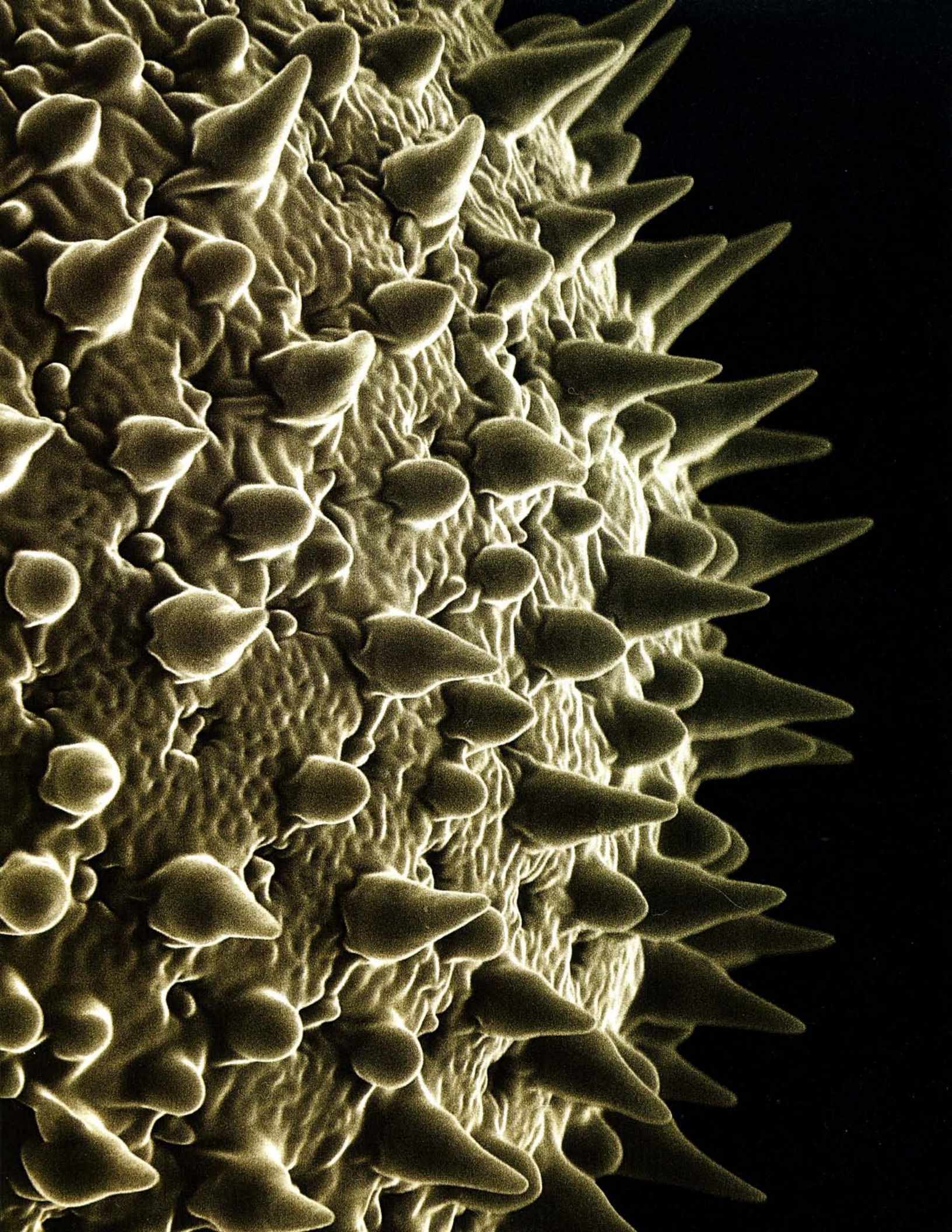


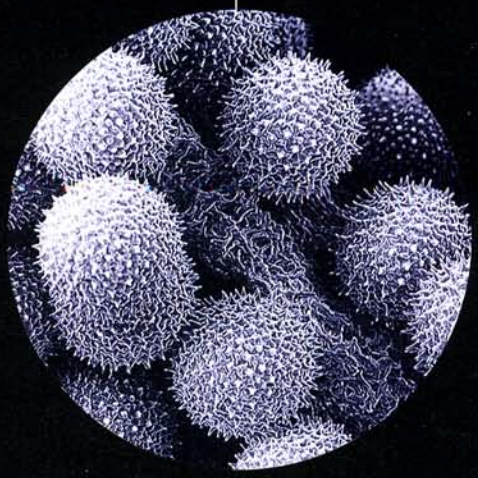
Micro Worlds:

Scripps Analytical Facility Provides New Views of Science

One would be hard-pressed to name an instrument that has advanced knowledge of the natural world more than the microscope. With it, 17th-century naturalists revealed organisms that were, until then, too small to see, thus pioneering the exploration of the microbial world.

