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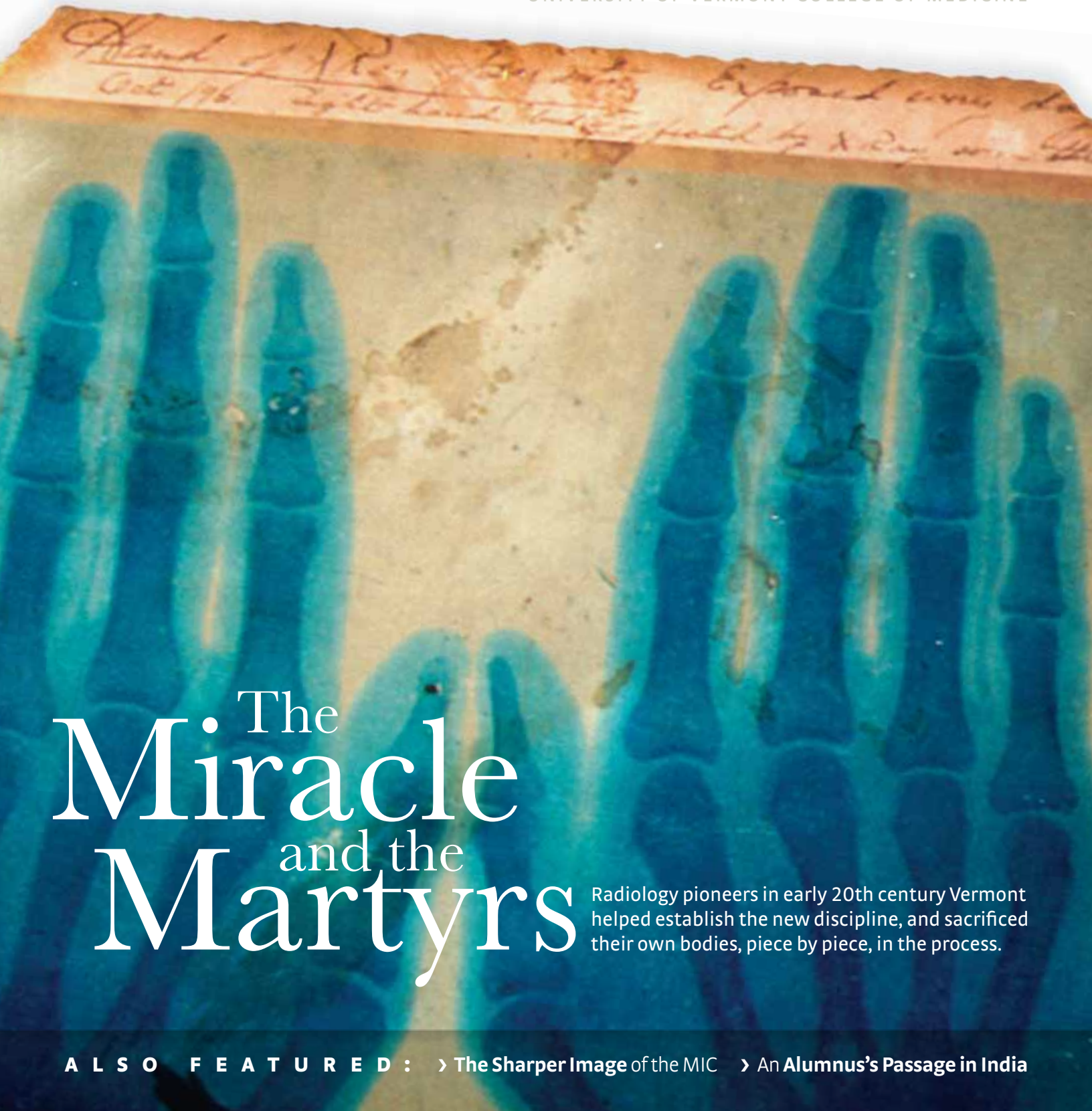


The Miracle and the Martyrs

Radiology pioneers in early 20th century Vermont helped establish the new discipline, and sacrificed their own bodies, piece by piece, in the process.

the sharper image

by **Jenny Blair, M.D.** | photography by **Mario Morgado**

Researchers from across the region come to the Microscopy Imaging Center, the only place in Vermont to find a wide collection of leading-edge instruments that open windows on the hidden "nanoworld" around us.

