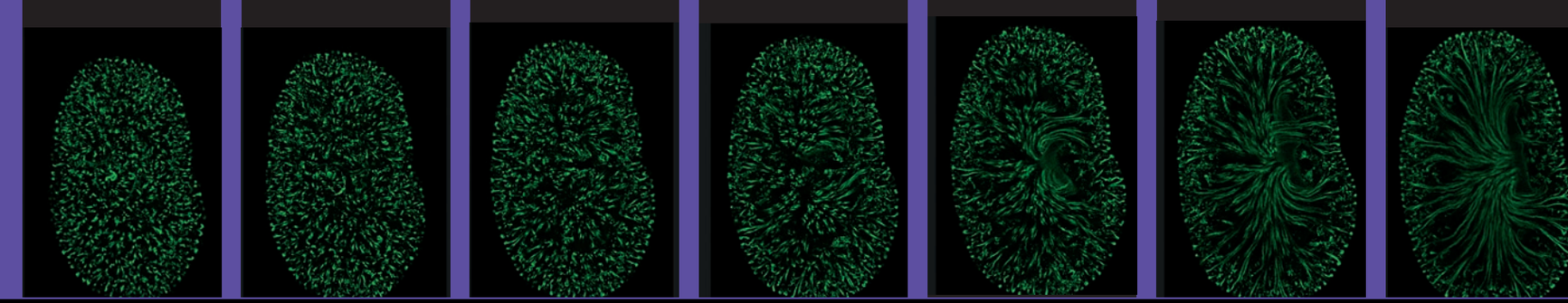
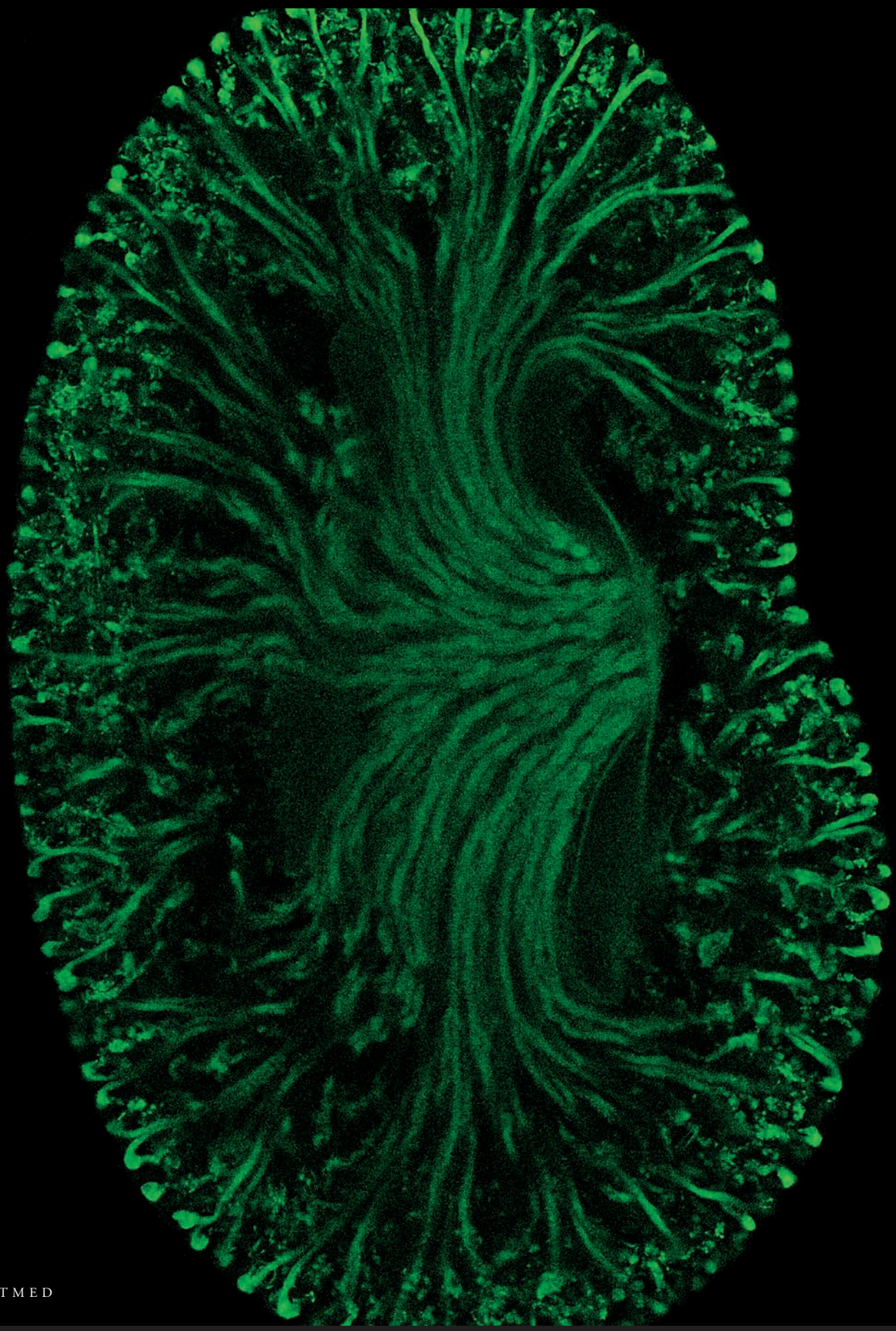
