

Self-Supervised Learning for Single-Molecule Localization Microscopy Denoising

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Abstract

We evaluate the ability of self-supervised deep learning for Poisson denoising of Single-Molecule Localization Microscopy (SMLM) in addition to the impact denoising can have on the ability to locate molecules within the Single-Molecule Localization Microscopy images. SMLM images are predominantly corrupted with Poisson noise. There are currently existing methods for producing super-resolved SMLM images. However, there is a need for more accurate SMLM images in order for scientists to gain a better understanding of the functions of live cells at the nanoscale. By denoising SMLM images prior to super-resolution, we create a less corrupted version of SMLM images. As a result, the exact locations of the molecules in the images can be determined with more accuracy and precision. We denoise SMLM images utilizing only the original noisy images as training data with a Self-Supervised Deep Learning model. By modifying the previous Self-Supervised techniques that have been successful in denoising images with Gaussian noise, we remove Poisson noise from SMLM images.

Introduction

Since a majority of biological processes occur at the nanoscale, the ability to study cell and molecular behavior at this scale is critical for scientists. Specifically, the knowledge of nanoscale functions can help medical researchers design tools, treatments, and therapies that are more precise and personalized than conventional ones (Nano.gov).

Due to the effects of diffraction, classic optical microscopes are only able to resolve structures larger than 200nm (Allen et al. 2016). Single Molecule Localization Microscopy is a key technology for creating nanoscale images with optical microscopes. Using both super-resolution techniques and statistically locating individual molecule's blinks to sub-pixel resolution, SMLM is able to produce a new image which compiles the individually detected molecules together. The more exact SMLM can be in detecting and locating individual molecule blinks is a determining factor in how concise the location of the molecules can be determined. So, there is still room for improvement in locating individual molecules with more accuracy than is currently possible from noisy SMLM images due to the fact that the

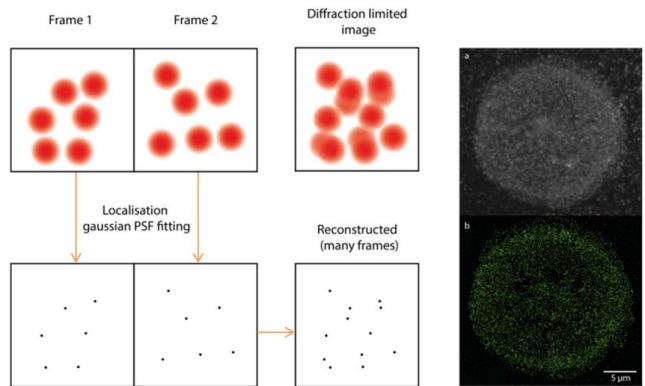


Figure 1: Process of SMLM localization and reconstruction to surpass the diffraction barrier (Shannon et al. 2015).

noise within an image makes pinpointing each molecule's true signal difficult.

SMLM images are taken as light is absorbed by the molecules, and, as a result, the molecules randomly emit light of a larger wavelength in small blinks which are captured by the camera. Because the blinks only emit a small number of photons, the resulting images are very noisy. Due to this dynamic blinking and low light levels in SMLM images, only a single noisy instance of a signal is available for a given instance of an image. Without a clean version of an image's signal, the use of a traditional deep learning approach that trains by mapping noisy images to the corresponding clean ones is not possible.

A current state-of-the-art super-resolution technique which utilizes deep networks is DeepSTORM, a CNN that trains on simulated and experimental data (Nehme et al. 2018). However, this method, although it has strong super-resolution performance, does not use any localization techniques which are vital in SMLM. Therefore, this method is not a practical solution for locating molecules on the nanoscale.