Few mosquitoes meet their end this way: gilded, mounted on a pedestal, and entombed in a vacuum chamber. Michelle von Turkovich, a research technician in the Department of Pathology, has prepared and dried this mosquito, then placed it in a sputter coat machine, which covers every crevice of the creature with a thin layer of gold and silvery-white palladium. After sputter coating, the mosquito looks something like Han Solo in carbonite. She slides fly and pedestal into the cylindrical body of the scanning electron microscope, switches on the vacuum, and takes hold of a dial. That's when things get wondrous.

"I think of it like landing on the surface of Mars," she says, twirling the dial. We focus down on the creature, then

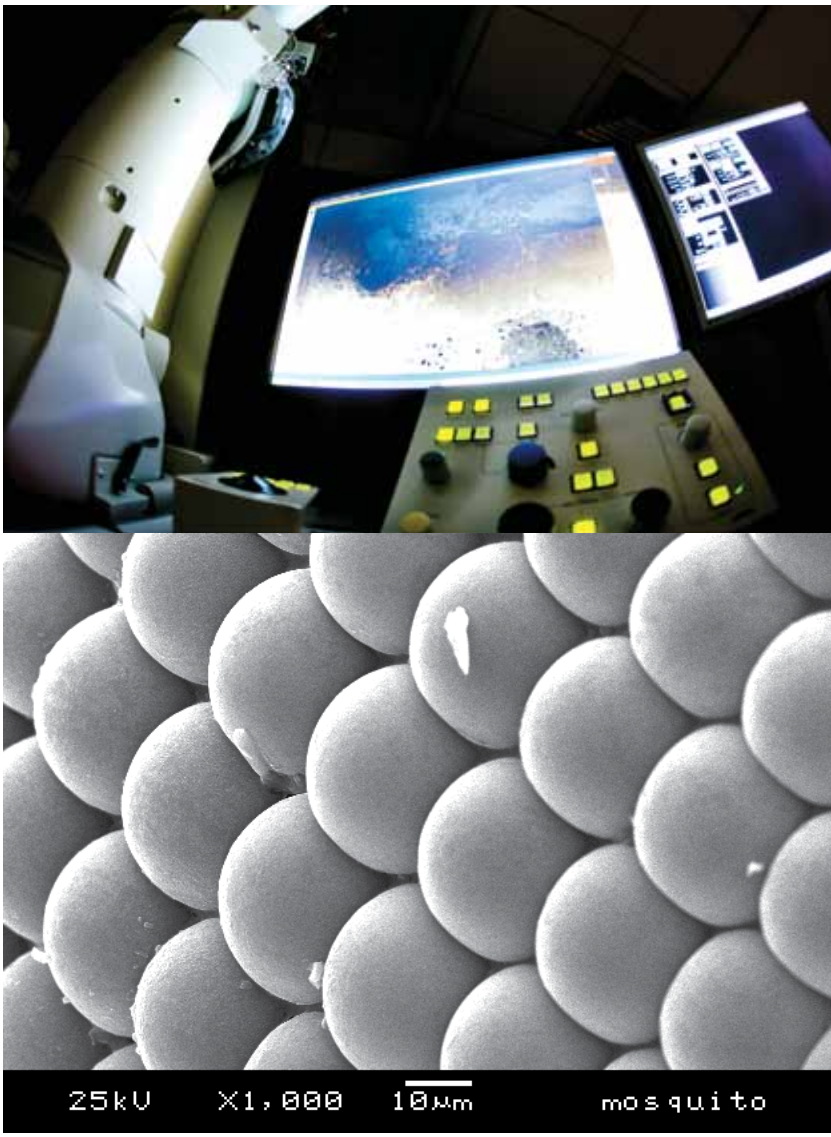
zoom in closer. Legs, hairs, a compound eye, all in opaque and ghostly gray. Closer. A carpet of fine hairs pops into view on what had looked like a smooth body. Closer, up to one hair, which reveals a subtle ribbed texture.

