# A technique for generating colour scanning electron microscope images

By Ted Kinsman

For a number of years, I have been imaging samples with the scanning electron microscope (SEM) and have used the images to illustrate books and magazines. In the competitive world of photography, editors are only interested in colour images so I have spent a lot of time experimenting with colorisation techniques that are quick and easy. This procedure I have come up with is the easiest path from two black and white images to an image that has color and is interesting to the eye. There are a number of techniques that involve hand coloring of images, while these techniques can yield wonderful images the time required is extensive.

This technique works from low magnifications to about 500x or higher with conductive samples. The microscope should not have any setting changed between the different modes. One good image is taken with secondary backscattered electrons, and with the same beam conditions a second image of back scattered electrons are collected. Due to the nature of the specimen these images usually align quite well at lower magnification settings. At magnifications higher than 1000x this technique might not work either due to problems of alignment between images, or due to charging of the sample, or just the inability to get two separate images under the same electron beam condition. Give this technique a try with different samples and see what happens. It is easiest to understand the procedure if you start with two sample images and work through the procedure yourself.

The Procedure that I have found most helpful is shown below. It might seem like a lot of complicated steps, but with a little practice the reader can combine two different images into a full colourised image in about less than 10 min.

1. File > Scripts > Load files into stack.
2. Select the two files that will be used to colourize the image and then click the open button.
3. The load layers window will pop open and ask if you want to add more source files, since you have already selected the two files, click the OK button.
4. The two files will now be displayed as two different layers. High light the two layers by clicking on the layers and the shift key at the same time.
5. Make sure the two layers are highlighted, then go to Edit > Auto-Align Layers.
6. The Auto-Align Layers box will open and you will want to select the Auto Projection button then hit the OK button.
7. Now select the bottom layer. Unlicked the eye in the box on the top layer – this will make the layer invisible. Once the bottom layer is highlighted, Go to Layer > New Adjustment Layer > Hue Saturation.
8. A New Layer box will open and be sure to click the Use Previous Layer to Create Clipping Mask button, then hit the OK button.
9. The Hue Saturation control panel will pop open. Be sure to click the box next to the colourization control. Now when the hue slider is moved the image visible on the screen will take the hue from the slider. Adjust the Hue, Saturation and Lighten sliders until you are happy with the image. The layer is an adjustment layer, and can always be changed in the future if you are not happy with the current colour.
10. Now click on the top layer and make sure the eye shows on the left side of the layer display. Now go to the Select > All control. The image should be outlined with a flashing dotted line. This means that the whole image in the layer is selected.
11. Go to Edit > Copy The layer is now copied to a clip board to be pasted in later.
12. Go to Layer > New Fill Layer > Solid Colour The Colour Picker (solid Colour) window will pop up and pick a colour you like for this layer and Click OK. Keep in mind that you can always return to the layer to change the colours to get it the way you like it.
13. This new colour layer will have a while box on the layer. This white box is the layer mask. What is white in the mask will be added to the layer below. To copy the second layer SEM image to this mask, use the mouse and click on the white mask as you hit the alt key (PC) or

