
Denoising Diffusion Probabilistic Models for Single Molecule Localization Microscopy

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Abstract

Single-molecule localization microscopy (SMLM) techniques are a mainstay of fluorescence microscopy and can be used to produce a pointillist representation of living cells at diffraction-unlimited precision. Classical SMLM approaches leverage the deactivation of fluorescent tags, followed by spontaneous or photoinduced reactivation, which can be used to achieve super-resolution reconstructions. Standardized SMLM inference algorithms require tight control of activation and reactivation to maintain sparse emitters, presenting a tradeoff between imaging speed and labeling density. Recently, denoising diffusion probabilistic models (DDPMs) have been adapted conditional super resolution tasks, demonstrating promising results in detail reconstruction. Here, we adapt DDPM to the task of single molecule localization, and demonstrate that DDPM approaches the Cramer-Rao lower bound on localization uncertainty over a wide range of experimental conditions.

1 Introduction

Single molecule localization microscopy (SMLM) relies on the temporal resolution of fluorophores whose spatially overlapping point spread functions would otherwise render them unresolvable at the detector. Common strategies for the temporal separation of molecules involve transient intramolecular rearrangements to switch from dark to fluorescent states or the exploitation of non-emitting molecular radicals. Estimation of molecular coordinates in SMLM is achieved by modeling the optical impulse response of the imaging system. However, dense localization suffers from the curse of dimensionality - the parameter space volume grows exponentially with the number of molecules, which is often unknown a priori. Exploration of this high dimensional parameter space in dense SMLM is often intractable.

A common approach to this issue has been to predict super-resolution images or coordinates using deep neural networks (Ouyang 2018; Barth 2020; Chen 2023; Nehme 2020; Speiser 2021). Previous deep methods have proven quite useful, yet the lack of a clear physical origin to the produced super-resolution images creates a need for a more physically-constrained class of models. Here, we present a novel diffusion model for single molecule localization microscopy. The first stage of our algorithm performs interpolation by computing second order coherence of pixel pairs. Subsequent stages cast localization as a conditional image refinement task, realized by a U-Net model trained on denoising at various noise levels. This is followed by coordinate refinement by a gradient-based Markov Chain Monte Carlo (MCMC) scheme, known as Langevin dynamics.

2 Background

2.1 Single Molecule Localization Microscopy

In a coherent state, photon arrivals at a pixel follows Poisson statistics, with expected value

$$\omega = i_0 \int O(u) du \int O(v) dv \quad (1)$$

where $i_0 = \eta N_0 \Delta$. The optical impulse response $O(u, v)$ is often approximated as a 2D isotropic Gaussian with standard deviation σ (Zhang 2007). The parameter η is the photon detection probability of the sensor and Δ is the exposure time. N_0 represents the number of photons emitted.

Using the common definition $\text{erf}(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-t^2} dt$,

$$\int O(u) du = \frac{1}{2} \left(\text{erf} \left(\frac{u_k + \frac{1}{2} - u_0}{\sqrt{2}\sigma} \right) - \text{erf} \left(\frac{u_k - \frac{1}{2} - u_0}{\sqrt{2}\sigma} \right) \right) \quad (2)$$

For the sake of generality, the number of photoelectrons at a pixel k , \mathbf{s}_k , is multiplied by a gain factor g_k [ADU/ e^-], which is often unity. The readout noise per pixel ζ_k can be Gaussian with some pixel-specific offset o_k and variance σ_k^2 . Ultimately, we have a Poisson component of the signal, which scales with N_0 and may have Gaussian component, which does not. Therefore, in a single exposure, we measure:

$$\mathbf{x}_t = \mathbf{s}_t + \zeta \quad (3)$$

What we are after is the likelihood $p(\mathbf{x}_t|\theta)$ where θ are the molecular coordinates. Fundamental probability theory states that the distribution of \mathbf{x}_k is the convolution of the distributions of \mathbf{s}_k and ζ_k ,

$$p(\mathbf{x}_t|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\omega_k} \omega_k^q \frac{1}{\sqrt{2\pi}\sigma_k} e^{-\frac{(\mathbf{x}_k - g_k q - o_k)^2}{2\sigma_k^2}} \quad (4)$$

where $P(\zeta_k) = \mathcal{N}(o_k, \sigma_k^2)$ and $P(S_k) = \text{Poisson}(g_k \omega_k)$, A is some normalization constant. In practice, (4) is difficult to work with, so we look for an approximation. We will use a Poisson-Normal approximation for simplification. Consider,

$$\zeta_k - o_k + \sigma_k^2 \sim \mathcal{N}(\sigma_k^2, \sigma_k^2) \approx \text{Poisson}(\sigma_k^2) \quad (5)$$

Since $\mathbf{x}_k = \mathbf{s}_k + \zeta_k$, we transform $\mathbf{x}'_k = \mathbf{x}_k - o_k + \sigma_k^2$, which is distributed according to

$$\mathbf{x}'_k \sim \text{Poisson}(\omega'_k) \quad (6)$$

where $\omega'_k = g_k \omega_k + \sigma_k^2$. This result can be seen from the fact the convolution of two Poisson distributions is also Poisson. The quality of this approximation will degrade with decreasing signal level, since the Poisson distribution does not retain its Gaussian shape at low expected counts. Nevertheless, the quality of the approximation can be predicted by the Komogonov distance between the convolution distribution (4).

