

HIGH-SPEED, MULTI-COLOR LIVE CELL NANOSCOPY USING MUSICAL

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High-speed, multi-color nanoscopy of sub-cellular structures in live cells opens several possibilities to investigate biological phenomena. Localization microscopy (LM) can provide resolution of ~ 20 nm. However, the need for thousands of frames for one LM image limits the imaging speed, and different customized chemical environments for each color restrict multi-color nanoscopy [1]. Lastly, toxic photochemical environment used in LM is uncondusive for live cell imaging.

Recently proposed Multiple Signal Classification Algorithm (MUSICAL) provides support for all the above mentioned desirable characteristics [2], albeit with a resolution of ~ 50 nm. MUSICAL does not require special photochemical environment, thus supports multi-color nanoscopy. Further, 50 to a few 100 frames are sufficient for supporting nanoscopy, permitting high speed nanoscopy. Here, we present results on diverse cellular systems, such as *E. coli* bacteria (Fig. 1), cardiomyoblasts (Fig. 2), and macrophages to illustrate multi-color live cell nanoscopy using MUSICAL. In Fig. 1 and Fig. 2, the frame acquisition rate for each color channel is 100 frames/sec (5 ms exposure time per frame per color channel in sequential mode). Images are taken using a DeltaVision OMX microscope with 1.42 NA. The net acquisition time for a two-color MUSICAL image is 1 second (100 frames per label) in Fig. 1 and 2 seconds (200 frames per label) in Fig. 2. The ability of MUSICAL to image the intercrystal space of mitochondria is evident in Fig. 2, indicating significant resolution enhancement over the mean image. Thus, MUSICAL supports high speed time lapse super-resolved multi-color imaging of living cells.

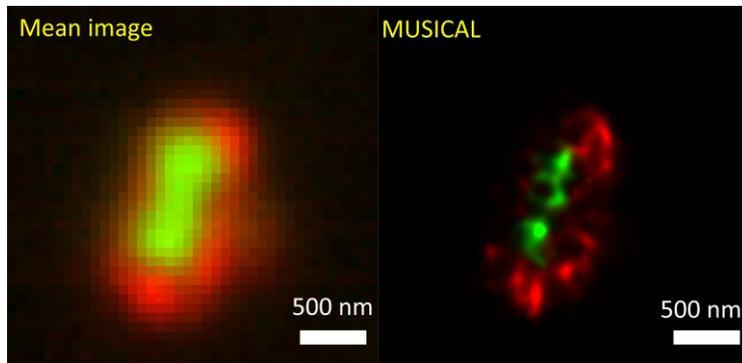


Fig. 1: The inner membrane and DNA of a living *E. coli* bacterium are labeled using MitoTracker Deep Red (red) and PicoGreen (green) fluorescent probes, respectively. The MUSICAL image is generated using 100 frames (exposure time 5ms).

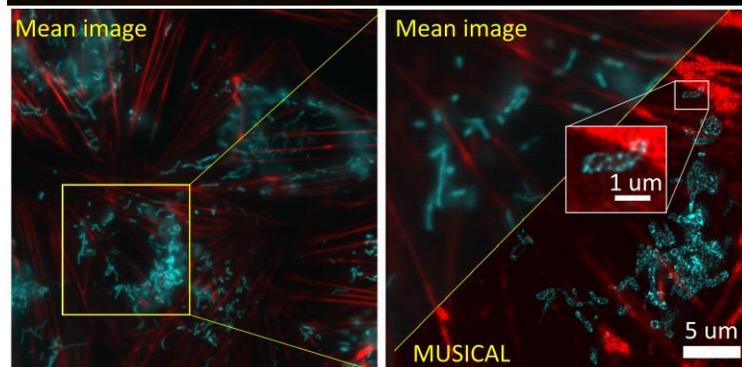


Fig. 2: F-actin and mitochondria in living cardiomyoblast cells (H9C2 cell line) labeled using SiR-Actin (red) and MitoTracker Green (cyan) fluorescent probes, respectively. The MUSICAL image is generated using 200 frames (exposure time 5ms).