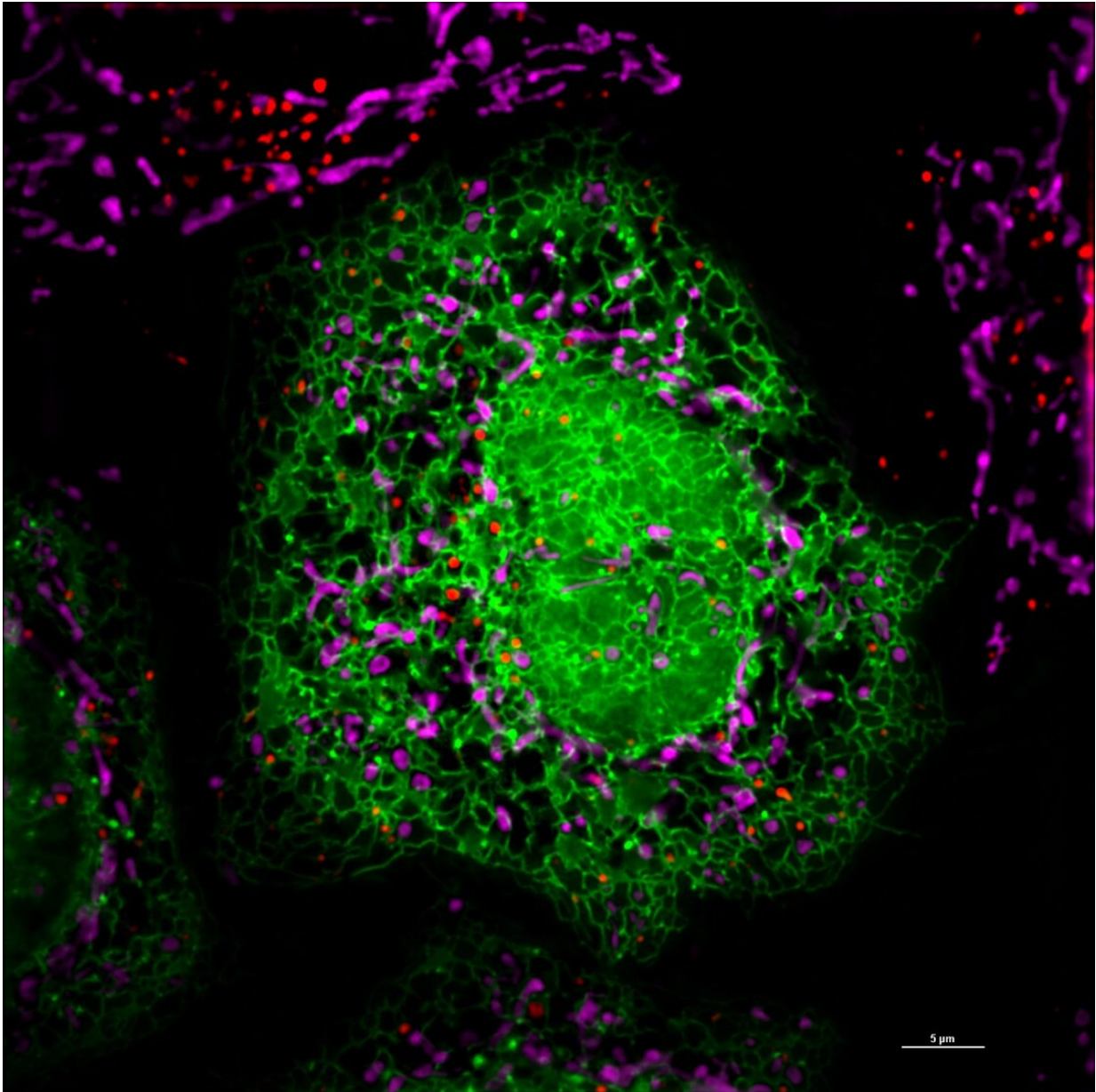# Advancing our view at the subcellular level

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A super resolution image captured in Diao's lab shows the endoplasmic

reticulum in green, lysosomes in pink and mitochondria in red. Credit: Jiajie Diao.

In the field of scientific research, details matter. The minutia of processes and structures are explained with specificity, data points are reported to the most precise decimal and seeing is believing.

Now, University of Cincinnati cancer biologists have developed a new piece of technology and a new imaging technique that will help researchers glean more detailed data points and see cells in more precise detail when studying the development of cancer and neurodegenerative diseases.

Jiajie Diao, Ph.D., associate professor in the Department of Cancer Biology in UC's College of Medicine, recently published an article detailing the progress in the journal *Advanced Healthcare Materials*.

