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Deep Learning-Empowered Virtual Channel Separation Structured Illumination Microscopy

Doazheng Gong, Ashesh Ashesh, Florian Jug, Norbert F. Scherer

Machine learning and deep learning methods are becoming powerful tools in image analysis. Feature identification is made possible by multiple characteristics; color, morphology, context, etc. In this research we investigate the robustness of feature identification of sub-cellular (biological) structures with beyond diffraction-limit spatial resolution using a new multi-layer context-sensitive neural network method developed by our collaborators Ashesh Ashesh and Florian Jug at the Human Technopole in Italy. In particular, we employed this method on "super-resolution" image data obtained from a novel structured illumination microscope (SIM) developed at the University of Chicago[1], demonstrating a color-separation super-resolution imaging capability that allows spatial correlation of multiple intracellular structures and the measurement of dynamics on 100msec timescales.

SIM has become increasingly important in biological imaging offering a good balance between spatial and temporal resolution. The "super-resolution" aspect of SIM arises from the Moiré effect: the sample is illuminated (excited) with a known periodically structured pattern of light generated with, e.g., a digital micromirror device (DMD). This high-spatial frequency pattern is used in computational reconstruction of the sample's high spatial frequency information from the measured (e.g., fluorescence) image; i.e., the Moiré pattern. While multi-color SIM imaging is feasible, it usually requires complex alignment and trading off area on the array detector or (at considerable cost) using a separate detector for each color. Instead, we use machine learning to distinguish distinct intracellular structures for super-resolution microscopy data from "gray scale" imaging data, having accomplished this in the research funded by the *Data Science Institute.*

Figure 1 shows 3-color channel separation results we have obtained using the µSplit algorithm [2,3]. The model was trained with 32 images (2048 x 2048 pixels each) for 25 epochs. Structure similarity (SSIM) is used as a metric to measure the performance of the model's prediction. We obtain excellent separation in the actin (SSIM = 0.913) and mitochondria channels (SSIM = 0.860).

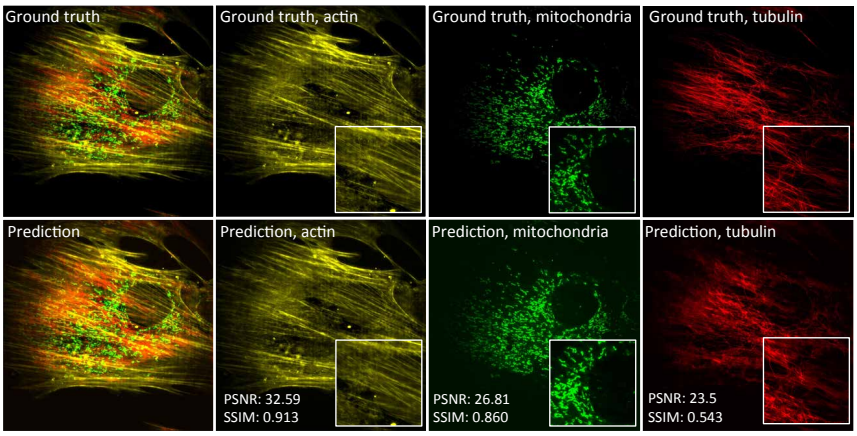

Figure 1. Preliminary multi-color virtual color separation and feature identification of SIM data using the µSplit deep learning approach. (left) 3-color superimposed experimental data (top) and computationally predicted (bottom). Other columns show experimental SIM data (top) and the computational feature prediction (bottom) for actin (yellow), mitochondria (green) and microtubules (red). Structure similarity (SSIM) is a measure of agreement: SSIM = 1 is perfect.

References: [1] Gong, et. al.; Multi-scan structured illumination microscopy for rapid and efficient volumetric super-resolution imaging; Optics Letters, under review (2025).
[2] Ashesh, et. al., *MicroSplit: Semantic Unmixing of Fluorescent Microscopy Data*, Nature Methods, manuscript in preparation (2025).
[3] Gong, Ashesh, Jug, Scherer, manuscript in preparation (2025).