Strange New Worlds Come into View Through Electron Microscopes

Expansion of the Samuel Roberts Noble Laboratory has given the University's scientific faculty the capability of developing an important research center, while Sooner students are receiving invaluable hands-on training with sophisticated equipment.

By DIANNE BYSTROM

New worlds are unfolding under the beams of electron microscopes.

Simplest forms of life such as viruses, which could not be seen before, can be examined visually with transmission electron microscopes. Scanning electron microscopes provide scientists with vivid, seemingly three-dimensional pictures of surfaces which are often quite beautiful in their explicit topographical detail.

Such microscopes, which have helped advance scientific research for over 20 years, now are available to University of Oklahoma faculty and students through the recent $500,000 expansion of the Samuel Roberts Noble Laboratory of Electron Microscopy. New equipment and research facilities provided by the lab will help place OU in the forefront of scientific research.

"With our new equipment, we'll be up with anybody in the United States," said Dr. John Skvarla, OU professor of botany and director of the laboratory. "Electron microscopy has become so important that a good university can't function without some type of electron microscope. Many universities don't have the type of equipment that we have at OU. We're really first class."

Magnified approx. 3,600 times is Zoologist Paul Bell's transmission electron microscope view of the cytoskeleton of a whole chick embryo. Large dark object is the cell nucleus.

Through a grant from the Samuel Roberts Noble Foundation of Ardmore, OU has purchased two new transmission microscopes and a scanning electron microscope with an X-ray attachment to enhance its capabilities. Also purchased were three ultramicrotomes to slice extremely thin tissue specimens and an image analysis system to assist in analyzing results recorded by the instruments.

In addition, the grant funded construction of a 10-room second-story facility on top of the animal laboratory, located between the botany-microbiology and zoology buildings at OU. The new laboratory, which opened in February, houses both the new equipment and a scanning electron microscope inherited from the Oklahoma Geological Survey.

The additional microscopes will allow students, as well as faculty, to conduct research.

"Prior to getting the money, the electron microscopes that we had were not available to students," Skvarla explained. "Now, one of the transmission microscopes and one of the scanning microscopes will be designated for student training, while the others will be used for faculty research.

"This hands-on training with electron microscopes will not only help our students with their research, it also will give an added dimension to their marketability. Knowing how to operate an electron microscope will enhance their chances of finding jobs."

Electron microscopes have been available to OU faculty since the 1940s, when the Noble Foundation answered the appeal of then-President George Lynn Cross to supply the first piece of equipment.

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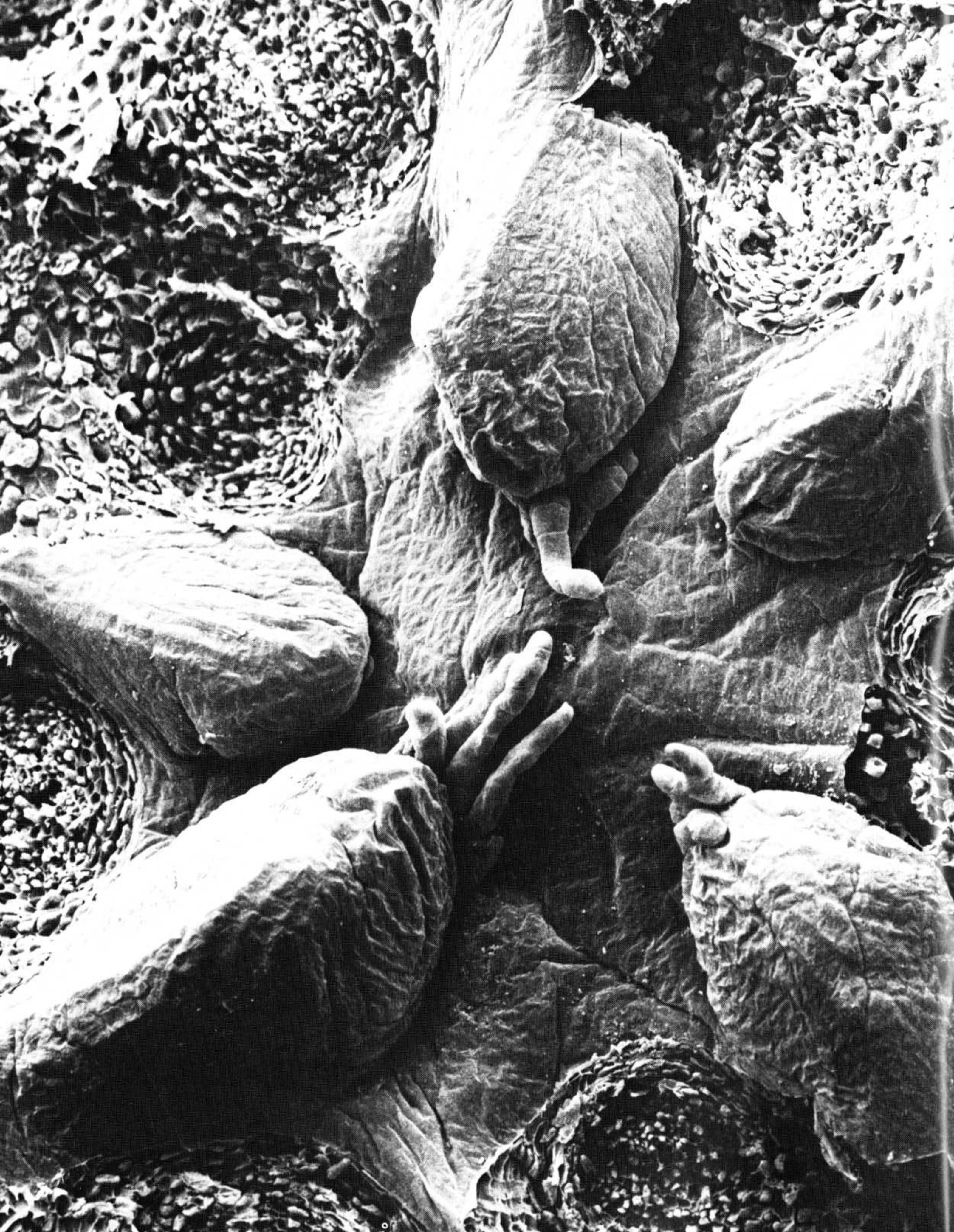