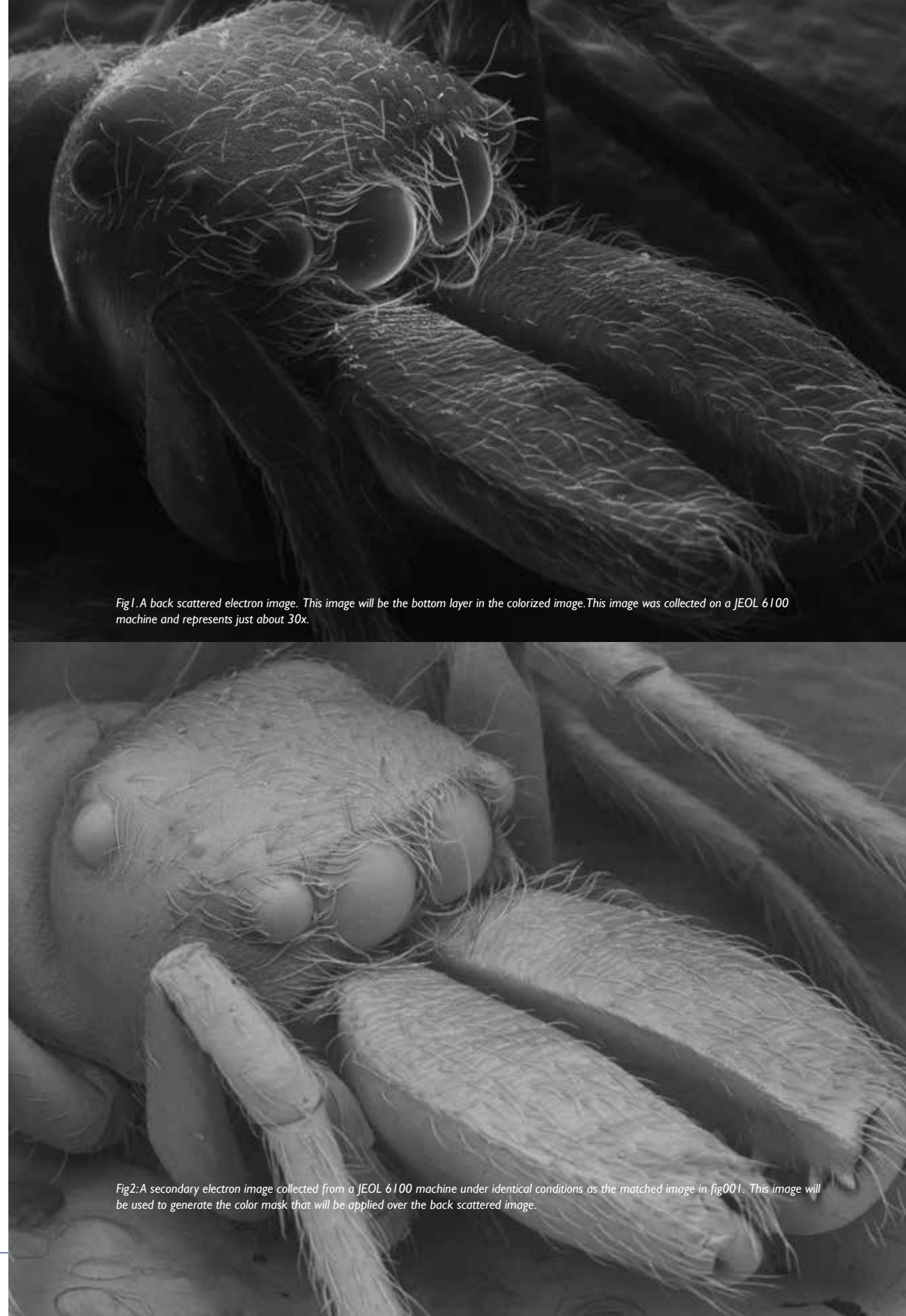


*Fig1. A back scattered electron image. This image will be the bottom layer in the colorized image. This image was collected on a JEOL 6100 machine and represents just about 30x.*

*Fig2: A secondary electron image collected from a JEOL 6100 machine under identical conditions as the matched image in fig001. This image will be used to generate the color mask that will be applied over the back scattered image.*

*Fig3: The resulting image made from the two different images. Total time for a finished image can be under 10 minutes, although the technique works best at lower magnifications. The time it takes to collect a second image with back scattered electrons saves you an hour in the colorization process.*

the option key (mac) the screen will now show the full mask which is pure white. Use Edit > Paste to copy the image from the clipboard to this mask. The layers should now look similar to Fig-step015.

14. Now click on the layer below that holds your second or top image and turn it off. The image will now show several colours from each layer shown on the screen.

15. In the layer control panel change the attribute of the top layer from Normal to Screen. The opacity of this layer can also be changed from 100% down to a pleasing level. Note that the screen attribute is only one of a number of different layer attributes that yields pleasing results. The reader might also like to try the colour setting, and even the normal setting will work if the opacity is lowered.

16. At this point you will want to return to the colour setting for each layer by selecting them and adjusting to achieve colours that are pleasing for the image.

17. Save the file as a PSD or Photoshop file that keeps the layers and can be changed at any time in the future.

18. To use the image in print and web media, go to Layer > Flatten Image and now save the image as a JPG format.

To make this procedure a bit easier I have made a video which is available at http://www.youtube.com/watch?v=aOrj3HpBwB0

Keep in mind that this is only one technique, there are many ways to achieve similar results, feel free to experiment.

I hope this procedure will allow colour image to be made a bit easier than the laborious hand colouring techniques.

