Lifetime Denoising

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Outline

- Motivation and Noise type
- Options for lifetime denoising
- Block diagram at high level and low level
- Preprocess and post-process
- Results and metrics to compare
- Conclusions

Motivation

- Lifetime provides the additional contrast to the fluorescence intensity since it is independent of so many factors like power, concentration, movement of the animal etc.
- But the lifetime information is noisy with low-SNR.
- Only our setup has the lifetime information extraction capability.
- To get high SNR, lot of averages are required with in the same FOV, which takes more time and can affect photobleaching of the sample.
- Use machine learning model to perform lifetime denoising like the intensity denoising [1], but the lifetime noise type is not same as intensity noise (which is combination of Poisson noise and Gaussian noise) [2].

Then, from Eq. (14), we get the standard deviation of the lifetime,

$$\sigma_\tau = \frac{1+\tau^2}{2\sin(\pi a)}\left(\frac{1}{N_{\text{det}}}\frac{\pi a}{1+4\tau^2}\right)^{\frac{1}{2}} \times (2\pi a - \sin(2\pi a) + 10\pi a\tau^2 - 3\sin(2\pi a)\tau^2 + 8\pi a\tau^4)^{\frac{1}{2}}. \quad (51)$$

Consequently, the F-value is calculated as

$$F = \frac{1+\tau^2}{2\tau\sin(\pi a)}\left(\frac{\pi a}{1+4\tau^2}\right)^{\frac{1}{2}} \times (2\pi a - \sin(2\pi a) + 10\pi a\tau^2 - 3\sin(2\pi a)\tau^2 + 8\pi a\tau^4)^{\frac{1}{2}}. \quad (52)$$

By letting $a \to 0$, the F-value of a Dirac comb modulation can be directly obtained as

$$F = (1+\tau^2)\left(\frac{1+2\tau^2}{1+4\tau^2}\right)^{\frac{1}{2}}. \quad (53)$$

[1] Zhang, Yide, Yinhao Zhu, Evan Nichols, Qingfei Wang, Siyuan Zhang, Cody Smith, and Scott Howard. "A poisson-gaussian denoising dataset with real fluorescence microscopy images." In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, pp. 11710-11718. 2019.
[2] Zhang, Yide, Aamir A. Khan, Genevieve D. Vigil, and Scott S. Howard. "Investigation of signal-to-noise ratio in frequency-domain multiphoton fluorescence lifetime imaging microscopy." *JOSA A* 33, no. 7 (2016): B1-B11.

Lifetime noise type observations

- SNR of estimated lifetime is the ratio of mean lifetime and standard deviation of lifetime

$$SNR_\tau = \frac{\tau\_mean}{std(\tau)} = \frac{\tau}{\sigma_\tau}$$

- For Poisson dominated noise (ex: intensity), mean of noise is N_{det} and standard deviation of noise is $\sqrt{N_{det}}$ which gives the SNR value is $N_{det}/\sqrt{N_{det}}=\sqrt{N_{det}}$

where $\sqrt{N_{det}}$ is the number of detected photons. (i.e., $\sigma_I = \sqrt{N_{det}}$, and $I = N_{det}$ and $\frac{\sigma_I}{I} = \frac{\sqrt{N_{det}}}{N_{det}} = \frac{1}{\sqrt{N_{det}}}$)

- From JOSA paper, $\frac{\sigma_\tau}{\tau}$ is also inversely proportional to $\sqrt{N_{det}}$,

so we can assure that lifetime noisy type is also Poisson dominated noise with additional factor of true lifetime value.

- Also, from the F value which is independent of N_{det}, this also confirms that lifetime has Poisson dominated noise distribution.

Then, from Eq. (14), we get the standard deviation of the lifetime,

$$\sigma_\tau = \frac{1+\tau^2}{2\,\sin(\pi a)}\left(\frac{1}{N_{\text{det}}}\frac{\pi a}{1+4\tau^2}\right)^{\frac{1}{2}} \times (2\pi a - \sin(2\pi a) + 10\pi a\tau^2 - 3\,\sin(2\pi a)\tau^2 + 8\pi a\tau^4)^{\frac{1}{2}}. \quad (51)$$

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Options for machine learning lifetime denoising

Option1: Train the network from scratch with lifetime image dataset

- Need to capture the lot of images → only our setup has lifetime imaging capability
- Creation of dataset takes longer time and photobleaching on the samples for longer acquisitions
- Can not cover all the microscopic modalities like in intensity denoising (widefield and confocal are missing here)
- Need of image registration to get the ground-truth image in each FOV (stage movement is more)
- Cells types are limited to only BPAE, mouse kidney and mouse brain here for fixed wavelength (800 nm): Zebrafish has lot of auto-fluorescence at 800nm.
- Training a new model takes lot of time to find best tuning parameters (like learning rate, optimizer, machine learning NN model (ex: U-Net/noise2noise model)).
- Evaluation metrics and comparison with other methods takes more time.

