

3D Imaging of diatoms with Light Microscope. Empirical Amateur Case Report.

Robert Hill UK.

On the road down any hobby, perhaps particularly microscopy, there are many branches and side turnings that one sometimes wanders down and it's fun to experiment – to see if something will work. Of course these may be dead ends but no matter.

One thing that has struck me since I took up this hobby is how recent developments in technology have helped the amateur to the extent that some of the amateur pictures seen on *Micscape* are so much better than those from professional research departments of 10 years ago. Of the developments that have been of most help one would include:-

- Digital cameras
- Personal computers with image manipulation software
- LED lighting systems
- Image stacking software
- An appreciation of different ways of illuminating a specimen

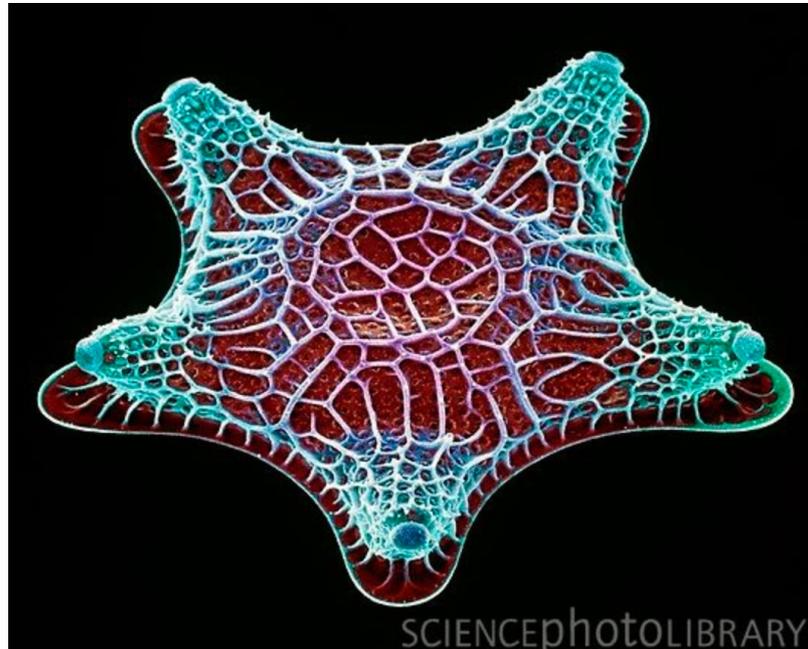
This is just a short report about some experimenting, (or fiddling as my wife might say), with images using some of the above.

Diatoms, after metallography, are my main microscope interest. They can be difficult to image being translucent and are of course 3 dimensional structures but down the light microscope one gets a distinctly 2 dimensional impression. As if to emphasise this my copy of "The Diatoms" by Round, Crawford and Mann, (2000 reprint, digital version 2007), is largely illustrated with scanning electron micrographs showing diatoms in 3D with an occasional light microscopy image serving to emphasise the difference with the 2D images of light microscopy. I don't say better but many might – it depends on your point of view.

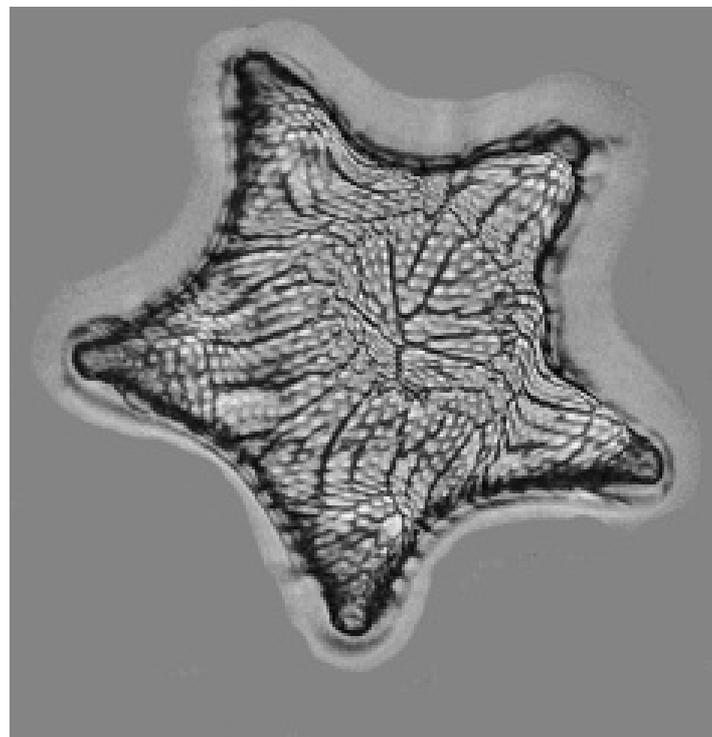
I did read "[A scanning electron microscope in the dining room](#)" by Ted Kinsman published in the September 2008 edition of *Micscape* and I have seen them advertised on eBay but ... well I didn't even bother to bring it up in conversation. Even I know my limits.

