Breakthrough imaging tool maps cells' composition in 3-D

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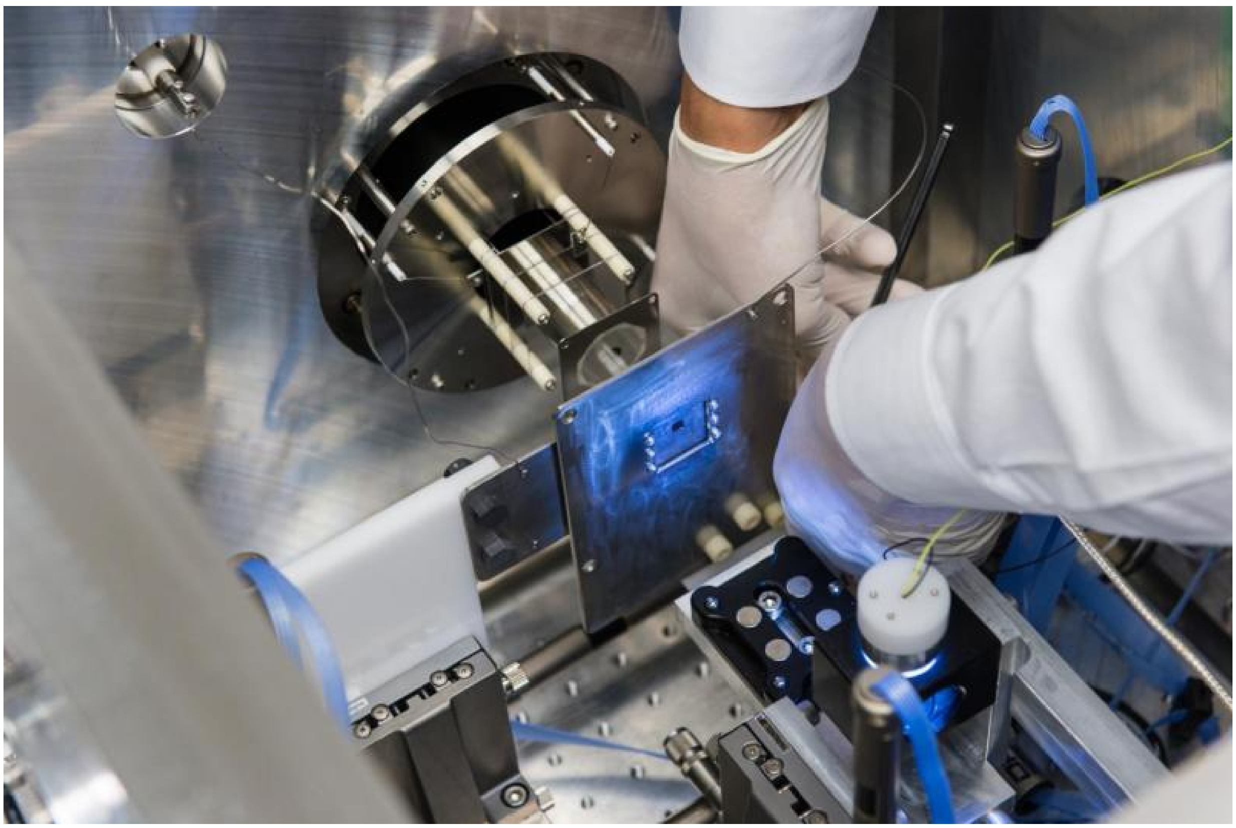


A sample has to be perfectly positioned in the instrument to gain proper readings. Credit: William Cotton/Colorado State University

A one-of-a-kind instrument built at CSU lets scientists map cellular composition in three dimensions at the nanoscale, allowing researchers to watch how cells respond to new medications at the most minute level ever observed.

The new mass-spectral imaging system is the first of its kind in the world and its applications are just beginning to surface, said Carmen Menoni, a University Distinguished Professor in the Department of Electrical and Computer Engineering.

A special issue of *Optics and Photonics News* last month highlights the CSU research among "the most exciting peer-reviewed optics research to have emerged over the past 12 months." Editors identified the imaging device among global "breakthroughs of interest to the optics community."

Menoni's group, in collaboration with an interdisciplinary group of faculty, devised and built the instrument with help from students. She found a partner in CSU's renowned Mycobacteria Research Laboratories, which seek new treatments for the global scourge of tuberculosis.

