Scanning Electron Microscope Photography

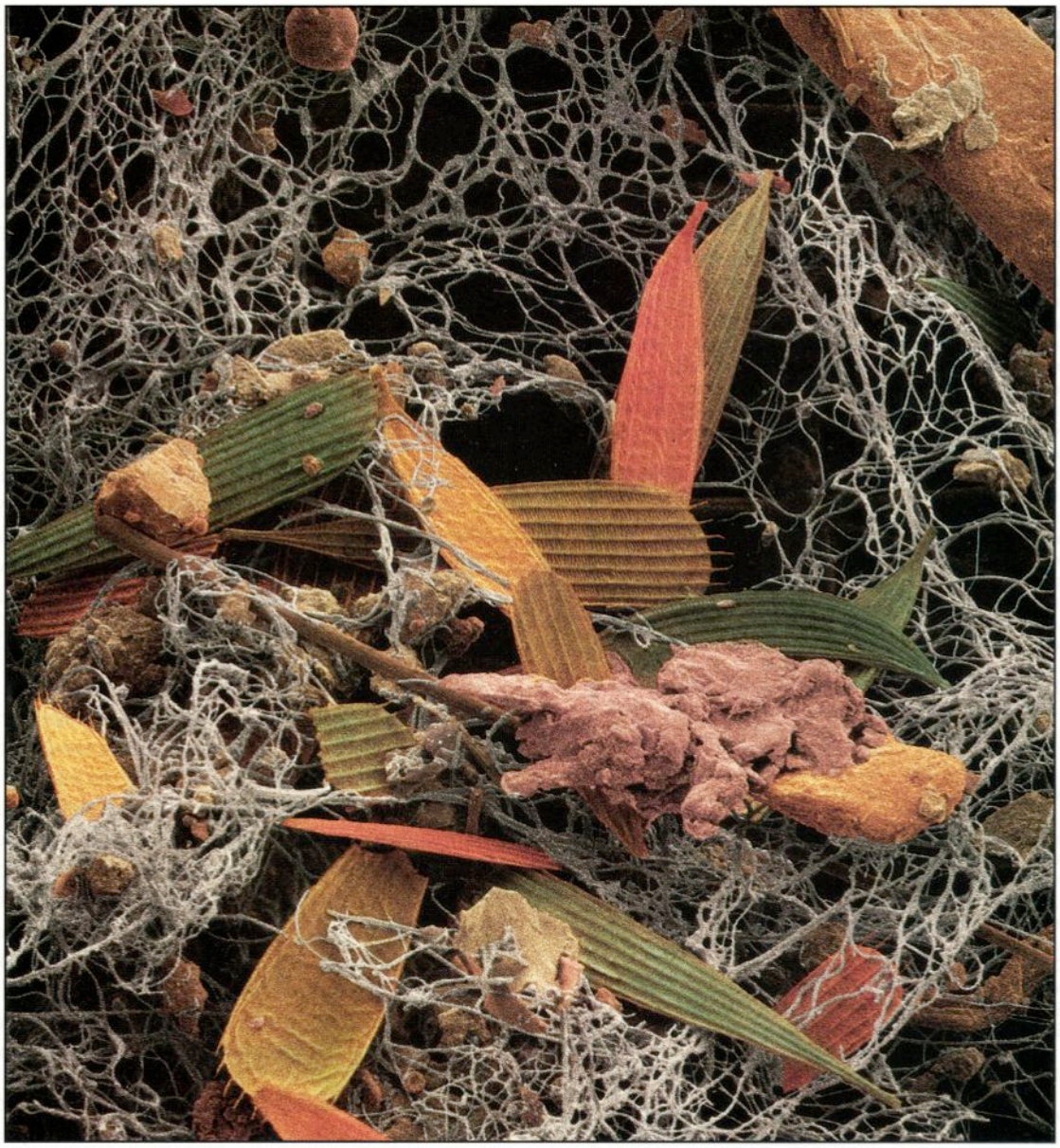
By

David Scharf

I suppose this would be considered by many to be the ultimate in high technology imaging. Most of my subjects are beyond the range of normal visual examination - they're too small - far too small. These subjects are illuminated with electrons rather than light, and the lenses used are magnetic rather than glass. As if that weren't enough, the process is taking place in a high vacuum chamber. My camera is a scanning electron microscope (S.E.M). The S.E.M. has the unique capability of being able to image objects from the very low macro range (around 1x to 4x) to the ultra micro range (from 100,000x to over 1,000,000x on the most powerful S.E.M.'s). Unlike the more historical, transmission electron microscopes (T.E.M.'s) which image thin sections of a subject, the S.E.M displays a three-dimensional, photographic-like image that shows the object's surface with great detail and depth of focus.

