3D Nanoscopy at 10,000 Cells a Day

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Single-molecule switching (SMS) nanoscopy techniques like STORM/(F)PALM circumvent the diffraction limit to enable 3D fluorescence imaging with resolutions in the range of 10-75 nanometers. This increased spatial resolution is achieved by sacrificing temporal resolution - typically requiring several minutes to reconstruct a single super-resolution image, which limits the number of cells that can be studied with SMS. While our lab has recently implemented high-speed SMS by using high laser power, sCMOS cameras, and sCMOS-specific localization algorithms [1], conventional analysis pipelines cannot handle the high data volumes of up to 70 TB/day this produces. Overcoming this, we have now developed a platform for high-speed and high-throughput SMS, which enables us to perform automated super-resolution imaging of ~10,000 3D fields of view a day.

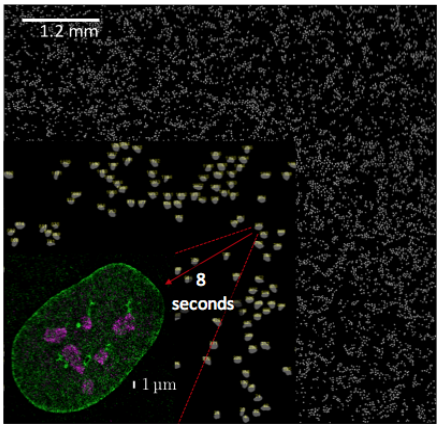

Figure 1: Overview image of nuclei on coverslip, with inset zoom of detected nuclei and example 8 second SMS image

Our platform is an integrated system, comprising of an automated biplanar astigmatism microscope specifically designed to produce high volumes of 3D and two-color SMS data as well as a computer cluster for real-time analysis. The system can record SMS images of whole nuclei (25 x 25 x 4 um) in 8 seconds, and can image autonomously for at least 30 hrs. The computer cluster runs PYthon Microscopy Environment (PYME) software, which we have developed to include a microscopy-specific compression algorithm and distributed storage framework to move data off of the instrument computer in real time despite recording at the full 800 MB/s bandwidth of sCMOS cameras. In order to truly enable high-throughput SMS microscopy, we additionally use the computer cluster to run our now fully GPU-accelerated sCMOS-specific localization algorithms to localize 39,000 emitters/s, which is real-time at our 800 Hz imaging for up to 49 emitters/frame. The distributed analysis backend in PYME additionally supports batch-processing post-localization analysis recipes over thousands of super-resolution images or point-clouds.

Our extended high-speed imaging with real-time localization and distributed post-localization analysis converts SMS imaging from a qualitative or small-scale quantitative tool into a valid imaging technique for large-scale quantitative hypothesis testing. After preliminary high-throughput demonstrations classifying cells into phenotypic subpopulations, we are now leveraging our advances to study the organization of the interphase nucleus, specifically studying the size and positioning of lamin-associated domains with the nuclear envelope.

[1] F. Huang, et al., "Video-rate nanoscopy using sCMOS camera–specific single-molecule localization algorithms", *Nat. Methods* **10**, 653 (2013)