One can easily stack images of diatoms but there are problems with this – basically the black hole or white hole dilemma. The problem is that stacking is good for subjects that are not translucent like my metallurgical specimens. If the specimen has depth, including lack of flatness greater than the depth of focus of the objective some bits will be out of focus but by stacking up the images you can get the entire specimen in focus. Well not so easy if it's translucent as what started as a black hole in one plane of focus becomes a white one in a different plane which tends to confuse the stacking software and affect the picture. Whilst playing with "Helicon Focus" my stacking software I noted the option to make a 3D image and wondered whether this would work or indeed be accurate. So I stacked up some images of a reasonably well silicified diatom and ran the 3D program and fiddled about with software of various types and then searched the internet for a 3D EM picture of the same or

roughly same specimen in the same genus to compare with. Our EM colleagues seem to have taken to colouring their diatom pictures with garish colours but I did have a way of doing something similar to my picture.



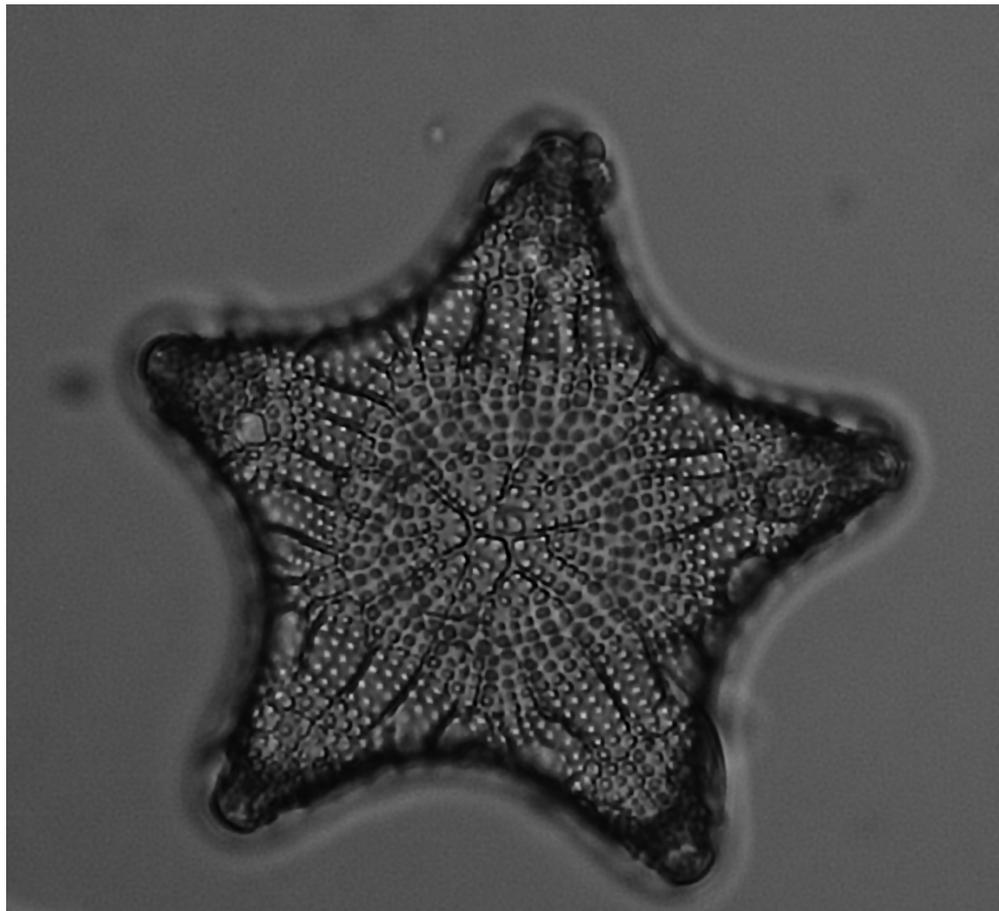
All acknowledgements to the [Science Photo Library](#) and to [Steve Gschmeissner](#) who took this picture but here is an SEM coloured picture of a specimen from *Triceratum* genus. Definitely 3 dimensional.



Here is my more modest 3D picture by Helicon Focus from 13 stacked images using an oblique lighting technique I have described previously.