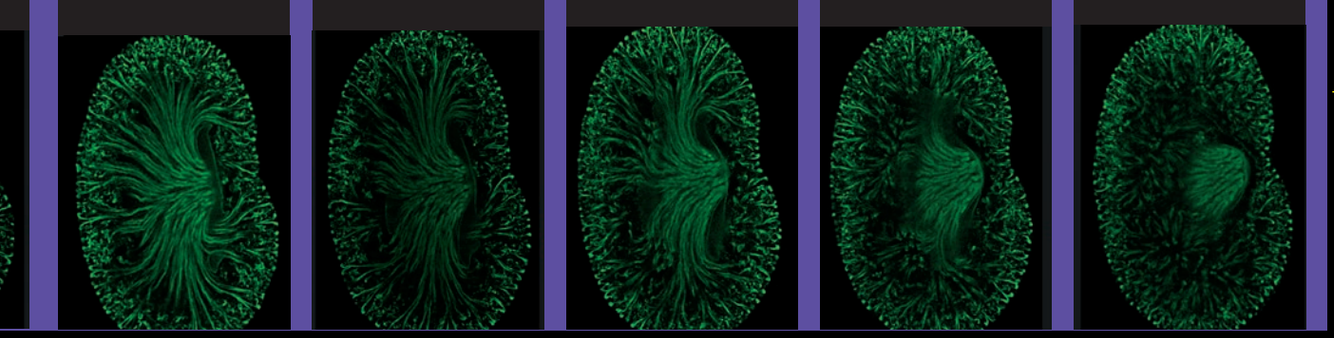