*Fig4: A scanning electron microscope image of the leaf of the lavender plant (Lavandula augustifolia ). The branching cells give added protection from insects. The pillow shaped structure produces the aromatic sent that gives lavender it characteristic smell. This image was made from a standard secondary electron detector, and from a second SE detector located inside the stack. An image created from two different detectors.*

*Fig5: SEM of Monarch Butterfly Egg (Danaus plexippus), aid on the underside of a common milkweed leaf (Asclepias syriaca). The milkweed plant serves as the primary food source for monarch butterflies as well as a host for the monarch's eggs and larvae. This images was collected at 50x and represents a field of view .5mm wide. The two detector image was then masked to make the background green.*

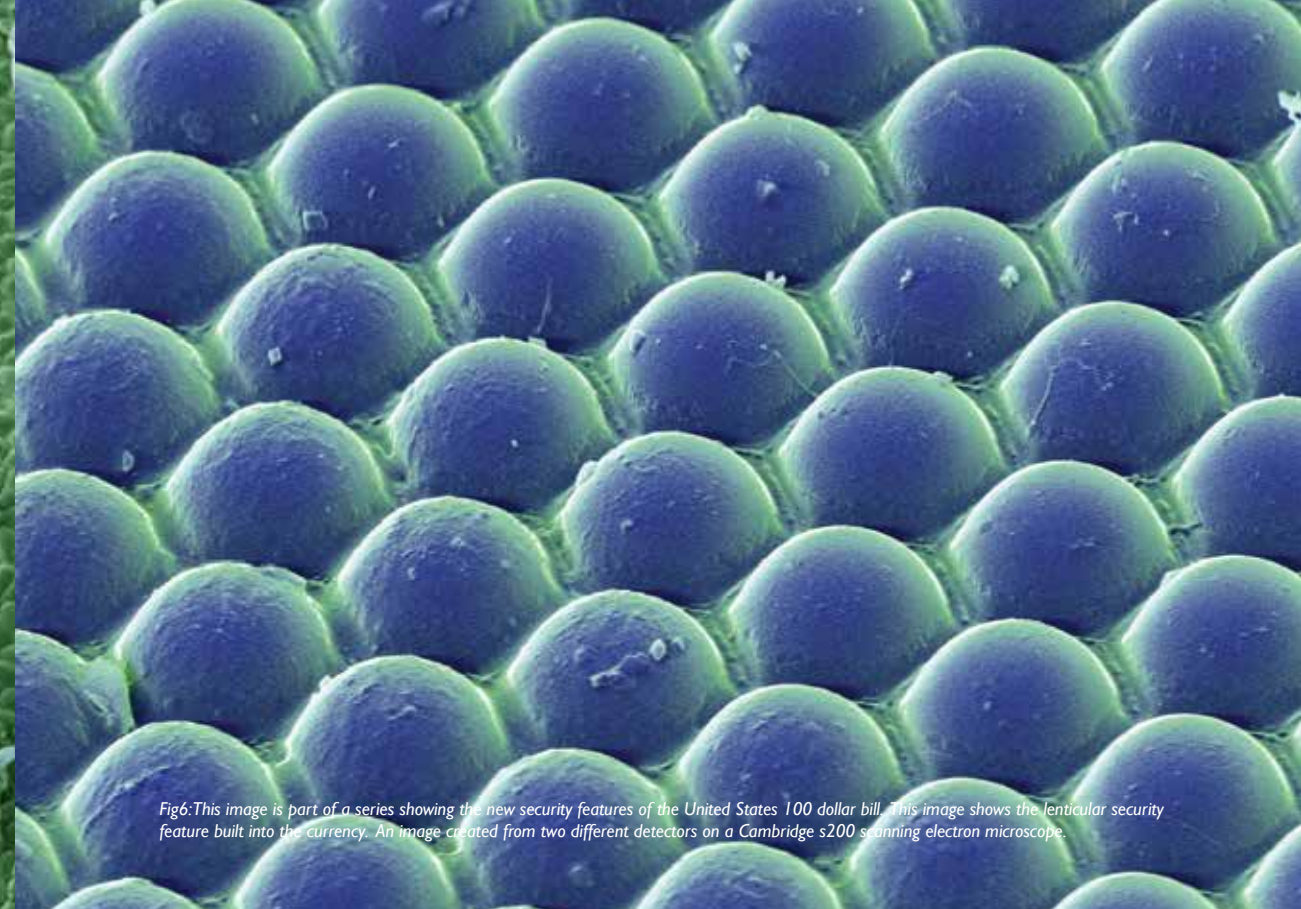


*Fig6: This image is part of a series showing the new security features of the United States 100 dollar bill. This image shows the lenticular security feature built into the currency. An image created from two different detectors on a Cambridge s200 scanning electron microscope.*

