

¹ Deep Learning for Single Molecule Localization Microscopy

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⁷ Summary

⁸ Single Molecule Localization Microscopy (SMLM) enables researchers to interrogate nanoscale
⁹ spatial details in a range of systems. Biological investigations benefit greatly from SMLM
¹⁰ due to its ability to quantitatively investigate details of great importance such as protein
¹¹ distribution in the cell membrane or protein-protein interactions([Baddeley & Bewersdorf, 2018](#)).
¹² However, SMLM set-ups can be quite expensive, imaging times can be lengthy and data
¹³ analysis can require expert knowledge. Recently, Deep Learning (DL) algorithms have been
¹⁴ developed to reduce imaging time and automate data analysis. Naturally each subsequent
¹⁵ model aims to address a different shortcoming of the prior work and so there is a family of
¹⁶ model architectures. However, there is not a singular location for researchers to have access
¹⁷ to these models dedicated to SMLM. We developed the Deep Learning for Single Molecule
¹⁸ Localization Microscopy (DL4SMLM) in Python using the PyTorch framework to democratize
¹⁹ access to these models and lower the barrier of entry to SMLM. DL4SMLM is a Python
²⁰ package that uses the Pytorch DL framework to implement Convolutional Neural Nets (CNN)
²¹ dedicated to SMLM. We created it for researchers and engineers who wish to use DL for
²² SMLM in whichever field they practice granted they have sufficient data.

²³ Statement of Need

²⁴ DL4SMLM is designed to automate the training, validation and testing of different machine
²⁵ learning models dedicated toward the single molecule localization task in a single software suite.
²⁶ Currently, such models are located in their respective code repositories and/or enmeshed into
²⁷ software plug-ins. While this makes their models publicly available it hinders rapid re-training,
²⁸ prototyping and comparison of different models on the same dataset. By providing this software
²⁹ environment, investigators can generate their training data (simulated or experimentally
³⁰ collected), train various models, and then decide which model is best suited for their task.
³¹ Because our software is built atop of the Pytorch framework, our functions allow the user
³² to designate which device, CPU or GPU, the inference process will occur on. We allow this
³³ flexibility on the inference process because we want investigators to be empowered to assess
³⁴ how their models will perform on the CPU of their choice especially where they wish to push
³⁵ their models onto compute limited devices such as mobile phones([Diederich et al., 2019](#)).

³⁶ Features

³⁷ Models

³⁸ Currently, there are six models: Super Resolution Convolutional Neural Network (SRCNN)([Dong](#)
³⁹ et al., 2015), Deep-Stochastic Optical Reconstruction Microscopy (Deep-STORM)([Nehme](#) et

al., 2018), Skip-STORM, Unet for STORM (U-STORM)(W. Yao et al., 2018), Deep Residual STORM (DRL-STORM)(B. Yao et al., 2020), and Fast Dense Image Reconstruction based Deep Learning STORM (FID-STORM)(Zhou et al., 2023). SRCNN and Skip-STORM are novel architectures to the SMLM field. While SRCNN has been applied to perform the super resolution task in more traditional image processing tasks, its utility in SMLM is unknown. Skip-STORM's architecture is similar to Deep-STORM but a skip connection between the initial image and final layer is introduced to enable it to have a spatial context during its reconstruction phase which could assist it in emitter dense images. U-STORM is inspired by U-Net and adopts a similar biphasic architecture where in the first half the initial image is max pooled while simultaneously increasing channel number and the second half involves the up sampling and reduction in channel. It also contains skip connections that connect the features of the first half to the those of second half during image reconstruction. These models are implemented using the object-oriented programming paradigm enabling researchers to instantiate multiple models for a single data set to allow downstream comparison of inference performance.

55 Loss Functions

56 We have implemented three loss functions: L1L2, weighted mean square error, and weighted
57 mean absolute error. Each loss function accepts a lambda parameter which controls the
58 sparsity of the predicted output. The lower the lambda value the sparser the output while a
59 higher lambda value preserves more of the signal from the original diffraction limited image.
60 The L1L2 loss metric was first introduced by Nehme et al. (2018) and involves a gaussian
61 convolution of the predicted spikes in the ground truth image. We chose to implement the
62 weighted mean square error and weighted mean absolute error in instances where the gaussian
63 convolution is unneeded in the ground truth image but the user still desires control over the
64 sparsity of the predicted image.

65 Helper Functions

66 A custom ImageDataset class has been implemented using PyTorch's Dataset functionality
67 to automate the loading and normalization of the noisy diffraction limited images and their
68 super resolved counterparts. This dataset can then be loaded into the DataLoader class
69 in PyTorch. To assist researchers in visualizing the emitter localization performance of their
70 model, we have implemented a function that visualizes the diffraction limited image of the
71 emitters, its super-resolved version, and the predicted super-resolved image according to the
72 trained model. This empowers the researcher to troubleshoot the performance of the model and
73 determine if the model should be retrained with different parameters or to proceed with the
74 experiment. To facilitate the development of powerful yet accurate models we have implemented
75 two functions that enable knowledge distillation between a teacher model and a student model:
76 Hint Learning and Knowledge Transfer. Hint training automates the learning process of a
77 student network to have its intermediate representation mirror that of a teacher network.
78 Knowledge Transfer uses the attentive imitation loss function from Saputra et al. (2019), the
79 teacher model, and the ground truth data set to optimize the student network.

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85 References

- 86 Baddeley, D., & Bewersdorf, J. (2018). Biological insight from super-resolution microscopy:
87 What we can learn from localization-based images. *Annual Review of Biochemistry*, 87(1),
88 965–989.
- 89 Diederich, B., Then, P., Jügler, A., Förster, R., & Heintzmann, R. (2019). cellSTORM—cost-
90 effective super-resolution on a cellphone using dSTORM. *PLoS One*, 14(1), e0209827.
- 91 Dong, C., Loy, C. C., He, K., & Tang, X. (2015). Image super-resolution using deep
92 convolutional networks. *IEEE Transactions on Pattern Analysis and Machine Intelligence*,
93 38(2), 295–307.
- 94 Nehme, E., Weiss, L. E., Michaeli, T., & Shechtman, Y. (2018). Deep-STORM: Super-
95 resolution single-molecule microscopy by deep learning. *Optica*, 5(4), 458–464. <https://doi.org/10.1364/OPTICA.5.000458>
- 97 Saputra, M. R. U., De Gusmao, P. P., Almalioglu, Y., Markham, A., & Trigoni, N. (2019).
98 Distilling knowledge from a deep pose regressor network. *Proceedings of the IEEE/CVF
99 International Conference on Computer Vision*, 263–272.
- 100 Yao, B., Li, W., Pan, W., Yang, Z., Chen, D., Li, J., & Qu, J. (2020). Image reconstruction
101 with a deep convolutional neural network in high-density super-resolution microscopy. *Opt.
102 Express*, 28(10), 15432–15446. <https://doi.org/10.1364/OE.392358>
- 103 Yao, W., Zeng, Z., Lian, C., & Tang, H. (2018). Pixel-wise regression using u-net and its
104 application on pansharpening. *Neurocomputing*, 312, 364–371. <https://doi.org/https://doi.org/10.1016/j.neucom.2018.05.103>
- 106 Zhou, Z., Wu, J., Wang, Z., & Huang, Z.-L. (2023). Deep learning using a residual decon-
107 volutional network enables real-time high-density single-molecule localization microscopy.
108 *Biomed. Opt. Express*, 14(4), 1833–1847. <https://doi.org/10.1364/BOE.484540>