# Image acquisition setup of Super-resolution dataset:

**Images**: The test images can be downloaded from here <https://curate.nd.edu/show/5h73pv66g4s>

**Citation for dataset**: Please cite the super-resolution dataset using the following format: Mannam, Varun, Yide Zhang, and Scott Howard. “Super-Resolution (SR) Fluorescence Microscopy Dataset.” Notre Dame, 9th Feb 2021.

or Mannam, Varun. 2021. “Super-Resolution (SR) Fluorescence Microscopy Dataset.” Notre Dame. <https://curate.nd.edu/show/5h73pv66g4s>

### The microscope setups are used to capture the complete super-resolution dataset: Two-Photon microscopy

All images were acquired with high-quality custom-built fluorescence microscopy and imaged with 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.All animal studies were approved by the university’s Institutional Animal Care and Use Committee.

To acquire noisy microscopy images for super-resolution 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 two-photon fluorescence microscope equipped with a Nikon 40×, 0.8 NA water immersion objective. The two-photon images were 256x256 pixels with a pixel size of 200 nm and a pixel dwell time of 12 µs. The fluorescence imaging with the BPAE cells, all three images were acquired simultaneously by using 800nm excitation wavelength and detector bandpass filter from 400 to 700 nm with excitation power: 6mW. For two-photon microscopy, the excitation was generated by a Spectra-Physics Mai Tai femtosecond laser (80MHZ, pulse width 100fs, pulse interval: 12.5nsec), and for all two-photon images, the laser power was set to 6mW, the PMT gain to 0.6volts (corresponds to 100000 gain), and the excitation wavelength to 800 nm.

For the imaging modality, BPAE sample was imaged with 16 different FOVs, and each FOV was repeatedly captured for 50 times as 50 noise realizations.

Target generation: using SRRF technique to generate the super-resolution using 50 noisy realizations.

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