Learning to unmix single-color SMLM data for multicolor SMLM imaging

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1) Motivation and goal

- Simultaneous multicolour SMLM imaging is technically challenging to achieve, especially with more than two colors: it requires a search for chromatically distinguishable photo-switchable probes to avoid spectral overlap [1], and where each individual probe needs to have specific properties.
- Sequential imaging allows us to visualize unlimited number of structures [2], but requires washing and registration.
- Goal: learn to discriminate the different structures to transform the single-color image, where multiple structures are labeled with the same dye and imaged simultaneously, into multicolor one.

2) Multiple structures imaged simultaneously

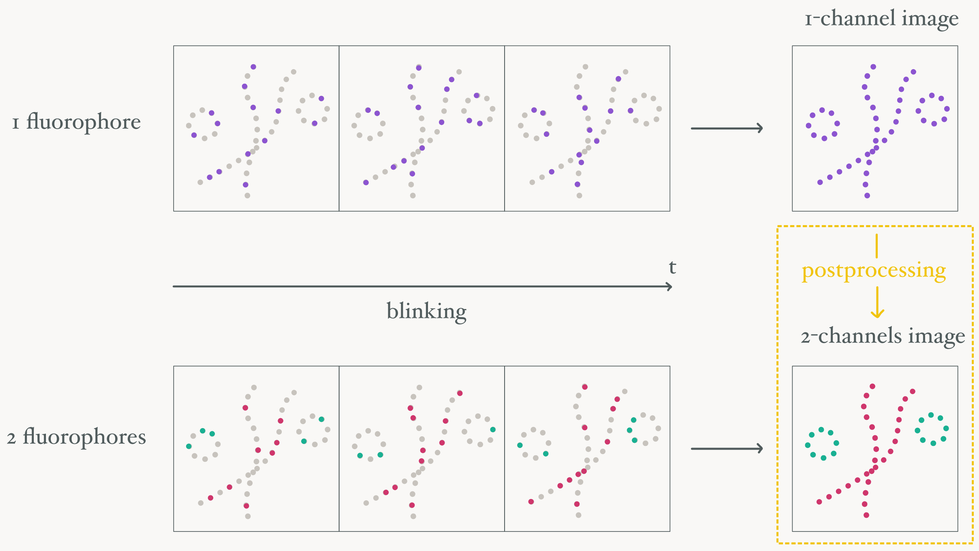

Figure: Our methodology aims to achieve SMLM multicolor imaging by using a single fluorophore, instead of using different fluorophore for each different structure. This new experimental methodology is coupled with deep learning techniques to achieve final unmixing.

3) Generating the dataset

The overlapping of single-color simulated or exprimental images give synthetic ground truth multicolor images. The input images are obtained by summing among the channels.