2.2 The Cramer-Rao lower bound

The Poisson approximation is also convenient for computing the Fisher information matrix and thus the Cramer-Rao lower bound, which bounds the variance of a statistical estimator of θ , from below (Chao 2016). Define the log-likelihood as $\ell(\mathbf{x}_t|\theta) = \log p(\mathbf{x}_t|\theta)$. The Fisher information is

$$\mathcal{I}_{ij}(\theta) = \mathbb{E} \left(\frac{\partial \ell}{\partial \theta_i} \frac{\partial \ell}{\partial \theta_j} \right) = \sum_k \frac{1}{\omega'_k} \frac{\partial \omega'_k}{\partial \theta_i} \frac{\partial \omega'_k}{\partial \theta_j} \quad (7)$$

3 Denoising Diffusion Probabilistic Model

3.1 Second order coherence interpolation

We now consider the case where readout noise of the detector is sufficiently low and fluorescence is non-stationary such that we can use spatial correlation functions in place of raw intensities. We consider the following normalized correlation function, for the intensity at two pixels \mathbf{x}_i and \mathbf{x}_j

$$\mathbf{g}_m = g_{ij}^{(2)}(0) = \frac{\langle \mathbf{x}_{i,t} \mathbf{x}_{j,t} \rangle}{\langle \mathbf{x}_{i,t} \rangle \langle \mathbf{x}_{j,t} \rangle} \quad (8)$$

where $m = (i, j)$. Fluorescent molecules can exhibit photoswitching behavior, wherein the emitter accesses a discrete set of states with unique fluorescent emission characteristics. We describe this process as a Poisson Hidden Markov Model (HMM), with state probabilities ξ_k and expectations μ_k at equilibrium. The zero-lag normalized second order coherence function then reads

$$\mathbf{g}_m = \frac{\omega_i^2 \omega_j^2 \langle \mu^2 \rangle + \mu_B (\omega_i^2 + \omega_j^2) \langle \mu \rangle + \mu_B^2}{\langle \mu \rangle^2 \omega_i^2 \omega_j^2 + \mu_B (\omega_i^2 + \omega_j^2) \langle \mu^2 \rangle + \mu_B^2} \quad (9)$$

where $\langle \mu \rangle = \sum_n \xi_n \mu_n$ and $\langle \mu^2 \rangle = \sum_n \xi_n \mu_n^2$. Let $\mathbf{y}_0 = \sum_{i=1}^n \omega_n(\sigma)$ be a density estimate of the molecular distribution. The *forward* process is the joint distribution $p_\theta(\mathbf{y}_{0:T})$, which is Markovian.

$$q(\mathbf{y}_t | \mathbf{y}_0) = \prod_{t=1}^T q(\mathbf{y}_t | \mathbf{y}_{t-1}) \quad q(\mathbf{y}_t | \mathbf{y}_{t-1}) = \mathcal{N}(\mathbf{y}_t, \sqrt{\alpha_t} \mathbf{y}_{t-1}, (1 - \alpha_t) I) \quad (10)$$

We optimize a denoising model f_θ which takes as input an interpolated low-resolution input \mathbf{y} and a noisy input \mathbf{y}_T .

$$p_\theta(\mathbf{y}_{0:T}) = p_\theta(\mathbf{y}_T) \prod_{t=1}^T p_\theta(\mathbf{y}_{t-1} | \mathbf{y}_t) \quad p_\theta(\mathbf{y}_{t-1} | \mathbf{y}_t) = \mathcal{N}(\mathbf{y}_{t-1}, \mu_\theta(\mathbf{y}_t, \gamma_t), \sigma_t^2 I) \quad (11)$$

where $\gamma_t = \prod_{i=1}^t \alpha_i$. Note that the model θ is not a function of t . The mean of the transition density reads

$$\mu_\theta(\mathbf{x}_t, \mathbf{y}, \gamma_t) = \frac{1}{\sqrt{\alpha_t}} \left(\mathbf{y}_t - \frac{1 - \alpha_t}{\sqrt{1 - \gamma_t}} f_\theta(\mathbf{x}_t, \gamma_t) \right) \quad (12)$$

4 Experiments