Digital Imaging and the Microscope

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Images are generated by an optical system – a microscope in our case – and then recorded by drawing, painting, photography, or nowadays by computer capture. All these processes could be included in the one blanket word "imaging". In the broadest sense of "image", it is a representation of a part of the universe as it was at one instant of time, and as visual images are the most valuable means of human communication, it may be of interest to consider very briefly some more ancient pictures.

When *Homo sapiens* was evolving, eventually to replace *Homo erectus*, perhaps 350,000 years ago, speech of some kind must have been part of the culture. However, the earliest known images date from only about 35,000 years ago, as cave paintings made about the same time as the earliest sculptures and carvings. Evocative images, such as the painted bull in Lascause Grotto in France, and the painted horse from the Alamira cave in Spain date from about 15,000 BC, but no written language had evolved at that time.

The ability to make written records, where various symbols replace reasonably accurate pictures, was first developed from about 3500 BC, and with the invention of moveable type at the end of the fifteenth century, accurate communication soon became widespread in books.

By the time the microscope was fairly widely in use, in the mid-seventeenth century, some accounts of discoveries made with it were illustrated, the high point being Hooke's *Micrographia* of 1665. The only method of including pictures in a book was to make a drawing of what was seen under the microscope, and make from this a plate for printing from. Unfortunately, many microscopists could not [and cannot] draw accurately, and some drew [and some still draw] what they think they should see rather than what is really there. Hooke had the great advantage of being a trained artist as well as a scientist, and this goes far to explaining the impact of his magic book.

Some aids to assist the artist in drawing ordinary subjects had been in use for two centuries before Hooke, such as a frame with threads stretched across to assist in maintaining correct perspective, mentioned by Leon Batiste Alberti and by Leonardo da Vinci.

Perhaps this idea was the basis of the net of silver wires used by Cuff as a micrometer in 1747. In the 1700s also the solar microscope was in use. This used a beam of sunlight as the illuminant to project a picture from a simple microscope onto a wall, for multiple viewing or for drawing round the outlines.

In 1807 William Hyde Wollaston invented the camera lucida, using a glass prism as the basis of a small instrument which could be carried round by an artist, and clamped in place to assist in the accurate outlining of a scene. In the later part of the same century a version was made for use with the microscope. A modern version is the drawing tube for use with current instruments.

The beginnings of applying a more objective method of image recording were seen in the later 1700s, when Wedgwood tried to produce an image with silver compounds, but it was not until 1802 that Humphrey Davy published these results, only to have them lie forgotten for another thirty years. In the late 1830s Daguerre announced his system of photography. Although this was slow and cumbersome, and produced only one copy of an image, it was rapidly applied to recording microscopical images. When Fox Talbot shortly afterwards announced his much more satisfactory system, the techniques of photomicrography had indeed been born for a wider audience. The advent of silver halide photography excited as much attention in its day as that of digital work has in ours. The art world was in a ferment, but the objective nature of the process, so important in science, was widely recognised, and as Lady Elizabeth Eastlake wrote in *The Photograph* for April 1857:

> . . . the camera could but give evidence of fact, as minutely and as impartially as, to our shame, only an unreasoning machine can give.

As dry plates were introduced in the 1870s, flexible films in the 1900s, cheaper colour in the 1930s, and instant-result Polaroid in the 1940s, so photomicrography went from strength to strength, hand in hand with the means to reproduce more and more cheaply half-tone pictures in print from the 1890s.

The moving image had been adequately recorded from the later 1880s. The possibility of so doing rested on the discovery of persistence of vision, made by Peter Mark Roget in 1824. The higher speed of photographic emulsions by about 1880, and improvements in camera shutters, made motion picture photography possible among pioneers such as the Lumière Brothers, Eastman, Edison, Acres, Le Prince, and, later, Pathé. In 1907 time-lapse photography was developed by Frank Percy Smith, a QMC member; his 1910 colour film *Birth of a Flower* remains a landmark.

In 1936 television transmissions began to a very limited London audience, but were halted by World War II. In 1946 they began again, and in the next twenty years reached a large audience in colour of excellent quality. The development of the electronic computer had begun in secrecy in the war, and with the invention of the transistor and its subsequent amazing development into integrated circuits by the later 1960s, the way was open for the personal computer explosion of the later 1980s.

Until recently the amateur microscopist had to have a range of technical knowledge and a deep pocket to use television as a recording medium, in place of silver halide processes. However, with widespread reception of television, demand for amateur TV cameras arose. Following the adoption by professional electron microscopists of TV techniques, which began widely to replace conventional photographic recording in the 1980s, a range of equipment became available.