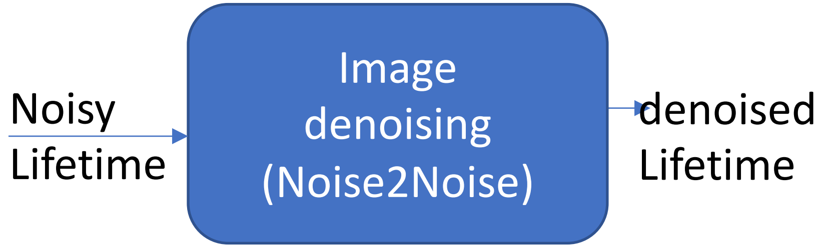
Cont

Option2: Use the existed intensity denoising network (even the lifetime noise is not combination of Poisson and Gaussian noise) and test the lifetime denoising performance

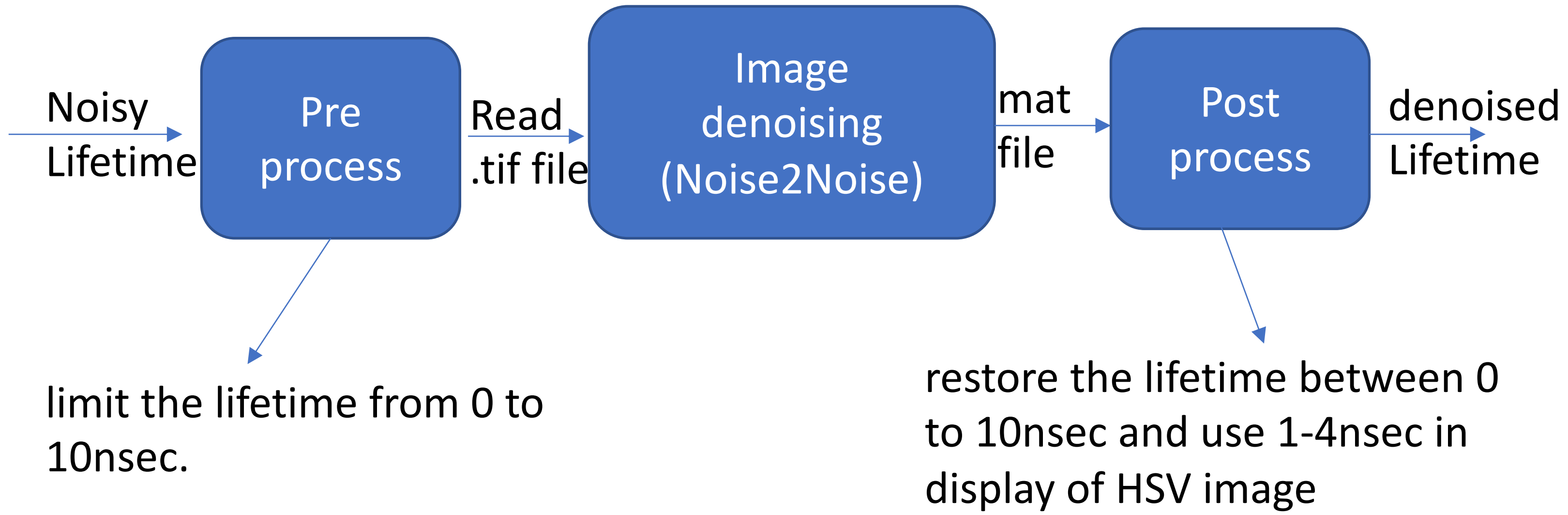
- Only inference is used here: collection of dataset and model training is avoided here
- Use the two-photon microscopy fixed cells (BPAE and mouse kidney)
- Possible with zebrafish samples (later)
- Use the pre and post process steps to convert the lifetime images for the network input and output
- Evaluation metrics are required, i.e., the ground truth lifetime is required which can be obtained by averaging the samples with in the same FOV.
- To get the ground truth image, image registration is required if the stage movement is more with in the same FOV.

Lifetime denoising: Option2

- High-level block diagram of lifetime denoising is here



- Low-level block diagram of lifetime denoising is here



Cont..