INTERSTITIAL TRAVELS: Pitt profs are aligning around immersive 3-D imaging technology to bring new understanding to their work.

For instance, Sunder Sims-Lucas and Jacqueline Ho, both assistant professors of pediatrics at Pitt, partnered with the Center for Biologic Imaging to generate these 3-D scans of a mouse kidney. The series shows a one-day-old transgenic kidney at different depths. The green channels highlight the duct system of the kidney, which collects urine from filtering nephrons.

No other university imaging center has the ability to create scans of entire organs revealing the cellular level at such high resolution.



VOYAGES FANTASTIC

20,000 VIEWS OF YOUR KIDNEY | BY EVAN BOWEN-GADDY

On any given day, Alan Watson is likely to be exploring an enclosure, moving through it with care, noting its contours. For a new perspective, he might reach up, pulling his body upward with one arm, then the other, like a rock climber scaling a wall. The spaces he surveys are not caves, nor are they man-made tunnels; they are blood vessels, the lining of an ovary or a lung, the white of an eye. The architecture of life-forms.

Alan Watson demonstrates the VR system at Pitt's Science festival.

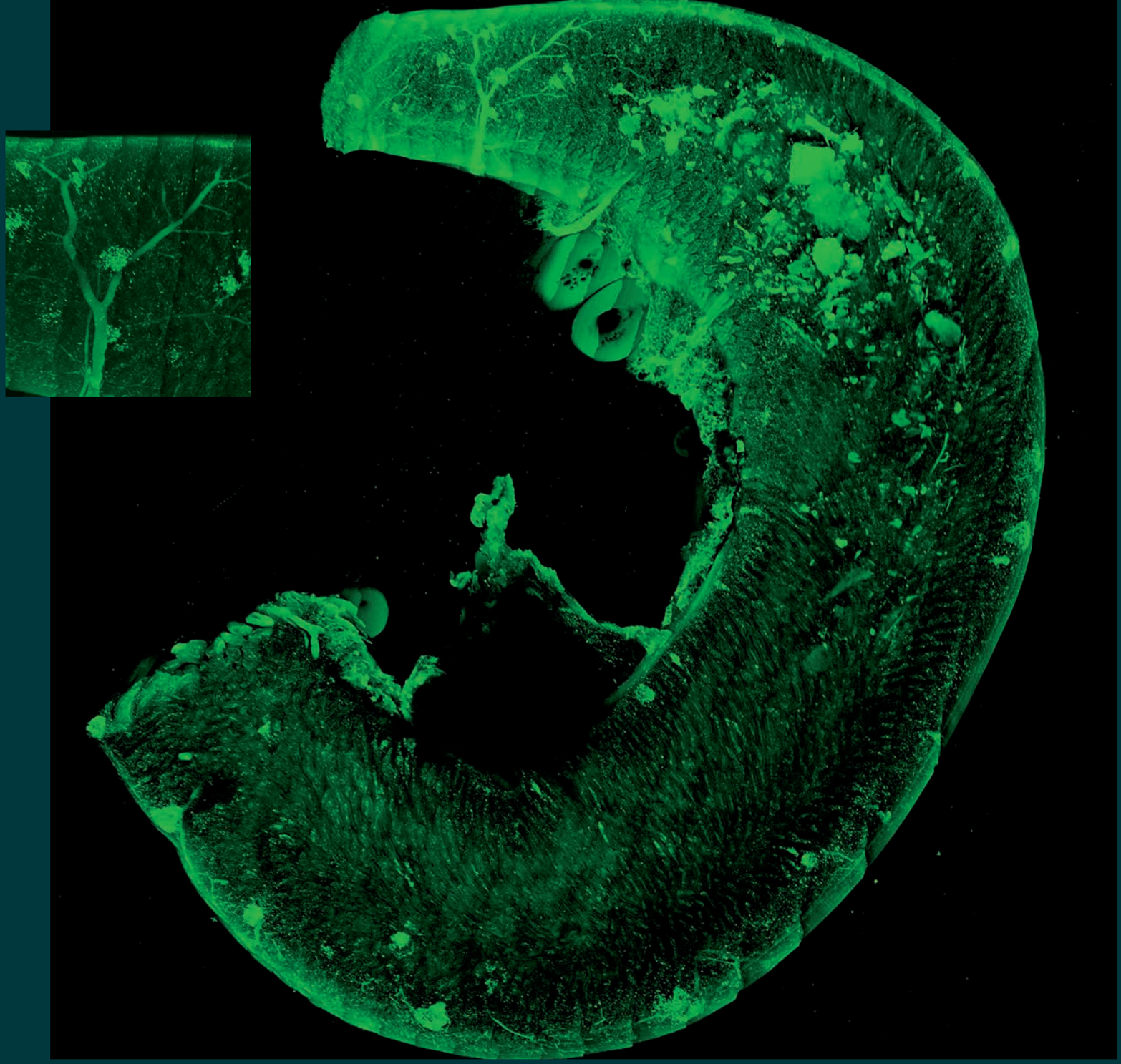
When Watson removes his virtual reality goggles, he might feel a little disoriented. But that's a small price to pay for an extraordinary view of an organ. Watson is a research assistant professor of cell biology at the University of Pittsburgh.

His fantastic voyages take place at Pitt's Center for Biologic Imaging (CBI), which is led by Simon Watkins, Distinguished Professor of Cell Biology.

In 2016, Watson was an immunology postdoc at Pitt's Center for Vaccine Research working with William Klimstra, associate professor of microbiology and molecular genetics and of immunology. They sought out the CBI with the intention of using one of the center's advanced microscopes to try to find a few virally infected cells within a mouse brain. This pursuit, Watson says, had him "staring at black."

IMAGES COURTESY CENTER FOR BIOLOGIC IMAGING AND COLLABORATORS

SIMS-LUCAS AND HO LABS



THIS PAGE: GREENBERGER LAB
OPPOSITE PAGE: BROWN LAB

Simon Watkins saw the trouble the young researcher was having, took the brain, and came back the next day with a high-resolution scan from deep into the brain, revealing hundreds of infected cells.

Watkins showed Watson what he'd used: a prototype of a unique "ribbon" scanner he'd been developing with Caliber ID, a company in Rochester, N.Y. The instrument is called a ribbon scanner because it's able to copy swaths of specimen at once. It'll do 16 scans of 1,000 by 1,000 pixels in a second; so it can put together massive 100,000 by 100,000 pixel swaths as much as 40 times faster "than anything else out there," notes Watson.