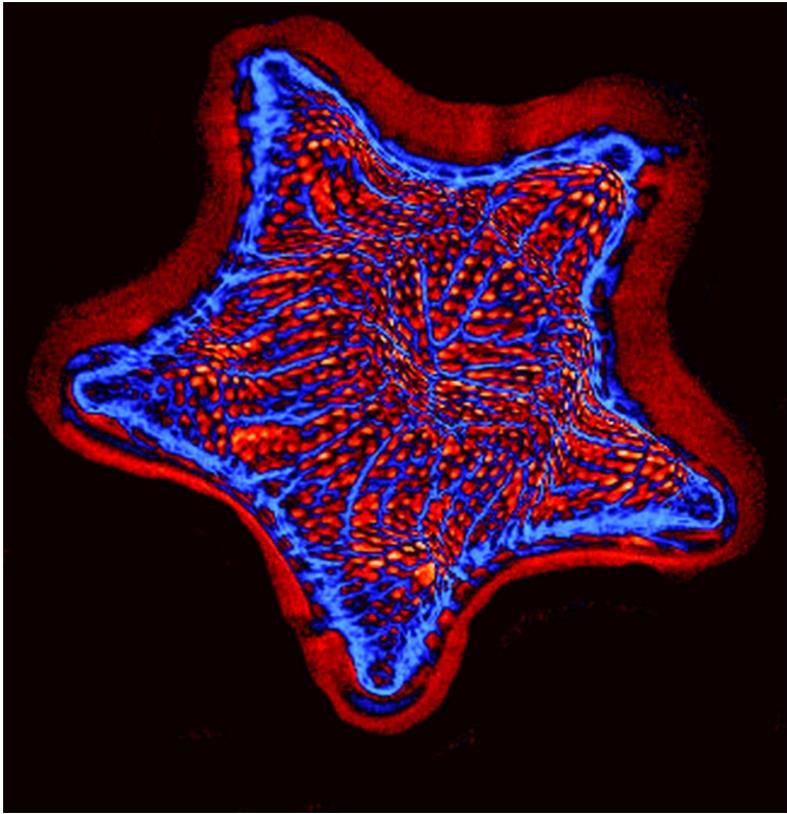
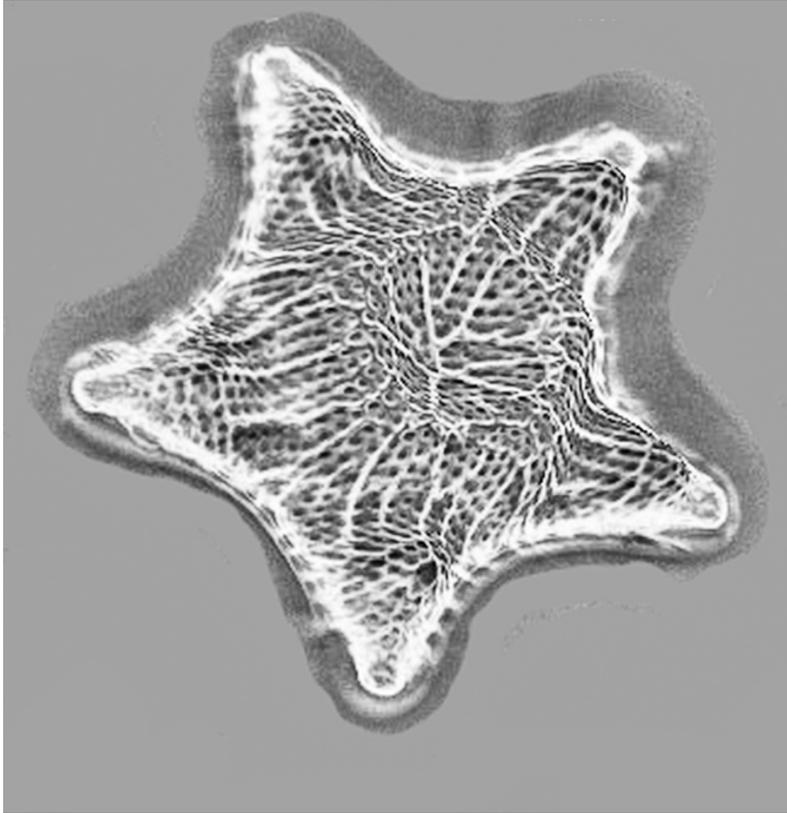
Well it looks roughly the same so that's good and means that it may be possible to rely on the pictures the system will produce but I am sure one has to be careful that one is not constructing a fantasy picture.

Here is a 2D picture of the same diatom using an experimental lighting technique – basically a form of oblique illumination which can itself give a 3D effect.



Hence my view that 3D is different rather than necessarily better and I do sense a potential conflict with the purists of light microscopy... Image heavily manipulated by software.

At risk of further potential conflict here are two more manipulated images to change the colour and make the image look a bit like the SEM at the top of the article – who needs an electron microscope in the dining room? Marital harmony reigns...



Comments to the author welcomed.
Email - robahill AT hotmail DOT co DOT uk

Published in the December 2018 issue of *Micscape* magazine.
www.micscape.org