

## **Electronic Supplementary Information for:**

# **Deep-Learning-Assisted Spectroscopic Single-Molecule Localization Microscopy based on Spectrum-to-Spectrum Denoising**

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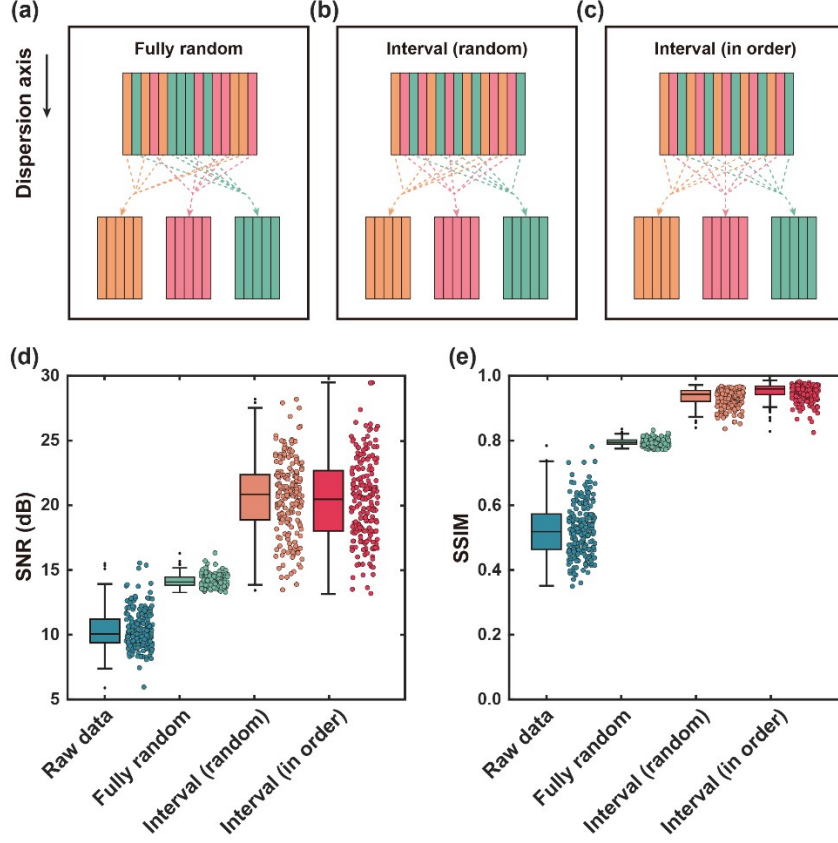
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## **Supplementary Note 1**

### **Spec2Spec denoising performance among different re-sampling strategies**

We quantitatively compared the denoising performance of different re-sampling strategies on the same experimental data. The training dataset contains 20,000 experimentally acquired single-molecule emission spectral images. Three re-sampling strategies were investigated, including fully random re-sampling strategy and two interval re-sampling strategies, as shown in Fig. S1. In interval re-sampling strategies, three adjacent lines with  $H \times 1$  pixels were separated either randomly or in order, and then concatenated into three sub-images with  $H \times W/3$  pixels. We used the signal-to-noise ratio (SNR) and structure similarity index measure (SSIM) to quantitatively evaluate the network performance on testing data containing 500 spectra. The average of 20,000 single-molecule emission spectra was served as the ground truth in the testing stage. According to the quantitative results of SNR and SSIM, we found that the random re-sampling strategy has worst results since this method leads to inconsistent signal intensity and noise level among three re-sampled spectra. In contrast, two interval sampling strategies achieve similar output SNR and SSIM results, and show much better network performance as re-sampled spectra have more consistent signal intensity and SNR level.



**Fig. S1** Spec2Spec denoising performance among different re-sampling strategies, including (a) fully random re-sampling, (c) interval re-sampling while three adjacent lines were separated randomly, and (b) interval re-sampling while three adjacent lines were separated in order; Statistical results of (d) SNR and (e) SSIM among different re-sampling strategies.

We further quantitatively compared the denoising performance using the training pair generated with different re-sampling intervals on the same experimental dataset. For interval re-sampling strategy generating two, three, and four sub-images, loss functions need to be modified, respectively, as below.

1. for two sub-images ( $X, Y$ ):

$$Loss = |F_{Spec2Spec}(X) - Y|_1 + \|F_{Spec2Spec}(X) - Y\|_2^2. \quad (S-1)$$

2. for three sub-images ( $X, Y1, Y2$ ):

$$Loss_1 = |F_{Spec2Spec}(X) - Y1|_1 + \|F_{Spec2Spec}(X) - Y1\|_2^2, \quad (S-2)$$

$$Loss_2 = |F_{Spec2Spec}(X) - Y2|_1 + \|F_{Spec2Spec}(X) - Y2\|_2^2, \quad (S-3)$$

$$Loss_{Total} = Loss_1 + Loss_2. \quad (S-4)$$

3. for four sub-images ( $X, Y1, Y2, Y3$ ):