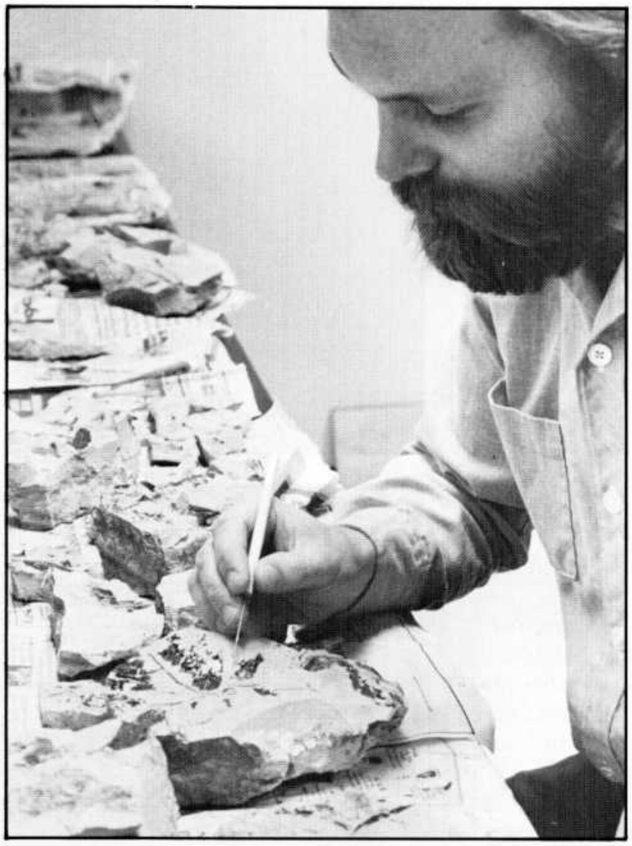

Left, OU Botanist Charles Daghlian carefully removes fossil pollen from rock, where it has been embedded for millions of years, for study under the electron microscope. On the opposite page is a fern shoot apex, magnified, 1,160 times, showing the 5-leaf primordia, some with hairs. The smooth, low dome of cells is the apical meristem.

"During the 1960s, when electron microscopes were becoming important, Dr. Cross helped obtain funds to buy a modern electron microscope for OU," Skvarla said. "He envisioned establishing a lab that would really train students in electron microscopy."

The lab was opened officially in 1965 and was named in honor of Samuel Roberts Noble, the father of OU Regent Lloyd Noble, the Ardmore oilman who had established the Noble Foundation. Even after his retirement, Cross continued to promote the laboratory's cause and was instrumental in obtaining the recent Noble grant.

The OU Provost's office became another important ally of the electron microscopy lab. "The provost's office has been marvelous in making the lab go," said Skvarla, who was brought to OU in 1965 to direct the Noble laboratory's activities.

