IMAGING

Attention to detail

Computational processing allows sub–diffraction-limit resolution imaging with a standard wide-field microscope.

Gordon Wang was working during a break at a conference when a group of astronomers noticed the pixelated dots on his laptop screen. They asked if he was looking at a field of distant stars. "I said, 'No—this is actually the brain!'" recalls Wang, a postdoc in Stephen J. Smith's lab at Stanford University. The similarities between the data would prove greater than expected.

Smith's group pioneered a method called array tomography, in which ultrathin brain slices are arranged on a slide and imaged serially, enabling researchers to position fluorescently labeled molecules in a three-dimensional reconstruction of the tissue. But imaging artifacts make it hard to tease apart adjacent signals at the single-molecule scale, particularly given the resolution boundaries imposed by the diffraction limit.

Wang's astronomer friends suggested a computational image-processing technique called Richardson-Lucy (RL) deconvolution. RL can eliminate artifacts under conditions where signal characteristics and sources of noise can be accurately modeled—as when imaging space, or in ultrathin tissue sections that minimize heterogeneity. "The way we're imaging with fluorescence is actually a lot like taking in a field of stars at infinity," says Wang. "We have extremely low background; the two data sets are actually very similar."

If the data can be fitted well to the deconvolution model, one can even image at sub–diffraction-limit resolution with a standard wide-field microscope. Wang and Smith demonstrated that RL could accurately discriminate between two fluorescent beads 100 nanometers apart, which appeared as a single blob in standard wide-field imaging. In subsequent experiments with clumps of microtubules or cortical sections in which the protein synapsin was fluorescently labeled, RL-deconvoluted wide-field images achieved resolution and imaging accuracy comparable to those of true super-resolution microscopy.

Wang is excited about being able to delve into the fine details of protein localization and interaction at synapses and other complex biological structures. "This algorithm actually allows you to estimate the colocalization distance at each putative synapse," he says. Similar deconvolution should be possible with other thin-section imaging techniques. "A lot of people in the microscopy realm are starting to think about computational super-resolution," says Wang.

Michael Eisenstein

RESEARCH PAPERS

Wang, G. & Smith, S.J. Sub-diffraction limit localization of proteins in volumetric space using Bayesian restoration of fluorescence images from ultrathin specimens. *PLoS Comput. Biol.* **8**, e1002671 (2012).