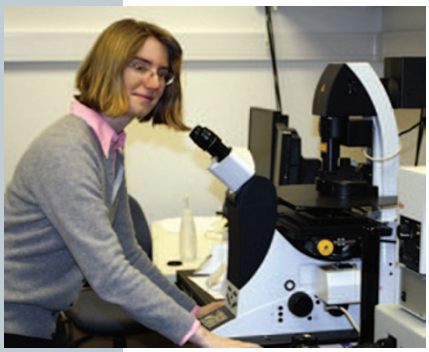
THE AUTHOR FILE

Susan Cox

Using Bayesian statistics to speed super-resolution microscopy

When Susan Cox set out to develop a way to get more information from super-resolution images, she found that the work required three crucial ingredients: late-night trains, take-away food and a large whiteboard.



As a new research associate in Rainer Heintzmann's laboratory at King's College London, Cox was working on super-resolution microscopy, which lets researchers observe structures once considered too small to see with light. Her job was to assign single fluorophores to precise positions. This is an established approach that requires activating fluorescent molecules sparsely so that their signals can be reliably separated. As she began analyzing data, Cox encountered a common problem: signals from fluorophores frequently overlapped, forcing her to discard many images. If she wanted to watch cells change over time, she would have to either accept limited information or find a way to pinpoint individual fluorophores amid overlapping signals.

Cox decided to consult acquaintances who studied computer vision. She took her laptop and headed west to Oxford University, then north to Cambridge University. "I took it round to a lot of people, and mostly they said it was too difficult," recalls Cox. Finally, Ed Rosten, a former colleague (and fellow first author of the paper on p195), agreed to collaborate. At the time, says Cox, neither realized that they were about to embark on a three-year project that would largely be conducted on nights and weekends, since it wasn't initially part of either scientist's official duties.

To localize fluorophores' signals, Cox and Rosten needed statistics. More specifically, they needed an inference method that could model how many fluorophores bleach and blink. One approach was precise but impractical: calculations to find positions of even fifteen fluorophores would take years, says Cox. Another approach was much faster but less accurate. Cox and Rosten constructed a hybrid method that would gain speed from the second approach but accuracy from the first.

They spent months working out a series of proofs to show that the hybrid model was sound. This was difficult, not least because descriptions of the two approaches used conflicting notations: the same symbols meant different things. It was like bringing together two pieces of music, one written in treble clef, one in bass clef, and then writing them down in yet another register, says Cox.

Cox and Rosten worked out the math separately over holidays but ultimately found that they could progress faster bouncing ideas off each other in person. Cox began traveling to Cambridge to work with Rosten on a whiteboard, having food delivered so that they could argue about math without breaking for dinner. It became standard routine, says Cox. "I always associate takeaway pizza with Bayesian maths."

Once the mathematical proofs were finally worked out, they needed to try their analysis on biological specimens. Serendipitously, Gareth Jones, also at King's College, approached Heintzmann around this time to enquire about using super-resolution microscopy in his study of podosomes, formations on the cell membrane that allow cells to migrate and adhere to each other. The structure of podosomes and the speed with which they move made them perfect candidates for Cox's and Rosten's analysis method, dubbed 3B, for Bayesian analysis of bleaching and blinking.

Initial results, says Cox, were "not spectacular." Then Rosten suggested a slight tweak—rather than displaying the maximum likelihood of each fluorophore's position, they would map a range of possibilities. With that adjustment, 200-nm structures, previously seen as faint smudges, came into clear view. "Our jaws just dropped," says Cox. "We took it to Gareth, and his jaw just dropped, and that's when we all knew we had something really special. That's when we realized this method would make a difference."

Ultimately, they were able to watch podosomes in live cells, collecting 50-nanometer-resolution 'snapshots' every 4 seconds. (In practice, each 'snapshot' is constructed from 200 images, enough to allow reliable localization of the fluorophores.) Cell biologists can use this method on a standard microscope to get super-resolution data, says Cox. For scientists already performing super-resolution techniques, the method should allow them to collect more information on faster time scales. The technique works for video data and for two-color experiments.

The researchers have released the code for the analysis technique, but Cox admits that it will be difficult for most biologists to implement. However, she and colleagues are currently working on a plug-in for ImageJ, a popular image analysis program, and that should make the technique much more accessible.

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Cox, S. *et al.* Bayesian localization microscopy reveals nanoscale podosome dynamics. *Nat. Methods* **9**, 195–200 (2012).