Modern developments in science and technology continue to advance knowledge at an ever-accelerating rate. With the scanning electron microscope (SEM) located in the Scripps Analytical Facility, UC San Diego scientists can magnify objects up to a half million times their size; this is many thousands of times greater than the capabilities of even the most advanced light microscopes.

In a conventional microscope, light rays are reflected by the object being observed, and the image produced is magnified and focused through a combination of lenses. The SEM, first developed in the early 1960s, projects a beam of focused electrons across an object's surface. Due to the much shorter wavelength of electrons compared with visible light rays, images are resolved to a much higher degree under the SEM, with a greatly improved depth of field. For this reason, highly detailed images of very great magnification can be produced, with uniform focus over the entire surface of the object. This combination of three-dimensionality and very high magnification power sets the SEM apart from other investigative tools.



Hollyhock pollen as imaged using the scanning electron microscope. **Facing page,** Shown 4,625 times actual size; **Above,** Magnified 170 times.

The Scripps scanning electron microscope, one of only two at UC San Diego, shares space in the Scripps Analytical Facility with many other specialized pieces of equipment, all of which are available to both Scripps and other UC San Diego researchers. According to Kevin Walda, director of the facility, "Our mission is to advance science by providing researchers with access to and training on state-of-the-art equipment. The main purpose of the training we provide is to help researchers get their science done."

BY JOE HLEBICA

On a day-to-day basis, graduate students compose the single largest group using the facility, which also is valued by postdoctoral researchers and faculty. Time demands and limited access mean that the scanning electron microscope is booked eight hours a day, five or more days a week.

Because Scripps provides good accessibility to its SEM, many researchers from other departments at UC San Diego rely on the Scripps facility. One such person is UC San Diego postdoctoral researcher Cristina Ferrandiz, a plant biologist from Spain.

Ferrandiz is studying the development of plants of the genus *Arabidopsis*, a member of the mustard family, comparing mutants produced through genetic manipulation in the laboratory to wild plants. Applications of her research may include improvements in agriculture—providing greater yields over shorter generations in a variety of flower- and fruit-producing plants.

Arabidopsis has one of the smallest flowers of any plant; Ferrandiz is concentrating on the reproductive organs within the flower. According to her, "It is a good plant for laboratory study because it is small and produces many generations over a relatively short time." Even though it is visible to the naked eye, and large enough to be easily examined under a light microscope, the flower is still best viewed through the SEM because it can be studied intact.

"We are looking at irregularities in the structures of the organs. These irregularities are important to the understanding of differences between our mutants and wild specimens. If we use a light microscope, we must cut the flower into flat sections, and if we do that, important characteristics are destroyed," says Ferrandiz.

This illustrates a basic drawback of viewing larger objects with a conventional light microscope, because a sample must be flat and thin enough to mount on a slide and also must be permeable to light. The SEM does not require such preparations.

Ferrandiz has worked in other facilities, and she is quick to point out that the Scripps Analytical Facility "is a very good place to work because the hands-on software is so easy for me to use and the facility is so accessible."

Charles Graham is the SEM operator. He explains the essential steps in the preparation of a biological sample, such as Ferrandiz's flowers, for viewing. "Most tissue samples must go through a special preparation process that includes chemical fixing and dehydration before being placed in the vacuum chamber." He refers to the compartment at the base of the SEM where samples are placed for viewing. "A filament produces the electron beam, which can only be generated in a total vacuum, the same principle as in a light bulb. Biological samples need special preparations to hold up under the conditions of a vacuum."

