

Advanced photomicrography: construction of a 3D Diatom model saved as an image or movie

(by Stefano Barone, Diatom Lab)



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Microscopic photography (or photomicrography) often has to deal with depth of field: for example if you want to capture a diatom - especially at medium or high magnification - you will realize that the single shot will hardly allow you to focus all the details of the frustule as the latter develops on different focal planes. Few know that in the thirties of the last century (and therefore when solely analogue photography) this problem was already addressed and solved by P. Lefébure (*Bulletin de la Societe Française de Microscopie* , Vol. 1, 1932), who elaborated the *Méthode des poses successives* (technique with successive poses), which consisted of executing several shots on different focal planes, while maintaining the same photographic plate! A successful example of this technique appears on page 15 of the *Panorama du Micro-monde, Technique Moderne de la Microphotographie* by Louis-Jacques Laporte (Paris, 1949), where we find a beautiful photomicrograph of radiolaria in black and white with all the fine details.

The digital revolution has made photography with the microscope undoubtedly easier and more or less within the reach of everyone, even if to produce beautiful and accurate images you must first of all have a certain mastery with this tool. It is also a duty to recognize that the concept underlying the *Méthode des poses successives* has never left us, even though eighty-seven years have elapsed! In fact, today we have the most refined focus stacking or Z-stacking: it is a digital photographic technique that uses a series of shots of the same subject taken on different levels of focus, which are processed by editing software (for example Helicon Focus, Zerene Stacker, Combine ZP and Adobe Photoshop in the CS4, CS5 and CS6 versions). This software selects and combines the areas in focus of the various shots in order to create a single final image with a considerable depth of field compared to any single shot. Both in the thirties and in modern times, even if the technology has changed, a *sine qua non condition* is also valid, that is the minimization of vibrations inside the microscope system, camera adaptor and camera (in other cases a microscope camera), which cause a displacement of the frame that some software can correct only if it is not excessive.

Focus stacking is therefore almost essential for the microscopist who for pleasure or profession has to work with the wonderful diatoms and in any case with microscopic objects with a significantly detectable thickness: each shot of the series is obtained with a minimum advancement of the fine focus knob (which obviously does not have to be touched while a single photomicrograph is realized), generally we start from the highest focal plane (that coincides with the highest part of the diatom) and we proceed gradually to the lower focal plane, but we can also operate in reverse. The important thing is to capture a good number of focal planes to avoid the appearance of blurred areas in the final image generated by the software (for diatoms with a considerable thickness, it may take more than a hundred shots!).

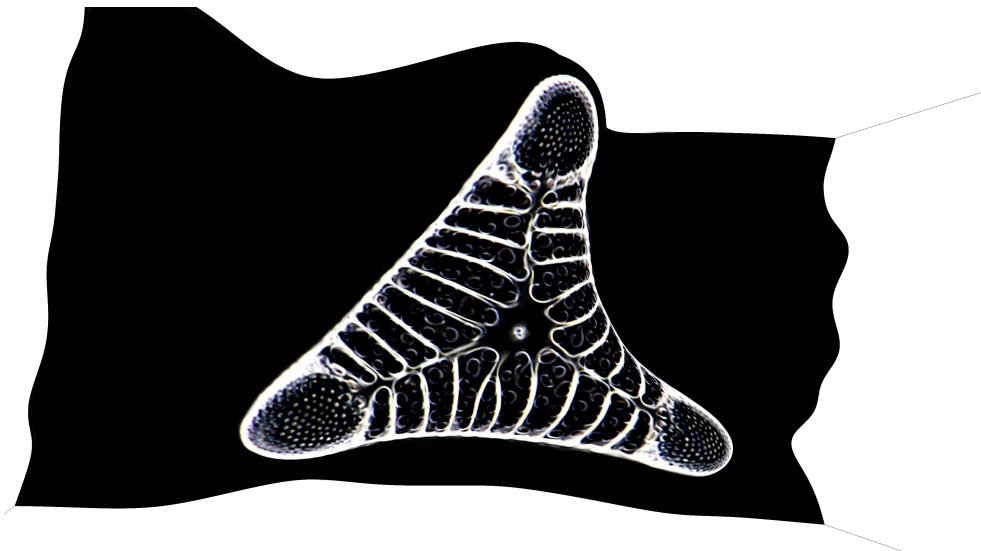
If the concept behind the *Méthode des poses successives* was actually from the thirties as we have seen, the digital revolution must recognize the advent of 3D models, which can be realized for example with an advanced function of Helicon Focus: it is an evolution / implementation of the focus stacking where the image generated by the latter is in turn transferred onto a virtual three-dimensional surface. The 3D model obtained like this is never static, but with simple movements of the mouse can be rotated in every direction: in the case of Helicon Focus the file can be saved in .jpg or in the form of an anaglyph (stereogram) enjoyable with the appropriate glasses, or – *last but not least* - you can save it in motion as a movie. Who knows how much the diatomists of the past would have paid in order to have the chance to realize and admire these fabulous 3D models! In the case of diatoms, the 3D model often has a remarkable scientific value, because the mental reconstruction that our brain realizes is not always accurate when we operate under a microscope: by moving the fine focus knob it is natural to imagine the three-dimensional aspect of the observed diatoms, but often only after having examined the relative 3D model will we have a total and real cognition of the frustule in question, especially in the case of surfaces that have elaborate differences in height: try to believe!

