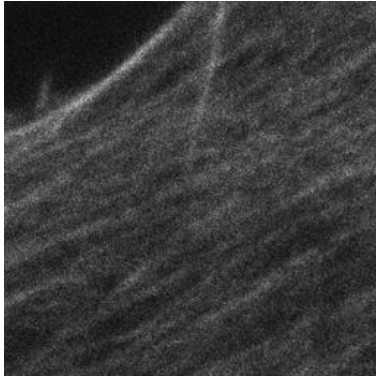
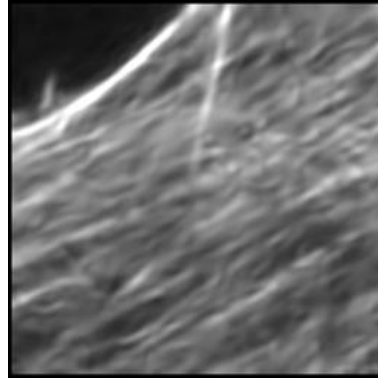


## Denoising Fluorescence Images

The BLS-GSM is one of the most powerful denoising algorithms based on rigorous statistical criteria and multi-resolution image analysis. However it takes some efforts to implement the method and calibrate the noise parameters in order to receive optimal performance. The outcome can be astounding when the method is applied properly. The procedure requires the MATLAB software and a camera with well-behaved noise characteristics.



Before denoising



After denoising

### Installation and Testing Procedure

1. The modifications below are necessary for removing intensity-dependent Poisson noise (approximated as Gaussian noise).
2. Download BLS-GSM Image Denoising Toolbox version 1.03 from [https://github.com/ricedsp/D-AMP\\_Toolbox/tree/master/Packages/BLS-GSM](https://github.com/ricedsp/D-AMP_Toolbox/tree/master/Packages/BLS-GSM)
3. Create a folder “denoise”, and subfolders with the names corresponding to those in the package.
4. Download matlabPyrTools from <https://github.com/LabForComputationalVision/matlabPyrTools>. Create a subfolder “matlabPyrTools” to place the files.
5. In the subfolder “Added\_PyrTools”, disable mirdwt.dll and mrdwt.dll by renaming them as mirdwt\_bak.dll and mrdwt\_bak.dll respectively.
6. In the subfolder “denoising\_subprograms”, disable denoi\_BLS\_GSM.m by renaming it as denoi\_BLS\_GSM\_bak.m.
7. Download <http://yuliwang.bme.cmu.edu/Methods/Imaging/BLS-GSMmod.zip> and unzip the files into the denoise folder and subfolders. Do not change the file destination.
8. Add the following folders in the exact order to the MATLAB path:  
denoise\denoising\_subprograms (at the top)  
denoise\matlabPyrTools\MEX  
denoise\matlabPyrTools  
denoise\Simoncelli\_PyrTools  
denoise\Added\_PyrTools
9. Set denoise as the current folder
10. Test run denoise.m. The images shown above should appear if the program is executed successfully.

## Calculating Noise Parameters

1. Proper determination of noise parameters is critical. The values may be slightly dependent on the exposure time. The image shown above was collected with an Andor Ixon DU-887 EMCCD camera.
2. Prepare a sample of faint uniform intensity, e.g. a thin layer of fluorescent dyes at a very low concentration.
3. Take a dark image—without illumination, at the same exposure as what was used for the target image to be denoised. All image files should be either uncompressed or compressed with a non-lossy algorithm.
4. Take a series of images with different intensities of illumination but the same exposure, covering the range of intensities as seen in the target image. Avoid areas with any bright debris.
5. Measure the average and standard deviation of intensities of each image using a program like ImageJ, enter the values into the spreadsheet noise.xls columns A and B. Values from the dark image should be entered in Row 2.
6. Fix the data range in cells B21 and B22.
7. Values in B21 (A), B23 (DV), B24 (B) should be entered into the program denoise.m lines 8-10. The name of the raw image is entered in line 13.

## Please Cite the Following References

1. Portilla, J., Strela, V., Wainwright, M.J. & Simoncelli, E.P. (2003) Image denoising using scale mixtures of Gaussians in the wavelet domain. *IEEE Trans. Image Process.* **12**, 1338–1351.
2. Wang, Y.-L. (2007) Noise-induced systematic errors in ratio imaging: serious artefacts and correction with multi-resolution denoising. *J. Microscopy* **228**, 123-131.