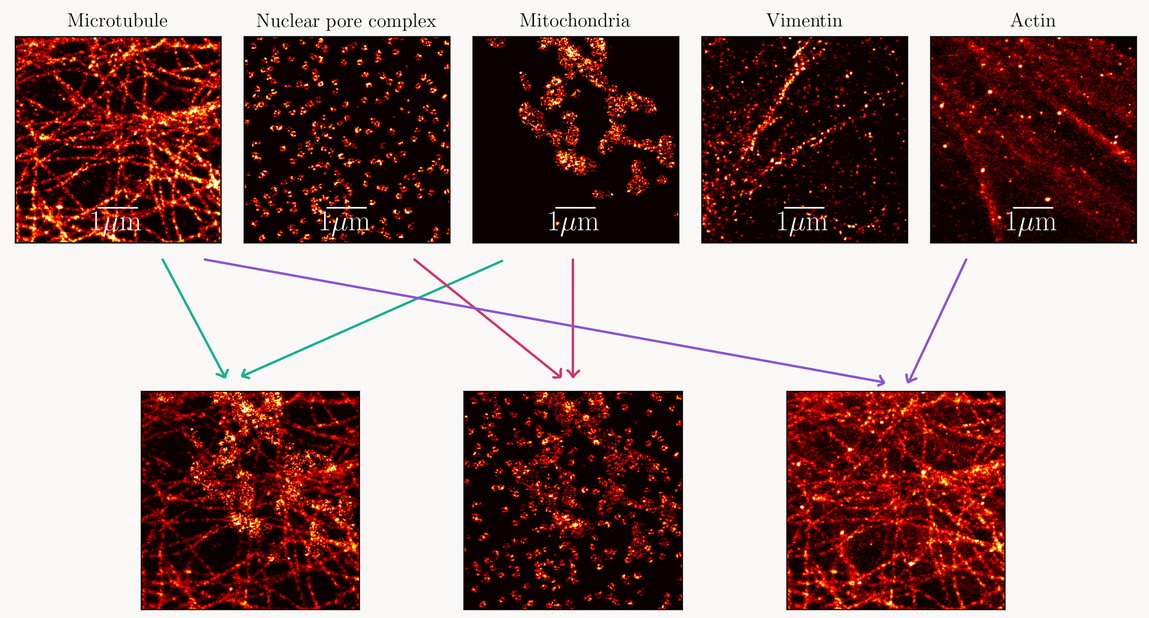

Figure: Composite images created from the sum of different independent structures.