- Preprocess step: replace the pixels whose lifetime is above 10nsec and below 0nsec with 0nsec save to a .tif file or read the .tif file and limit lifetime values between 0 to 10nsec (valid range) in python.
- In mouse kidney, the number of pixels **within the valid range is 98%** and less than 2% is out of the range and in BPAE cells, the number of pixels within the valid range is 60% and around 40% is out of the range.
- Divide the lifetime image by 10e-9 to get the image with in 0-1 range and passthrough the network for the denoised image
- Postprocess step: add the value of 0.5 and multiply with 10nsec to get the denoised lifetime image. (within valid range)

- Use the lifetime of 1-4nsec to display the results in HSV mode.

Image registration

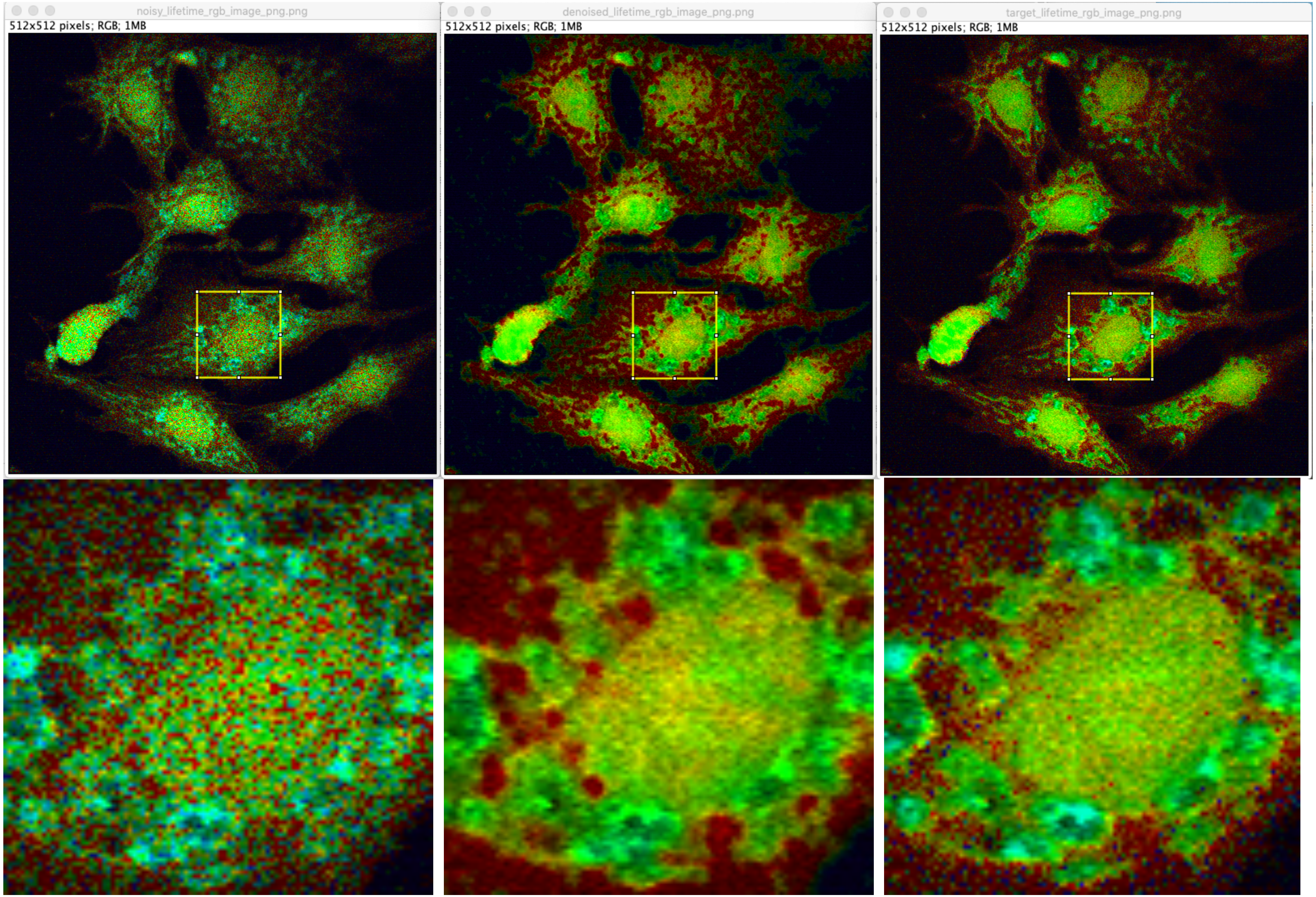
- Small PSNR improvement is observed in the denoised result, since to the target image is also noisy due to the stage movement.
- Image registration is required to align the images by correcting the stage movement.
- methods:
  - Register virtual stack -> imageJ (need input of valid range)
  - Correct_3D_drift -> imageJ (need input of valid range)
  - imregister -> Matlab (need input of valid range)
- We used Matlab based image registration function (imregister()) with parameters:
  [optimizer, metric] = imregconfig('monomodal') (all images are captured using the same sensor)
- Where optimizer: RegularStepGradientDescent and Properties: GradientMagnitudeTolerance: 1.000000e-06 -> epsilon value to say it is reached the convergence, MinimumStepLength: 1.000000e-06 -> lr_min (1e-8), MaximumStepLength: 1.000000e-02 -> initial lr -> 1e-3, MaximumIterations: 500 -> number of iterations (epochs), RelaxationFactor: 1.000000e-01 -> learning rate (eta) and metric is MSE.

