ECE594Q: Computational Biomicroscopy Sandeep Bhat
Critique on "Dynamic far-field fluorescence nanoscopy [1]" sandeepkbhat@ece.ucsb.edu

Context:
Dynamic far-field nanoscopy addresses the following challenges. Firstly, the lateral resolution of any lens-based (far-field) optical microscope is limited by diffraction to $\lambda/(2n \sin \alpha)$, where λ is the wavelength of light, n is the index of refraction, and α is the semi-aperture angle of the lens (1873, Ernst Abbe). Secondly, the imaging technique needs to be sufficiently fast to achieve the frame rates required to capture the motion of the particles accurately. Lastly, the imaging of dynamic structures at nano resolution results in fewer photons being acquired per image.

Method description:
Stimulated emission depletion (STED) microscopy achieves super-resolution through intelligent use of light and fluorophore properties. The fluorophores are excited from the ground state (S_0) to the fluorescent state (S_1) using an ordinary beam. The beam is co-aligned with a wavefront-modified beam producing a doughnut-shaped focal spot, which depletes S_1 by stimulated emission. This confines the fluorescence to a smaller region. The FWHM of the resultant fluorescence spot follows $d \approx \lambda/(2n \sin \alpha\sqrt{(1 + I / I_{sat})})$ where I is the intensity of the STED-beam at the doughnut crest and I_{sat} denotes the saturation intensity. As $I / I_{sat} \rightarrow \infty$, we see that $d \rightarrow 0$. Therefore, the spatial resolution can theoretically be improved up to the molecular scale. Practically spatial resolutions of 16-80nm have been achieved. But so far this technique has been applied only to static samples. This paper proposes a rapidly scanning STED microscope for imaging of dynamic structures. The technique achieves frame rates that are three times greater than video frame rates. Fast scanning along x-axis is achieved by use of a 16kHz resonant mirror. Scanning along y and z axis is done by use of piezo stage featuring a digital piezo controller with an internal feedback loop to minimize scan errors during rapid, repetitive scans. High photon count rates are achieved by dividing the light due to fluorescence with a 1:4 fiber coupler onto four avalanche photo diodes featuring a quantum efficiency of over 65%. The resulting bright images enable high frame rates. This method allows bidirectional nano resolution frame acquisition at up to 80 frames per second (fps). Its capabilities are demonstrated by applying the technique to visualize and quantify the Brownian motion in a highly concentrated suspension of 36 nm particles.

Features and limitations:
Comparison of the STED images with those acquired from confocal microscopy reveals the advantages of the technique. Unlike the confocal images, the STED images (after linear deconvolution using Wiener filtering) clearly show the individual diffusing particles. In fact, the confocal images fail to even discern the individual particles. Unlike other super-resolution techniques like PALM, STORM or Structured Illumination which are slow (or extremely slow), STED is fast and is therefore able to produce the high frame rates required to image the dynamic particles. The images captured still need to be analyzed offline by using automatic localization and tracking algorithms to assess speed, trajectory and other statistics of interest. However, the image acquisition itself does not need any special computation and the resolution improvement is purely physical in nature (i.e. it is still based on diffraction). The Continuous Wave STED (CW-STED) technique is limited by the high cost of the pulsed lasers required.

Proposed improvements:
1. The technique suggested in the paper applies only a single local zero (the fluorescent region that remains after stimulated depletion) in the focal STED intensity distribution. By illuminating multiple spots and using arrays of zeros the technique could be parallelized thereby increasing the speed of the system.
2. The fluorescence signal can be increased 2-fold by collection with two objectives in a 4Pi configuration. The effective PSF of the system would be far narrower in the axial direction compared to the single objective configuration. The resultant improvement in the axial resolution would thereby enable better tracking of particle motion along the axial direction.
3. A-priori knowledge of the features in the sample can be used to reduce the amount of signal (photons) needed to detect them and hence improve the overall speed of the system.

Applications:
STED microscopy has unraveled protein agglomerations on the nanoscale inside cells and on cell membranes. But these samples have mostly been 'static'. The technique suggested in the paper would enable sub-diffraction resolution images of live cells.
In fact the simplicity of the technique (use of regular lenses and visible focused light) coupled with its capability to image at high speeds makes this technique ideal for far-field optical nanoscopy of any dynamic sample.

Conclusion:
Given the simplicity and the advantages of this method, I would definitely use (and recommend) this technique as a primary tool for capturing nanoresolution images of dynamic structures.

References:

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