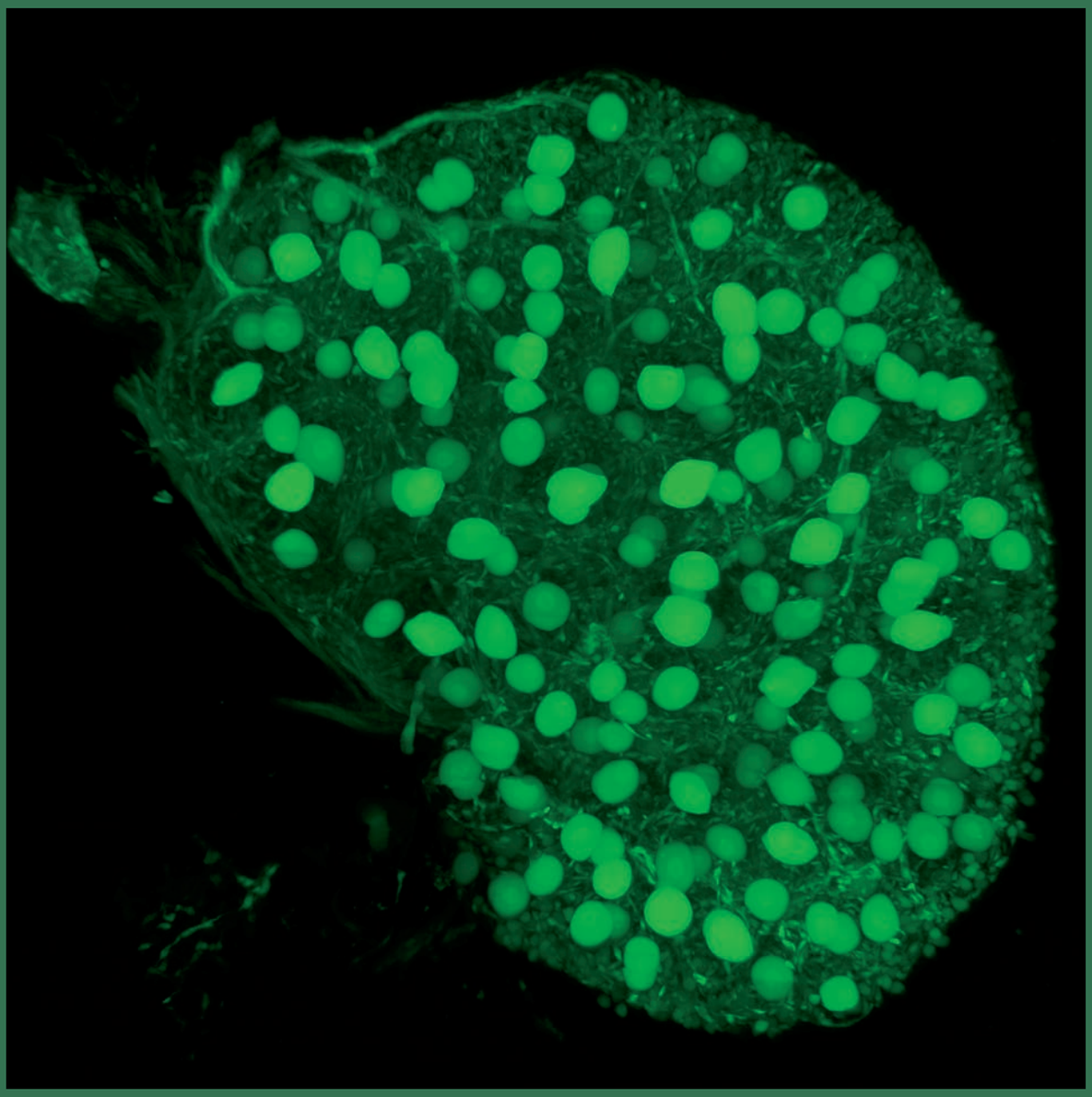
Watson was stunned by what he was able to see; and soon after, he started collaborating with Watkins. Watson is now on the CBI faculty.

The two scientists have since souped up the ribbon scanner. They added a powerhouse lens that Nikon gave Watkins to test. The lens is able to look deep, as well as deliver superb resolution. Usually you give up one capability for the other, says Watson.

"There are only three lenses like this in the world," Watkins says. CBI is the only imaging center with one.

Now the scientists are scanning *entire organs* in 3-D and at the cellular level.

No cellular biologist would try scanning whole organs with an ordinary microscope, says Watkins: "You just couldn't do it." (By "ordinary," Watkins is speaking of the type of high-end biologic research scopes of which Pitt's CBI has several.)



OPPOSITE PAGE: Joel Greenberger, Pitt's Claude Worthington Benedum Professor and chair of radiation oncology, is developing new drugs to counteract radiation from a nuclear disaster. By looking at the entire bowel of a mouse, Greenberger found that after radiation exposure, those circular green portions, called Peyer's patches, saw an influx of neutrophils, a type of immune cell. The neutrophils are the tiny green dots that make up the larger green circles in the image.

Greenberger is a big fan of using 3-D imaging in his studies: "Prior to Simon's techniques, all you had was the ability to sample organs. This also greatly reduces the need to repeat experiments."

THIS PAGE: To make organs transparent for 3-D scanning, Watson optically clears them using a chemical technique he developed. This 3-D scan of a cleared 12-day-old mouse ovary allowed Michael Buckenmeyer, a graduate student from the laboratory of Bryan Brown, assistant professor of bioengineering, to track individual follicles. Brown's lab focuses on regenerative medicine for unmet clinical needs in women's health.

By collaborating with colleagues at another scientific technology firm, Watkins and Watson have married these 3-D images to VR setups. They're making the system available to medical scientists throughout Pitt, who seem to be unanimously astonished by what they're learning from the immersive experiences. The researchers are suddenly able to see things no one else has seen.

In the less than two years that they've been applying the technology, Pitt people have already gained new understanding about vision loss, aneurysms, and other conditions.

Although its new capabilities capture every single pixel in 3-D, the CBI's approach is not about minutia.

"Imagine if you went to a football game, and you saw someone in a red hat," says Watkins. "If you were a bad scientist, you'd say, 'Every human being wears a red hat.' You have to see a thousand red hats to say, 'Well, 10 percent of these people wear red hats.'"