The scanning electron microscope (SEM) that von Turkovich pilots is one of ten advanced microscopy systems available to researchers at the UVM Microscopy Imaging Center (MIC), a facility that has benefited from a substantial investment in equipment over the past few years. Headed by Professor of Pathology Douglas J. Taatjes, Ph.D., the MIC is a core facility of the College of Medicine, but it serves science and engineering departments all over the University as well as researchers from Middlebury College and nearby companies such as General Dynamics. "Because the cost of even a single advanced microscope can be prohibitive, they come to use equipment...that may not find its way into a single investigator's laboratory," says Taatjes. MIC staff first train researchers on the use of the equipment, which allows them to examine a wide range of experimental objects — everything from live cells to asbestos crystals to shrapnel. In addition to experienced scientists and physicians, some of those researchers are still in high school: participants in the Governor's Institutes of Vermont collected the mosquitoes that were later sputter-coated and placed in the SEM, and they learned how to use the instrument from von Turkovich. "They left here in awe," she recalled of the most recent class.

a fleet of scopes

Like the SEM, most of the imaging center's fleet of microscopes are not the glass-slide-and-coverslip kind familiar to decades of students. The classical light microscope has its limitations, offering at best about a 200-nanometer resolution due to the fundamental properties of visible light. But there are ways around that barrier. For example, instead of visible light, the SEM bounces electrons off the specimen. The center's transmission electron microscope (TEM) sends electrons straight through a sample, while the atomic force microscope physically probes the specimen to map out its topography. The electric cell-substrate impedance sensing system isn't a microscope, but it provides submicroscopic information. The system cultures live cells on a slide that contains gold electrodes; these measure changes in a cell's electrical processes, which allows a researcher to examine their physiology in real time — a little like checking an EKG on a patient in the clinic.

Then there is the confocal laser scanning microscope, which allows researchers to focus on an object at a specific depth, without interference from foreground or background. Like an advanced CT scanner, it "sees"