Equipment which became available soon required less technical knowledge in its use, and cost rather less. QMC members such as Ken Jones, and Ven and Vaughan Dodge demonstrated their own gear to the Club in 1988, and in due time they and others used digital cameras and output them to computers.

This brings us to the amateur scene of today, when analogue techniques are giving way to digital.

There is no doubt that, magic as it seemed twenty years ago, the analogue signal with its varying voltages is liable to distortion and interference. The digital signal, a series of on/off pulses, is virtually free from both.

In a digital signal the information is carried in bundles of on/off sequences, each called a bit. Eight bits give a byte of 256 instructions, each assigned a function. As more functions are needed, so must more bits be put into each bundle – 4 bits give 16 instructions, 8 give 256, 12 give 4096, 16 give 65,536, 24 give 16, 777,216, 32 give 4,294,967,296, and so forth.

The microscope image as viewed is analogue in character, but a modern video camera, with its pixel array, produces a record of it as a digital message. This can be output direct to a personal computer. This digital record is immune from interference, and can be transmitted electronically at high speed over great distances if required, arriving as a true replica of the original. Further, the record from each individual pixel of the several million making up an image can be altered in character in the computer, and/or moved to a new position. *Any such alteration no longer produces a true copy, and therefore is a false image.*

My own outfit is a standard personal computer, with mouse, keyboard, monitor and printer, with attached microscope and video camera. To process the image I have inserted a graphics and tuner card and their drivers, working under Windows 95, and with Adobe Photoshop and Paint Shop Pro installed.

I do not fit a lens to the camera [which is recommended practice], but neither do I use an eyepiece on the microscope [which is not]. Further, I use a tube length of only 80mm.

Now, any higher-power objective is computed to work with an eyepiece at a fixed tube length [usually 180mm] if it is to yield an adequate image. The expedients I have adopted are forced on me to get all of my image [from a 20x/NA 0.40 Russian achromat] onto the pixel array in the camera. If I did use an eyepiece or a longer tube-length only a very small part of the image would fall on the array. The set-up may not be ideal, but it works for me as I am not seeking technical perfection, which I know to be well beyond my resources.

The video camera signal is modulated to be compatible with the computer, and the video card is pretuned to it, when the specimen is set up on the microscope, and adjusted to give the required area of image on the monitor. The initial image is slightly over-enlarged, giving an exaggerated pixelation, and is copied to the Windows clipboard file. After this the microscope and camera play no further part in the presentation of the image.

The original image shown [fig. 1] is the diatom *Triceratium secedens* from Oamaru, mounted in