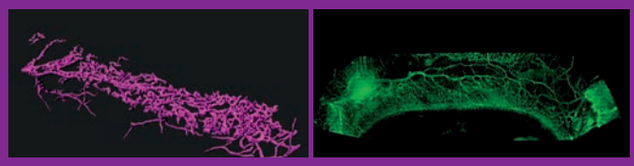
The ability to explore entire organs at a cellular level is unique to Pitt, Watkins believes. And it will be crucial to furthering our understanding of biology: "We need to look at whole systems," he says. "When people look at subsamples, they miss things. When you see everything, you can make much more sophisticated observations."

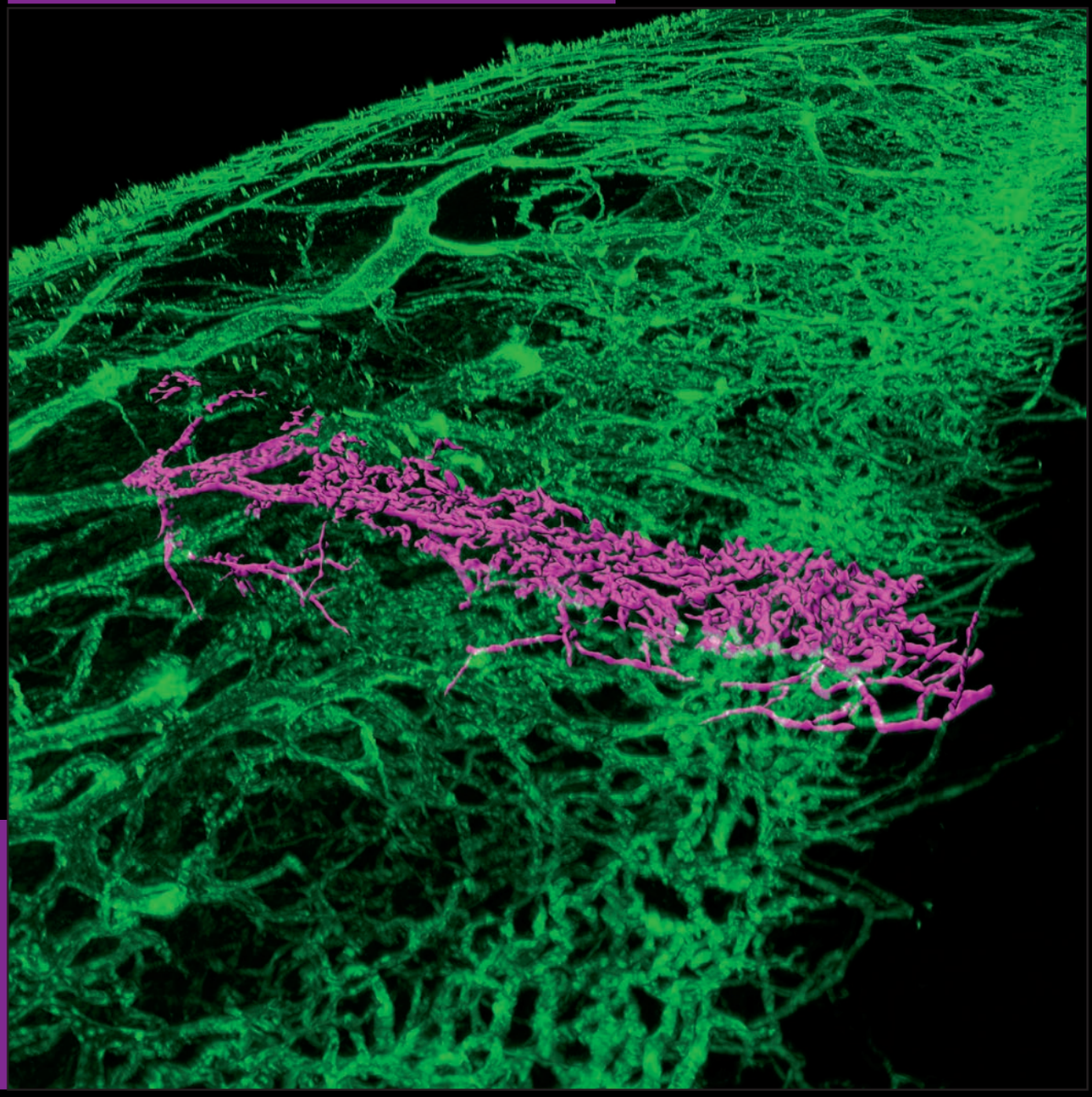
It's a December morning at CBI, and George Spirou, codirector of the Rockefeller Neuroscience Institute at West Virginia University, sits with a VR headset, waiting to meet with Pitt researchers. He has a packed schedule ahead of him.

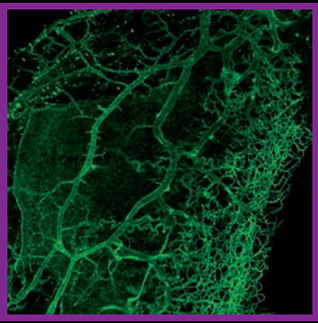
The ability to walk through Watson and Watkins's high-resolution scans comes with the help of syGlass, Spirou's West Virginia–based company that designs VR software to work with high-resolution microscopy. Spirou wants to find out more about how researchers are using the technology, as well as what else might be useful to them.

