exceedingly poor illustration of this diatom. To justify this criticism, I have intentionally made a photomicrograph of *Petrodictyon gemma* (Figure 1) with techniques that might be called "retro" instead of "advanced," as follows: perfectly central ordinary bright-field illumination; white light, completely unfiltered to avoid masking the residual optical shortcomings of the optics used; "dry" condenser, effective NA circa 0.9; and the objective was a Reichert achromatic oil immersion 100/1.3 manufactured around 1912.

The valve of this diatom is always strongly vaulted, digital stacking would be required to obtain a sharply focused image over the entire valve, but the differences in focus nicely illustrate the "white dot" versus "black dot" settings. Resolution of the *striae* into *puncta* is excellent—the results of the "advanced" techniques used for the Piper and Chmela paper were obviously inferior to those that can be obtained with an objective of a century ago.

References

[1] JT Quekett, *A practical treatise on the use of the microscope*, Hippolyte Bailliere, London, 1848.

[2] W Smith, *Annals and Magazine of Natural History* 9(2) (1852) 1–12.

[3] A Schmidt et al., *Atlas der Diatomaceenkunde*, Aschersleben-Leipzig, 1874–1959.

[4] J Tempère, J Brun, P Bergon, PT Cleve, E Dutertre, E Grove, P Miquel, and H Peragallo, *Le Diatomiste: journal spécial s'occupant exclusivement des Diatomées et de tout ce qui s'y rattache*, vol. 1 and 2, M. J. Tempère, Paris, 1890–1895.

[5] E Frison, *L'évolution de la partie optique du microscope au cours du dix-neuvième siècle*, Rijksmuseum voor de geschiedenis der Natuurwetenschappen, Leiden, the Netherlands, 1954.

MT

Authors' Response

Jörg Piper* and Gunther Chmela

* JPu.MP@t-online.de

Unfortunately, the letter from Mr. Sterrenburg contains some passages that seem to be rather polemical and not very objective. Nevertheless, we hereby give our comments to his letter.

When reading our article, it is clear for everyone to see that the standard optical equipment and illumination modes we used (glass lenses, condensers, oculars, digital cameras, bright field, oblique light) were not presented as "advanced," but rather the various monochromatic astronomy filters described (H-beta, O-III, Solar continuum) were considered advanced. Moreover, it should be noted that mirror objectives (reflecting objectives) are special lenses that can lead to "advanced" optical results in some fields because of their particular optical properties (some catchwords: luminance contrast, achromatism, great working distances, enhanced depth of field).

In our view, a method, tool, or technique can be regarded as "advanced" if it leads to improved or "better" results when compared with conventional means. It also can be called "advanced" if it can improve an already existing method. In our article, the following findings or techniques were mentioned as being "advanced":

1. Monochromatic astronomy filters are well-suited for improvements of many observations and photomicrographs, especially when very fine and low-contrasted details have to be visualized. The optical design of such extremely narrow band filters is "advanced" (see further explanations below).
2. Green light sources of 546 nm or 540 nm lead to only modest enhancements of resolution and contrast, although they are most commonly used. Narrow-band filters of 500 nm or 480 nm should be preferred for observations in visible light because they lead to greater improvements in image quality (resolution, sharpness, contrast). For most tasks, the blue-green, 500-nm filter will lead to the most balanced results (optimized contrast and resolution).
3. Astronomy narrow-band filters used for our technical evaluations cannot be compared with or replaced by "modern" green LEDs because such LEDs do not enhance resolution and contrast in a relevant manner even when declared "monochromatic."
4. Enhancements of image quality with these filters are superior when compared with the optical effects achievable with immersion condensers.
5. In many cases, the condenser aperture diaphragm can remain wide open, even for very low-contrasted specimens because of the contrast enhancement achievable by monochromatic light filtering with narrow-band filters. Thus, the respective specimens appear in adequate contrast even though the aperture diaphragm is wide open so that any reductions in lateral resolution resulting from a reduced condenser aperture are avoided.
6. For particular tasks, mirror lenses can be used for illumination in luminance contrast. This is a new and "advanced" technique awarded the "*Microscopy Today* Innovation Award" in 2010 [1].

Of course, these "advanced" methods can also be used for other tasks that are not related to diatoms. As clearly explained in our paper, diatoms were just selected as instructive examples in order to demonstrate the potential of the light filters and mirror lenses. Moreover, the described improvements of image quality achievable by our filters are relevant for all optical equipment. Mr. Sterrenburg and other users may work with

doi:10.1017/S1551929511000630

archaic, old, newer, or newest lenses; in all cases, the optical outcome will be improved.

The astronomy filters used for our experiments are an advanced and rather new optical development. Designed as modern interference filters, they are optimized with regard to their optical design. Their transmission band is very narrow, the amplitude of the transmitted wavelengths is maximized, and the transmission of aberrant light spectra is minimized. Any reflection of light is minimized by an anti-reflection coating consisting of seven different layers. Because of their particular properties, these filters are advanced and high-tech. "A century and a half" ago such filters were not available. Might it be possible that Mr. Sterrenburg does not know the difference between such high-tech filters and simple colorized glass plates?