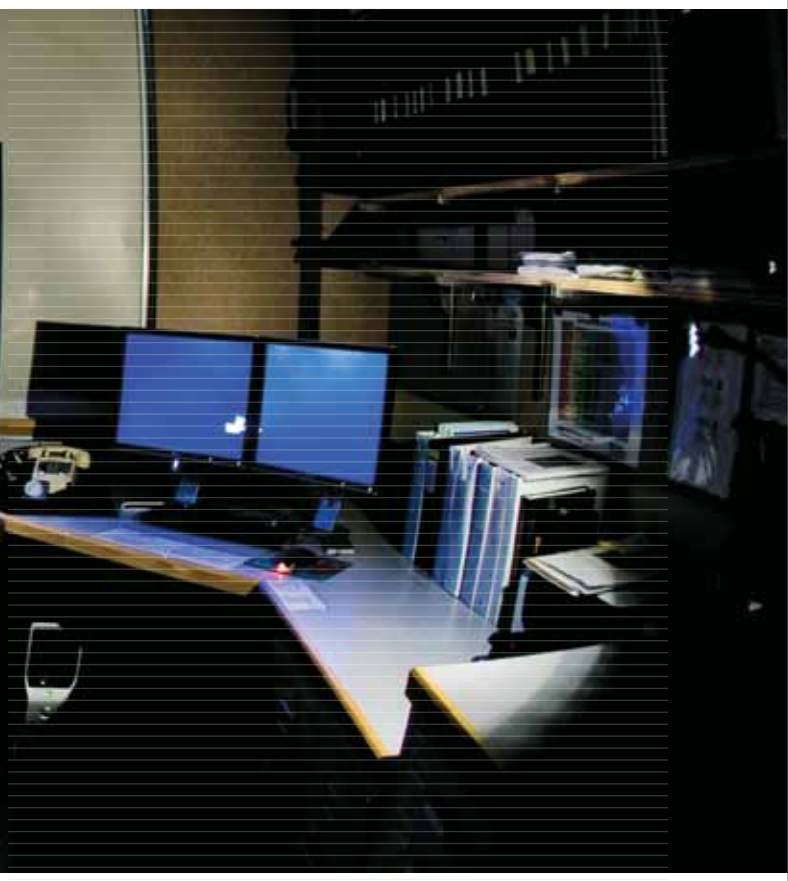


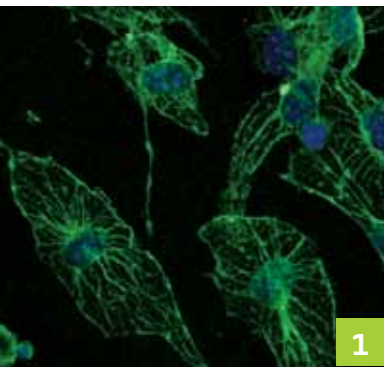
"Like landing on the surface of Mars," is how research technician Michelle von Turkovich describes using UVM's scanning electron microscope (top) to focus on minute objects such as the cell structure of a mosquito eye (above).

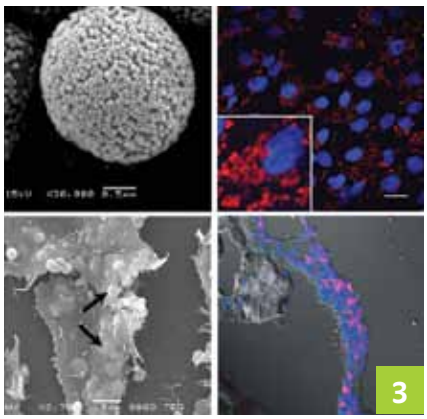
"The confocal microscope allows you to look at the whole sample without cutting it, and it allows you to take optical sections...

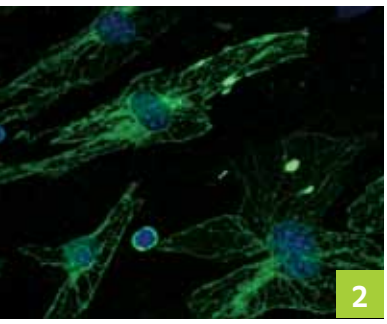
—Helene Langevin, M.D.
Professor of Neurology

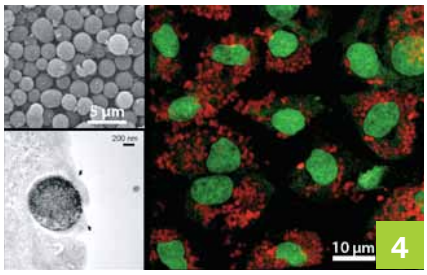
Mosquito eye image courtesy Michelle von Turkovich











Housed in a vibration-dampening shield, the probe on the atomic force microscope (top) taps across a sample to reveal textures a thousand times smaller than can be seen with conventional light microscopes. (Above left) Images 1 and 2 show connective tissue being stretched during research by Helene Langevin, M.D. (Above right) Images 3 and 4 show confocal microsope views of of the hollow silica spheres Christopher Landry, Ph.D., and Brooke Mossman, Ph.D., are testing as a new system of drug delivery.

deep into specimens and captures images of individual planes; researchers can opt to create three-dimensional reconstructions with imaging software. And like a CT scanner, it can examine live tissues and organisms — no slicing or fixation required.

