Bayesian Localization Microscopy with Denoising Diffusion Probabilistic Models

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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 estimate of the density of a tagged biomolecule in cellular compartments. Standard SMLM localization algorithms based on maximum likelihood estimators or least squares optimization require tight control of activation and reactivation to maintain sparse emitters, presenting a tradeoff between imaging speed and labeling density. Deep models have generalized SMLM to densely labeled structures, yet uncertainty quantification is still lacking. Recently, denoising diffusion probabilstic models (DDPMs) have been adapted conditional super resolution tasks, demonstrating promising results in detail reconstruction, while directly providing uncertainties in model predictions. 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 acheived 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.

Previous approaches to this issue has been to predict super-resolution images from a sparse set of localizations with conditional generative adversarial networks (Ouyang 2018) or direct prediction of coordinates using deep neural networks (Nehme 2020; Speiser 2021). However, diffusion models are an appealing alternative because they infer a distribution of deconvolved images that are compatible with an observation. Although conditional VAEs and conditional GANs can provide a distribution of deconvolved images, both are known to suffer from mode collapse and produce insufficient diversity in their outputs. Diffusion models are a recently developed alternative to VAEs and GANs that excel at producing diverse samples and have been successfully applied to solve inverse problems. Here, we present a novel diffusion model for deconvolution in single molecule localization microscopy. The first stage of our algorithm performs interpolation by computing second order coherence of pixel

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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 Model of image formation

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

$$\omega = i_0 \int O(u)du \int O(v)dv \tag{1}$$

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 $\mathrm{erf}(z) = \frac{2}{\sqrt{\pi}} \int_0^t e^{-t^2} dt$,

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

For the sake of generality, the number of photoelectrons at a pixel k, $\mathbf{s}_k$, is multiplied by a gain factor g_k $[\mathrm{ADU}/e^-]$, 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:

$$\mathbf{x}_t = \mathbf{s}_t + \zeta \tag{3}$$

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,

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

where $P(\zeta_k) = \mathcal{N}(o_k, \sigma_k^2)$ and $P(S_k) = \mathrm{Poisson}(g_k\omega_k)$, 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,

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

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

$$\mathbf{x}'_k \sim \mathrm{Poisson}(\omega'_k) \tag{6}$$

where $\omega'_k = g_k\omega_k + \sigma_k^2$. This result can be seen from the fact the 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

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

3 Denoising Diffusion Probabilistic Model

Model selection techniques for localization microscopy is challenging, due to the unknown number of fluorescent particles when computing the likelihood. The likelihood can be avoided by instead solving an image restoration problem, where a high resolution kernel density estimate is reconstructed from a low resolution image.

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.

$$q(\mathbf{y}_t|\mathbf{y}_0) = \prod_{t=1}^T q(\mathbf{y}_t|\mathbf{y}_{t-1}) \quad q(\mathbf{y}_t|\mathbf{y}_{t-1}) = \mathcal{N}\left(\mathbf{y}_{t-1}, \sqrt{\alpha_t}\mathbf{y}_{t-1}, (1-\alpha_t)I\right) \tag{8}$$

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

$$p_\theta(\mathbf{y}_{0:T}) = p_\theta(\mathbf{y}_T)\prod_{t=1}^T p_\theta(\mathbf{y}_{t-1}|\mathbf{y}_t) \quad p_\theta(\mathbf{y}_{t-1}|\mathbf{y}_t) = \mathcal{N}\left(\mathbf{y}_{t-1}, \mu_\theta(\mathbf{y}_t, \gamma_t), \sigma_t^2 I\right) \tag{9}$$

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

$$\mu_\theta(\mathbf{x}_t, \mathbf{y}, \gamma_t) = \frac{1}{\sqrt{\alpha_t}}\left(\mathbf{y}_t - \frac{1-\alpha_t}{\sqrt{1-\gamma_t}} f_\theta(\mathbf{x}_t, \gamma_t)\right) \tag{10}$$

3.1 Second order coherence interpolation

For certain setups, such as one with nonstationary fluorescent emitters and detectors with negligible readout noise, we can use spatial correlation functions in place of raw intensities. This can be useful because such technologies typically have larger pixel pitch, and lower resolution according to Nyquist sampling. We consider the following normalized correlation function, for the intensity at two pixels $\mathbf{x}_i$ and $\mathbf{x}_j$

$$\mathbf{g}_m = g_{ij}^{(2)}(0) = \frac{\langle \mathbf{x}_{i,t}\mathbf{x}_{j,t}\rangle}{\langle \mathbf{x}_{i,t}\rangle\langle \mathbf{x}_{j,t}\rangle} \tag{11}$$

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

$$\mathbf{g}_m = \frac{\omega_i^2\omega_j^2\langle\mu^2\rangle + \mu_B(\omega_i^2+\omega_j^2)\langle\mu\rangle + \mu_B^2}{\langle\mu\rangle^2\omega_i^2\omega_j^2 + \mu_B\left(\omega_i^2+\omega_j^2\right)\langle\mu^2\rangle + \mu_B^2} \tag{12}$$

where $\langle\mu\rangle = \sum_n \xi_n\mu_n$ and $\langle\mu^2\rangle = \sum_n \xi_n\mu_n^2$.

4 Experiments