4) Deep Learning Architecture

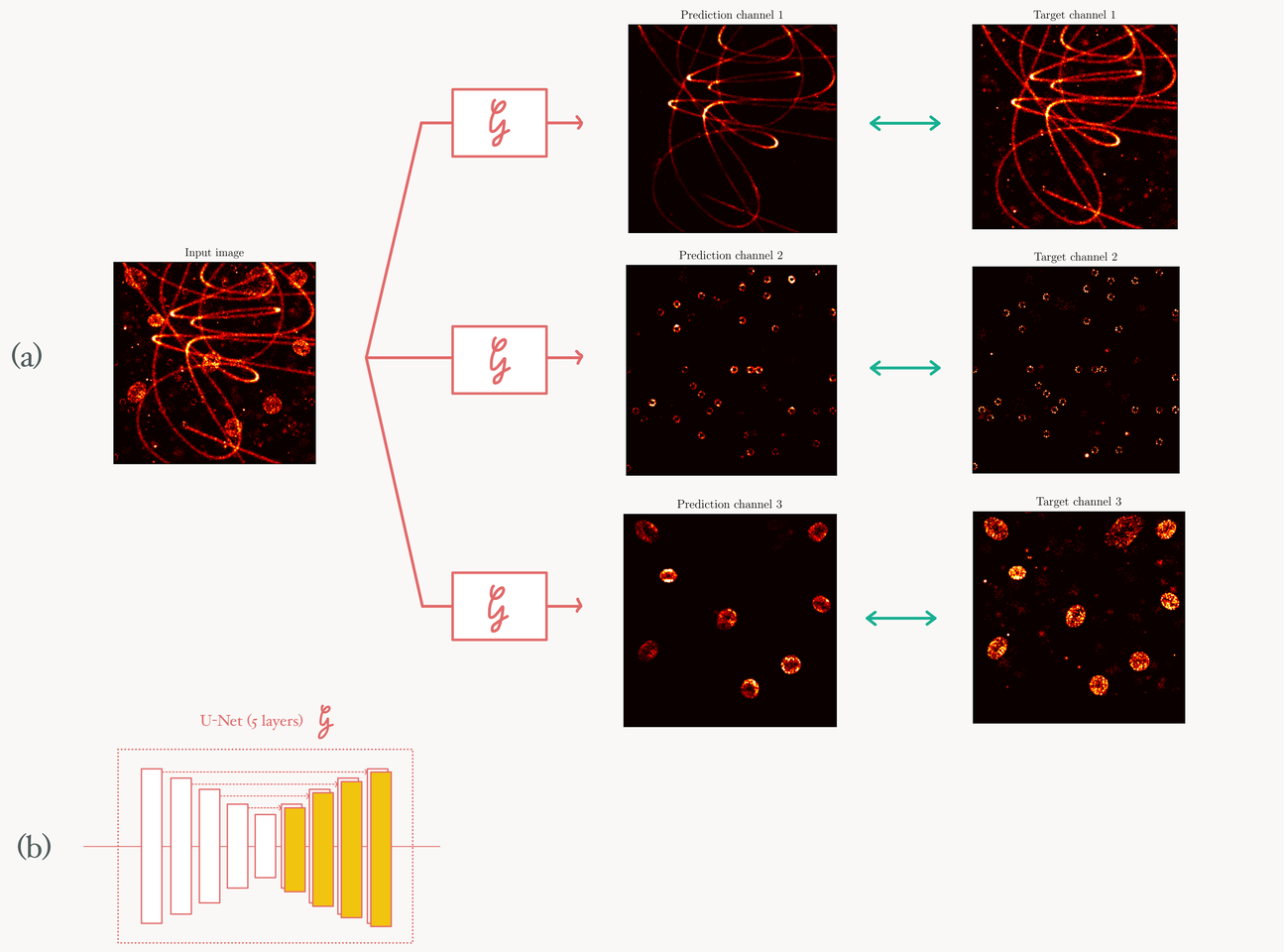

Figure: (a) Methodology to unmix the single color image into a multicolor image, here in a three-color simulated image. Input and targets images are two noisy samples computed from the same localization table such as the network learns to denoise while unmixing, on the same model as Noise2Noise [3]. (b) For each channel, a generator U-Net [4] $\mathcal{G}$ is trained, which consists of an encoder-decoder network with skip connections.

5) Results on simulated data

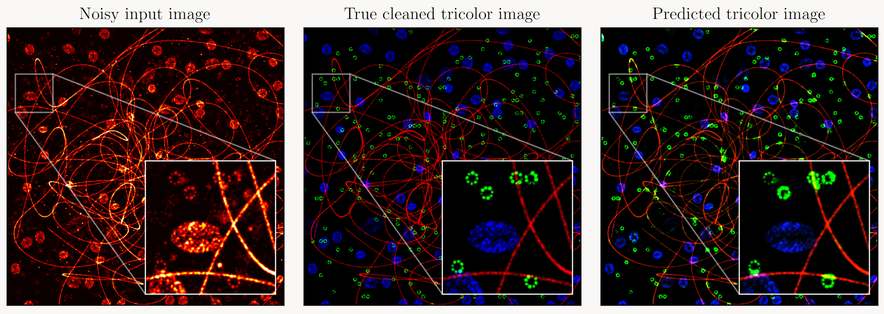

Figure: Output of the network evaluated on overlap of simulated nuclear pore complexes, microtubules and mitochondria.

6) Preliminary results on experimental bicolor data

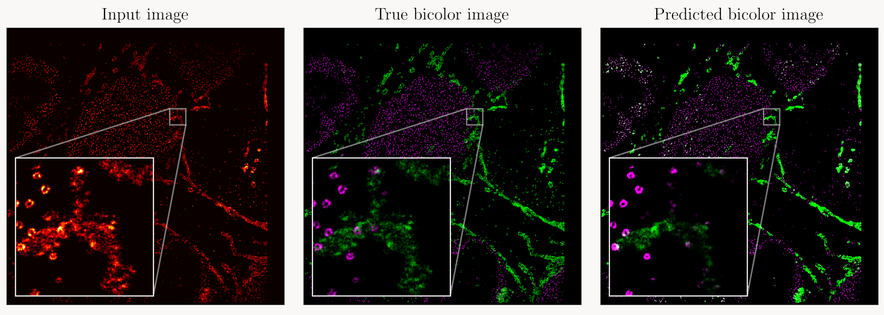

Figure: The single color image is constructed by summing among the channels. We observe limitation on the intersection of the two structures.

9) Conclusion and perspectives

- The proposed methodology effectively unmixes (and denoise) single-color simulated images and gives promising results when applied to experimental imaging of nuclear pore complex and mitochondria.
- Limitation 1: Reconstruction quality remains limited at intersetction
- Limitation 2: The method works only when the images are slightly blurred, such as the generator can learn continuous spatial patterns.
- Limitation 3: Poor results when structure have very different density (from example fir microtubules and nuclear pore complexes).
- Perspectives: Adressing limitations and extending to more than two colors on experimental data.

10) Acknowledgements

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INCEPTION

11) Bibliography

[1] Dempsey, Graham T, Vaughan, Joshua C, Chen, Kok Hao, Bates, Mark, & Zhuang, Xiaowei. 2011. Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging. *Nature methods*, **8**(12), 1027–1036.

[2] Klevanski, Maja, Herrmannsdoerfer, Frank, Sass, Steffen, Venkataramani, Varun, Heilemann, Mike, & Kuner, Thomas. 2020. Automated highly multiplexed super-resolution imaging of protein nano-architecture in cells and tissues. *Nature communications*, **11**(1), 1552.

[3] Lehtinen, et al. 2018. Noise2Noise: Learning image restoration without clean data. *arXiv preprint arXiv:1803.04189*.

[4] Ronneberger, Olaf, Fischer, Philipp, & Brox, Thomas. 2015. U-net: Convolutional networks for biomedical image segmentation. 234–241.