Regarding the diatoms we used, the schematic in Figure 2 of our article was used to show morphological considerations. The image taken at the highest magnification demonstrated that perforated and non-perforated zones indeed exist (this was not a product of our imagination). The image in Figure 1 below shows this morphology in a high-resolution scanning electron microscopy image. It is clear to see that the repetitive non-perforated stripes in *Amphipleura pellucida* are broader than the perforated linear patterns. This situation was shown in our drawing. Our article deals with technical methods; it does not deal with the particular aspects of the morphological variance in diatom shells. Thus, it can be regarded as adequate that we showed the situation graphically for one of the specimens.

In logical accordance with the highly resolved real structure shown in Figure 1, the position of visible dark and white stripes was similar in all diatom specimens in our light microscopy observations. We could not see any "modulation" of their appearance associated with the plane of focus. When Mr. Sterrenburg describes a different appearance determined by focusing, it can be expected that this effect was caused by diffraction, that is, by an imaging artifact. Thus, Figure 1 here should verify that our explanations with regard to the morphological structures are not "completely mistaken" and "erroneous" as suggested by Mr. Sterrenburg.

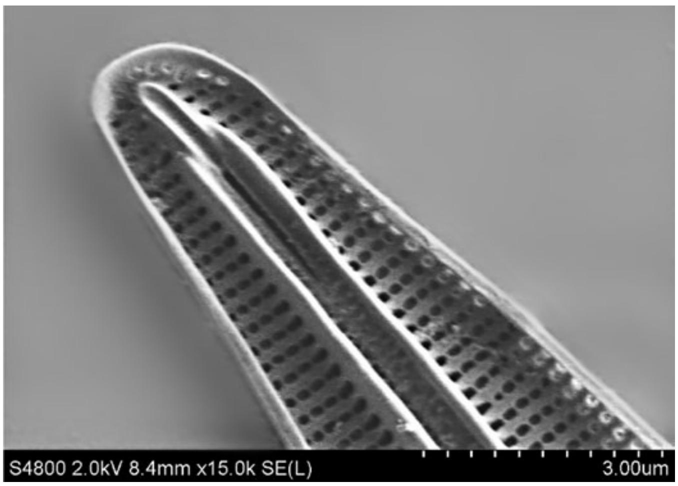

Figure 1: *Amphipleura pellucida* imaged in the scanning electron microscope. Alternating stripes, with and without perforations, can be clearly seen (photograph by Peter Höbel, web source: www.mikroskopie-ph.de).

The preparations used for our evaluations were selected by Mr. Chmela, who is a biologist. He intentionally chose very low-contrasted pieces that were just barely visible in standard white light (so-called "problem-specimens"). In these preparations the existing perforations were indeed invisible for all light microscopy techniques carried out (bright field, dark field, phase contrast, interference contrast, polarized light). We do not think that the optical equipment used by Mr. Sterrenburg is better than our equipment manufactured by Zeiss and Leitz/Leica.

When Mr. Sterrenburg shows us a diatom shell resolved better but taken in unfiltered white light with historic equipment, it is possible that the native contrast in his preparation could be much higher than in those selected for our evaluations. The refractive index of the embedding medium is fundamental for the contrast and clarity of such low-density, unstained specimens. We do not know which kind of embedding medium was used by Mr. Göke for preparing our slides. Unfortunately, Mr. Göke has been deceased for several years so that we cannot ask him for further information. Thus, it might be possible that the preparation used by Mr. Sterrenburg was prepared with a "better" embedding medium so that more details are visible in normal circumstances.

When reading Mr. Sterrenburg's letter, we can learn that he "cannot remember having seen such an exceedingly poor illustration" of Surirella (=Petrodictyon) gemma—"even in 60 years of diatom studies." This sounds rather arrogant—at least in our ears. Because of this statement, we did a short web-based research about Mr. Sterrenburg's activities. We found an article from him published in 2005 [2] containing just a few photomicrographs from diatoms arranged in a table. These images are indistinct, and in our personal opinion, such "poor" material should not be published.

Finally, we learn that taxonomy seems to be one of Mr. Sterrenburg's favorite "hobbies." Several articles of his published deal with this topic. Our article was not a taxonomic article, but solely a technical contribution. Even so we feel the need to provide a last short remark concerning the names of the specimens. We used the names written onto the slides by the preparator, Mr. Göke. To make the points in our article, it was not relevant whether *Surirella gemma* had been renamed *Petrodictyon gemma*, or not. It was also not relevant for us whether this renaming took place "yesterday" or "20 years ago." Moreover, the "old" name "Surirella" is neither "wrong" nor "illegal." The "new" name resulted from a diversification of the parent species "Surirella." Nevertheless, the "old" name "Surirella" is widely used and well known. Thus, for this reason also, the criticism from Mr. Sterrenburg is not relevant for our article and our technical messages.

Acknowledgment

We thank Mr. Peter Höbel, Germany, for his allowance in using his high-resolution diatom images.

References

[1] CE Lyman, *Microscopy Today*, 18 (5) (2010) 32–37.
[2] FAS Sterrenburg, *Proceedings of the California Academy of Sciences*, 50(14) (2005) 156–61.

MT