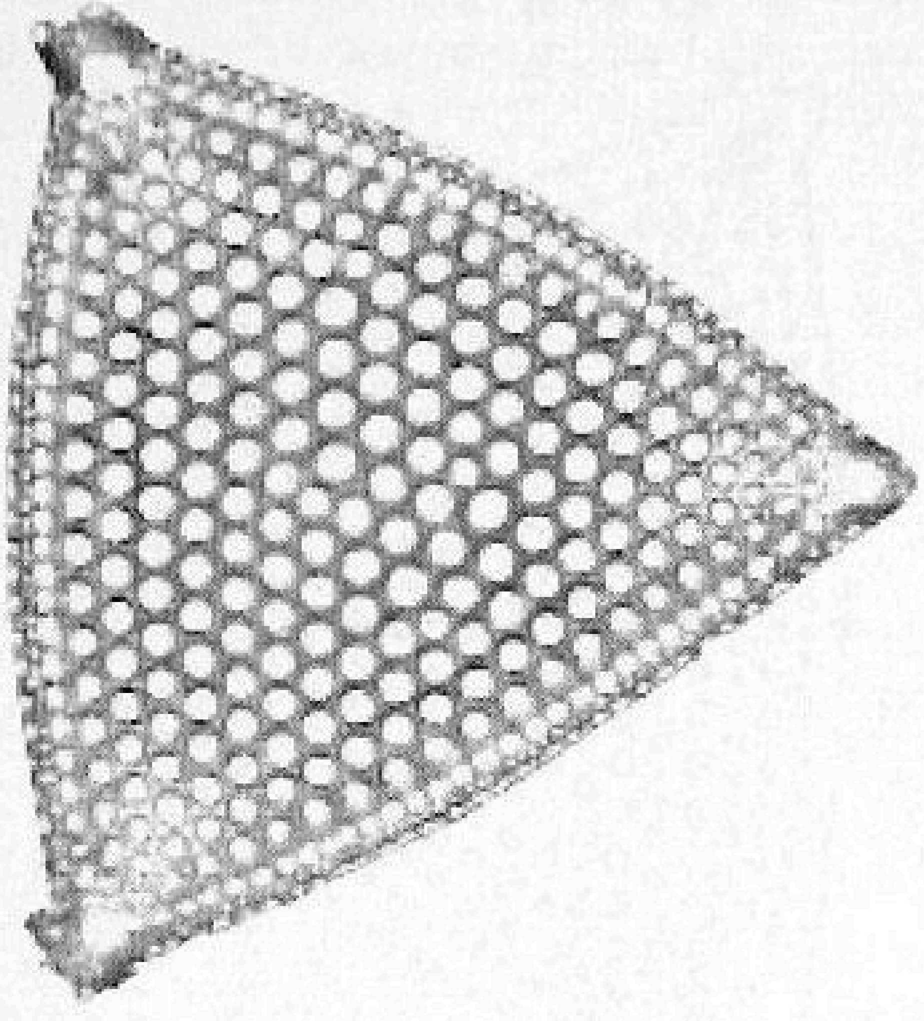

FIG. 1. The diatom *Triceratium secedens* as recorded without manipulation.

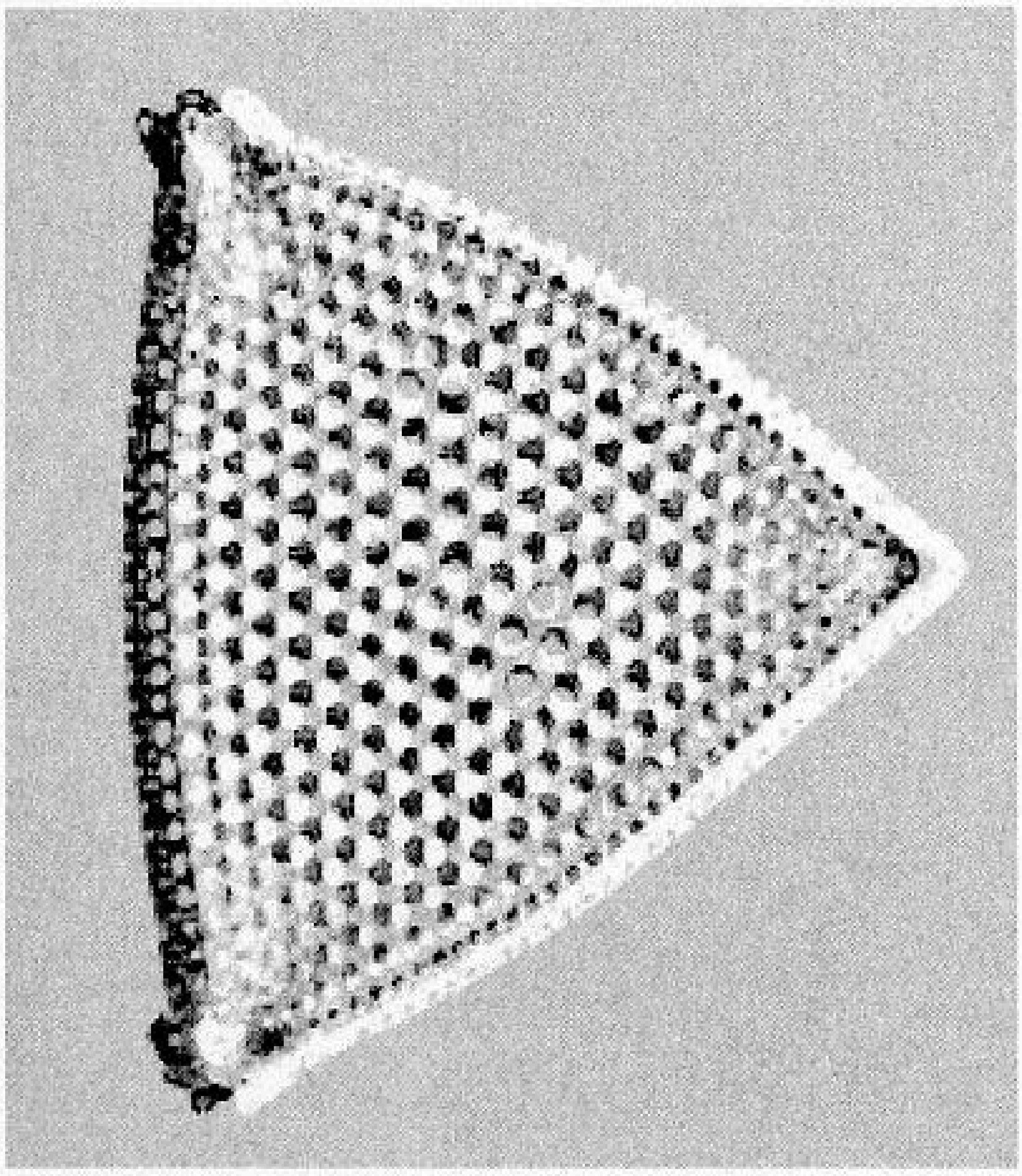

FIG. 2. The image after being filtered, stylized, and embossed, at an angle of 180^0, with a height of 10 pixels, and an amount of 100%.