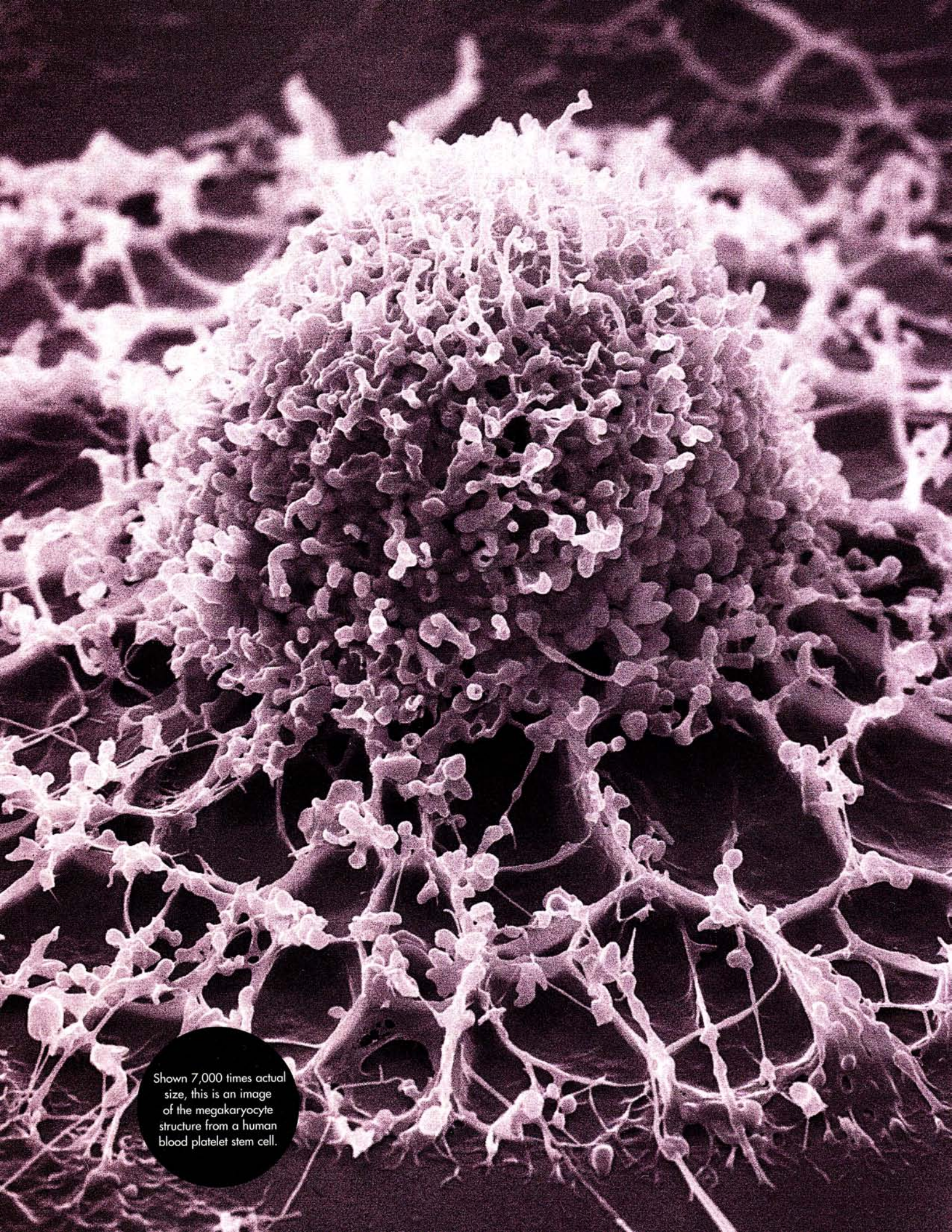
Walda is working to acquire a new type of instrument, called an environmental SEM, which allows biological samples to be observed in a more natural state with less preparation and variable dehydration. With this instrument, according to Walda, "The hydration [water content] of samples can be varied so that we can start looking at dynamic biological processes, rather than at lifeless samples frozen in time. This will change the SEM's role from that of a static observational tool to one that will allow us to monitor changing environmental processes."

"The main purpose of the training we provide is to help researchers get their science done."

Although most samples examined with the SEM are not biological, practically all samples require preparation of some kind. One necessary step, in most cases, is a process in which the sample is coated with a thin layer of a highly conductive precious metal such as gold or palladium. This reduces the charge on the sample's surface so that the electron beam is evenly scattered rather than concentrated on a single spot.

The resulting images, viewed on a computer monitor, can be startling in their clarity, detail, and magnification. They also can be manipulated a number of ways using the computer program that

Above, Scripps Analytical Facility director Kevin Walda instructs graduate student Mark van Zuilen in the use of the inductively coupled plasma/optical emission spectrometer.



Shown 7,000 times actual size, this is an image of the megakaryocyte structure from a human blood platelet stem cell.

An *Arabidopsis* flower
shown 170 times actual size.

operates the microscope. Magnification can be changed, sections can be isolated, various measurement scales can be placed on the images, and X rays can be generated to examine the elemental composition of samples.

Peripheral features of the SEM include a Polaroid camera for taking instant snapshots of images and a computer disk for digital storage of images. Intricately colorized versions also can be generated.