I began doing this type of work around 1973 while I was employed as a researcher in a vacuum physics laboratory. Working after hours, long into the night, I

Above - Mediterranean Fruit Fly - Male. Photograph © David Scharf, 1980.



Above - Dust Ball - Containing Spider Webbing, Moth Scales, etc. Photograph © David Scharf, 1986.

educated myself on the operation of the scanning electron microscope which was there in the lab. Photography had been a hobby of mine since I was nine years old, so I had most of the basic skills which were needed for the learning and development process I was undertaking. I really had no idea what direction I was headed in at that time. I just knew that it was something intriguing, fantastic, and irresistible. Little did I know that I would develop a new and revolutionary technique in the process of exploring and learning.

DESCRIPTION OF THE S.E.M

The basic principles of the operation of a scanning electron microscope begins with the formation of an electron beam at the top of the electron-optical column. This beam is projected down a tube which is surrounded by lenses - magnetic lenses. The first and last of these lenses are for the purpose of focusing the narrow electron beam to a very small point on the surface of the sample, which sits below this column in the vacuum chamber. The next to last magnetic field encountered by the electrom beam before leaving the column and hitting the sample is that of the scanning coils which serve to deflect the focused point of electrons across the sample horizontally and vertically - precisely the way a television writes its picture on the picture tube. Now we are scanning the beam across the sample - what happens here is that the primary electrons from the beam knock other *secondary electrons* out of the orbits of the atoms of the samples surface. These negatively charged electrons, knocked out into the vacuum, are then attracted to the positive charge of the nearby electron detector. There are a few different types of secondary electron detectors, the most popular of which works by attracting the electrons to a phosphor coated *scinillator* disc which is at a a high positive potential - around

+10,000 volts. When an electron hits the phosphor, its energy is converted to a flash of light; so a continuous stream of electrons would continuously modulate the light being emitted from the scintillator according to the amount of electrons being knocked off of the sample. The scintillator is attached to a solid quartz rod called a light pipe, which conducts the light out of the vacuum chamber to a very sensitive device called a photomultiplier. It may seem odd, but this device converts the light back into an electronic signal. Now, however, the signal is contained within electronic circuitry and has been greatly amplified by the photomultiplier (which, by the way, has a variable gain that serves to control the gain/brightness/contrast). The next part of this process involves processing the signal for variables like brightness and contrast and whatever other kind of processing that one may have the capability for. Now the signal goes to a view screen, or C.R.T. (cathode ray tube), where an image is formed and viewed. In the S.E.M., the magnification is changed by varying the scanned area, since the view screen and film size is constant. For instance, the scanned head of an ant just fills the frame; now I scan just one hair on its head to fill the frame, thereby increasing the magnification; and so on. Incidentally, there is no color involved, since the subjects are illuminated with electrons, not light.

Even though the foregoing explanation is a great simplification of a somewhat complex process, it can be further simplified by imagining the analogy of having a view camera set on time exposure in the dark of night - let's say, in front of a barn. One would then proceed to shine a flashlight on the side of the barn, starting from the top, going side to side and down until the entire side of the barn had been scanned. An image of this structure would thereby have been written onto the film.