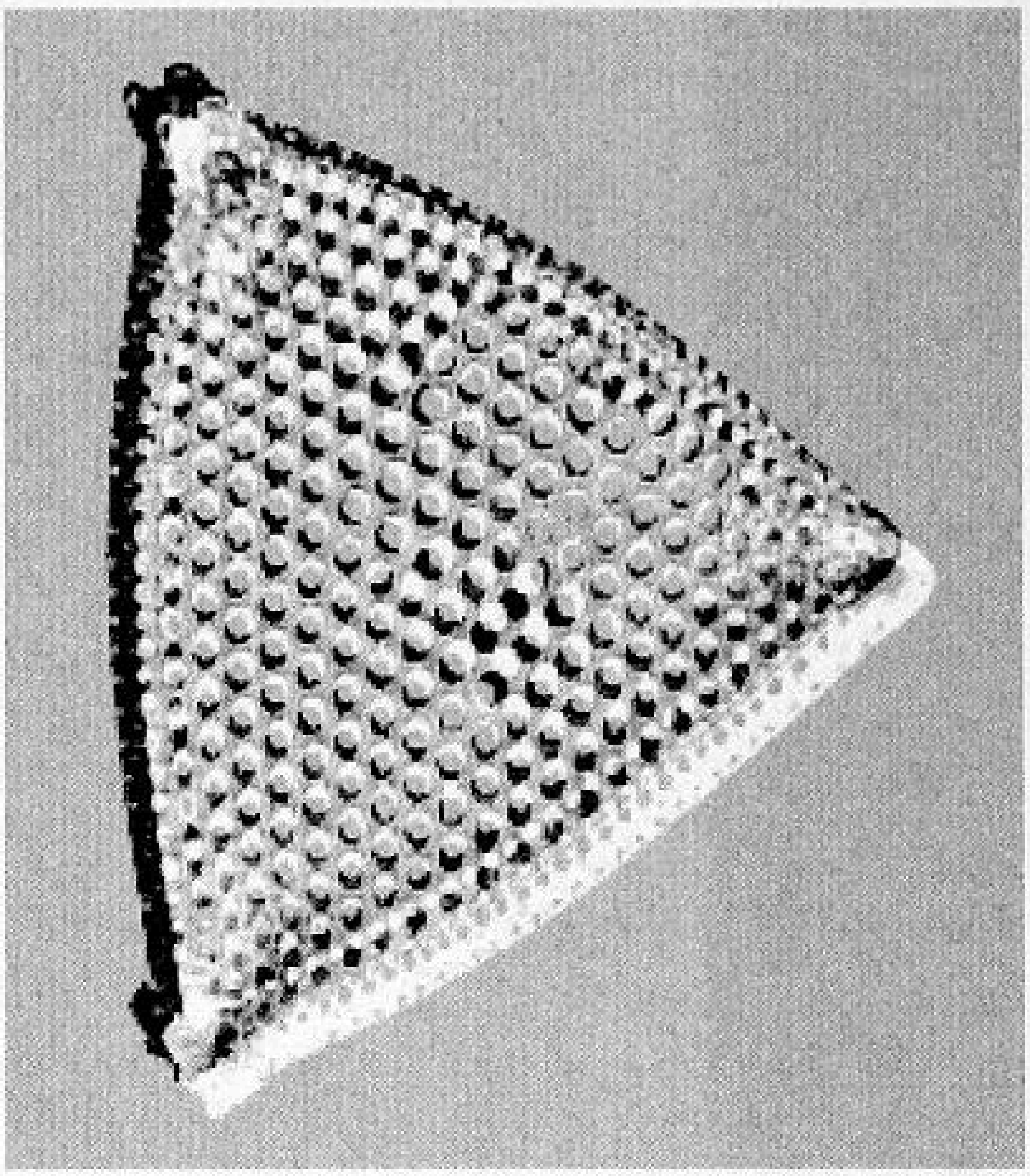

FIG. 3. The image treated as in figure 2, but at an angle of 135^0.

