IMAGING

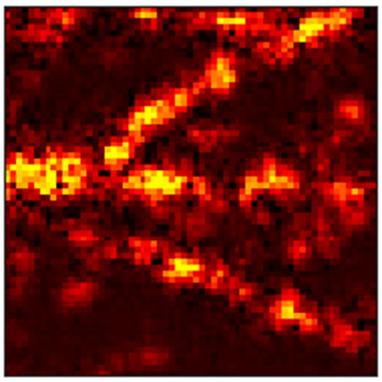
Quantum imaging in biological samples

The combination of image scanning microscopy and quantum imaging improves resolution up to fourfold compared with the classical diffraction barrier.

The diffraction barrier as defined by Abbe limits the achievable resolution to about one-half the wavelength of the light used. Several super-resolution microscopy methods have been developed in recent years to overcome the diffraction barrier. Dan Oron and his colleagues from the Weizmann Institute of Science in Rehovot, Israel, have now developed a method that combines image scanning microscopy (ISM) and quantum imaging.

ISM is very similar to confocal microscopy, but the confocal pinhole is replaced by a detector array with individual pinholes for each detector, resulting in a twofold enhancement in resolution compared with the diffraction barrier. ISM and most other super-resolution techniques treat light as a wave. In contrast, quantum imaging treats light as quantum particles (i.e., photons). Limitations of the detector hardware, which was too noisy and slow, compromised advances in quantum imaging. However, recent improvements in detector hardware, such as single-photon avalanche detectors (SPADs), have enabled Oron and his colleagues to demonstrate how the combination of quantum imaging and ISM (Q-ISM) can improve the resolution of biological samples.

Q-ISM works by recording both the conventional ISM information and the quantum information with a standard confocal microscope equipped with a modified detector. The fluorescence signal is imaged via a fiber-bundle camera consisting of 14 fibers, each coupled into a SPAD. These feed the signal to a time-correlated single-photon counting card to measure the arrival times of the detected photons. In addition to collecting spatially resolved information, this enabled the researchers to detect temporal correlations of arriving photons at fiber pairs. "The [correlation] data is there anyway, whether you measure it or not," Oron explained. A requirement for using this information is that "we have emitters that … emit just one photon [at a time]," which is the case for the fluorophores used in biological experiments, says Oron. This means that if coinciding photons are detected in a pair of fibers, they cannot have come from the same emitter, no matter how close the photons are. Furthermore, there are slightly fewer coinciding photon pairs than one would expect from the number of fluorescent molecules in the sample or the fluorescence intensity. "This small difference is proportional to the number of emitted pairs, and you try to quantify the pairs that don't arrive, the missing pairs. And that is our signal," says Oron, explaining the underlying principle of Q-ISM detection of missing coinciding quantum signals.



Q-ISM image of microtubules labeled with quantum dots. Adapted with permission from Tenne et al. (2019), Springer Nature.

The researchers demonstrated up to 2.5-fold enhanced resolution of Q-ISM in fixed cells by labeling microtubules with quantum dots. Quantum imaging works also in the axial direction, and provides substantially enhanced axial resolution compared with that of confocal microscopy.

As its only required modification to a standard confocal microscope is the detector, Oron expects their method to be an easy upgrade to an off-the-shelf confocal microscope, once the detectors become more affordable.

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Research papers
Tenne, R. et al. Super-resolution enhancement by quantum image scanning microscopy. *Nat. Photonics* **13**, 116–122 (2019).