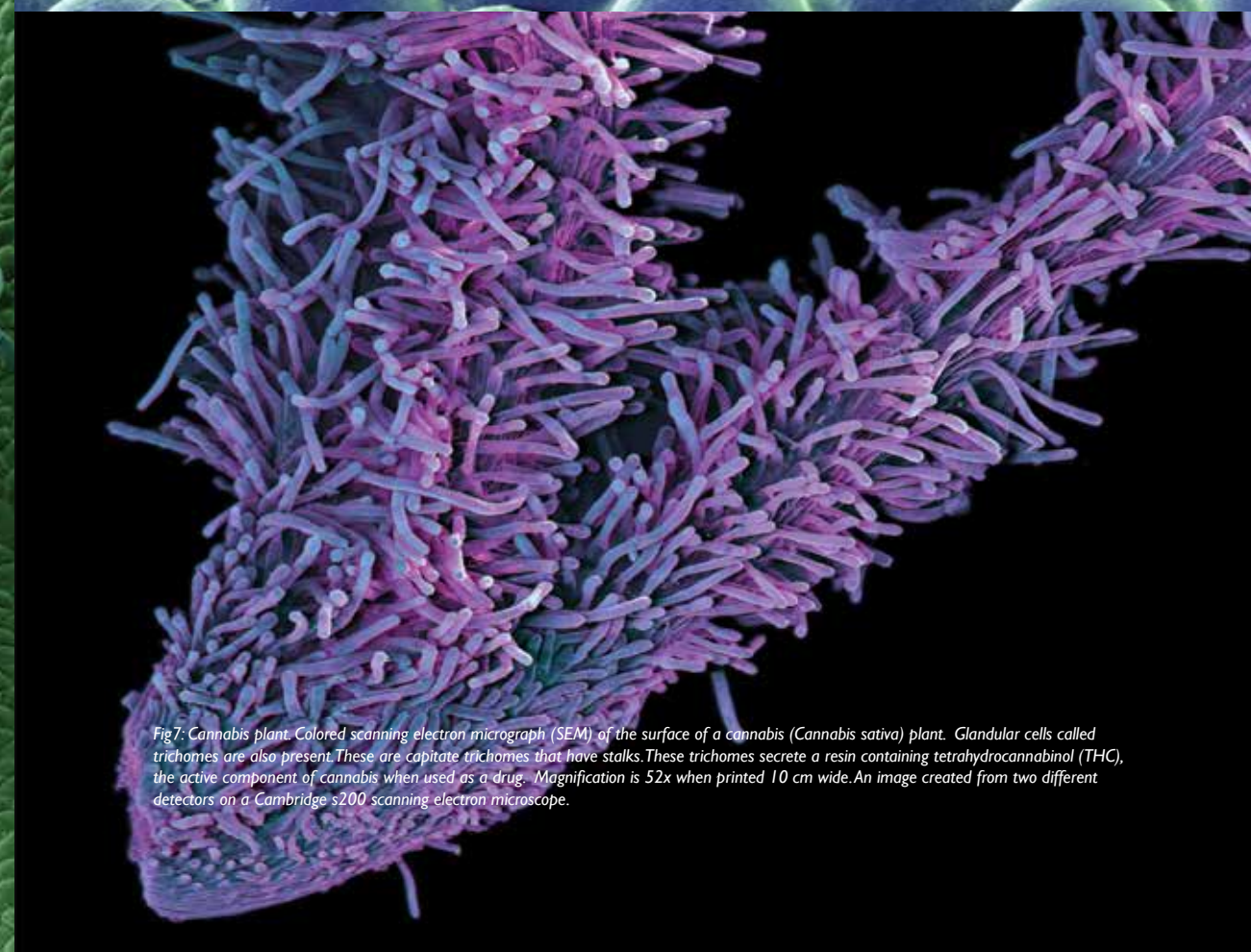


*Fig7: Cannabis plant. Colored scanning electron micrograph (SEM) of the surface of a cannabis (Cannabis sativa) plant. Glandular cells called trichomes are also present. These are capitate trichomes that have stalks. These trichomes secrete a resin containing tetrahydrocannabinol (THC), the active component of cannabis when used as a drug. Magnification is 52x when printed 10 cm wide. An image created from two different detectors on a Cambridge s200 scanning electron microscope.*