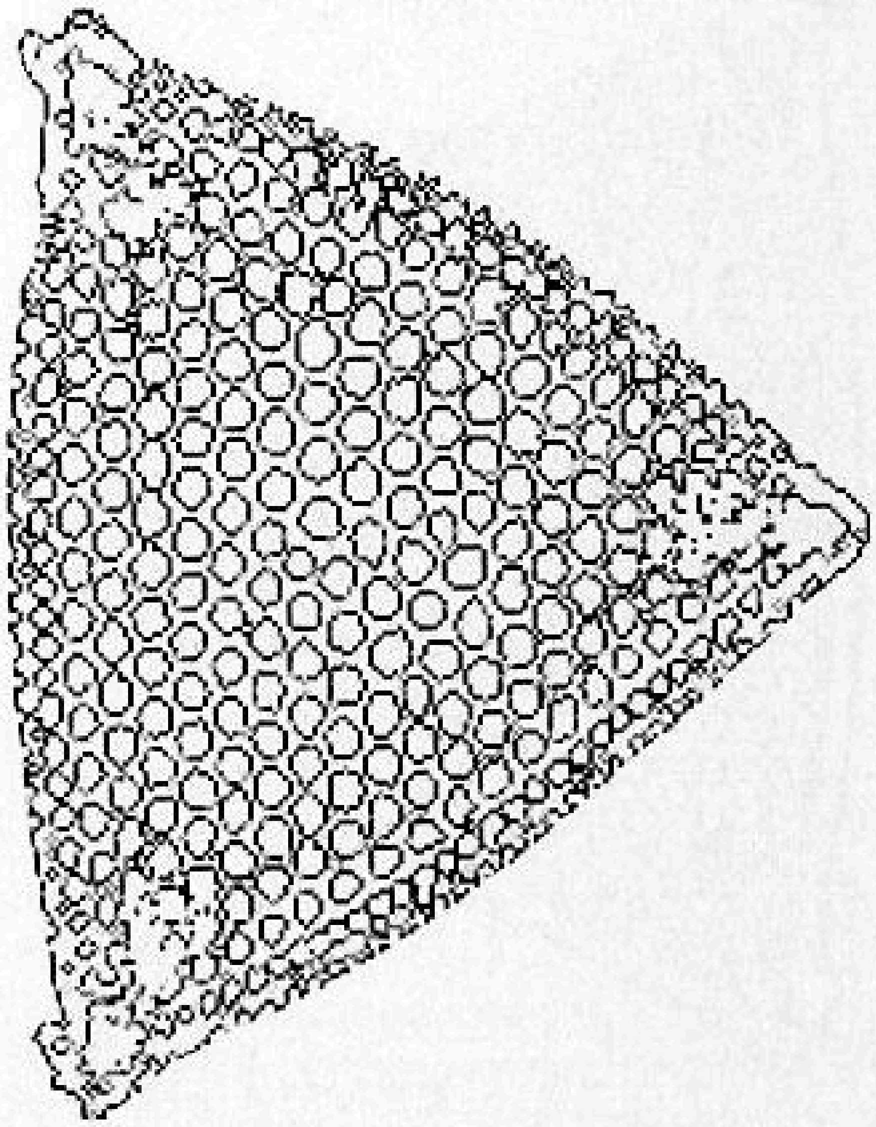

FIG. 4. The image processed using the trace contour control. The level selected was 170, for the upper edges.

Styraclor by Bernard Hartley in 1994. I decided to use some of the many controls offered by Paint Shop Pro to try to introduce a shadow, and if possible to measure depth by triangulation. Prior to setting up, a stage micrometer was captured as an image at the same scale as succeeding pictures, and processed in a similar manner. For the required image, from the menu bar I chose FILTER, then STYLIZE, then EMBOSS. This allows the selection of a height of between one and ten pixels, and adjustment of angle. Some surface details suggesting impact damage could be seen [fig. 2], and with an angle of 135^0 greater detail was visible [fig. 3].

