

¹ Deep Learning for Single Molecule Localization ² Microscopy

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⁴ Summary

⁵ Single Molecule Localization Microscopy (SMLM) enables researchers to interrogate nanoscale
⁶ spatial details in a range of systems. Biological investigations benefit greatly from SMLM
⁷ due to its ability to quantitatively investigate details of great importance such as protein
⁸ distribution in the cell membrane or protein-protein interactions([Baddeley & Bewersdorf, 2018](#)).
⁹ However, SMLM set-ups can be quite expensive, imaging times can be lengthy and data
¹⁰ analysis can require expert knowledge. Recently, Deep Learning (DL) algorithms have been
¹¹ developed to reduce imaging time and automate data analysis. Naturally each subsequent
¹² model aims to address a different shortcoming of the prior work and so there is a family of
¹³ model architectures. However, there is not a singular location for researchers to have access
¹⁴ to these models dedicated to SMLM. We developed the Deep Learning for Single Molecule
¹⁵ Localization Microscopy (DL4SMLM) in Python using the PyTorch framework to democratize
¹⁶ access to these models and lower the barrier of entry to SMLM.

¹⁷ Statement of Need

¹⁸ DL4SMLM is a Python package that uses the Pytorch DL framework to implement Convolu-
¹⁹ tional Neural Nets (CNN) dedicated to SMLM. We created it for researchers and engineers who
²⁰ wish to use DL for SMLM in whichever field they practice granted they have sufficient data.
²¹ Currently, there are six models: Super Resolution Convolutional Neural Network (SRCNN)([Dong
et al., 2015](#)), Deep-Stochastic Optical Reconstruction Microscopy (Deep-STORM)([Nehme et
al., 2018](#)), Skip-STORM, Unet for STORM (U-STORM)([Yao et al., 2018](#)), Deep Residual
STORM (DRL-STORM)(?), 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 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. Additionally,
we have implemented the L1L2 Loss metric first introduced by Nehme et al 2018 and used
for all subsequent architectures implemented in this library. Other features include functions
that automate the Training and Validation process for a user-set number of epochs in addition
to a function that automates the inference procedure of a trained model on a test data set
and reports the Structured Similarity Index Measure (SSIM) and the Normalized Mean Square
Error (NMSE). These metrics are often used to report the inference performance of models
dedicated to SMLM. To assist researchers in visualizing the performance of their model, we

⁴⁴ have implemented a function that visualizes the diffraction limited image of the emitters, its
⁴⁵ super-resolved version, and the predicted super-resolved image according to the trained model.
⁴⁶ This empowers the researcher to troubleshoot the performance of the model and determine if
⁴⁷ the model should be retrained with different parameters or to proceed with the experiment.

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