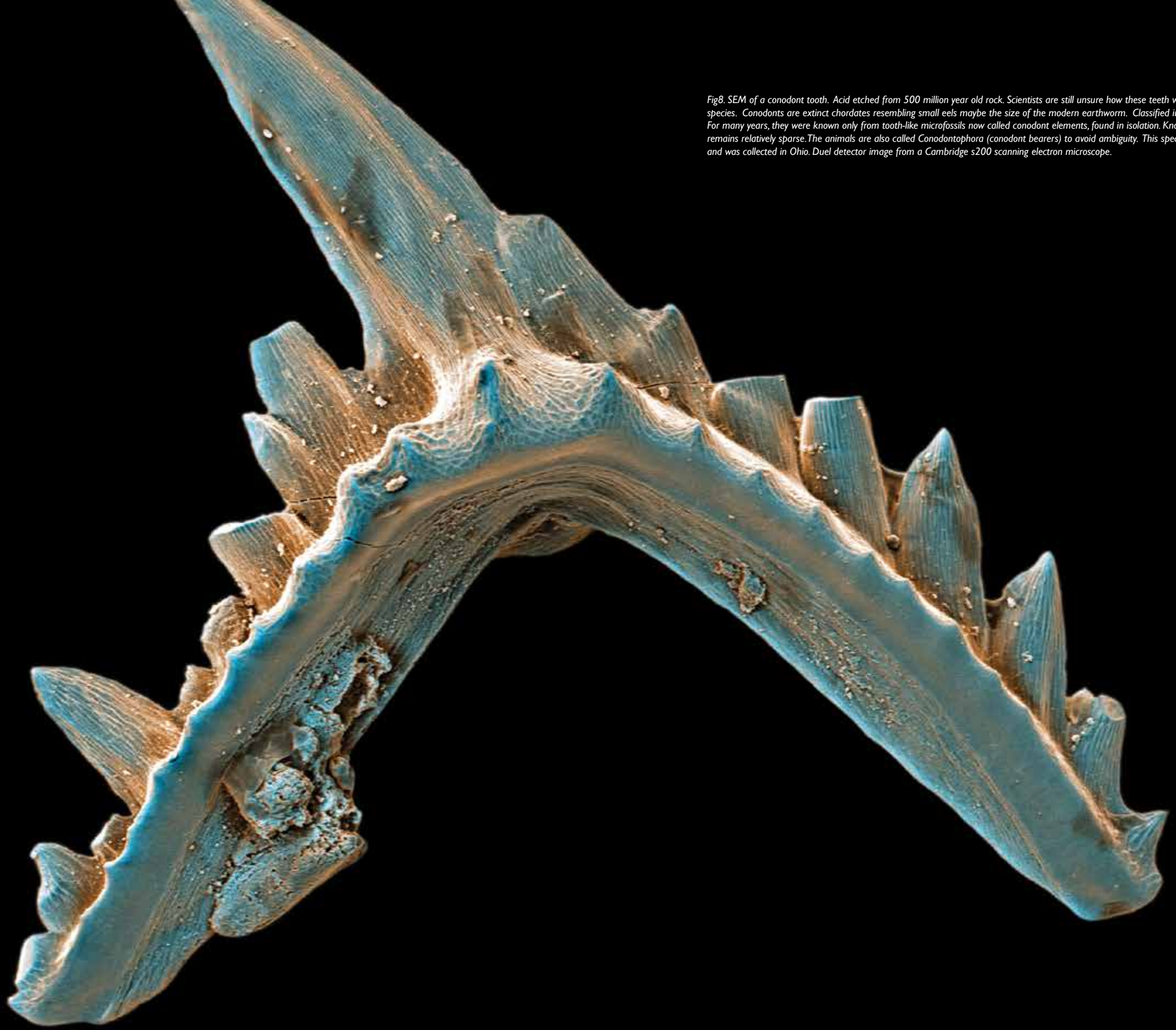


*Fig8. SEM of a conodont tooth. Acid etched from 500 million year old rock. Scientists are still unsure how these teeth were placed in most of the species. Conodonts are extinct chordates resembling small eels maybe the size of the modern earthworm. Classified in the class Conodonta. For many years, they were known only from tooth-like microfossils now called conodont elements, found in isolation. Knowledge about soft tissues remains relatively sparse. The animals are also called Conodontophora (conodont bearers) to avoid ambiguity. This specimen was 1.0 mm wide and was collected in Ohio. Duel detector image from a Cambridge s200 scanning electron microscope.*