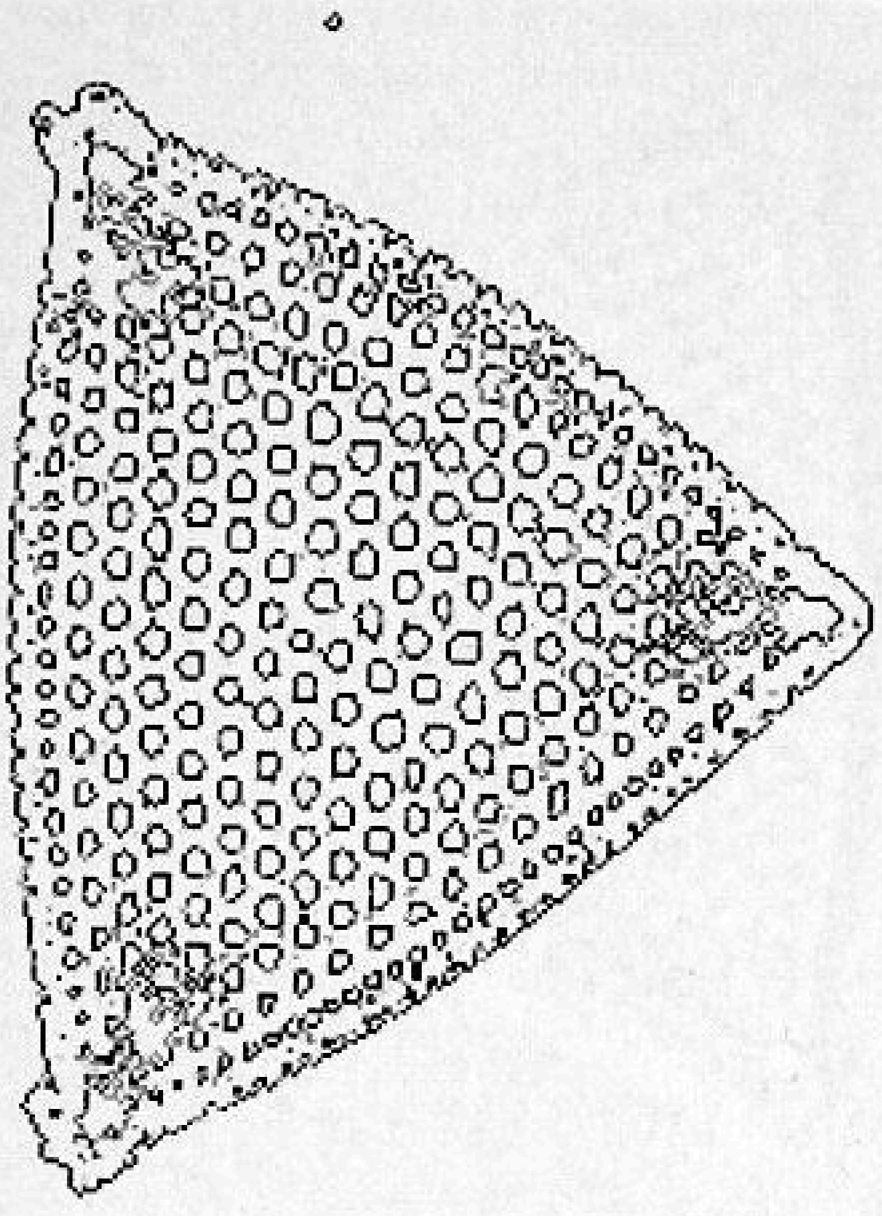

FIG. 5. As for figure 4, but for the lower edges.