SyGlass, established in 2013, helps scientists interact with 3-D scans in 3-D, rather than on a typical 2-D monitor. "Why would you want to see a photograph of a room rather than walk into the room?" Spirou asks. "Because as you walk around and look around, you're going to learn a lot more."

He shows off his software. It's like Google Earth, he explains, in that







THIS PAGE: By using VR to explore the white of an eye, Nils Loewen saw outflow tracts that he couldn't before. It turns out that the collapse of these tracts (highlighted in purple) leads to glaucoma.

LOEWEN LAB

"you can see the Earth, but then as you zoom in, you load more detail."

Since Watson started using VR, he needs to remind his kids (who like to visit him in the lab) that the headset is not for games. "It's for serious work," he says.

Among the people Spirou will be meeting with today is Nils Loewen, associate professor of ophthalmology at Pitt. Loewen is determined to find solutions for people with glaucoma.

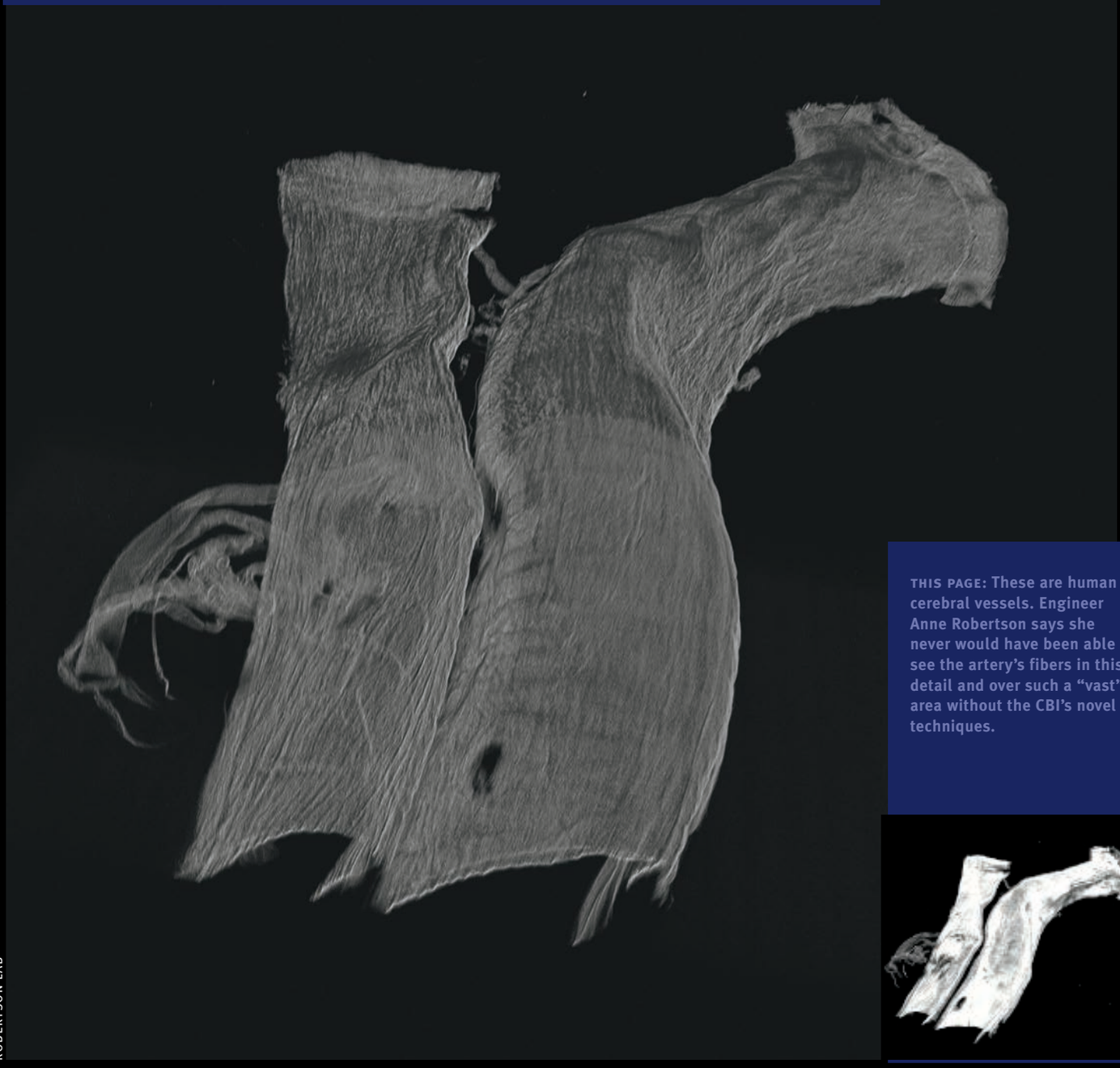
These patients have a plumbing problem, and it's happening somewhere deep in the eye, where even an ophthalmologist can't see.