PREPARARATION OF SPECIMENS

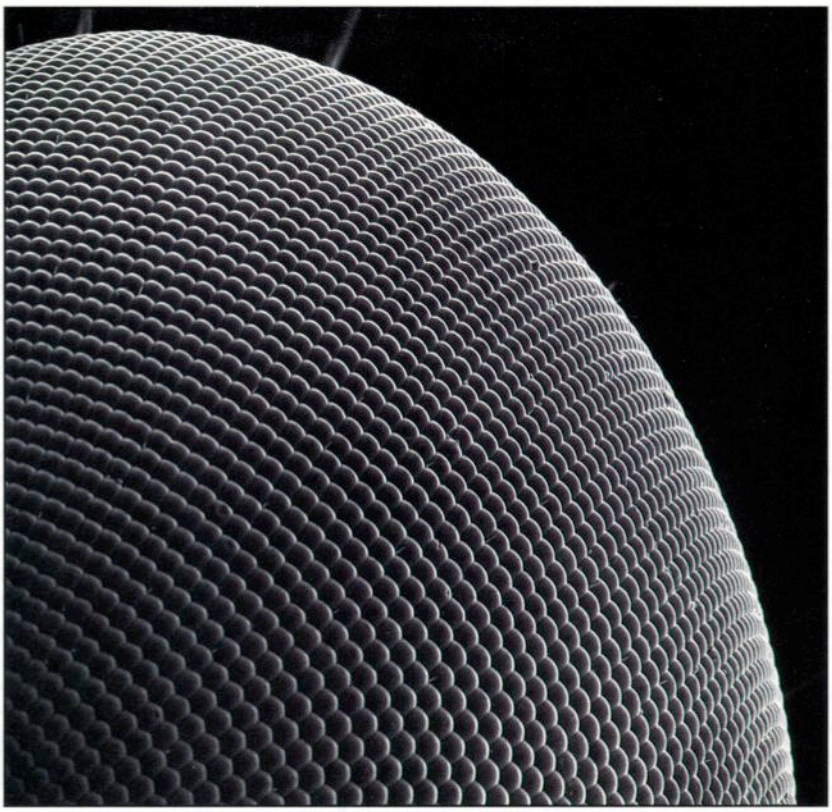
It is fitting here to mention that subjects viewed in the scanning electron microscope must be made conductive in order to dissipate the electrons which strike their surface. Traditionally, for biological specimens, this meant that they had to be killed (usually by chemical fixation), dehydrated (by substituting the water in the sample with pure ethyl alcohol by using a series of increasingly more concentrated baths and then critical-point drying), and lastly coated with a thin layer of conductive metal, such as gold (by a process of vacuum sputtering or evaporation).

During my early experimentation with the S.E.M., however, I developed techniques which allow me to image biolocigal specimens of insects and plants while they are alive and in their natural state by using the natural conductivity of living tissues - basically, by using a lower voltage electron beam (around 2 - 5 KV instead of 20 KV), setting up the equipment for maximum sensitivity, and working quickly. Many insect specimens survive the process and are released in the garden afterward. The difference in the imagery between dead and living subjects is spectacular and is one of the things that helps to set my work apart.

When I proceed to record a photograph from the S.E.M., I activate switches which send the image signal to a very high resolution record C.R.T. The image is scanned very slowly now (around 60 seconds for each frame) because it is writing over 2,500 lines for each plate and because slow scanning tends to average out electronic noise, which can be a problem and a limiting factor, especially at high magnifications. This part of the system is the film recorder. We are now finally out of the realm of electronics (usually) and into the art and science of photography.

The film recorder is a 1:1 camera for copying the image from the high resolution record C.R.T. onto film. My camera is equipped with an f1.9, 75mm Pi-Dynex lens from Opto Dynetics, Rochester, NY. The 75mm focal length gives sufficient coverage for my 3.5 x 3.5 image area to be recorded onto 4 x 5 film. The image of my subject in the S.E.M. is finely focused via three sets of focus controls, "coarse", "medium", and "fine", and astigmatism "magnitude" and "phase". However, it is essential that the film recorder have its own focus and astigmatism calibrated and set. This can be a very lengthy process if one is trying to squeeze every last bit of optical resolution out of the system. There are all sorts of voltages to be measured and set. The surface of the C.R.T. must be viewed with a 25x microscope when focusing the spot on the tube and the edge focus must be set independently. After the electronic focus is fine tuned, the critical focus distance must be set to the film plane - no easy task if you expect to get all of the information from the C.R.T. onto the film. Ground glass is not enough. The lens is opened to full aperture and focus approximated with the ground glass. Then a series of 10 to 20 plates are exposed, moving the film plane in .01" increments - half the plates are exposed moving out, and the other half moving in - recording and marking each plate. After development, the negatives are viewed under a microscope at 20x or so to find where the sharpness is at its peak. Sometimes it is easier to plot where the sharpness falls off and choose the point midway in-between. Fortunately, this process does not need to be done very often.