New probe

Some of Diao's research focuses on a tiny part inside cells, called a lysosome, that is involved in cell processes. A lysosome is an "organelle," or a specialized structure that performs various jobs inside cells. In the same way organs, such as the heart, liver, stomach and kidneys, serve specific functions to keep an organism alive, organelles serve specific functions to keep a cell alive. Diao's research centers on the lysosomes that act as the "recycling center" within cells, helping the cell reuse broken or malfunctioning building blocks for different purposes.

To accomplish its job, lysosomes need to be in an acidic environment and generally have a low pH value. However, abnormal pH levels within lysosomes have been associated with cellular malfunctions that can lead

to diseases like cancer and Alzheimer's disease.

In order to study how pH levels can change and affect lysosomes and cells, Diao and his team collaborated with Dojindo Laboratories and Yujie Sun, Ph.D., associate professor in UC's Department of Chemistry, to develop a new probe that attaches to the lysosomes and is specially designed to provide more details to researchers. Diao said the resulting "EC Green" probe is the next generation of lysosome probes and features several improvements from current industry standards.

As the name suggests, the probe is green and becomes a brighter shade of green when the cell environment becomes more acidic. This gives researchers more information than current probes, which do not change colors, and can help identify correlations between changes in acidity and cells becoming cancerous.

Diao said this unique probe enables multidimensional analysis of lysosome dynamics, including spatial, structural and pH information over time.

Many currently available probes attach within the lysosome, which Diao said is like placing a string inside a water balloon. If the lysosome bursts, the probe washes away and is detached from the lysosome, making it no longer useful for tracking purposes.

In contrast, the EC Green probe is anchored to the lysosome membrane. Even if the lysosome breaks, it stays secure in its position like a piece of string that remains attached to the outside of a popped balloon.

"So it will be very stable. You can put it there for several days," Diao said. "The commonly used commercial probe will disappear, but our probe will last forever because they get protected by the outer membrane."

The probe is also specially designed to emit a large number of photons so that it can withstand super resolution imaging under high laser intensity.

"When you use a high intensity, most of the probe will get photo bleached. When you stimulate something too hard, it will just die," Diao said.

While it provides a number of advantages, Diao said the most important facet of the probe is that it is useful.

According to Diao, EC Green is relatively inexpensive and extremely quick and easy for researchers to use. About 20-30 minutes after staining cells with the probe, the samples can be washed and placed under a microscope for observation.

"It's so simple and nobody would have a problem using them," he said. "That was another concept for us when developing the new probe. We don't want to just make everything so difficult; what we want is to make everything simpler."

Diao and Sun are already hard at work collaborating on the next generation of EC Green, which will provide even more details into pH levels by turning from green to red in different levels of acidity. The hope is to be ready to obtain a patent and publish another article on this new probe by the end of 2022.

Mapping the cellular landscape

In addition to the EC Green probe, Diao and Sun have also developed a new imaging technique that allows researchers to precisely measure the distance, shape and location of each organelle within a cell. This can help provide more information about the interaction between organelles

and how these interactions may lead to the development of diseases.

The technique builds upon and uses superresolution microscopy, which provides clearer images of particles at the subcellular level.

"So we measure the relative distance between organelles like lysosomes and mitochondria," Diao said. "We found that just by simply doing a surveying and mapping inside the cell using our superresolution, we can achieve and discover many unknown changes inside the cell."

In the first use of the new measurement technique described in the article, the researchers found that mitochondria, the organelle in cells responsible for energy production and respiration, become enlarged when developing into degenerative diseases.

Lysosomes were also found to tend to move closer to mitochondria when the mitochondria are damaged. Further research can help determine more about how organelles' sizes and placements in relation to each other can cause disease, Diao said.

Increasing capabilities with AI

Now that the measurement imaging technique has been developed, Diao said the team is beginning to work with computer scientists to harness the power of artificial intelligence to increase how it can be used.

"Nowadays, lots of measurements are made by people and by a simple algorithm, so we still need manpower," Diao said. "We are developing AI, the machinery, the intelligence to try to do everything by the machine."

Down the road, Diao said the hope is that an algorithm can be trained with a wide variety of images of healthy and diseased cells so that the

software can analyze a cell image and then predict whether or not it will become cancerous.

The technique combined with AI could also be used to study the effectiveness of drugs to treat diseases, for example to see if there are specific organelles that are contributing to a patient developing drug resistance to a certain medication.

"This will give us a next level ability," he said. "Currently, most people are looking at the tissue level or a higher level, but we can go down to the subcellular level."

Due to the purchase of a new microscope from the UC College of Medicine and Department of Cancer Biology, any researcher at the University of Cincinnati or Cincinnati Children's Hospital Medical Center is now equipped to take advantage of the innovations from Diao's lab in future studies.

"So that's what we actually are trying, to let more people use the most advanced probe and the most advanced imaging technique to do their study," Diao said.

Provided by University of Cincinnati