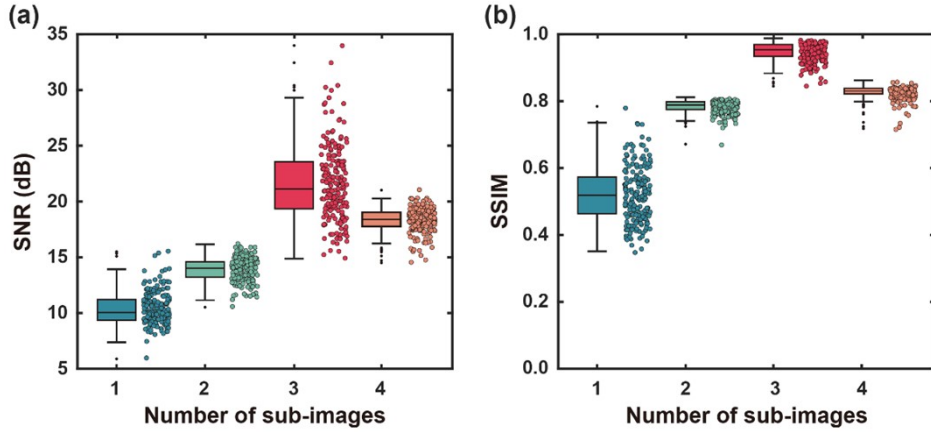
$$Loss_1 = |F_{Spec2Spec}(X) - Y1|_1 + \|F_{Spec2Spec}(X) - Y1\|_2^2, \quad (S-5)$$

$$Loss_2 = |F_{Spec2Spec}(X) - Y2|_1 + \|F_{Spec2Spec}(X) - Y2\|_2^2, \quad (S-6)$$

$$Loss_3 = |F_{Spec2Spec}(X) - Y3|_1 + \|F_{Spec2Spec}(X) - Y3\|_2^2, \quad (S-7)$$

$$Loss_{Total} = Loss_1 + Loss_2 + Loss_3. \quad (S-8)$$

In order to effectively learn noise patterns from re-sampled spectra, the self-supervised network needs a sufficient number of resampled spectra. On the other hand, when more sub-images are generated, the SNR of re-sampled spectra becomes lower, making it difficult to optimize the network parameters. Combining the above reasons, we find that the interval re-sampling strategy with three input sub-images results the best performance for the acquired data using the reported experimental system, as shown in Fig. S2.



**Fig. S2.** Quantitative evaluations of (a) SNR and (b) SSIM after Spec2Spec denoising using the training pair with different numbers of input sub-images.

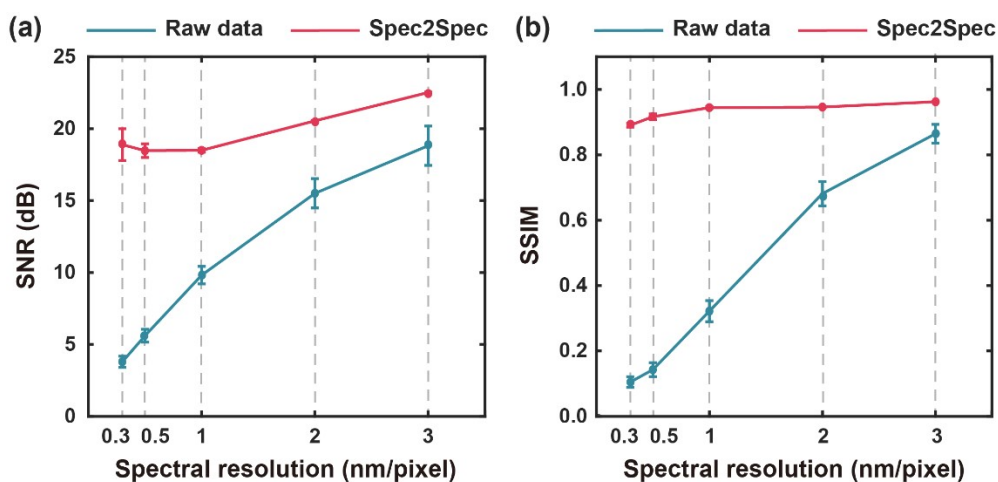
## Supplementary Note 2

### Spec2Spec denoising performance at different spectral resolutions

We quantitatively compared the denoising performance of Spec2Spec on simulated spectral data at different spectral resolutions. To synthesize single-molecule emission spectral images at different spectral resolution (ranging from 0.3 nm/pixel to 3 nm/pixel), we firstly produced a diffraction-limited point-spread-function (PSF) image, which was modeled as a two-

dimensional Gaussian function. Secondly, at a certain spectral resolution, the corresponding noise-free spectral image was generated by convoluting the PSF and the emission spectrum of a fluorescence dye (Alexa Fluor 647). A uniform background was further added to the spectral image. Thirdly, shot noise and read noise was added based on experimental conditions to generate noisy spectral images<sup>1</sup>. Note that, the photon budget and background level of single-molecule spectra images were consistent at different spectral resolutions.

Fig. S3 shows the quantitative evaluations of SNR and SSIM after Spec2Spec denoising at different spectral resolutions. As expected, single-molecule emission spectra with higher spectral resolutions generally result lower SNR and SSIM. Benefiting from the Spec2Spec, the SNR and SSIM of denoised spectra were significantly improved at all tested spectral resolutions. Especially, the advantages of Spec2Spec denoising at higher spectral resolutions are more pronounced. For example, Spec2Spec enables 3.9-fold improvement in SNR (3.85 dB versus 18.90 dB) and 7.1-fold enhancement in SSIM (0.11 versus 0.89) at a spectral resolution of 0.3 nm/pixel, whereas 0.2-fold improvement in SNR (18.83 dB versus 22.52 dB) and 0.1-fold enhancement in SSIM (0.86 versus 0.96) at a spectral resolution of 3 nm/pixel. It is important to note that the performance improvement results derived from this simulation are different from those calculated in the main text based on experimental data.



**Fig. S3** Quantitative evaluations of (a) SNR and (b) SSIM at different spectral resolutions after Spec2Spec denoising.

Reference:

1. K. H. Song, B. Dong, C. Sun and H. F. Zhang, *Rev. Sci. Instrum.*, 2018, **89**, 123703.