Since becoming available to scientists, electron microscopes have helped advance research beyond the realm of light microscopes such as those still used today in many high school laboratories.

"Prior to the 1960s, the main way we had of looking at cells was through a light microscope," Skvarla said. "Such microscopes were good, but they did not have the clarity nor the ability to distinguish between very fine structures."

At best, light microscopes magnify a specimen 1,500 times that seen by the human eye. Transmission electron microscopes can magnify specimens 600,000 times while scanning electron microscopes have a potential magnification power of 100,000 times.

"Under a light microscope you could see a sphere, but you couldn't see a surface pattern," Skvarla explained. "Now, all of a sudden, you can look at a specimen, and its surface pattern might look like spaghetti. We have the ability to see specimens much sharper."

Electron microscopy is a "sophisticated extension of light microscopy," he added, and provides visual access to the world between cellular and molecular levels. "We still use light microscopes. It's another means of getting data."

Light microscopes, which usually are desk size, use a light bulb source

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and glass lenses in magnifying specimens. Electron microscopes, which often weigh more than 2,000 pounds and sit on the floor, provide great magnification and clarity through the use of electrons and electromagnetic lenses.

In an electron microscope, a narrow beam of electrons is produced in an electron gun at the top of a vacuum column and is then focused on a specimen placed on the other end. Along its path, the electron beam passes through several electromagnetic lenses.

The electron beam passes through the specimen in a transmission microscope, scattering electrons in the process. Electrons scattered off the target provide contrast in the specimen, which is imaged on a fluorescent screen. The image is viewed by looking through a microscope eyepiece.

In a scanning electron microscope, the electron beam repeatedly scans the specimen and forms a raster — as seen on a television set. When the beam hits the specimen, it interacts with it and kicks out a second family of electrons and X-rays. A picture of the specimen is produced by a cathode-ray tube, similar to one used in a television set, and shows up on a black-and-white screen.

Both types of electron microscopes have cameras to record the specimen image.

While transmission and scanning electron microscopes operate basically the same, each has different uses and advantages in pursuing scientific research.

"Transmission microscopes have great magnification and great clarity — or resolution," Skvarla said. "They are used to analyze and get a one-dimensional picture of the inside of tissues taken primarily from biological materials." Tissue slices viewed through a transmission microscope must be very thin, "so thin that you would need a microscope just to see them. Learning how to make the slices is tedious work. It takes several months to learn the process."

The lab's new ultramicrotomes are used to make the tissue slices. An automated device, the ultramicrotome uses a two-millimeter-long cutting knife of either glass or diamond to cut a thin slice from a mounted specimen. After being cut from the specimen, the tissue floats on a small channel of water located behind the cutting tool. Small, round copper discs are used to retrieve the tissue from the water.

With another piece of equipment purchased with grant money, the scientists are able to make glass cutting knives used for slicing biological materials. The diamond knives — which are used to slice harder specimens — cost about $3,000 to $5,000 apiece and must be replaced when they wear out.

"We spend a lot of time in specimen preparation," said Dr. Mary Whitmore, an OU assistant professor of zoology who helped write the grant proposal for the electron microscopy laboratory. After a tissue specimen is sliced, it must be stained to enhance its contrast. Preparation techniques vary, because of the physical and biological properties of the specimen. "We try to preserve the specimen's characteristics as near normal as possible by careful fixation," Whitmore said. "It's a real challenge, but very exciting."

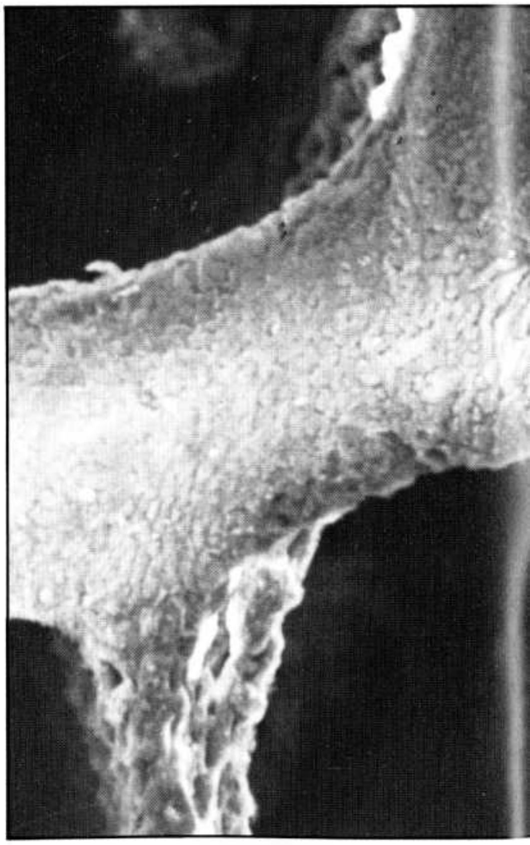
While transmission microscopes look through thin slices of tissues, scanning electron microscopes are used to analyze specimen surfaces and content.

