THE AUTHOR FILE

Rohit Bhargava and Carol Hirschmugl

Multiple synchrotron beams make infrared imaging faster and clearer.

Rohit Bhargava expected that 12 beams from a synchrotron would boost the quality of his imaging data, but he still was not prepared for what he saw. Bhargava, a bioengineer at the University of Illinois at Urbana-Champaign, uses a technique called infrared spectroscopic imaging, which detects various chemical groups in a sample based on their absorbance of infrared light. It is a specialized technique, valued not for its ability to produce stunning pictures but because it can yield molecular-level information without the need for labels. Normally, though, that means detecting lipids, fats and carbohydrates with a resolution of 5 micrometers or worse. Hence Bhargava's surprise at results from the new technique: "You could start to see details that you are used to seeing only in optical microscopy," he recalls. "The crispness, the details were comparable." In fact, the pixel size is only a half micrometer in diameter, a hundredth the size of current state-of-the-art infrared imaging.

Carol Hirschmugl and Rohit Bhargava

"Even to this day, every time we take data, I'm shocked by the quality of the images," says Carol Hirschmugl, a physicist at the University of Wisconsin–Milwaukee who, with Michael Nasse and others, developed a technique that uses multiple synchrotron beams to illuminate samples with infrared light. Not only is resolution improved, the imaging technique is also faster than methods that rely on single beams or on heat sources to produce infrared radiation. Data that would normally take 11 days to collect can now be acquired in 20 minutes.

The idea for the project began when Hirschmugl learned about a 'weekend experiment' at Brookhaven National Laboratories. A team of scientists set four beams onto nonbiological samples and showed that resolution improved, she says, but they did not take the project further to complex biological samples, largely because getting access to a beamline is difficult.

Hirschmugl was intrigued, so she approached the scientists at the Synchrotron Radiation Center at the University of Wisconsin–Madison, which was built to produce beams with considerably less noise than other synchrotrons. The director offered access to a bank of 12 beams—provided Hirschmugl could get the necessary funding.

When the funding came in, she and the engineers got access to the beamline within 2 years (a timeline so short Hirschmugl refers to it as "miraculous"). To figure out how to harness the setup for infrared imaging, the team spent weeks, sometimes working "morning to morning," testing algorithms to align the beams and focus the 48 mirrors.

Their technique could be used to take great pictures of polystyrene beads, but to learn whether the method would be useful for biological imaging, Hirschmugl's team had to find a biologist who could ask the right kinds of questions. That led her to Bhargava, who had worked on some of the earliest prototypes of infrared microscopy, including theoretical research on how to acquire data to get informative images.

"Out of the blue I got a call from Carol," Bhargava recalls. She said she had an interesting instrument and invited him to try it out. "It was very clear from the theoretical work that this would be something different," he says, "but I couldn't have anticipated the nice results we would get." For the first time, the researchers could distinguish the collagen-dense interface between epithelial and stromal cells using infrared imaging, and could distinguish between cancerous and healthy tissue in fixed slides of prostate and breast samples.

But that is just the beginning, the collaborators say. Any samples that have chemical organization at the micrometer scale can be imaged in this facility: projects under way include studying stem-cell differentiation, malarial parasites inside cells and even the pigment and oil layers of 500-year-old paintings. Theory-based research can also expand. Work developed for wide-field imaging with optical techniques can be applied to infrared imaging, and experiments on the synchrotron may show ways to improve desktop infrared imaging instruments.

Hirschmugl plans to invite more collaborators to the facility and even to build facilities onsite to enable experiments on living cell cultures. That, however, may depend on new sources of funding: in the same month this paper in *Nature Methods* was accepted for publication, the US National Science Foundation cut funds for the Synchrotron Radiation Center. "Now that we have these beautiful results, [the National Science Foundation] is not funding the running of the synchrotron," says Hirschmugl. "It's been an up and down time." But perhaps, she says, it represents a new opportunity; she and colleagues are looking for funding sources to reinvent the Synchrotron Radiation Center as a dedicated infrared imaging center.

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Nasse, M.J. *et al.* High-resolution Fourier-transform infrared chemical imaging with multiple synchrotron beams. *Nat. Methods* **8**, 413–416 (2011).