The confocal microscope is central to Professor of Neurology Helene Langevin, M.D.'s research. Langevin studies connective tissue, the substance that fills the spaces between muscles, nerves, and other types of tissue. Connective tissue, which hitherto has been the subject of relatively little research, is difficult to slice into thin sections, as it tends to shear. "The confocal microscope allows you to look at the whole sample without cutting it, and it allows you to take optical sections," she says; it also enables her to study how live connective tissue reacts over several minutes while being stretched. This is a key component of her research into acupuncture, in which she studies the possible effects caused by stretching of tissue around the needle insertion point. "We couldn't do this research without the Microscopy Imaging Center," Langevin says.

Professor of Chemistry Christopher Landry, Ph.D., a specialist in materials chemistry, has relied on the MIC for research into new systems of drug delivery he conducts in

1, 2, courtesy Helene Langevin; 3,4, courtesy Christopher Landry

association with Professor of Pathology Brooke Mossman, Ph.D.'77. Landry and Mossman synthesize hollow, microscopic, silica spheres whose shells are full of holes, like a Wiffle ball. Guided by antibodies, the spheres carry chemotherapy drugs to tumors, releasing their cargo inside tumor cells soon after their arrival. Because it targets only problem cells, Landry and Mossman's technique could allow for much lower doses of chemotherapeutic medications, many of which are more toxic in higher doses. On one study that involved students, the group used the confocal and transmission electron microscopes to prove that the spheres and their cargo, the drug doxirubicin, had reached the insides of tumor cells. These confocal images glow with color from fluorescent labels that track the spheres and cells.

The atomic force microscope was Taatjes's instrument of choice to study antiphospholipid syndrome (APL), a disease that leads to blood clots and repeated miscarriages. Situated inside a metallic vibration-dampening shield, the microscope nudges its way along the surfaces of specimens, gently tapping them with a probe that can resolve textures some 1000 times smaller than the best light microscope can reveal. Taatjes and his colleagues used it to make images of an important protective protein called annexin, which crystallizes in a two-dimensional pattern like a chain-link fence. They then added the harmful antibodies found in APL and captured images of the antibodies as they disrupted the fence, thus providing the first visual evidence of the destructive interaction they suspect underlies the disease. These interactions could only have been followed with an atomic force microscope, said Taatjes, in part because it allowed them to occur in a live, hydrated state.

a broad focus

Along with basic scientists, clinical and translational researchers also make regular use of the MIC. Several times a week, the Fletcher Allen pathology department sends kidney biopsy specimens to Senior Laboratory Technician Janet Schwarz. She slices each specimen, adds a stain, embeds it in resin, then prepares sections to examine through a transmission electron microscope at magnifications of up to 50,000×. The best images are captured digitally and sent back to the hospital for diagnosis by pathologists; Schwarz and the physicians often go over the cases in person together. Because the MIC handles patients' specimens, it is licensed by the College of American Pathologists, whose strict standards are listed in a dozen thick ring binders that line a shelf in the lab. Taatjes and his colleagues carry those standards over to research applications as well; their thorough record-keeping, he says, has impressed many a visiting researcher.

the MIC in focus

The Microscopy Imaging Center (originally called the Cell Imaging Facility) was established as a core facility in the College of Medicine in 1993. Originally consisting of a transmission electron microscope, a confocal scanning laser microscope, and an image analysis system, the MIC has expanded as a core facility to meet the diverse and expanding needs of the research base at UVM. Although the MIC is located within, and administered by, the College of Medicine, it serves the imaging needs of researchers throughout the University, as well as from outside of the University. The facility relocated to the Health Science Research Facility in the summer of 2001. Concurrent with this move, the facility was renamed the "Microscopy Imaging Center" to more accurately reflect the diverse microscopy-based imaging research carried out there. The MIC currently offers these imaging systems and equipment for research use:

- JEOL 1400 transmission electron microscope
- JEOL 6060 scanning electron
- Olympus BX50 research microscope
- BioRad MRC 1024ES confocal scanning laser microscope system
- Zeiss LSM 510 META confocal scanning laser microscope
- Asylum Research MFP-3D-BIO atomic force microscope station
- CompuCyte Laser Scanning Cytometer
- Arcturus PixCell II Laser Capture Microdissector system
- Olympus IX 70 inverted light microscope
- Applied BioPhysics Electric Cell Substrate Impedance Sensing System
- Caliper LifeSciences IVIS Lumina II Whole Animal In Vivo Imaging System
- Dell Precision T7400 workstation for image analysis and processing
- Dell Optiplex GX260 computer with Universal Imaging MetaMorph image analysis software
- Histology Lab Core

All of the imaging systems are connected to the internet, allowing sharing of images within the facility, as well as transferring digital images off-site. Extensive image processing and analysis software packages located on the various computers housed within the MIC, including the central imaging workstations, can then be utilized for analyzing digital images.

Tim Hunter (at left) demonstrates microarray equipment to visiting community members.

core facilities at UVM

In order to conduct today's molecular cellular research, biomedical scientists require access to a range of state-of-the-art equipment, including high-powered microscopes, scanners, technology for measuring the DNA in cells and the mass of chemicals in a laboratory sample. Called cores, these facilities are available to all members of the research community. Tim Hunter manages two cores at the University of Vermont — the Vermont Cancer Center (VCC) DNA Analysis Facility and the UVM Microarray Facility (Hunter is also the assistant director of the Translational Technologies Unit of the Vermont Center for Clinical & Translational Science). UVM has been a regional leader in core facilities development and administration, including hosting the Northeast Regional Life Sciences Core Directors (NERLSCD) in Burlington, which brought nearly 150 scientists from 60 institutions across North America to the UVM campus.

UVM CORE FACILITIES ARE:

Bioinformatics Core — builds biomedical research capacity throughout the state by promoting faculty and student research at Baccalaureate Partner Institutions

Biostatistical Bioinformatics Facility — provides support covering biostatistics, statistical genetics, and epidemiology for biomedical and health-related research

Cryoelectron Microscopy Facility — research focuses on the three-dimensional structure determination of macromolecular assemblies using electron microscopy

Flow Cytometry Facility — a resource for high speed analysis and sorting of cells

Laboratory for Clinical Biochemistry Research — integrates epidemilogy, biochemistry, and molecular biology to help assess cardiovascular risk factors

Mass Spectrometry — measures stable isotopically labeled compounds to study metabolism in humans

Microscopy Imaging Center — imaging in the biomedical and materials sciences from tissues and surfaces to molecules

MRI Center for Biomedical Imaging — a research-only facility that specializes in functional and static brain imaging

Neuroscience COBRE Core — includes Imaging and Physiology, Cellular/Molecular, and Translational Cores

Vermont Cancer Center Core — includes a **DNA Analysis Facility, Flow Cytometry Facility,** and **X-Ray Crystallography**

UVM Microarray Facility — Comprehensive support for assessing the expression of genes in DNA and RNA

Facilities for Transgenic Mice and Animal Care Management

The MIC makes it a point to connect with the community. Schwarz leads Project MICRO events, in which she visits local middle schools to give many children their first look through a microscope (profiled in"You're Never Too Young to Learn" in the Winter 2011 issue of *Vermont Medicine*). Some of the high school juniors who use the SEM during Governor's Institutes are so entranced by its possibilities that they return to use the instrument for projects during their senior year. In partnership with the Vermont Health Department, the MIC has also signed on as a satellite lab in case a bioterrorist attack overwhelms government facilities; one of its technicians trained at the Centers for Diseases Control and Prevention in Atlanta to learn to spot pathogens like smallpox and anthrax. "We're there in the background, just in case," says Taatjes.

two decades of service

Taatjes has run the MIC from its inception in 1993. Before college, he said, he liked science, but had no particular interest in microscopes. That changed in an instant after his undergraduate advisor invited him to do a project on an electron microscope. As Taatjes looked at the fantastically detailed images of cell structures for the first time, he recalls, "I knew then and there, 'This is what I'm going to do.'"