"The SEM is the one machine everybody loves because you can walk away from it with a photograph of what you were looking for. Students using our facility have provided images that have appeared on the covers of such noted science journals as *Nature* and *Science*, and even on popular magazines like *Time*," Walda points out proudly.

But the SEM serves purposes other than image capture. For Mark van Zuilen, a Scripps graduate student from the Netherlands who is working with renowned Scripps geochemist Gustaf Arrhenius, the SEM is but one step in a long investigative process.

Van Zuilen is examining graphite flakes he has extracted from a sample of one of Earth's oldest known rocks. Found by Arrhenius in southwest Greenland during the summer of 1997, the rock is 3.7 billion years old. It was collected as part of a project in which Arrhenius and others were searching for evidence of Earth's earliest life forms.

Van Zuilen uses the SEM to check the purity of the graphite flakes he has extracted. The next step is to determine their composition through a spectral analysis using an instrument known as the ICP/OES (Inductively Coupled Plasma/Optical Emission Spectrometer). The goal is to find out whether or not the graphite is biogenic, that is, whether it provides evidence of life.

"The SEM is really good for taking a closeup look at a sample like this and determining how well I've managed to dissolve all other mineral phases to acquire a pure graphite flake," explains van Zuilen.

As he uses the SEM keyboard, the enthusiastic student explains that the rock came from a marine formation known to have been deposited when the ocean was very young. "We believe biological materials are capable of concentrating specific trace metals, and we speculate that a biogenic graphite would contain these metals, while a nonbiogenic graphite would not. We're looking for these metallic biomarkers in the rock."

He points out that fossil evidence suggests that the window in time during which life first emerged on Earth was relatively brief, somewhere between 3.8 and 4.5 billion years ago. "This rock is right at the edge of that window."

"If we use a light microscope, we must cut the flower into flat sections, and if we do that, important characteristics are destroyed."

Van Zuilen is joined in the ICP/OES laboratory by Walda, who will coach him in the use of the equipment. Walda explains how it works.

"Essentially, the machine heats up the elements in a sample to the point where each element emits its own unique wavelength of light. Separating the elements by wavelength, the machine then indicates on the monitor the identity and amount of each element present. It's unique among machines of this type because it can simultaneously identify and quantify a variety of elements in a sample."

This capability is invaluable to van Zuilen because he is looking for a large number of trace metals, among them iron, cobalt, nickel, zinc, and copper. These are all potential evidence of biogenic activity written in rock billions of years old.

In order to prepare his graphite flake for analysis in the ICP/OES, van Zuilen has burned the flake to

Above, UCSD department of biology postdoctoral researchers Elena Alvarez-Boyalla (left) and Cristina Ferrandiz examine tiny flower structures on the SEM.

produce an ash, and this ash has been dissolved in highly concentrated acids. As vials containing these acid solutions are loaded onto a tray, Walda explains how the clear solutions are pumped into the machine. A plasma chamber of highly durable material receives the solutions in a very fine spray mist. There they are incinerated by an argon torch at a temperature of 10,000° Kelvin, one and one-half times the temperature of the Sun's surface. Nothing remains but the identifying glow of metal atoms, indiscernible to the eye but unmistakable to the machine.

The spacious Nuclear Magnetic Resonance (NMR) installation, another important part of the Scripps Analytic Facility, is found downstairs from the small ICP/OES lab. Unlike the ICP/OES upstairs, the NMR allows a researcher to determine the structure of a compound, not merely what it is made of. Because of this capability, the NMR facility is very important to research chemists and is heavily used by them, up to 20 hours a day, six days a week.

"Structures influence properties. It is very critical to know the structure of a compound. One small change in a compound's structure can drastically alter its properties," says Walda.

Walda is quick to point out that a researcher must interpret the information a machine provides even when working with a device as advanced as the NMR. Many of the facility's machines are equipped with search-and-match software. However, Walda asserts, no software yet devised can replace a trained operator.

"A computer just doesn't have the intuition of a human. It may be hundreds of years before computers can be programed to search and match compounds as effectively as a trained operator.

"Graduate students often come to the facility with their minds made up that they are going to see one thing, and we suggest that maybe they should be looking for something else. By modifying their procedures, they can still achieve their goals, and perhaps do so more effectively. Rarely is the premeditated strategy the best one, and then often only as a result of luck."

New developments in analytical technology and methods continue to revolutionize science, just as the first generation of microscopes did centuries ago.

One investigative technique notably advanced by analytical facilities such as the one at Scripps is that of gene sequencing. Sophisticated DNA analysis is helping microbiologists understand the evolutionary relationships among bacteria, for example. In the case of much larger organisms, such as dolphins and whales, this type of analysis has allowed precise identification of specific populations, information essential to understanding the ecology of these and other threatened groups.