The 3D models obtainable in this way have some similarities with the images provided by the scanning electron microscope (SEM), but they also present important differences: omitting the issues related to the resolving power, the SEM stops almost at the surface of the microscopic objects, while the optical microscope is able to reveal even the most internal structures if they are not opaque, thanks to the light beam that penetrates them.

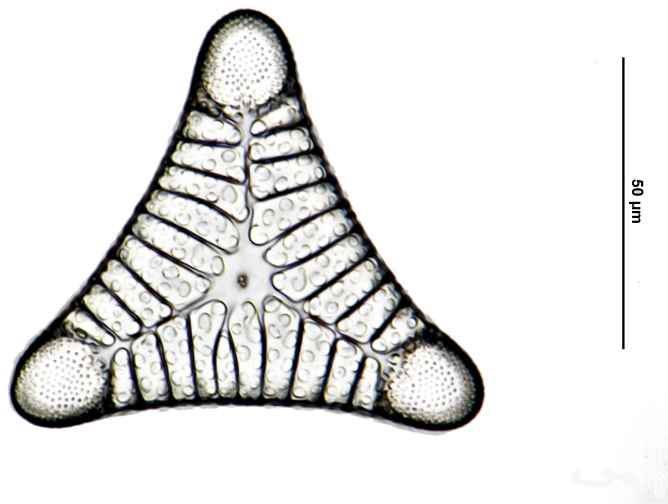
Description of the images:



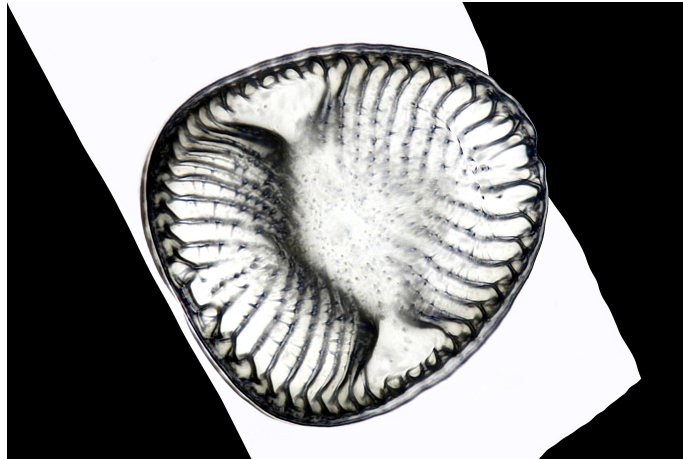
3D model of diatom *Triceratium venosum* Brightwell, rotated at will. Brightfield, 40x dry lens. It is a very rare and splendid fossil diatom from Oamaru (New Zealand). Micromanipulation, mounting and imaging by the author



