

Image restoration for fluorescence microscopy using optimal sparsity and camera modeling

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Abstract: In fluorescence microscopy, the quality of the acquired images determines the extent of observable biological phenomena. To address the different noise sources degrading these images, we introduce a model-based framework compatible with several microscopy systems independently from the detector used. © 2023 The Author(s)

Main Text

Fluorescence microscopy is a critical tool in biological research, but the extent of observable biological phenomena is determined by the quality of the acquisition process[1]. To address the different noise sources that degrade this process, we introduce an algorithm for multiscale image restoration through optimally-sparse representation (MIRO). This is based on a deterministic framework that models the acquisition process and uses pixelwise noise correction to improve image quality. Our studies demonstrated that this approach yields a significant improvement of the image quality for a wide range of microscopy systems, regardless of the detector used (e.g., EMCCD, sCMOS, or PMT). This enables fast, low-light optical microscopy, accurate image analysis, and robust machine intelligence when integrated with deep neural networks, expanding the range of biological knowledge that can be obtained from fluorescence microscopy.

Digital sensors are composed of different elements that convert the impinging photons (S) into electrons and electrons into a digital number (DN). Each of these elements introduces uncertainty to the detection process, which theoretically can be modeled as:

$$DN(p, t) = g(p) \cdot (\mathcal{P}\{S; p, t\} + P\{I_D \tau; p\} + \mathcal{G}\{0, \sigma_R^2; p\} + \mathcal{G}\{0, \sigma_{FPN}^2; p, t\}) + o(p) \quad (1)$$

where $\mathcal{P}\{\cdot\}$ indicates the Poisson distribution, $\mathcal{G}\{\cdot, p\}$ the heteroskedastic Gaussian distribution, I_D the dark current, τ the camera exposure time, $g(p)$ the pixel gain, $o(p)$ the offset, and σ_R^2 and σ_{FPN}^2 the variance of readout and fixed pattern noise, respectively.

The MIRO image restoration comprises three main stages. First, it corrects the pixel output using a calibrated map of the offset, gain, and variance for each pixel[2]. This step removes the uncertainty correlated with the spatial variance of the pixel response across the sensor, σ_{FPN}^2 , which results in the non-uniform collection efficiency for each pixel[3, 4]. The total noise contribution can then be modeled as a heteroskedastic Gaussian distribution:

$$I(p, t) \approx \mathcal{G}\{S, S + I_D \tau + \sigma_R^2; p, t\} + o'(p, t) \quad (2)$$

where $o'(p, t) = o(p) + g(p) \cdot I_D(p) \tau$.

Next, to correct the time-dependent noise component and retrieve the signal S , MIRO employs the optimal sparsity conferred by the shearlet transform[5] (**Figure 1**). Shearlets are an emerging extension of wavelets that allow for multiscale analysis with the efficient encoding of anisotropic features, particularly relevant to biological phenomena[6-8]. Indeed, shearlets provide optimally sparse representation for a large class of multi-variable, multi-dimensional functions, allowing for simplified mathematical analysis and fast algorithmic implementation[9-11]. Exploiting the shearlet transform for fluorescence images, we then conducted transform-domain thresholding to restore the input signal. Importantly, we derived a microlocal analysis to estimate and correct noise for each voxel in

the shearlet space, avoiding any assumptions about the noise uniformity. Finally, lingering noise can be further reduced by taking advantage of the non-local similarity of the imaged sample.

To characterize the performance of MIRO, we used both numerical and experimental datasets of varying signal-to-noise ratios (SNRs), resolution, and sampling rates. Specifically, we simulated fluorescence acquisitions in the presence of all the microscopy-relevant noise sources and restored the images using MIRO under different SNR conditions. We observed that MIRO allows for the removal of fixed-pattern, dark, and readout noise and a significant reduction of photon shot noise with an improvement of $\sim 90\%$ over the ideal camera behavior (i.e., containing pure photon shot noise) even at low photon counts (< 3 photons/pixel). Experimental data processed under similar conditions showed robust and accurate noise correction in accord with the simulated results. Moreover, we assessed using both numerical and experimental data that MIRO can effectively restore the image quality without feature smoothening. On the contrary, our results showed a significant resolution recovery even at low SNRs due to the reduction of the detrimental noise influence that deteriorates resolution.

Furthermore, we observed improved image restoration compared to several state-of-the-art denoising methods and reliable denoising of fluorescence images compatible with a wide range of microscopy systems independently from the detector used. Finally, the MIRO framework could be feasibly synergized with existing learning-based algorithms to enhance performance. We validated this hybrid concept by processing the 3D image datasets of single-cell and organoid specimens acquired with lattice light-sheet microscopy and confocal microscopy, respectively. We anticipate that MIRO would be a valuable toolkit to improve data visualization and processing pipelines in digital microscopy[12-14].

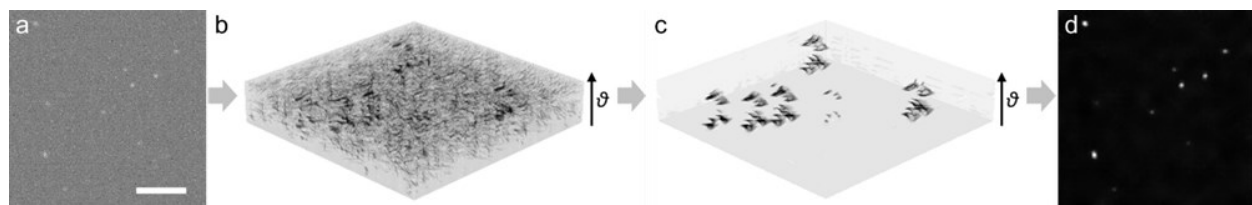


Figure 1: (a-d) Concept of microlocal shearlet shrinkage for MIRO image restoration. The input fluorescent image (a) is transformed to the shearlet space (b), where the spatially overlapping noise and signal features become microlocally distinguishable across shearlet components (θ). Thus, MIRO pipeline leverages this feature together with camera modeling to perform microlocal pixelwise shrinkage and restore image quality without any assumption about noise uniformity (c, d).

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