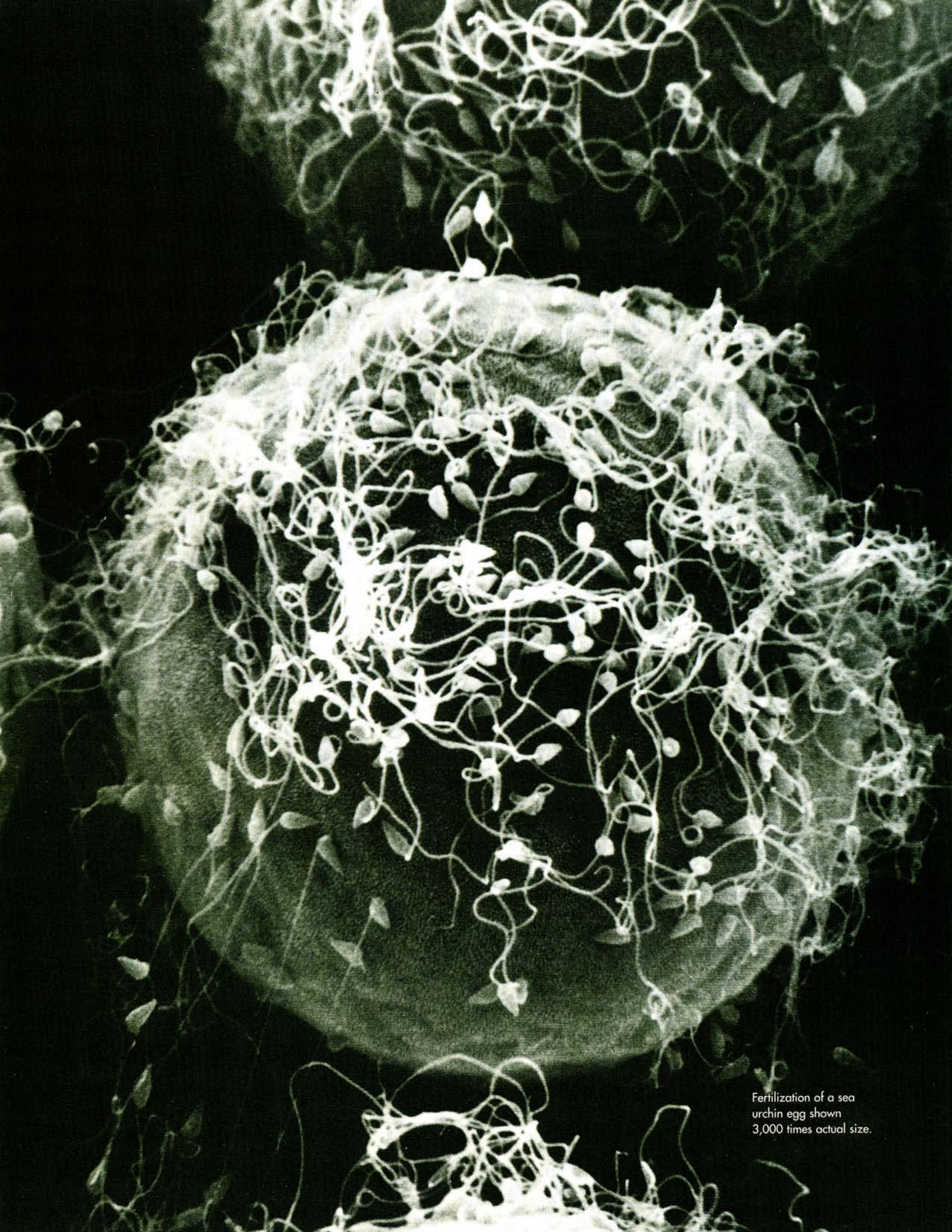
While instruments such as the SEM, ICP/OES, and the NMR can provide spectacular results, which have earned them the technological equivalent of celebrity status, all of the various equipment and procedures available to researchers in the Scripps Analytical Facility are contributing to the advancement of science.

"There is no magic wand in this field, not one machine that does it all. Each provides a piece of the puzzle."

"There is no magic wand in this field, not one machine that does it all. Each provides a piece of the puzzle," concludes Walda.

A complete listing of the instruments available in the Scripps Analytical Facility can be found on the Scripps Web site at http://sioaf.ucsd.edu/.

Above, Analytical Facility research associate Charles Graham uses this device to coat samples with reflective metal in preparation for viewing on the SEM.



Fertilization of a sea urchin egg shown 3,000 times actual size.