performance. We conclude that deep convolutional neural networks are an accurate method that require less curation time, are generalizable to a multiplicity of cell types, from bacteria to mammalian cells, and expand live-cell imaging capabilities to include multi-cell type systems.

## P68

**Board Number: B268**

### Live 5D hyper-spectral fluorescence imaging of developing zebrafish.

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The advent of fluorescent proteins (FP) has revolutionized the use of fluorescence microscopy in biology. The color palette of fluorescent proteins has grown over the years covering the entire spectrum from blue to near infra-red. The use of multiple FPs has enabled studies of spatio-temporal interaction of proteins, cells and tissues in vivo within living cells or developing embryos. Multiple labels have been imaged within the same sample, however, timelapse imaging of multiple labels remains challenging. Limiting factors such as noise, photo-bleaching and -toxicity greatly compromise signal quality and throughput can be limited by the time required to unmix multiple labels. In this work, we report a method for rapidly denoising and unmixing multiple spectrally overlapping fluorophores while maintaining reduced negative photo-effects, in a low signal-to-noise regime. We successfully applied the method to 4D datasets of Zebrafish embryos co-expressing multiple labels, separating a total of 7 different FPs and intrinsic tissues autofluorescences, unmixing. Taking advantage of the technique's enhanced signal collection and fast processing, we expanded the multi-dimensionality to include time, obtaining 5D datasets (XYZ,time,label), which often fails in other techniques due to the challenges of photo-damage and bleaching. We successfully performed long-term imaging vessel sprouts transgenically labeled zebrafish embryos (Tg(ubiq: membrane-Cerulean-2a-H2B-tdTomato);Tg(kdrl:eGFP), expressing fusion proteins of two endosome components, Rab9 and Rab11 (YFP and mCherry respectively). The rapid processing and denoising properties of our approach permitted the clean separation of the FP signals from one-another and from autofluorescence, using low laser power that allowed for unaffected development, permitting 5D imaging of 7 clearly distinctive components.

## P69

**Board Number: B269**

### Engineering a Light Activated Caspase to use Temporally and Locally in vivo.

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