Traditionally, the best performance in deep learning approaches for image restoration and the denoising of corrupted images has been done using a supervised deep network that utilizes pairs of corresponding corrupt and clean images. In particular, there are content-aware image restora-

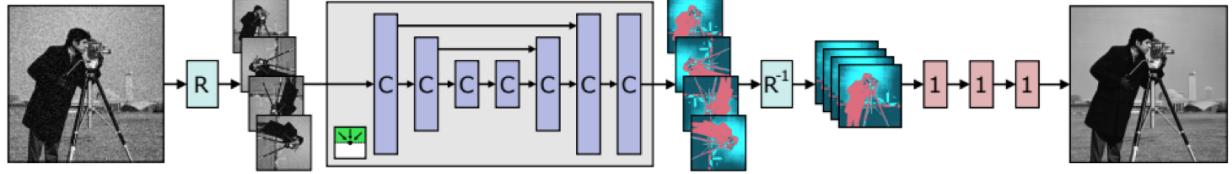


Figure 2: Proposed blind-spot network architecture for denoising SMLM images (Laine et al. 2019)

tion (CARE) networks that have proven useful for denoising fluorescence microscopy data given noisy and clean images (Weigert et al. 2018). Techniques such as these are rendered useless when considering SMLM data which has no way of obtaining clean images.

Related Work

New findings have shown that despite the limitations of solely individual noisy SMLM images, denoising to the performance level of supervised deep networks could be possible. The work of Lehtinen et al. (2018) shows that it is no longer necessary to provide a clean instance of an image. In NOISE2NOISE (N2N), they show by training using pairs of two corresponding noisy images that share the same signal, it is possible to achieve equal quality, if not higher quality (when training with finite data), performance for denoising. This method still requires at least 2 noisy realizations of an image's signal.

Building off the ideas from N2N, NOISE2VOID (N2V) shows that one can build a model for denoising using only individual noisy images for training data (Self-Supervised) (Krull, Buchholz, and Jug 2018). N2V introduces the use of a blind-spot network which masks a pixel's data from the network's receptive field and allows the data surrounding the said blind-spot to effectively predict the the pixel's signal. The N2V model is limited because it assumes every pixel's signal is dependent of the signals surrounding it.

To address the limitation of N2V, Laine et al. (2019) designed a different blind-spot architecture that uses 4 different receptive fields to allow more pixels to contribute to the loss function while maintaining the blind-spot. In order to predict the blind-spot pixel's signal, they apply a maximum a posteriori estimation (MAP) denoising procedure during testing that allows for the prediction of the clean signal to take into account the masked pixel. This method can perform on par with the supervised traditional deep denoising models under the assumption of Gaussian noise in the data.

Laine et al. does include a model for Poisson noise, but does use a Gaussian approximation to represent the Poisson noise distribution. However, given the signal from SMLM is low, this approximation does not accurately represent the noise present in SMLM images.

More continued work since N2V also attempts to predict the blind-spot pixel without the assumption of a Gaussian noise model (Krull, Vicar, and Jub 2019). PROBABILISTICNOISE2VOID (PN2V) model trains a probability distribution for 800 possible output values for a given pixel.

PN2V is able to out perform N2V using the same U-Net architecture, but does not perform to the level of traditional supervised techniques.

Research Questions

1. How well can self-supervised learning denoise SMLM images?

Hypothesis: By modifying the work which successfully denoises images with Gaussian noise, it will be possible to denoise SMLM images to the levels of other self-supervised denoising models.

2. Does denoising SMLM images prior to localization techniques and super-resolution improve the ability to locate molecule positions compared to just using current localization and super-resolution techniques on noisy images?

Hypothesis: Given SMLM currently depends on the noisy signals from the blinking molecules, by denoising the images prior to detecting and locating the molecules, our localizing abilities will be more precise. This is because the signal from the blinking molecules will be more representative of the molecules exact position in an image that is not corrupted with noise.

$$\text{Poisson PMF: } P(Z = z) = \frac{x^z \exp(-x)}{z!} \quad (1)$$

$$\begin{aligned} P(Z_i = z_i) &= \int_0^\infty P(Z_i = z_i | X_i = x) P(X_i = x) dx \\ &= \frac{\beta^\alpha}{(1 + \beta)^{\alpha+z_i}} \cdot \frac{\Gamma(\alpha + z_i)}{\Gamma(\alpha)} \cdot \frac{1}{z_i!} \end{aligned} \quad (2)$$

$$\begin{aligned} \mathcal{L}_i &= -\alpha \log(\beta) + (\alpha + z_i) \log(1 + \beta) \\ &\quad - \log(\Gamma(\alpha + z_i)) + \log(\Gamma(\alpha)) + \log(\Gamma(z_i + 1)) \end{aligned} \quad (3)$$

Method

By utilizing the blind-spot architecture designed by Laine et al. (Figure 2), which rationally obscures the middle pixel, while also implementing an adjusted method for training the model and predicting the blind-spot signal's output, we are able to remove Poisson noise from SMLM images. In the

process described by Laine et al. (2019), Poisson noise is approximated by considering a Gaussian distribution with $\sigma^2 = \lambda$. This estimate will only provide a strong approximation when the standard deviation within the Poisson noise is small and the image's signal is large.

