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Letter to the Editor

Advanced Techniques for Visualization of Diatom Structures?

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In *Microscopy Today*, January 2011, Piper and Chmela published an article called "Advanced Techniques for Observation and Photomicrography of Subcellular Structures in Diatom Shells." This paper clamors for some comments of a fundamental nature.

The techniques presented in the paper are called "advanced," but it is difficult to understand why this should be so. The paper describes observations with the conventional bright-field techniques—including oblique illumination—used since the late nineteenth century. The optics used were a 120/0.9 water immersion and a planapochromatic oil immersion 160/1.4, both somewhat unusual as regards their magnification, but not "advanced" in the sense that they would be optically superior to the 50/1.00 water or oil immersions and 100/1.4 or 63/1.4 (plan)apochromatic oil immersions that have been on the market for decades. As will be shown, any such "advanced" performance of the lenses used is not borne out by the photomicrographs presented.

Where the paper is completely mistaken is in the description, interpretation, and iconographic documentation of the fine structure of the diatom exoskeleton. As regards description and interpretation, consider the following quote:

> "Within the respective frustules, the bright stripes result from small perforations separated in very short distances that cannot be resolved in light microscopy. The neighboring dark stripes correspond to small zones that are not perforated . . . "

The actual situation is as follows:

- In the particular species illustrated, the valve displays fine "lines" (*striae*) consisting of rows of fine "dots" (*puncta*). The statement that these *puncta* cannot be resolved with the light-microscope is false, as will be discussed further on.
- The *puncta* are indeed perforations of the valve (as evidenced by the electron microscope), and thus the statement that they form "the bright stripes" intuitively seems convincing. In reality, it is erroneous: whether the rows of *puncta* appear dark or bright solely depends on how the microscope is focused—it is an optical effect. The standard manner in which diatomists have always illustrated the *puncta* (in both drawings and photomicrographs) is the "black dot focus," which gives the best contrast.

It is difficult to understand how the authors concluded that the "perforations"—the *puncta*—cannot be resolved in the light microscope. For a century and a half, resolving the "dots" has been a popular sport among microscopists, and thousands of diatom test slides have been marketed to allow them to test their equipment and their mastery of the microscope. An early, and famous, description of resolution into *puncta* is that by Quekett [1], who resolved *Pleurosigma angulatum* into *puncta* in 1848 (*sic!*). The reverend W. Smith published good illustrations of resolved species of this difficult genus a few years later [2], and in the later Victorian era a massive collection of perfectly resolved diatom images was already available (for example see references [3, 4]).

The diatom "*Surirella gemma*" discussed and illustrated by Piper and Chmela is a good example of the errors in their paper. Since twenty years, its correct name and authorship have been *Petrodictyon gemma* (Ehrenberg) D.G. Mann 1990. Resolution of its *striae* into *puncta* was obtained in 1870 [5], and for the past century it has been a standard test for an objective with an NA around 1.0.

It is, therefore, difficult to understand how the images in Figure 5 of the paper in question have been obtained with modern—let alone "advanced"—optics. In fact, in sixty years of diatom studies, I cannot remember having seen such an

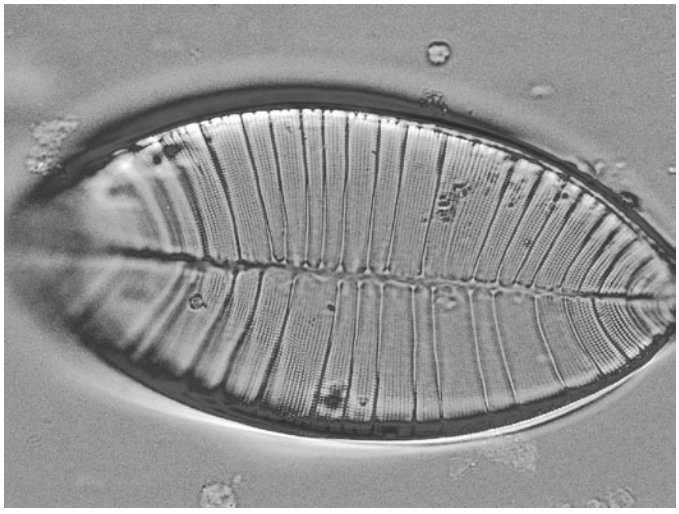

Figure 1: The diatom *Petrodictyon gemma* fully resolved in unfiltered white light with an oil immersion objective manufactured a century ago.

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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

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Authors' Response

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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