Early on, I experimented with many film/development combinations. My preference is Ilford FP-4 for most work. Kodak's Plus-X is almost as good (the image is about as good as FP-4, but I have experienced pinhole bits of emulsion flaking off here and there with Plus-X), and Agfapan 25 for those times when I do ultra high density scans (3,000 lines and over) - all developed in Acufine. I have found these to be very fine grain combinations and I appreciate the extra film speed contributed by the Acufine. (I am currently experimenting with Kodak T-Max 100, but haven't completed my tests yet.) Of course I don't use a light meter and once a standard development time is decided upon, the contrast and density can be adjusted with the film recorder's calibration controls. These will be only minor adjustments, because the C.R.T. has an optimum brightness range



for utilization of the maximum dynamic range of the tube's phosphors. The next and last adjustment is the f-stop. I end up using an f11 setting and developing the 4 x 5 FP-4 sheets in Acufine for around 8 minutes at 70° F using a Jobo daylight tank.

In 1980 I started "hand" coloring some of my photographs, using a combination of toners and masking. Since I had no clear idea of how to colorize photographs, I purchased every photographic toner available and various types of masking media and began experimenting. I discovered some very interesting interactions between toners. Most of these interactions were undocumented since the toners are from different manufacturers. Rather than trying to cover the entire subject, I will describe the process for the photographs accompanying this article only. However, common to all of my toned photographs is the use of a non-hardening fixer on Ilford Multigrade III R.C. (That's right, R.C.!) I use 11 x 14 paper, developed in Kodak Dektol at 2:1 dilution. The density and contrast needed for the print depends upon the toners to be used, since there can be either gain or loss of contrast, darkening or lightening. It is essential to run test prints and color test strips in order for me to form the strategy necessary to get the final result I am after.

After the 11 x 14 print has been toned, it is usually copied to transparency using the same lens that was used to enlarge it (a 135mm f5.6 Rodenstock Rodagon). I use daylight balance 4 x 5 Ektachrome 64 sheet film, illuminating with 4 strobes on the copy stand. Final color correction is achieved by placing CC filters over the strobes. I use polarizing filters over the strobes as well (orientation is critical), although a polarizing filter on the lens is not ususally necessary for control of stray reflections. Contrast control is achieved by "flashing" the film with appropriate neutral density filters and a white sheet in place of the photograph.

David Scharf is a scientist and a professional freelance photographer in Los Angeles, CA specializing in S.E.M. photography.

Above - A Fly's Eye (Lesser Housefly). Photograph © David Scharf, 1977.

Toning Information: Medfly Portrait (male)

1. Toned entire print short time in Berg Golden/Yellow. Not too yellow.
2. Toned entire print short time in Kodak Sepia (bath "B" no bleach.
3. Masked body and background black, leaving eyes bare.
4. Toned eyes in Berg Brilliant Blue.
5. Highlighted eyes in diluted Berg Red-1 on cotton Ball.

Toning Information: Dust Ball with Spider Webbing, Butterfly Scales, and Dirt

1. Soaked entire print in Berg Activator.
   (Activator + Sepia or +K. Brown = shades of brown.)
2. Masked webbing and things to be shades of brown.
3. Toned in Berg Copper/Brown for dirt to be copper color.
4. Mask things to remain copper color.
5. Q-tip Berg Colors on scales.
6. Mask off things to be (Activator + K. Brown) shades of brown.
7. Toned in Kodak Brown (to completion).
8. All masking off.
9. Re-masked spider webbing and things to remain copper and Activator + K. Brown shades. Leave exposed, things to be other (Activator + K. Sepia) shades of Brown.
10. Toned in Kodak Sepia (solution B, no bleach).