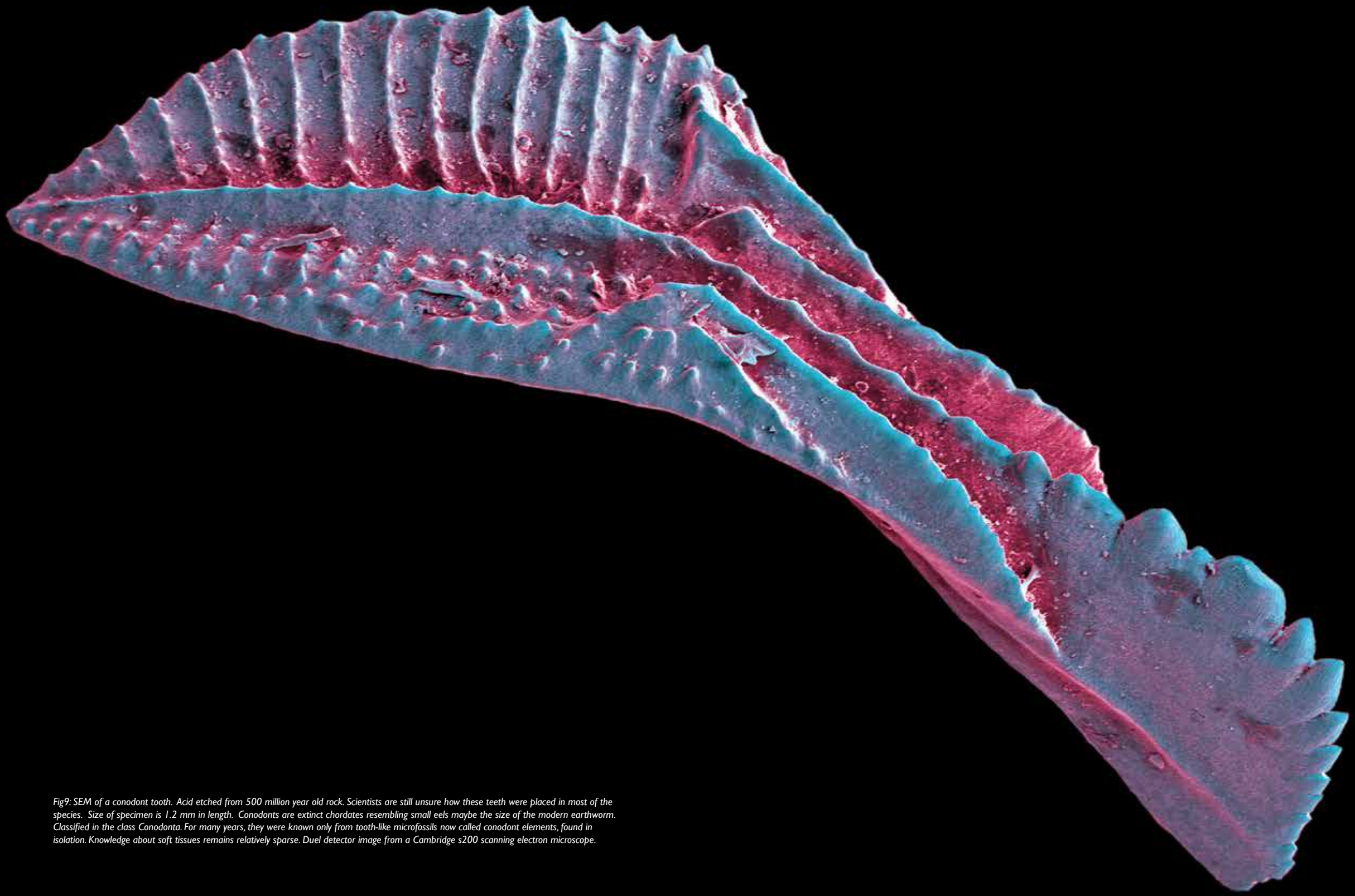