Glaucoma is the poor drainage of fluid through channels in the sclera, the white of the eye. Pitt's ophthalmology department has a whole host of strong microscopes, yet Loewen says they aren't powerful enough to see "the finer, more intricate parts of the outflow tract."

So Loewen turned to CBI. He now has a 1.2 terabyte scan of a pig eye that Alan Watson created from the ribbon scanner.

When he first saw the scan, Loewen wasn't sure what he was looking at. No one had ever seen outflow tracts in such detail.

By examining the scans with VR, Loewen's team learned that the tracts sometimes collapse. They're like the inflatable tube men you might see in front of gas stations



ROBERTSON LAB

THIS PAGE: These are human cerebral vessels. Engineer Anne Robertson says she never would have been able to see the artery's fibers in this detail and over such a "vast" area without the CBI's novel techniques.

or carpet stores, says Loewen. If there isn't enough pressure, the structures are not able to stay upright.

The utility of immersion has surprised Loewen. He was so taken with the experience, in fact, that he ended up buying a PlayStation and VR set for his home. (He's now a fan of the game *Bound*—"an uncanny amalgamation of modern dance, architecture, and psychoanalysis," he says.)

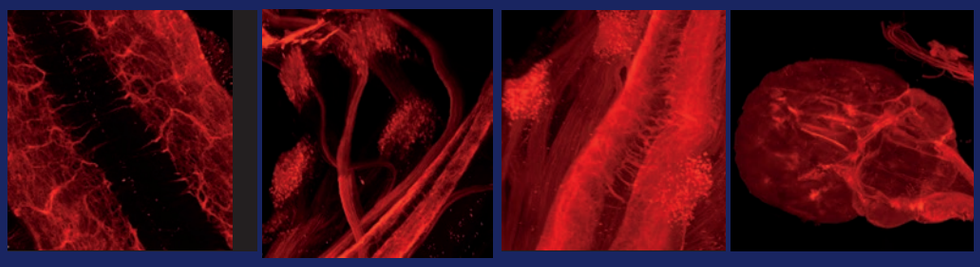
By the way, if you wanted to put Loewen's 1.2 terabyte scan data on CDs, you'd need 1,800 of them, or maybe a drawer full of thumb drives. Imagine trying to upload and download terabytes of information on the Cloud as a routine. It's a nonstarter, Watkins will tell you.

So CBI decided to build its own 3-D file storage system, aided by a supplement from the Defense Threat Reduction Agency awarded to Klimstra and Watkins.

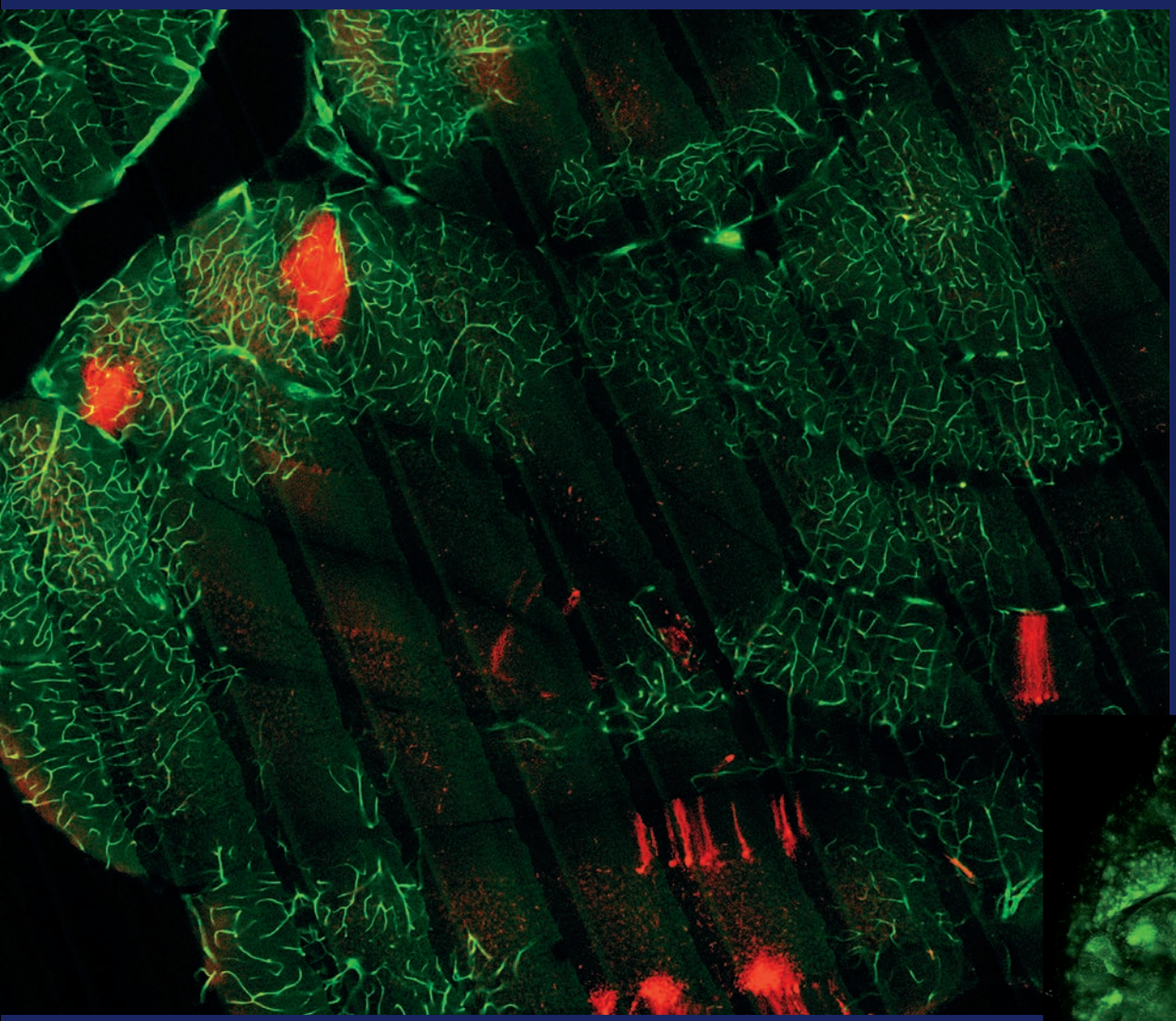
"We call it our hive storage solution," says Watson.