To better train our network and more accurately estimate a masked pixel's signal while considering the Poisson noise present in SMLM, we created a process that can model the Poisson noise while producing non-discrete clean signal values. Since we only have the noisy image signals, Z_i , our loss function must be representative of these known values. To do this we introduced the conjugate prior for the Poisson Distribution, the Gamma distribution. By representing the distribution of possible clean image signals, X_i by the Gamma distribution, it is possible to marginalize out the clean realization of our image which is unknown in SMLM. (Equation 2) Using this marginalized equation, the negative log likelihood loss function can be calculated without using an approximation. (Equation 3)

In order for the obscured pixel value to contribute to the clean pixel estimation, the best results were obtained using the Gamma posterior mean. This preformed better than the a the Gamma MAP estimation which tended to weight the noisy pixel value too heavily.

$$\text{Gamma Posterior Mean: } x_i = \frac{\alpha + z_i}{\beta + 1} \quad (4)$$

Evaluation

To train and validate our model we used single molecule microscopy datasets of yellow fluorescent protein (YFP)-tagged growth factor receptors taken from human Epithelial carcinoma A431 cells expressing mCitrine-ErbB3 (Luke et al. 2018). Since this data set uses YFP-tagged receptors rather than traditional dye, the molecules emit even less photons. So, the YFP datasets have low signal to noise ratio making them good candidates for denoising. We will also evaluate our model using simulated tubulin data that came out of the 2015 benchmark challenge for SMLM software (Sage et al. 2015). This simulated data will be useful for testing how accurate the localization from our denoised data are.

To evaluate how well our model is able to denoise the SMLM images we looked at the signal to background ratio (SBR). In order to find the SBR, there need to be a way of distinguishing the background from the foreground. By implementing an automatic iterative threshold selection commonly used for gray-scale image thresholding, it was possible to determine a signal threshold to separate the signal from the background (Svoboda, Kybic, and Hlavac 2007). We hoped this metric would give a marker of how well our model is denoising given we have no clean image comparison to compare our results to. However, even when the results qualitatively appeared to improve and the localization uncertainty lowered, the SBR of a resulting image was not necessarily greater than its noisy pair. Using the signal to background ration is also not an ideal way to generally assess denoising because our model could successfully denoise the black background without having the same

effect across the entirety of the image. For these reasons, we solely look at the qualitative results from the denoising model prior to utilizing our super-resolution and localization techniques. Then, if there is improvement in the ability to locate molecules in comparison to the localization ability on the images without denoising, it can be assumed that the denoising our model achieved occurred across the entirety of the image.

Once we have determined our model's ability to denoise the SMLM images we then evaluated the effect the denoising model had on the ability to locate individual molecules within the images. For this we used ThunderSTORM. ThunderSTORM is a SMLM image processing, analyzing, and visualizing tool(Ovesn et al. 2014). ThunderSTORM can be implemented as a plugin for the image processing application ImageJ (Schneider, Rasband, and Eliceiri 2012). It is particularly helpful in assessing a threshold for detection of molecules in raw and filtered images. So, in order to evaluate a potential improvement in the denoised images threshold for detection, we will first run the raw YFP data through ThunderSTORM and record the results. Then, we will denoise the YFP data with our Self-supervised denoising model. Finally, we will run the denoised data through ThunderSTORM once more and compare the threshold for detection results between the raw and denoised data.

