Self-Supervised Learning for Single-Molecule Localization Microscopy Denoising

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Abstract

We propose to evaluate the ability of self-supervised deep learning for Poisson denoising on Single-Molecule Localization Microscopy (SMLM). SMLM images are predominantly corrupted with Poisson noise. There are currently existing methods for producing super-resolved SMLM images. 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 are hoping to increase the accuracy of the super-resolved SMLM image, thus allowing more exact molecule locations to be determined. We can 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 will 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. (Nan)

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 precision from the noisy SMLM images.

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 of 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, not use any localization techniques which are vital in SMLM. Therefore, this method is not a practical solution for locating molecules on the nanoscale.

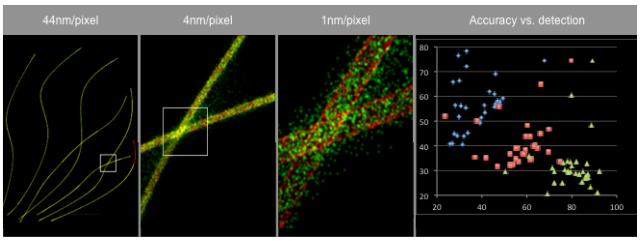

Figure 1: Results of SMLM super-resolution using traditional resolution techniques. (Bio)

Traditionally, for the best performance deep learning approaches to 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. Specifically, there are content-aware image restoration (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 as 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) design 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 MAP desnoising 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 uses a Gaussian approximation to represent the 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 Gaussian noise model prediction (Krull, Vicar, and Jub 2019). PROBABILISTIC-NOISE2VOID (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?
   (a) 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?
   (a) 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.

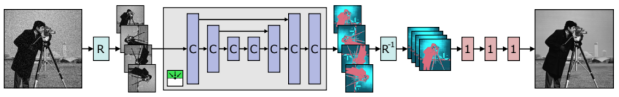

Figure 2: Proposed blind-spot network architecture for denoising SMLM images (Laine et al. 2019)

Method

By utilizing the blind-spot architecture designed by Laine et al. while also implementing an adjusted method for training the model and predicting the blind-spot signal's output, we will be able to remove 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 need 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 will introduce 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. (2) From there our negative log likelihood loss function can be calculated without using an approximation. (3) By also utilizing the conjugate prior for the MAP estimation, the clean estimation of a masked pixel will be representative of the surrounding pixel values as well as masked pixel's noisy value.

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

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

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

Evaluation

To train and validate our model we will use a single molecule microscopy datasets of Yellow fluorescent protein (YFP)-tagged growth factor receptors taken from human cells. (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.

To evaluate how well our model is able to denoise the SMLM images we will look at the signal to noise ratio (SNR) in a random area in the background of the image and compare it to the SNR of the noisy input image. This will give a marker of how well our model is denoising given we have no clean image comparison to compare our results to. Using the background SNR is 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. However, it should give an idea of how the model performing before moving onto the localization and super-resolution 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 will then need to evaluate the effect the denoising model had on the ability to locate individual molecules within the images. For this we will use ThunderSTORM. ThunderSTORM is a SMLM image processing, analyzing, and visualizing tool. (Ovesn et al. 2014) 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.

Conclusion

We will develop and evaluate a self-supervised deep learning model for denoising Single Molecule Localization Microscopy images which are corrupted with Poisson noise. To do this, we will build 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, we anticipate an improvement in detecting and locating individual molecules within SMLM images.

Timeline

1. Week 2: Finalize proposal and choose data sets to evaluate on. Figure out our specific method to evaluate denoising capabilities based on background SNR.
2. Week 3: Add to the blind-spot network code to include Poisson/Gamma denoising techniques for loss function and MAP denoising.
3. Weeks 4-7: Begin implementing denoising of SMLM and evaluating progress.
4. Weeks 6-9: Apply current super-resolution method to denoised images and evaluate performance. (Both Quantitatively and Qualitatively)
5. Weeks 9 and 10: Finalize evaluations and write final paper.

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