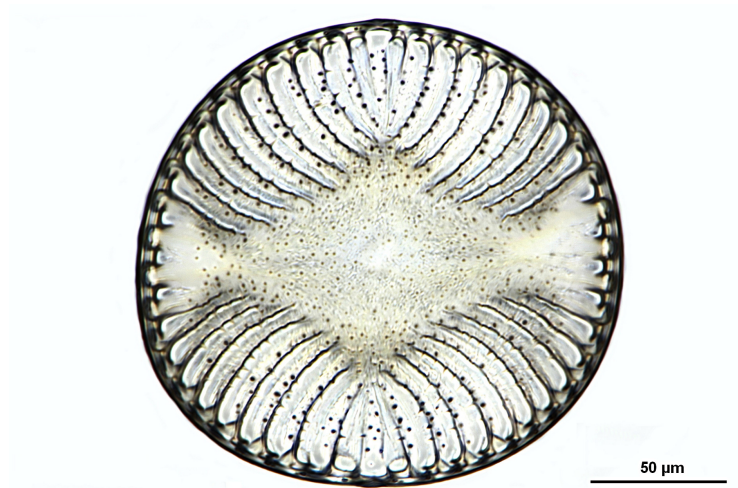
3D model of the same diatom *Triceratium venosum* Brightwell in darkfield



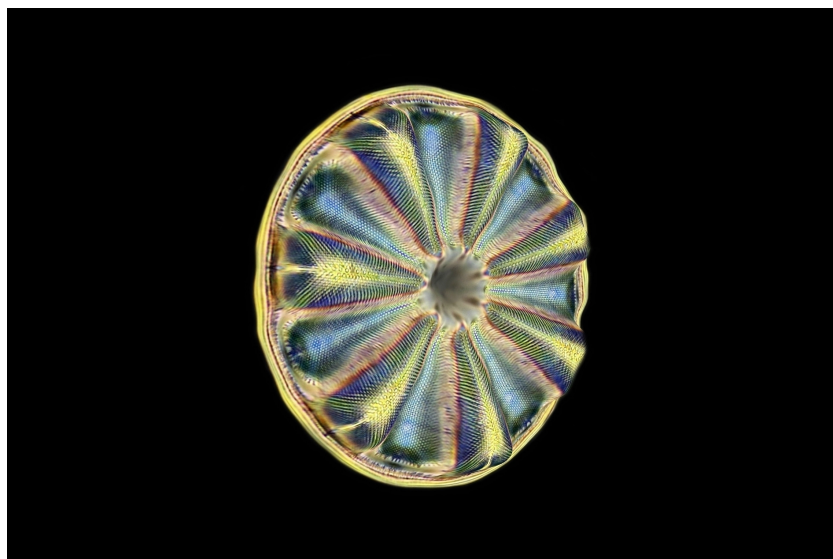
Diatom *Triceratium venosum* Brightwell itself, brightfield image obtained by focus stacking



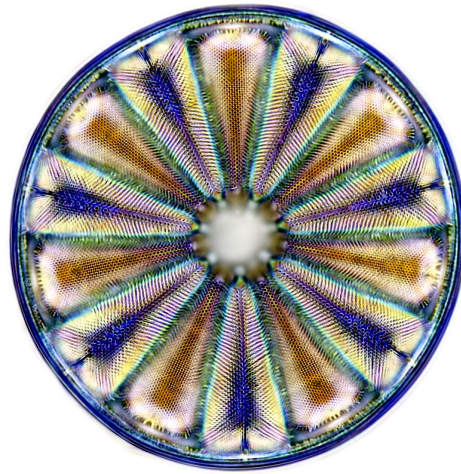
3D model of diatom *Campylodiscus hibernicus* Ehrenberg, rotated at will. Brightfield, 40x dry lens. Rare Pleistocene specimen of fresh water. Micromanipulation, mounting and imaging by the author



The same diatom *Campylodiscus hibernicus* Ehrenberg, image obtained through focus stacking



3D model of diatom *Actinoptychus splendens f. glabrata* (Grunow in Van Heurck) Pantocsek, rotated at will. Phase contrast, 40x dry lens. Rare Miocene marine specimen. Micromanipulation, mounting and imaging by the author



100 μ m

The same diatom *Actinoptychus splendens f. glabrata* (Grunow in Van Heurck) Pantocsek, image obtained by focus stacking

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