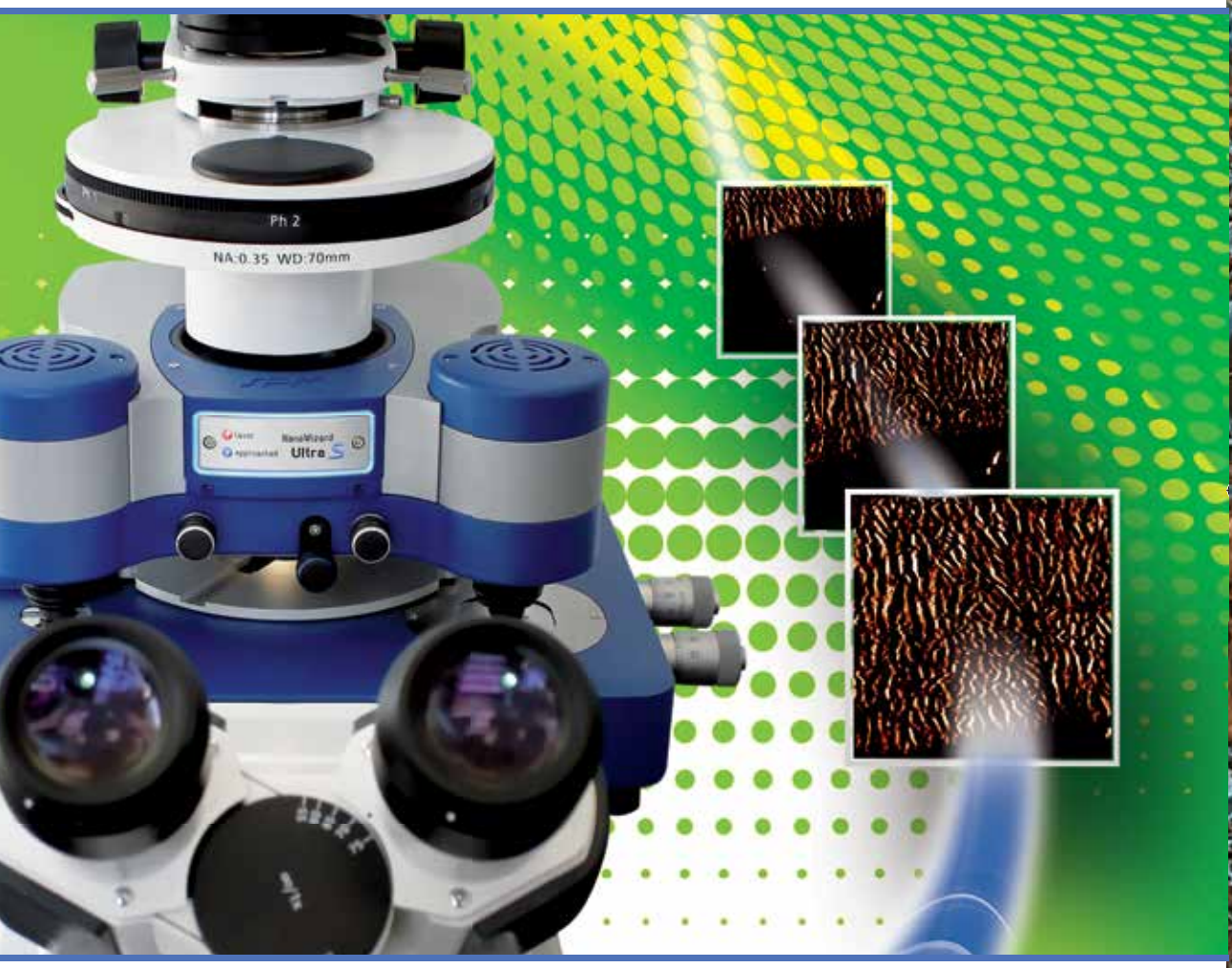