After an expansion funded by the Department of Cell Biology, the hive can now hold a petabyte and a half (that equates to about 2.3 million CDs, or 1.5 quadrillion bytes). It clocks in at 10 gigabits per second.

Alan Watson, who started his career peering at brain



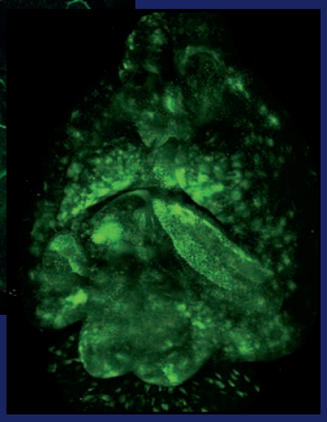
ALBERS LAB



OPPOSITE PAGE: Kathryn Albers, a professor of neurobiology at Pitt, is interested in learning more about how the peripheral nervous system develops in relation to the central nervous system and, ultimately, to the brain. These 3-D scans gave her a view of the systems that she'd never had before.

Using CBI's 3-D technology, Albers's team plans to investigate how certain biomolecules (neurotrophic growth factors) expressed in peripheral tissues influence the development and growth of nerves.

The approach should let her explore how the entire nervous system is affected by these factors. "Connecting the periphery to the brain," says Albers, "has always been a very difficult thing to do because of the complexity and size" of the nervous system. "This is a technology that lets us put it all together."



THIS PAGE: Before Watson joined the CBI, he was a postdoc in William Klimstra's lab studying viral infection. This series shows a mouse brain infected with Venezuelan equine encephalitis virus (red). When Watson first came to the CBI, he says he was "pecking and pecking here and there" to find a few infected cells. By scanning entire organs, Klimstra's team was able to identify infected regions throughout the brain. The investigators learned that collapsed microvasculature may contribute to the severity of the disease.

tissue, muses on what would be required to collect and scan data on an entire human brain at the cellular level.

Such an effort would probably take a year using the ribbon scanner (or a dozen with a more conventional scope); it would yield thousands of petabytes of information. That project is out of reach for the moment.

"We will do it eventually," Watson says. "Just not tomorrow." Until then, there is plenty to learn.

Anne Robertson is an engineer. She's interested in how blood vessels change after cerebral aneurysms strike.

Before she started working with Watson and Watkins, she'd section arteries to image them. That left her with a bunch of tiny donut-shaped samples that gave her hints about how structure relates to function but didn't tell her much about how fibers within the artery fit together.

The Pitt professor of mechanical engineering and materials science and of bioengineering wondered, *How do you know whether one slice is representative?*

After she learned about the CBI's immersive techniques, she said to herself, *We can ask completely different questions!*

Looking specifically at the fibers of a longer section of artery (shown on p. 17) helped Robertson see how the fibers are intertwined, as well as their orientation. Examining calcification in the aneurysm wall revealed that the fibers wend around calcification rather than going through it or ending abruptly at its boundary. By surveying the structure before running computations, her team can develop a more accurate and sophisticated model for the artery.

Robertson was one of Spirou's many appointments that December day. During their meeting, she used the syGlass/CBI VR setup once again; this time she "entered" an aneurysm wall. Her assessment: "phenomenal!"

"When you're in 3-D, you all of a sudden see these things you've been trying to see in your head." ■