2.5 to 5 µm. They oscillated slightly, too. Sometimes they were shifted a short distance. Every now and then they tilted and could

be seen in side view. The surface and margin appeared as if covered with small nodules or warts (Figs. 9 and 10).

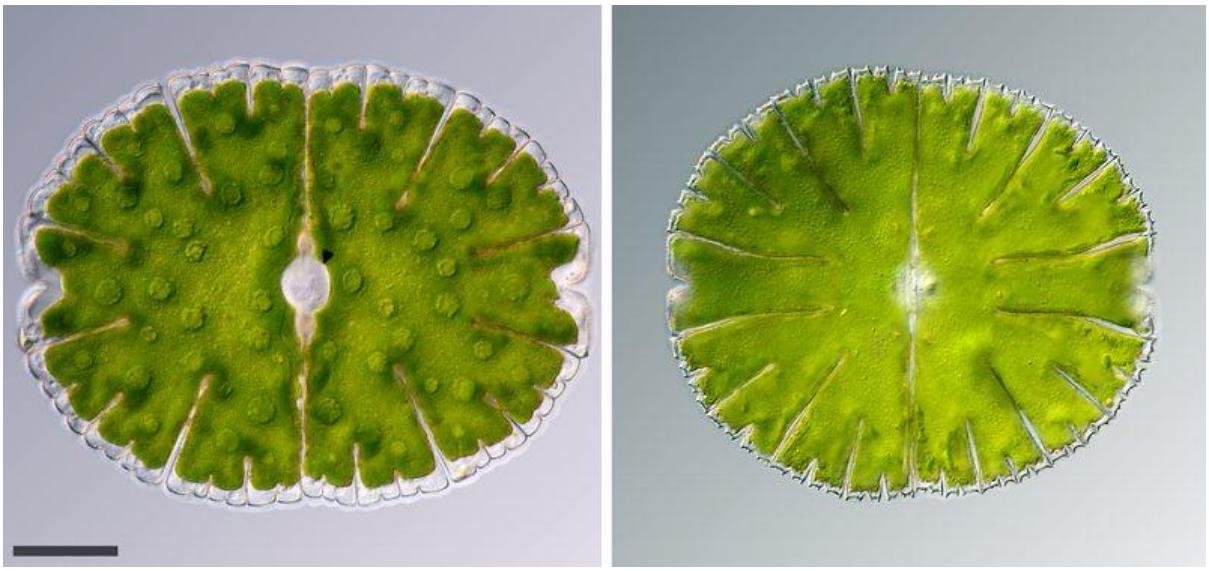
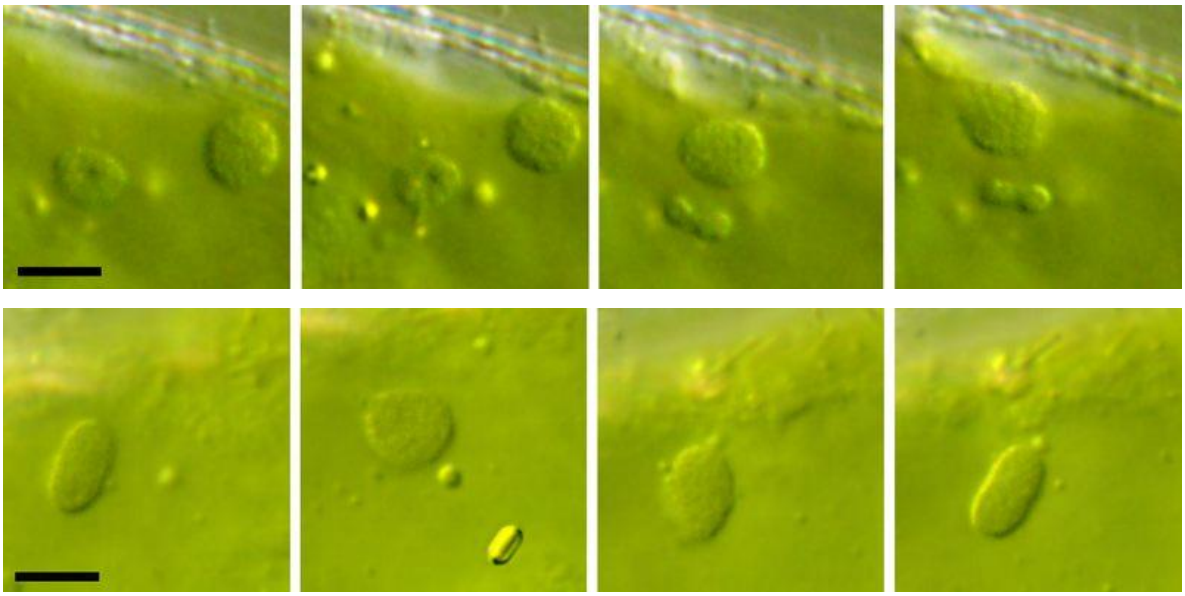


