New microscopes reveal live, developing cells in unprecedented 3-D clarity

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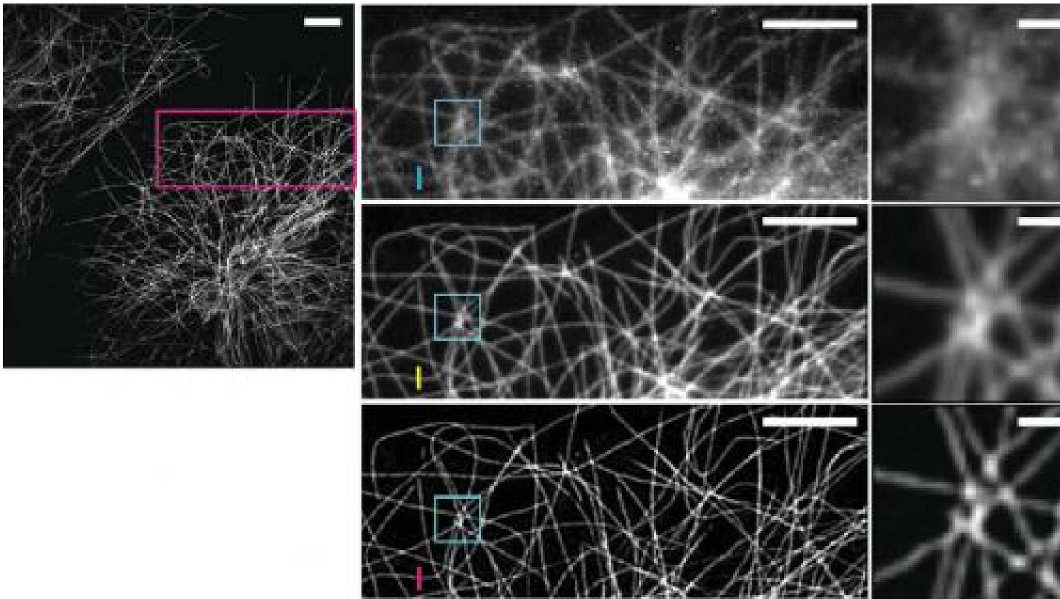


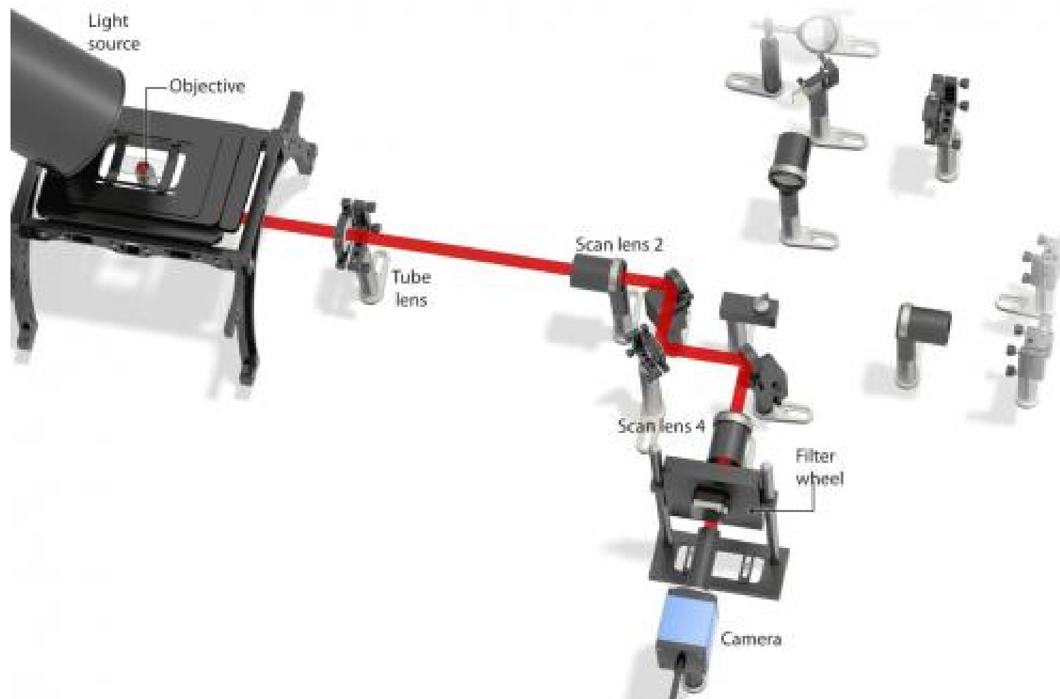
The resolution difference between standard microscopy and SIM technology can be clearly seen. Credit: NIBIB

Researchers at NIH have developed two new microscopes, both the first of their kind. The first captures small, fast moving organisms at an unprecedented rate and the second displays large cell samples in three dimensions while decreasing the amount of harmful light exposure to the cells. Both microscopes surpass in clarity any other currently on the market.

The first microscope allows researchers to obtain fast moving images at double the spatial resolution of a conventional microscope. This provides a vastly clearer picture, enabling cell components that were once quite blurry to now become sharply defined; the difference is similar to that of a 1990's-era standard TV set versus today's high-definition TVs. The microscope is also 10 to 100 times faster than traditional technologies.

