

Synergistic collaboration leads to new strategy for biomedical 3-D imaging

January 12 2021, by Russell Dickerson



3-D reconstruction of fluorescent stained cotton fibers. The blue, green, and red panels are slices of the object from x—y, y—z, and x—z slices, indicated by the colored rectangle in the main figure on the right. Scale bar equals 60mm. Credit: Randy Bartels



When it comes to getting a three-dimensional look at cells in the human body, it is not much different than figuring out precisely where a firefly is in a field at night. We can tell which direction it is in, but it is challenging to know how far away it is.

A firefly emits luminescent, incoherent light. The <u>light waves</u> spread out without propagating along a particular direction, which makes determining the exact location of the firefly difficult.

A bat flying through the night sky would not have the same problem. It can easily locate that poor firefly by launching a sound wave in the direction of the fly and listening for the return echo. The bat's <u>sound</u> <u>wave</u> is coherent and directional, allowing her to pinpoint the location of the firefly with the backscattered sound waves.

Similar coherent wave scattering is used in all sorts of everyday technologies, including <u>ultrasound scans</u>, sonar, radar, and coherent optical diffraction. All of these methods require coherent waves, with well-behaved peaks and valleys of the wave as it propagates. In the world of optics, lasers exhibit the same wave coherence.

Under support of funding from the National Institutes of Health, electrical and computer engineering Professor Randy Bartels' group, in collaboration with Professor Ali Pezeshki, Dr. Jeff Field, Colorado School of Mines Professor Jeff Squier, and graduate student Patrick Stockton, found a way to treat incoherent light emission as if it were coherent light. This new technology allows the team to collect incoherent light emitted by <u>fluorescent molecules</u> and reconstruct 3-D digital models of the <u>object</u>.

"We now have a completely new way to figure out where fluorescent



light is coming from that wasn't accessible before," said Bartels.

Creating a model from incoherent light

Published in the journal *Optica*, Bartels' group combined optics and mathematical computations to develop a new strategy that shapes incoherent fluorescent light emitted by an object to form a high-resolution 3-D image.

Bartels compares the strategy to ultrasound imaging that creates an image of a cell or other object within the human body. Ultrasound uses the oscillations of sound waves reflected off an object to create an image, using <u>mathematical computations</u> to work out the differences in distance and time it took to return a wave back to the detector.

The problem with fluorescent light, often used in optical microscopes, is that the light is incoherent. The incoherent fluorescent emission scrambles the phase of the emitted light, which hides the location of the fluorescent emitters.

The collaborative team employed a strategy that mimics coherent light scattering in an image of incoherent light emission, by transferring differences in the phase of spatially coherent beams into a temporal variation of fluorescent light emission. Using a spatial and temporal modulation of the illumination light, along with a mathematical model of the signal formation, the team created a higher resolution 3-D model through computational inversion of the data.

The process mimics the preservation of coherent oscillation of light in the scattering process, returning measurements of the precise location and brightness of objects emitting incoherent light.

"We have a sequence of shaped light that we use to illuminate the object



and then we simply measure the power of the fluorescent coming out of the object. These data when combined with a mathematical model allow us to figure out the 3-D distributions of molecules," said Bartels. "This process mimics coherent scattering much like ultrasound imaging."

Combining math and optics to create models

Taking all of those measurements of light gives data, but it is only useful if the right model can be built to interpret it.

CAT scans and MRIs use similar mathematical models to take data that are low-dimensional representations of the object to build a detailed 3-D image. To use incoherent light to create a 3-D digital <u>model</u> requires a new mathematically-driven strategy.

That's where electrical and computer engineering Professor Ali Pezeshki comes in.

Using data from the total power measurements of shaped <u>light</u> coming out of a fluorescent object, Pezeshki's mathematical models keep noise managed and valuable information from being buried. The threedimensional distributions of molecules can then be collected as if they were coherent.

Synergistic collaboration

This work is one of the highlights of a productive multidisciplinary collaboration between Bartels' group and the Squier group at the Colorado School of Mines.

"It becomes a synergistic collaboration," said Bartels. "It has to be a conversation between people of different expertise to understand the



limitations of the different domains."

Since 2016, the groups have collaborated on nearly a dozen published publications, with more being written. The interdisciplinary efforts of mathematics, science, and engineering enable them to push the boundaries of optical imaging with applications from advanced manufacturing to neuroscience.

"Students really get to see problems from the different perspectives supplied by Randy, Jeff Field, Ali and myself," said Squier. "We have made advances in imaging I suspect none of us foresaw until we launched this collaborative effort and are now applying it across domains that we hadn't envisioned previously."

More information: Patrick A. Stockton et al. Single-pixel fluorescent diffraction tomography, *Optica* (2020). DOI: 10.1364/OPTICA.400547

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