After earning his Ph.D. from the University of Basel in Switzerland, Taatjes joined the UVM faculty in 1987, and was recruited to run the pathology department's single-transmission electron microscope. Several years later, then-Associate Dean of Research John Evans, Ph.D., and Chair of Pathology Edwin Bovill, M.D., decided to centralize the university's microscopy resources, so they bought a new electron microscope and made it the heart of a new facility. A confocal microscope arrived soon after, the first of many grant-funded acquisitions over the years. Thanks to shared instrumentation grants from the National Institutes of Health (NIH) — which go to fund equipment for at least three NIH-funded researchers who will share it — and funding from other sources, the MIC's instruments now comprise a formidable lineup.

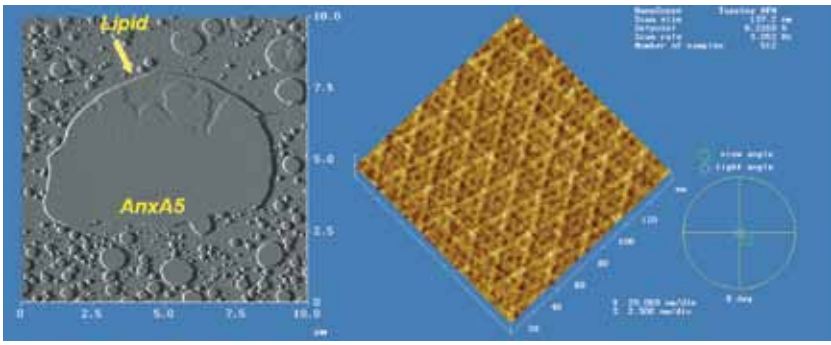
Landry recalls asking to visit the Cell Imaging Center during his 1996 job interview. (The Cell Imaging Center is the MIC's former name; it was changed in 2001 because so many researchers were using it to study other objects.) Now, as a frequent search chair for new faculty members, Landry always arranges for candidates to tour the MIC. "In one way or another, most of the new chemists that we're hiring in our department will be making some use of the imaging facility," he said. "It's rapidly becoming an important tool for departments outside medicine."

Raj Chawla, UVM Med Photo

"When I interview potential faculty... I always hear 'Wow, you've got basically everything I need.'"

—Douglas J. Taatjes, Ph.D, Professor of Pathology

Taatjes hopes soon to bring a groundbreaking new instrument to the MIC. The super resolution microscope is a light microscope that breaks the 200-nanometer limit, one which was thought for at least a century to be unbreakable. (200 nanometers is about 1/500th the width of a human hair.) The super-resolution microscope resolves objects ten times smaller than that without resorting to the fixing and staining that higher-resolution electron microscopes require. "What you're seeing [with electron microscopes] is a snapshot of what the cell was doing when you dumped the fixative on it," said Taatjes. With super resolution microscopy, by contrast, researchers can watch live cells in action at nearly the same scale. That's thrilling, in part because so many cell organelles are smaller than 200 nanometers. "[We can] begin to look at dynamic interactions between molecules and cells," said Taatjes. "Super resolution is really a revolution right now in cell biology, and we want to get this technology on campus." Researchers are eagerly anticipating its arrival. During a recent seminar to discuss the super-resolution microscope, Taatjes recalled, the room was "unbelievably packed.... People were sitting in the aisles."



(At top) Professor of Pathology Douglas Taatjes, Ph.D., director of the Microscopy Imaging Center, has been involved with imaging at UVM for nearly 25 years. (Above) The atomic force microscope reveals the chain-link fence structure of the protein annexin that Taatjes is studying, which is involved in APL syndrome.

If the grant he is writing is successful, the super resolution microscope will become the MIC's eleventh microscopy-based imaging system, giving researchers the freedom to design entirely new experiments. "When I interview potential faculty members," Taatjes said, "one of the things that I always hear is 'Wow, you've got basically everything I need.' ...We're pretty unique, I think." VM

annexin images courtesy Douglas Taatjes.