"It's a really neat sort of system," Skvarla said. "With the scanning electron microscope you can get a three-dimensional picture of a variety of surfaces ranging from biological materials, to rocks and highway road samples, to the surface of a razor blade. And, by hooking up the X-ray attachment, you can identify and locate various elements contained in the specimen — gold, aluminum particles, silver, copper, whatever."

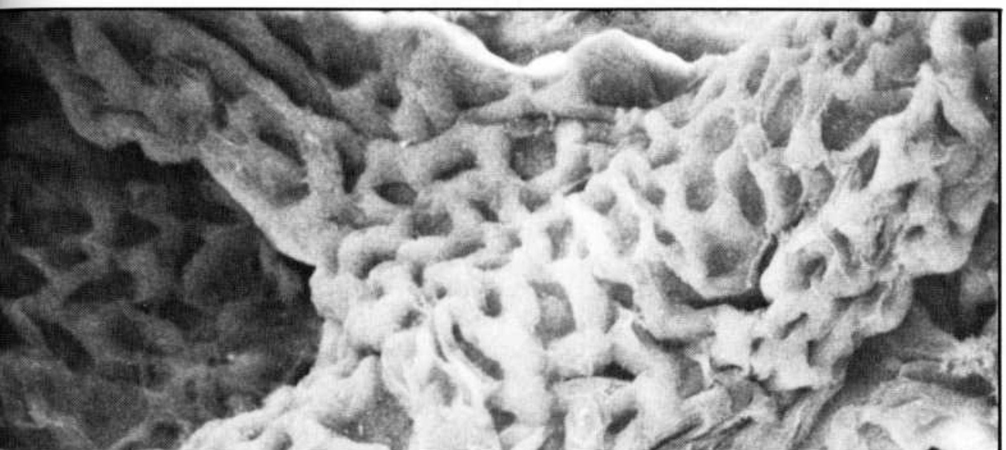
Researchers in Texas have used the scanning electron microscope to analyze the content of mitochondria bodies found in the cytoplasm of all cells, Whitmore said. "They have found that calcium builds up in the mitochondria after a heart attack."

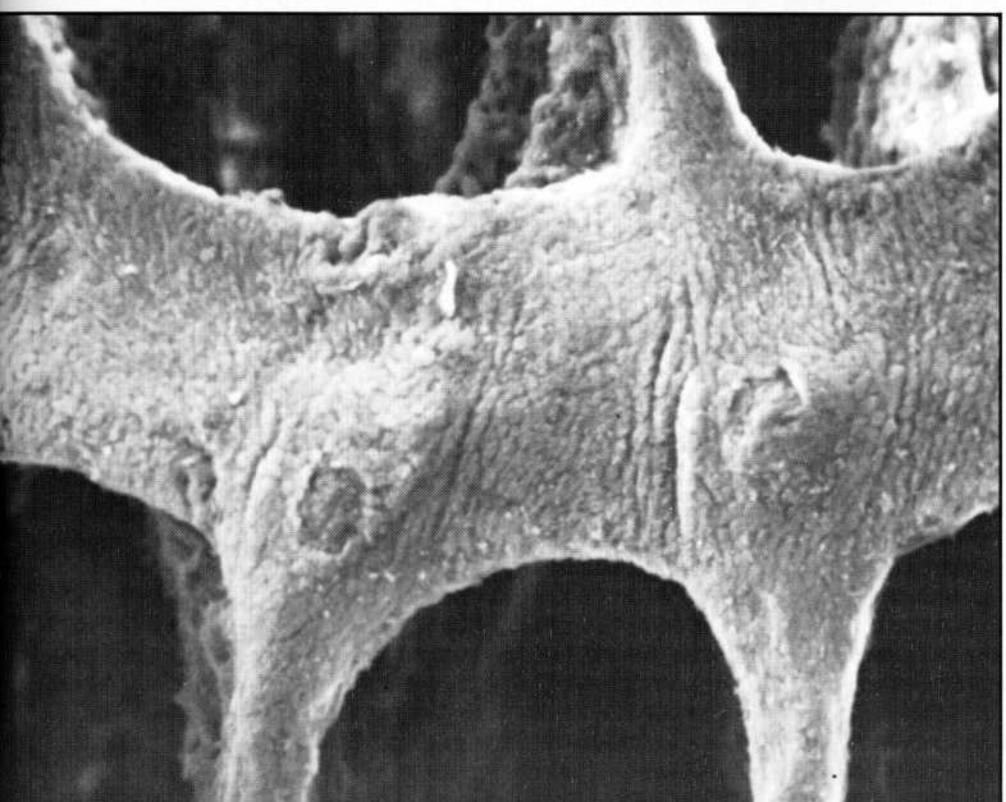
Scanning electron microscopes also are used by law enforcement agencies to analyze hair, skeletal remains and poisons. Space researchers examined rock samples from the moon under a scanning electron microscope.