Fig. 7: *Micrasterias denticulata*: Synoptic image of shape, chloroplasts with pyrenoids plus central nucleus (arrow head). 20 frames manually stacked. – Fig. 8: *Micrasterias thomasiana* var. *notata*, showing shape and surface structure. 35 frames manually stacked. Scale bar indicates 50 µm.

I had the opportunity to discuss these observations with Professor Dr. Werner Herth, cell biologist at the University of Heidelberg. He explained that the images in Figs. 9 and 10 most likely show the ordinary dictyosomes (those that do not produce mucus for motility) of the desmids studied.



Figs. 9 and 10: Continuous shootings of ordinary dictyosomes of *M. denticulata* (9) and *M. thomasiana* var. *notata* (10). The typical tilting movements, which are often mentioned in the literature, are recognizable. Scale bar indicates 5 µm.

Summary and acknowledgements

The *Micrasterias* species with a cell diameter of about 200 µm gave me interesting insights into the interior of cells. Never before had I been able to observe mitochondria with such clarity. A very special experience was the discovery of the different types of dictyosomes, whose visibility under the light microscope was completely unknown to me. For the observations the following devices were used: Zeiss Universal with DIC optics, plan apochromats 40/1,0 oil and 63/1,4 oil, photo eyepiece S-KPL 10x, digital compact camera Olympus C7070.
On the way from the first step of the discoveries up to the finished report described above, there were many prolific discussions and a number of shared observations with Dr. Detlef Kramer, cytologist at the University of Darmstadt, who accounted valuable contributions to this report. To him I am primarily grateful. He discussed our observations with the cytologists Prof. Dr. Eberhard Schnepf and Prof. Dr. Werner Herth (University of Heidelberg). I am also grateful for their critical evaluations and encouragement. Furthermore, many thanks go to Prof. Rupert Lenzenweger (Ried im Innkreis, Austria) for sending desmids samples, the stimulating discussions on the taxonomy and ecology of desmids and generous support with specialist literature, as well as to Dr. Jens Hallfeldt for the samples of *Micrasterias thomasiana* var. *notata*.

Last but not least a statement of Dr. Detlef Kramer:
"The large *Micrasterias* species show a number of small and tiny cell components in the light microscope with unusual clarity, which can usually only be shown clearly in the electron microscope. It is fascinating to perform these observations on living organisms and to experience the characteristic dynamics, which is not possible with the electron microscope due to the preparation. And the ambitious amateur can do this with equipment that, with a little effort, is now available on the used market at a fraction of the new price. The differential interference contrast according to Normarski offers possibilities that are being forgotten more and more in the life sciences. In this way, bridges can be built from purely static cytology, which is operated with electron microscopy, to a fascinating, living observation of the inner life of the cell. I'm thrilled to have stumbled upon this topic after 37 years of work on electron microscopy!"

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