The partners described the system in a paper published earlier this year in *Nature Communications*.

Unprecedented detail

Dean Crick, a professor who researches tuberculosis, collaborated with Menoni to refine the mass spectrometer imaging system. He said the instrument will allow him to examine cells at a level 1,000 times smaller than that of a human hair - about 100 times more detailed than was earlier possible.

This will give researchers the ability to observe how well experimental drugs penetrate and are processed by cells as new medications are developed to combat disease.

Crick's primary research interest is tuberculosis, an infectious respiratory disease that contributes to an estimated 1.5 million deaths around the world each year.

"We've developed a much more refined instrument," Crick said. "It's like going from using a dull knife to using a scalpel. You could soak a cell in a new drug and see how it's absorbed, how quickly, and how it affects the cell's chemistry."

The earlier generation of laser-based mass-spectral imaging could identify the chemical composition of a cell and could map its surface in two dimensions at the microscale, but could not chart cellular anatomy at the more detailed nanoscale and in 3-D, Crick said.

Possible applications

In addition to observing how cells respond to new drugs, he said, researchers could use the technology to identify the sources of pathogens propagated for bioterrorism.

The instrument might also be used to investigate new ways to overcome antibiotic resistance among patients with surgical implants.

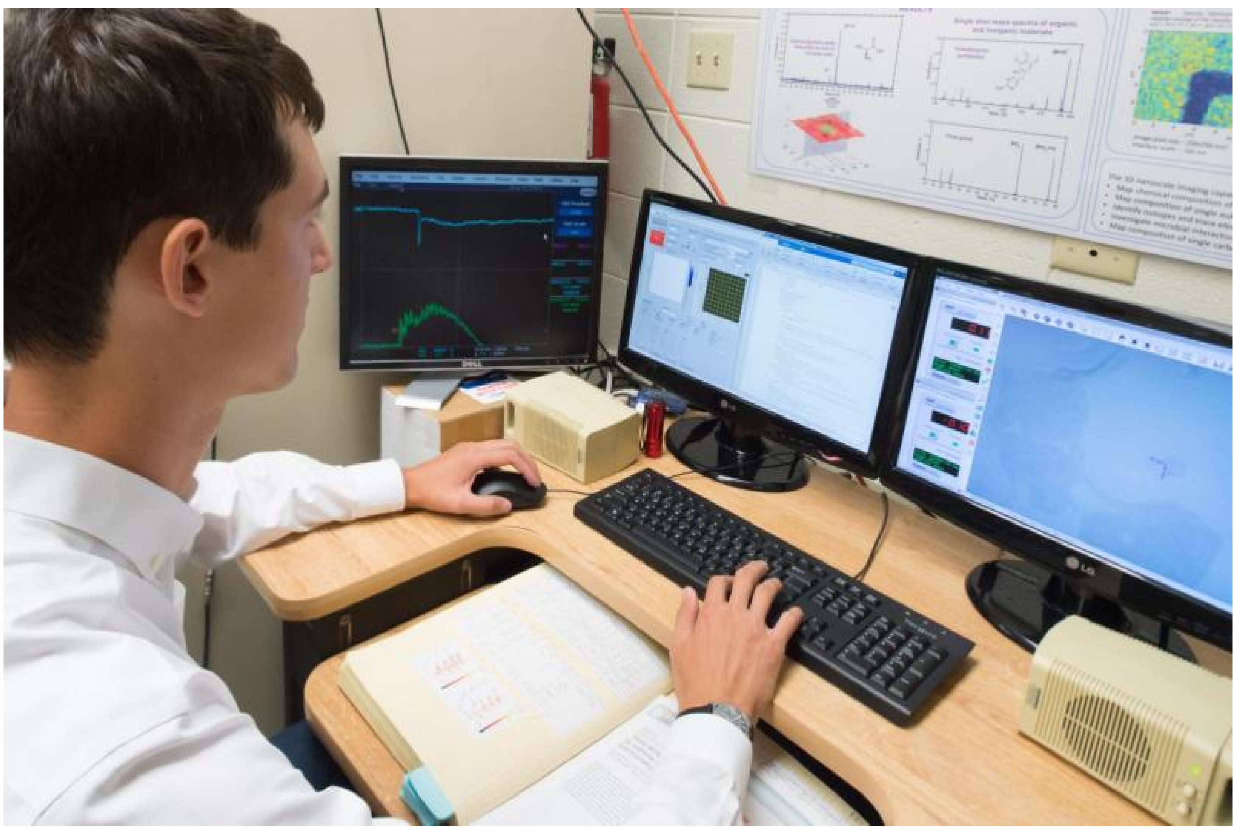
"You might be able to customize treatments for specific cell types in specific conditions," Crick said.