"The great thing about a scanning microscope is that you can analyze a specimen without destroying it," Whitmore said. In addition, specimens don't require elaborate prepara-



(1) Botanist John Skvarla and Zoologist Mary Whitmore operate the new scanning electron microscope, which Whitmore used for two views of the interior of snake lungs, showing the surface of posterior air sacs at (2) approx. 1,680 times and (3) approx. 4,800 times actual size.





(4) Another Whitmore photograph of the snake lung interior shows detail of the respiratory surface, illustrating the capillary network, magnified approx. 744 times. (5) The "honeycomb" spaces of the lung appear in this low-power view, 400 times actual size.

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Mary Whitmore's transmission electron microscope view of nerve fiber, showing layers of myelin (fatty material) is magnified approx. 105,300 times.

tion before they are scanned. Generally, non-living materials require a cleaning, mounting and coating with a conductive material. Plant and animal cells can be viewed in their living state, but are often dehydrated in a critical point dryer.

"The dryer enables us to freeze dry cells and keep their normal contours," Whitmore said. "Instead of drying a cell by air, they are freeze dried under pressure using carbon dioxide."

Specimen preparation and analysis can be completed rather quickly using a scanning electron microscope. "We could have an analysis of a highway road sample out in an hour," Skvarla said.

On a limited contract basis, to help defray expenses, the OU lab has offered its services to public and private enterprises. "We have analyzed road samples for cracks and pores for the Oklahoma Highway Department and drilling samples for oil companies," Skvarla said. "We also have analyzed the remains of human skulls. Through this service work, we are able to underwrite some lab costs."

The service work is performed by Bill Chissoe, chief laboratory technician, who has been with OU for 11 years. Chissoe, who has an office in the new facility, also maintains the instruments and assists in training.

Most of the work of the lab is devoted to the research efforts of OU botanists, microbiologists, zoologists and geologists. "With the basic equipment in the lab, we hope to respond to anyone's research needs," Whitmore said.

"A central laboratory with versatile equipment . . . no other place in Oklahoma has the quality and number of instruments."

Skvarla uses the electron microscopes to compare tiny grains of pollen — both modern pollen and fossil pollen found in rocks. Through this comparison, he hopes to learn more about the evolution of plants.

"I'm involved in a classification study of pollen," he said. "I've found that you can separate closely related plant groups on the basis of their pollen. If we can compare the modern pollen with the fossil pollen, we may be able to trace the history of plants through time."

Whitmore, who has an office and laboratory in the new electron microscopy facility, uses the equipment in her comparative study of lung tissue. "I'm interested in the evolution of lungs in amphibians, reptiles and fish," she said. "I look at the structure of the lung tissue, try to interpret it and determine how it is related to other lung tissues."

Three other zoology professors currently are using the electron microscopes in their research. The structure of fruit flies is being examined by Jim Thompson while Paul Bell is looking at the cyto-skeleton of tissue cells. Tim Yoshino is examining blood parasites which cause schistomiasis, a debilitating disease which afflicts humans. OU geologists plan to use the electron microscopes to examine fossil remains and skulls.

The new electron microscopy lab will help OU recruit other researchers and faculty members. "Now that we have the facility, we can recruit faculty around it," Skvarla said. The new facility already has attracted the services of Charles Daghlian, an electron microscopist and botanist. Daghlian, who will study pollen with Skvarla, has his office and laboratory in the new facility.

With the expansion completed, the Samuel Roberts Noble Laboratory of Electron Microscopy is on its way to becoming a major research and teaching center.

"No other place in Oklahoma has the quality and number of instruments that we have," Whitmore said. Other universities in the region — such as the University of Texas may have more electron microscopes than OU, but their equipment is scattered throughout various departments.

"We're building a central laboratory with versatile equipment," Whitmore said. "We anticipate providing space and facilities for researchers, faculty and advanced students from all areas in the biological sciences. Research activities and training will be the primary goals of the new laboratory."