Preliminary Results

During our preliminary testing using the self-supervised denoising model on YFP-tagged data, there have been improvements in both the reduction of noise in the image (evaluated qualitatively) and the ability to locate individual

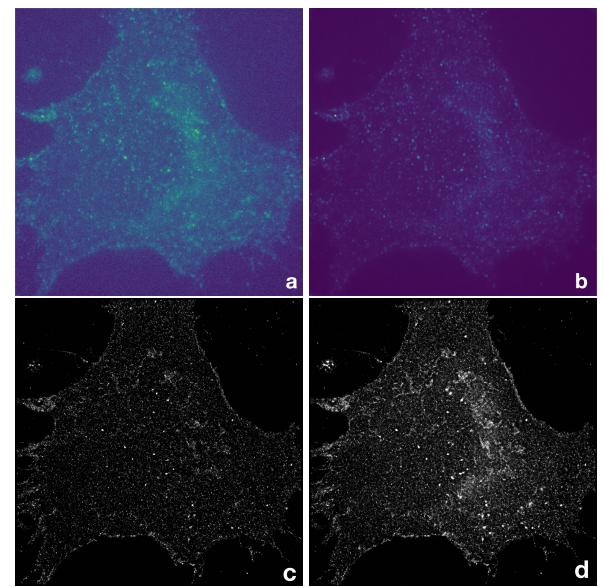


Figure 3: Denoising visual results: (a) Single noisy YFP image. (b) Single corresponding denoised YFP image. (c) Image compiled from molecule localization from noisy images. (d) Image compiled from molecule localization from denoised images (Luke et al. 2018).

| | Molecules Located | Mean Sigma [nm] | Standard Dev. Sigma [nm] | Mean Uncertainty [nm] | Standard Dev. Uncertainty [nm] |
|-----------------------|-------------------|-----------------|--------------------------|-----------------------|--------------------------------|
| Noisy YFT-Dataset3 | 364,509 | 104.8981 | 62.35194 | 17.43365 | 5.770702 |
| Denoised YFT-Dataset3 | 882,188 | 111.466 | 60.64917 | 13.46961 | 6.124521 |

Table 1: Molecule localization data obtained using ThunderSTORM with default settings + EM Gain = 150.0. Post-processing with ThunderSTORM: Duplicate removal and drift correction.

molecules within the SMLM images.

Despite the Signal to Background Ratio metric not being a quality measure of the noise in the images, in Figure 3, images (a) and (b) demonstrate the models ability to pick out the signal amongst noise. Image (b) does not appear to have as much signal as its noisy counterpart image (a). This is due to the noise being amplified in areas of signal in image (a). The ability to get rid of the excess noise around the signal allow for individual molecules to be pinpointed. In an area where more than one molecules are close together, their respective noise can blur together making it unfeasible to locate each molecule individually with any certainty.

To locate each molecule in an SMLM image, ThunderSTORM fits a Gaussian distribution to the signals from the image. Using the data from the Gaussians at each point, it can be determined whether a molecule is present, where it is present, and a location uncertainty value.

Table 1 shows the results of the analysis of the original noisy image set in comparison with analysis of the denoised image set. Using ThunderSTORM it was possible to locate more than double the amount of molecules with the denoised data compared to the original corrupted data. It was able to locate more molecules with less uncertainty. Once the molecules are located in each of the images from the dataset, the located molecules are compiled into a new final image. In Figure 3, images (c) and (d) show the new final compiled images.

Conclusion

We have developed and began to evaluate a self-supervised deep learning model for denoising Single Molecule Localization Microscopy images which are corrupted with Poisson noise. To do this, we built off recent research in self-supervised denoising models and fit the techniques to denoise images taken in low light conditions (images containing large amounts of Poisson noise). As a result of the denoising, there has been no correlation with the Signal to Background metric. However, utilizing the ImageJ plugin, ThunderSTORM, we have been able to see an improvement in detecting and locating individual molecules within SMLM images. We will continue to reproduce these results with other datasets (including simulated SMLM data) in addition to making improvements to our model as outlined in the Timeline below.

Timeline

1. Weeks 7: Further attempts to improve denoising model by adjusting parameters. Continue to familiarize with ThunderSTORM tools to attain the optimal super-resolution and localization.

2. Weeks 8: Test model on simulated data to confirm accuracy of localization performance.
3. Weeks 9 and 10: Finalize evaluations on multiple datasets, write final paper, and present results.

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