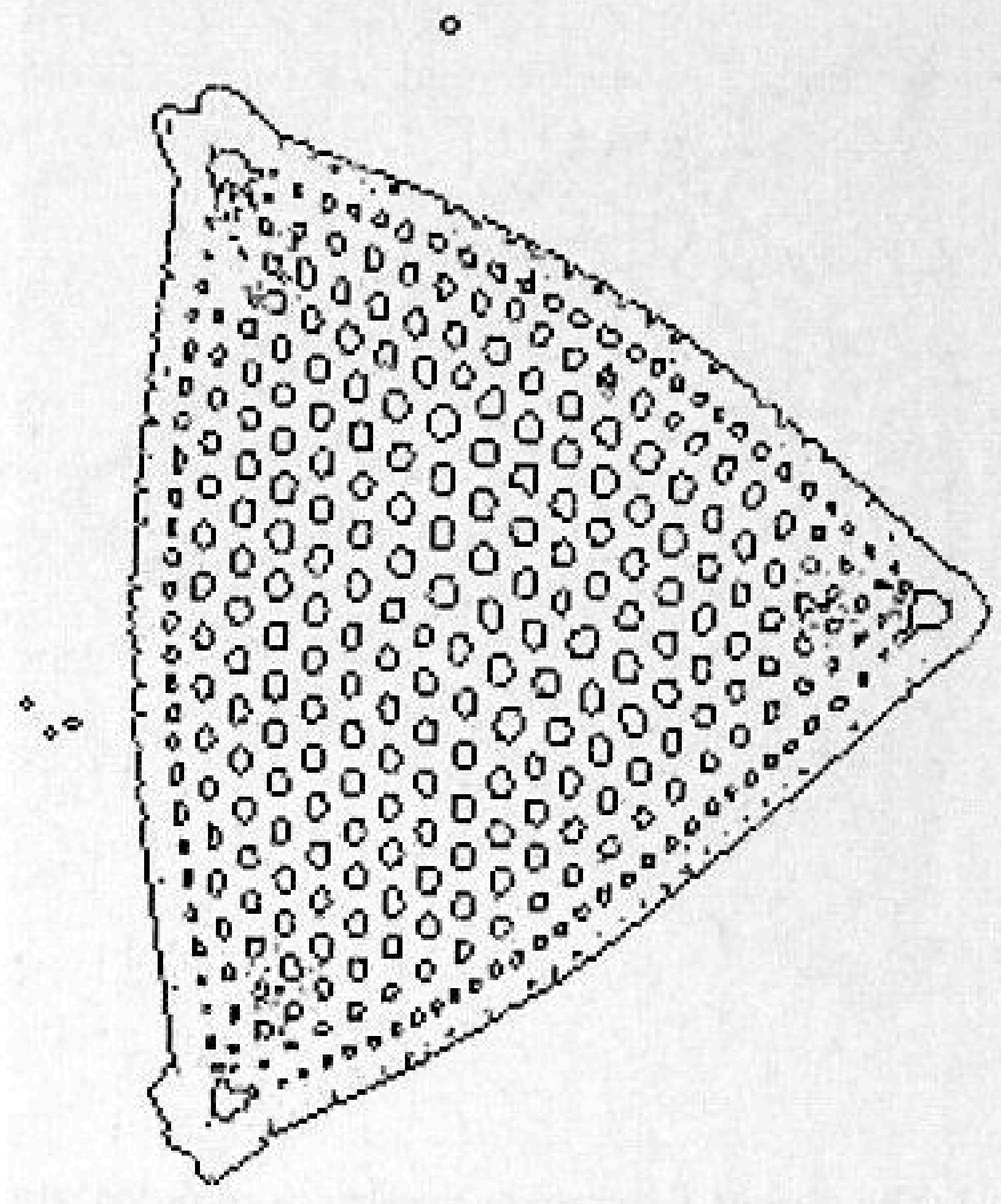

FIG. 7. As for figure 6, for the lower edges.

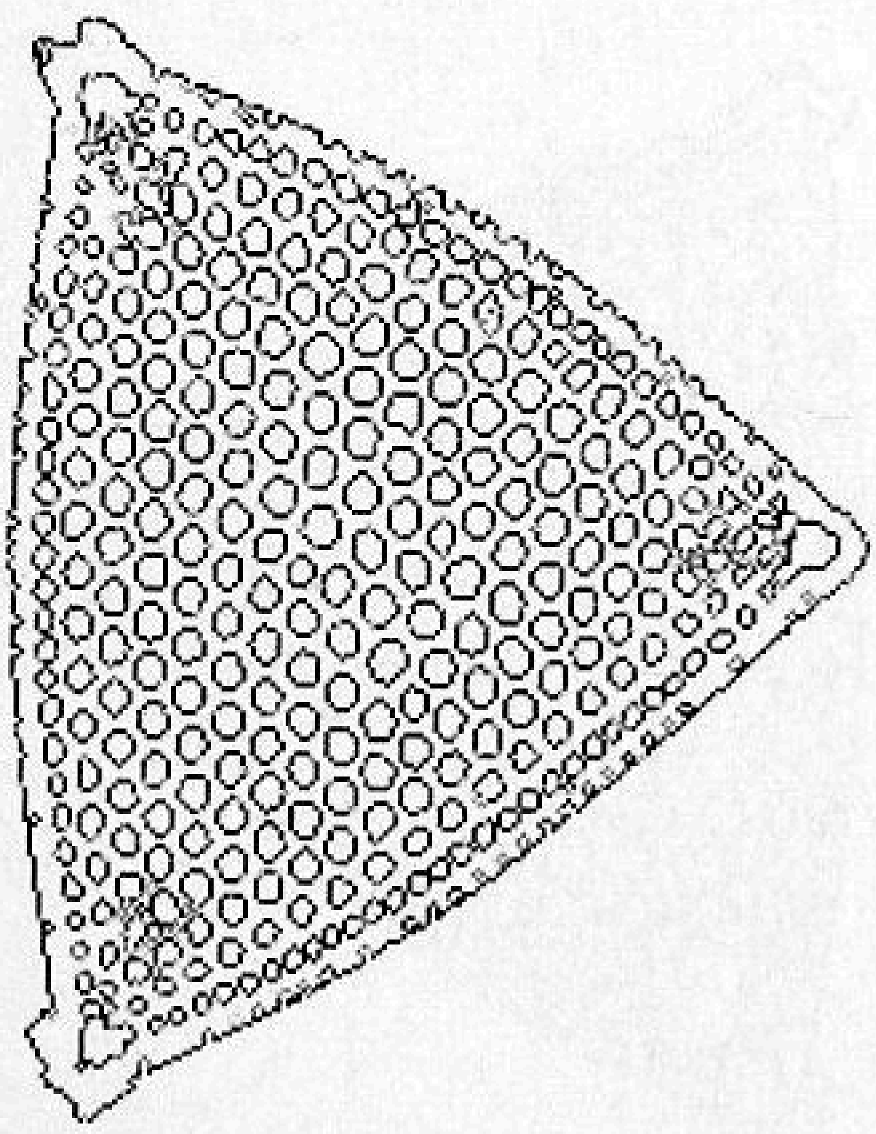

FIG. 6. Trace contour control applied again, but with a level of 220 for the upper edges.

