# Image acquisition setup of FMD dataset:

**Images**: The test images can be downloaded from here <https://curate.nd.edu/show/f4752f78z6t>

**Citation for dataset**: Please cite the FMD dataset using the following format: Mannam, Varun, Yide Zhang, and Scott Howard. “Fluorescence Microscopy Denoising (FMD) Dataset.” Notre Dame, April 21, 2019. <https://doi.org/10.7274/r0-ed2r-4052>. #DOI: 10.7274/r0-ed2r-4052

### Three microscope setups are used to capture the complete FMD dataset. 1. Confocal microscopy, 2. Two-Photon microscopy and 3. Widefield microscopy

Our FMD dataset covers the three main modalities of fluorescence microscopy: confocal, two-photon, and wide-field. All images were acquired with high-quality commercial fluorescence microscopes and imaged with real biological samples, including fixed bovine pulmonary artery endothelial (BPAE sample #F36924) cells [labeled with MitoTracker Red CMXRos (mitochondria), Alexa Fluor 488 phalloidin (F-actin), and DAPI (nuclei); Invitrogen FluoCells F36924], fixed mouse brain tissues (stained with DAPI and cleared), and fixed zebrafish embryos [EGFP labeled Tg(sox10:megfp) zebrafish at 2 days post fertilization]. All animal studies were approved by the university’s Institutional Animal Care and Use Committee.

To acquire noisy microscopy images for denoising purposes, we kept an excitation laser/lamp power as low as possible for all imaging modalities. Specifically, the excitation power was low enough to generate a very noisy image, and yet high enough such that the image features were discernible. We also manually set the detector/camera gain to a proper value to avoid clipping and to fully utilize the dynamic range. Although pixel clipping could be inevitable because distinct biological structures with various optical properties could generate extremely bright fluorescence signals that could easily saturate the detector, we were able to maintain a very low number of clipped pixels (less than 0.2% of all pixels) in all imaging configurations.

## Microscopy setup:

The confocal and two-photon images were acquired with a Nikon A1R-MP laser scanning confocal microscope equipped with a Nikon Apo LWD 40×, 1.15 NA water immersion objective. The confocal and two-photon images were 512×512 pixels with a pixel size of 300 nm and a pixel dwell time of 2 µs. The A1R-MP microscope has multiple detectors (PMTs) in parallel, so for multi-channel (color) fluorescence imaging with the BPAE cells, all three images were acquired simultaneously. For confocal imaging, the excitation was generated by a LU4/LU4A laser unit, the pinhole size was set to 1.2 Airy unit, and the imaging conditions for different samples were as follows: BPAE nuclei, 405 nm excitation, 0.5% laser power, 110 PMT gain; BPAE F-actin, 488 nm excitation, 0.5% laser power, 110 PMT gain; BPAE mitochondria, 561 nm excitation, 0.5% laser power, 110 PMT gain; mouse brain, 405 nm excitation, 0.5% laser power, 115 PMT gain; zebrafish embryo, 488 nm excitation, 10% laser power, 140 PMT gain. For two-photon microscopy, the excitation was generated by a Spectra-Physics Mai Tai DeepSee femtosecond laser, and for all two-photon images, the laser power was set to 0.5%, the PMT gain to 130, and the excitation wavelength to 780 nm. Note that our dataset did not include two-photon images of the zebrafish sample because during two-photon imaging, very strong two-photon auto-fluorescence signals from the zebrafish were observed, which severely degraded the imaging quality.

The wide-field images were acquired with a Nikon Eclipse 90i wide-field fluorescence microscope equipped with a Nikon Plan Fluor 40×, 0.75 NA objective. The excitation was generated by a halogen lamp (with ND16 neutral density filter) and the images were captured by a DS-Fi1-U2 camera with an exposure time of 200 ms and a gain of 46. The raw image size was 1280×960 and the pixel size was 170 nm. These images were cropped to 512×512 before being processed for our dataset. Note that our dataset only covered wide-field images of the BPAE cells because widefield microscopy could not image well in animal tissues such as mouse brain and zebrafish embryo, where strong out-of-focus fluorescence would blur out the wide-field images. Since the BPAE cells were stained with three different fluorophores while only one detector (CCD camera) was available in the 90i microscope, we imaged three times for the same FOV, each time with a different filter block (DAPI for nuclei, FITC for F-actin, TRITC for mitochondria), to acquire the multi-channel (color) fluorescence image of the cells.

For any imaging modality, each sample was imaged with 20 different FOVs, and each FOV was repeatedly captured for 50 times as 50 noise realizations.

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