*Fig9: SEM of a conodont tooth. Acid etched from 500 million year old rock. Scientists are still unsure how these teeth were placed in most of the species. Size of specimen is 1.2 mm in length. Conodonts are extinct chordates resembling small eels maybe the size of the modern earthworm. Classified in the class Conodonta. For many years, they were known only from tooth-like microfossils now called conodont elements, found in isolation. Knowledge about soft tissues remains relatively sparse. Duel detector image from a Cambridge s200 scanning electron microscope.*

*Fig10: SEM of the cross section of a cannabis plant leaf. Duel detector image from a Cambridge s200 scanning electron microscope. Magnification is 50x.*

# NanoWizard® ULTRA Speed AFM

## Fast-scanning and super-resolution AFM on inverted microscope from the pioneers of BioAFM




- Fast scanning > 100 Hz line rate in air and liquid with excellent resolution
- Atomic resolution in closed-loop by lowest noise level
- QI™ mode for quantitative material property mapping
- DirectOverlay™ for most precise correlative microscopy
- Most flexible and upgradable with a broad range of modes and accessories

**www.jpk.com | e-mail: nanowizard@jpk.com**

JPK Instruments

*Nanotechnology for Life Science*

**Follow us on Facebook, Youtube and Linkedin.**

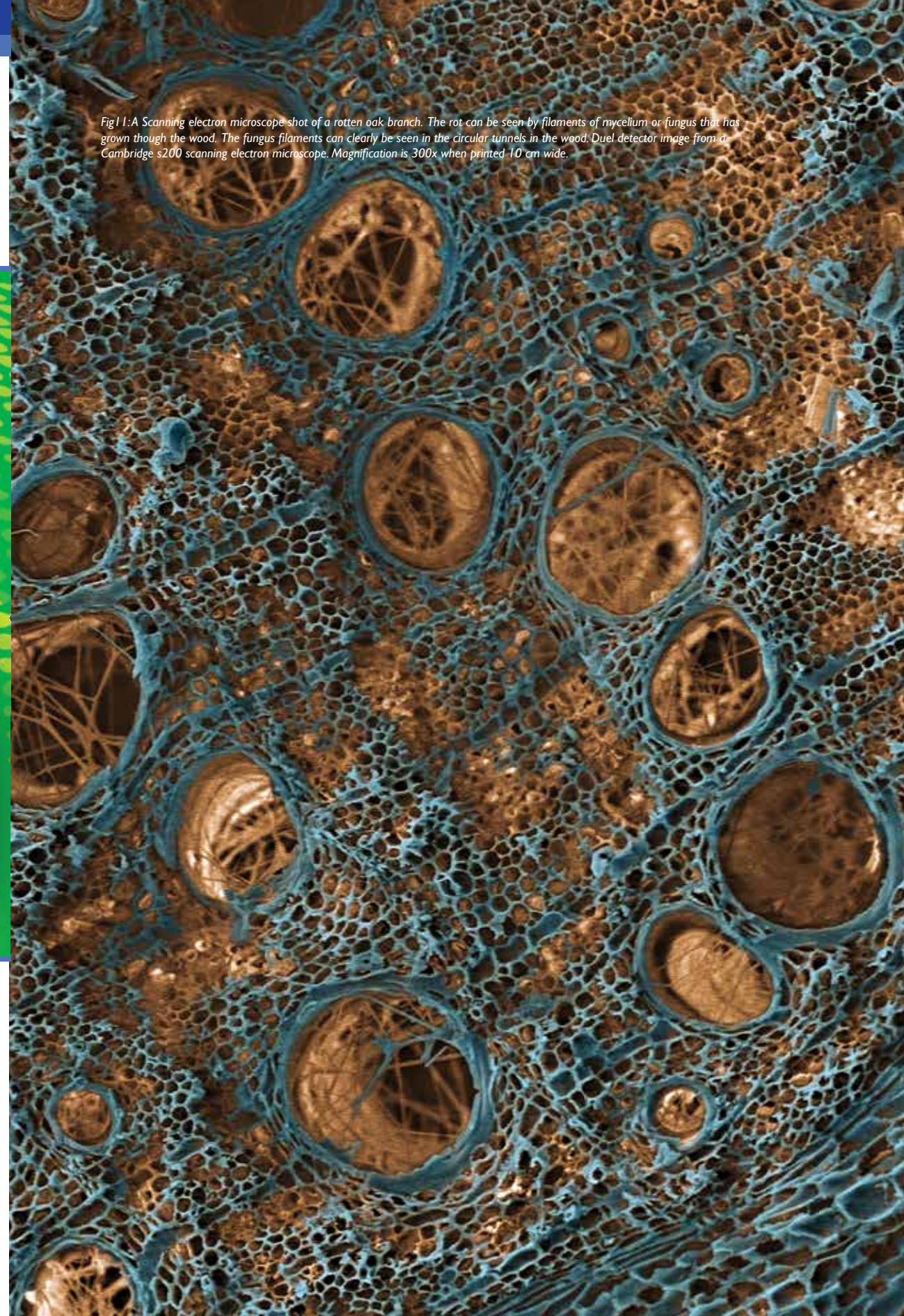



*Fig 1: A Scanning electron microscope shot of a rotten oak branch. The rot can be seen by filaments of mycelium or fungus that has grown though the wood. The fungus filaments can clearly be seen in the circular tunnels in the wood. Duel detector image from a Cambridge s200 scanning electron microscope. Magnification is 300x when printed 10 cm wide.*

*Fig 12: Scanning electron micrograph (SEM) of poppy seeds. Poppy seeds used in breads and sweets are the seeds of Papaver somniferum, "opium poppy," Duel detector image from a Cambridge s200 scanning electron microscope. The background was removed in photoshop and a false shadow was created.*

**About the author:**

Ted Kinsman is an assistant professor at Rochester Institute of technology (RIT) in Rochester, New York where he teaches in the Photographic Sciences Department. Kinsman has been involved with using images to teach since for a number of years, and continues to create images to facilitate the teaching of science.

He can be reached at emkpph@rit.edu