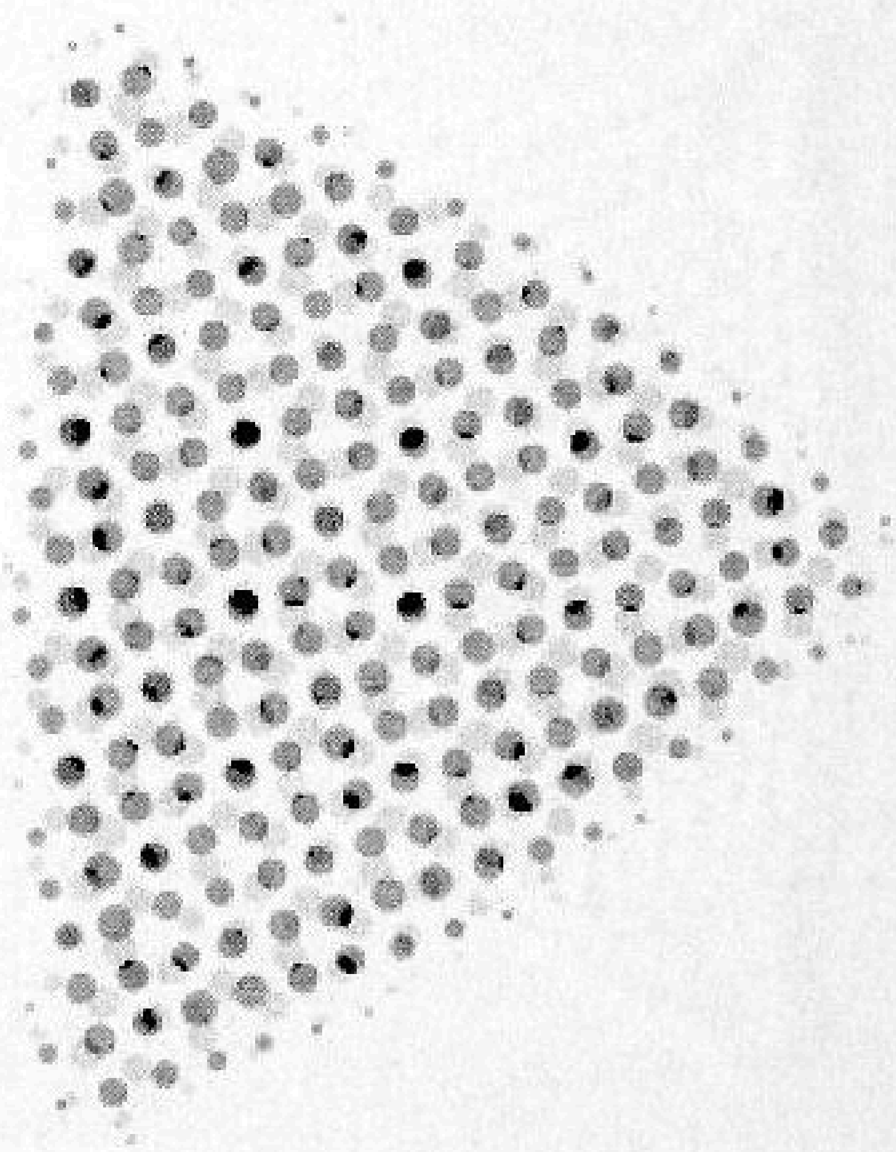

FIG. 8. Here the lower and upper edge trace contour images at the 220 level [figs. 6 and 7] have been superimposed.

The image was again processed, this time using the TRACE CONTOUR filter, which allows a level of outline tracing and selection of upper or lower edges of the specimen. With the level set at 170 [figs. 4 and 5] we can see interference at each corner, and similarly with the level set at 220 [figs. 6 and 7]. Now, if upper and lower edges at the 220 level are superimposed [fig. 8] we see a difference in area of the profiles, possibly suggesting a tapering within the holes.

These then are examples of image manipulation, of a relatively quite restrained extent. It is possible to manipulate images much more extensively, as reference to any of the many books on particular software will demonstrate. It is only too easy to produce an image which has little residual resemblance to the original. This is fine if one is working to make a design, but if one is working as an objective scientist it seems to me to be very important to observe two simple rules:

1. Always show the original unmodified image, and

2. State exactly how any modified results were obtained.

If these two simple rules are followed by all, amateur and professional, who publish their own photomicrographs, the objective nature of scientific recording will remain intact.

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