The CSU instrument would cover the average dining room table. Its central features are mass-spectral imaging technology and an extreme ultraviolet laser.

Jorge Rocca, also a University Distinguished Professor in the

Department of Electrical and Computer Engineering, created the laser attached to the spectrometer. Its beam is invisible to the human eye and is generated by an electrical current 20,000 times stronger than that of regular fluorescent tubes in ceiling lights, resulting in a tiny stream of plasma that is very hot and dense. The plasma acts as a gain medium for generating extreme ultraviolet laser pulses.

The laser may be focused to shoot into a cell sample; each time the laser drills a tiny hole, miniscule charged particles, or ions, evaporate from the cell surface. These ions then may be separated and identified, allowing scientists to determine chemical composition.



Ilya Kuznetsov examines the results generated by the instrument.

The microscopic shrapnel ejected from each hole allows scientists to chart the anatomy of a cell piece by piece, in three dimensions, at a scale never seen before, the scientists said.

The project was funded with $1 million from the National Institutes of Health as part of an award to the Rocky Mountain Regional Center of Excellence for Biodefense and Emerging Infectious Disease Research.

The optical equipment that focuses the laser beam was created by the Center for X-Ray Optics at the Lawrence Berkeley National Laboratory in Berkeley, Calif.

The CSU system recently received support for system engineering design from Siemens. The company gave the CSU team an academic grant for its NX software package, including 30 seat licenses, valued at $37 million.

Additional CSU partners

Other CSU faculty involved in the project include Feng Dong and Elliot Bernstein from the Department of Chemistry.

The lead author on the paper published in Nature Communications is Ilya Kuznetsov, a CSU doctoral student in Electrical and Computer Engineering.

"The whole system was built by students and post-docs," Menoni said. "This is something we pride ourselves on, that the students get an interdisciplinary experience. Having access to design software such as the Siemens NX package is critical for creating these instruments and for training students."

Key to the project has been collaboration among scientists who build high-tech devices and those who use them to solve global problems.

"It's been very interesting learning how to communicate with engineers," Crick said. "We don't think alike. They understand the biology about as well as I understand the engineering. But over the years we've learned how to talk to each other, which is nice. I can see the need for the instrument, but I have no idea how to build it. They do."

How it works:

At one end of the instrument is a special laser created in an argon gas-filled tube when a pulse of 60 kilovolts is discharged.

"It's like a lightning strike in a nanosecond," said Carmen Menoni, University Distinguished Professor in the Department of Electrical and Computer Engineering.

The laser is guided through chambers using mirrors and special lenses that focus it down to a diameter of less than 100 nanometers. In a chamber at the far side of the spectrometer, the laser hits a sample cell placed with the aid of a microscope.

"When you're trying to hit a single bacterium with a laser, it's tricky. You have to aim well," said Dean Crick, a CSU professor in the Department of Microbiology, Immunology and Pathology.

Once the laser drills a miniscule hole in the cell, charged ions emitted after the tiny explosion are drawn into a side tube using electrostatic fields. The larger mass the charged particle has, the slower it moves down the tube; the time it takes an ion to reach a detector gives scientists information about its mass.

"It's like you have a sports car and a big truck," said Ilya Kuznetsov, a doctoral student in Electrical and Computer Engineering. "Imagine you put the same motor in both—they will move at different speeds. And the more you allow them to go, the more they separate. That's why our tube is so long, to allow for that differentiation."

A set of special pumps creates high vacuum that sucks all air from the tube, to remove any foreign particles the sample might collide with and to ensure equally smooth sailing for all the ions.

"If you want to have a car race, you need to remove all traffic from the roads," Kuznetsov explained.

By keeping the charge and amount of energy applied to each particle consistent, the mass becomes the key signature that provides researchers with every ion's chemical identity.

A computer program developed in-house generates the data in a color spectrum of masses, which is then used to create a kind of topographical cell composition map.

Provided by Colorado State University