"It's always helpful to look at smaller and smaller things," said Hari Shroff, Ph.D., at NIH's National Institute of Biomedical Imaging and Bioengineering (NIBIB) lab chief of NIBIB's section on High Resolution Optical Imaging (HROI.) "Looking at a fixed cell at high resolution can tell you where different parts of the cell are at any given moment; but because much of biology depends on the movement of very small proteins finding each other and interacting, we really needed to look at how things move in a live cell."

The problem is that the higher the resolution, the harder it is to eliminate the blur from both light diffraction (the glow that sometimes occurs as light bends around objects) and the motion going on inside the live cell. Traditional linear structured illumination microscopy (SIM) cannot maintain the high resolution desired by researchers when the sample is moving quickly.



A lamp is used to illuminate the sample from above. Transmitted light is collected via the objective, passed through the Tube lens and Scan lens 2, and directed through Scan lens 4 via removable mirrors before imaging onto the camera. Credit: NIBIB

Shroff and his research fellow Andrew York, Ph.D., found an answer to these problems with their new instant linear structured illumination microscopy (iSIM), described in a paper published in *Nature Methods* on October 6th. Building on traditional SIM technology, the iSIM allows real-time, 3-D super resolution imaging of small, rapidly moving structures—such as individual blood cells moving through a live zebrafish embryo. This kind of imaging is impossible with other microscopes; the ones that are fast enough to record rapid movement do not have a high enough resolution to see inside the cells; and other microscopes with similar resolution are just too slow to capture that amount of motion clearly.

If a photographer wants to take a better photograph, he can either buy a camera with a better lens and higher pixels or he can modify the picture after it's taken, using Photoshop. The principle is similar in microscopy. Instead of approaching the problem by creating better imaging software that helps to increase the resolution after the fact, as most high resolution microscopes do, Shroff and his lab developed a microscope with better lenses and mirrors so that the higher resolution is captured in the original image.

"What we've essentially done is eliminate the need for extensive computer processing by creating a better microscope at every stage of data gathering," said Shroff. "Before, we relied on computer software and algorithms to do things like sort through hundreds of images, eliminate out of focus light, and combine the individual images together. Now, we can do most of that optically with the microscope itself." This means that researchers can skip the time-consuming steps in which computers process the massive amounts of data normally required for such high resolution imaging. Now they will be able to see the images instantly instead of waiting hours or sometimes days, and the data itself takes about 1% of the hard drive space as that produced by previous microscopes.

The second microscope, described in a paper published in *Nature Biotechnology* online on October 13, builds on selective plane illumination microscopy (SPIM). Traditional microscopes expose the whole sample to light even though they are only imaging one small section at a time. However, just as the sun can damage skin cells, too much light exposure can damage or even kill biological samples like embryos. SPIM uses a thin beam of light to illuminate only the single plane that is currently being imaged so the biological sample is not damaged by overexposure to light. However, the technology is limited because looking at a 3-D object from only one point of view does not provide a complete representation of the object—in the same way that

viewing a globe from one perspective gives no information about what is on the other side of the world. Traditionally, SPIM microscopes rotate the sample so that they can clearly see all the dimensions, but this severely limits the imaging speed and can increase the damage done to the cells from light exposure because of the many extra images taken at multiple angles. As a result imaging is also slowed down, and the ability to capture much of the fast moving cellular motion is lost.

In order to combat this problem, Shroff and NIBIB staff scientist Yicong, Wu, Ph.D., developed a dual-view SPIM (diSPIM) microscope with two separate detection cameras. The cameras are set at a 90 degree angle to capture perpendicular views of the sample. This perpendicular view results in undistorted 3-dimensional images, and since only two views are acquired, the microscope can still capture events at very high speed. Additionally, with relatively simple modifications, traditional single camera SPIM microscopes can be converted into the dual-camera diSPIM. The real challenge in developing this technology was to find a way to combine the two disparate images from the two cameras, which required the creation of a new post-processing software algorithm.

The increased speed at which the new dual microscope can image the cells allows for clearer images of even very fast moving viruses. Being able to see how a virus enters a cell, and once it's in, how it moves around, could go a long way towards scientists' understanding of how infections occur and potentially how to fight them more effectively. In the same way, observing the migration of cancer cells in a 3-D environment could unlock information on how cancer grows, finds nutrients, and spreads.

"Biology is three-dimensional, not two dimensional. The nucleus of a cell is spherical, not circular, and as scientists, it's up to us to find ways to observe cells as accurately as possible, Shroff said. "We're really moving biology into the third dimension with this microscope." There's a

lot of attention right now on how neurons fire and interact with each other, but the truth is, we don't even understand how a brain develops—even in the most simple of organisms like C. elegans, a worm with only 300 brain cells. We don't know why brain cells go where they do or what determines their organization. We can't understand more about this process without observing it, and that's something that these devices can help to provide."

The Shroff lab has already begun multiple collaborations with biological labs both inside the NIH as well as external institutions, including Yale, Sloan Kettering, and the University of Connecticut Health Center.

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