BPAE samples dataset

Results: Noisy and denoised images (HSV)

Full frame

ROI



Results: Metrics and comparison

Image	PSNR (dB) on the HSV data	SSIM (Structural Similarity index)
Noisy lifetime image	8.0485	0.0774
Denoised lifetime image (at index 43)	9.8047	0.1066

- difference in PSNR is 1.7561 dB
- power is decreased as time progress -> shift in Z plane

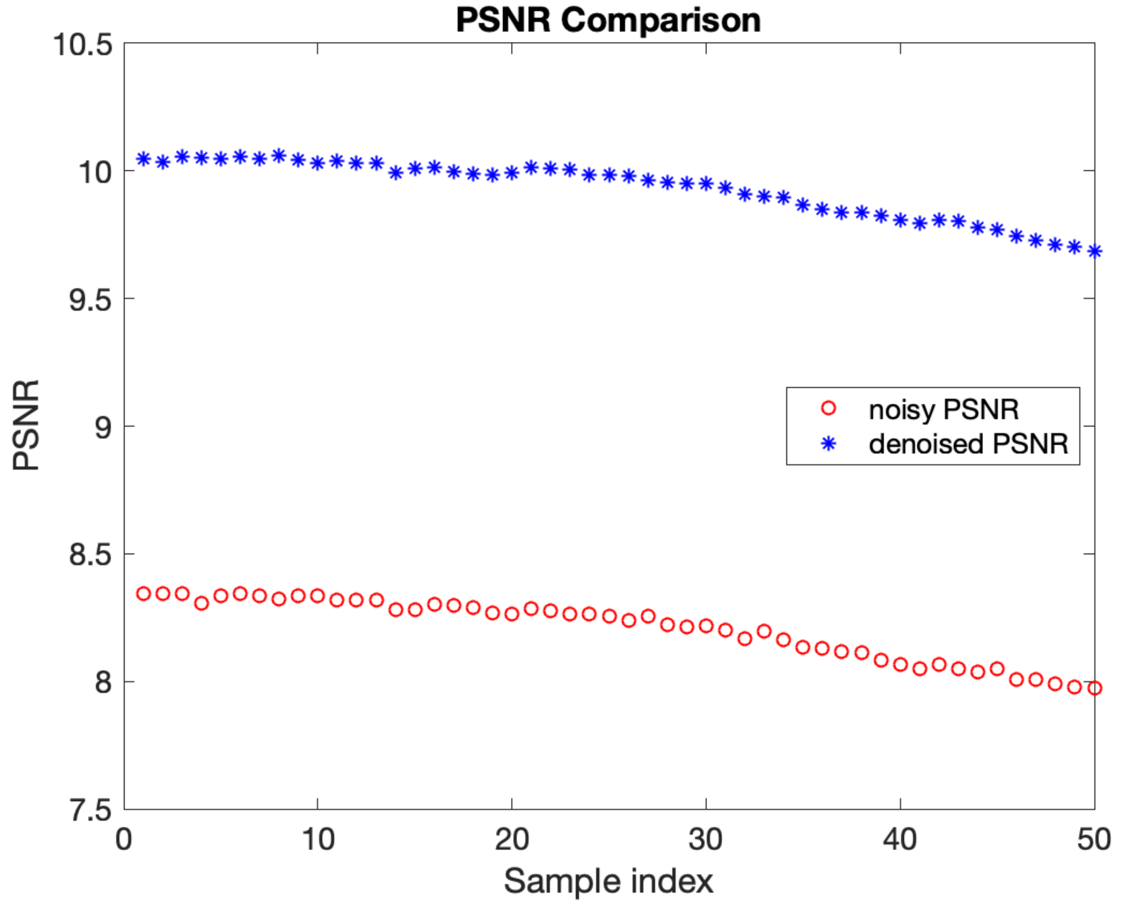


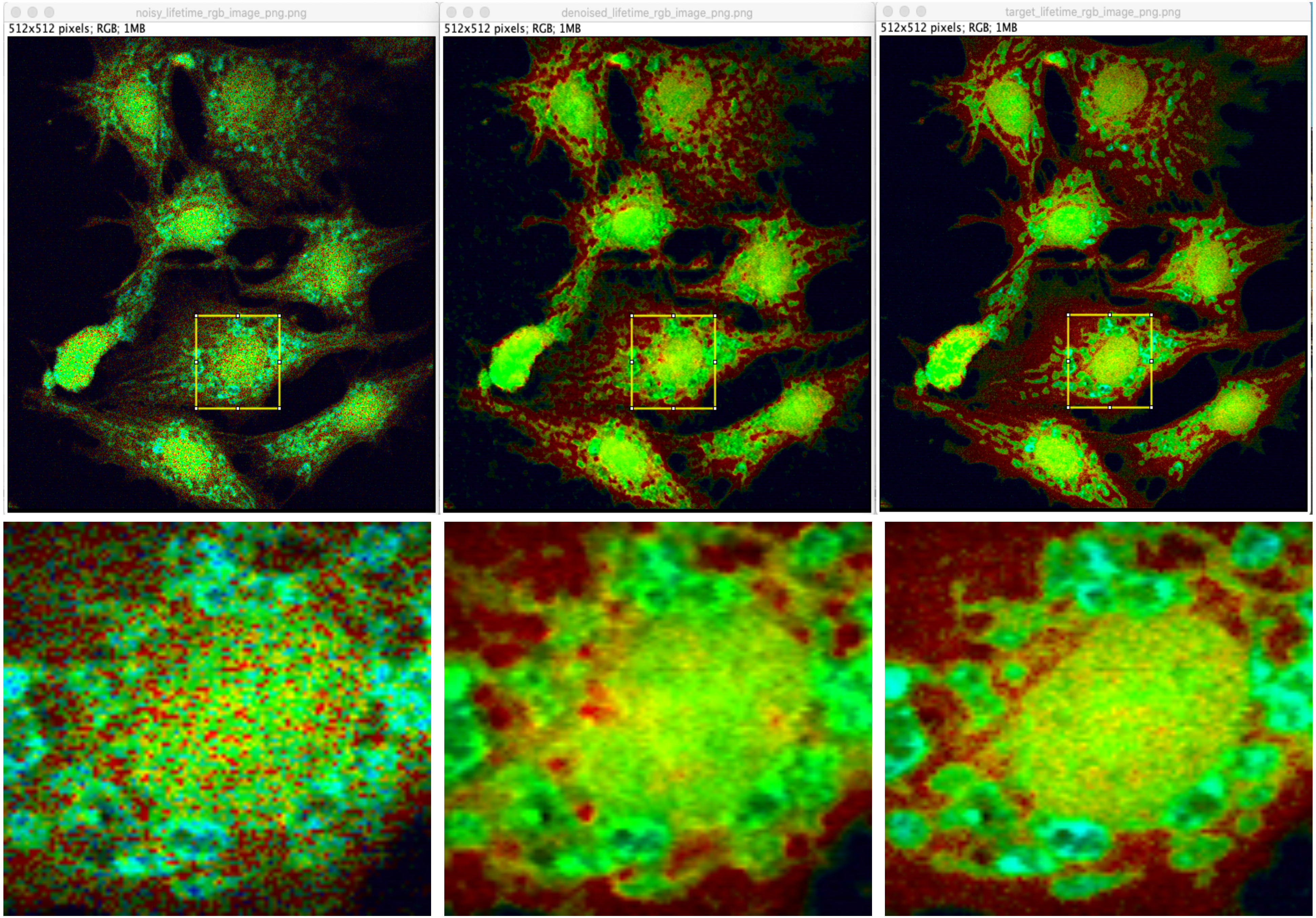
Figure: PSNR of the lifetime images in the same FOV

- Average (over 50 image) input PSNR = 8.2068 dB, denoised PSNR = 9.9311 dB with average difference of 1.7243 dB

Results: Noisy and denoised images (HSV) (with registration)

Full frame

ROI



Why the artifacts as red cells in denoised image within the ROI?

Results: Metrics and comparison

Image	PSNR (dB) on the HSV data	SSIM (Structural Similarity index)
Noisy lifetime image	12.9356	0.1209
Denoised lifetime image (at index 28)	24.7218	0.5351

- difference in PSNR is 11.7861 dB

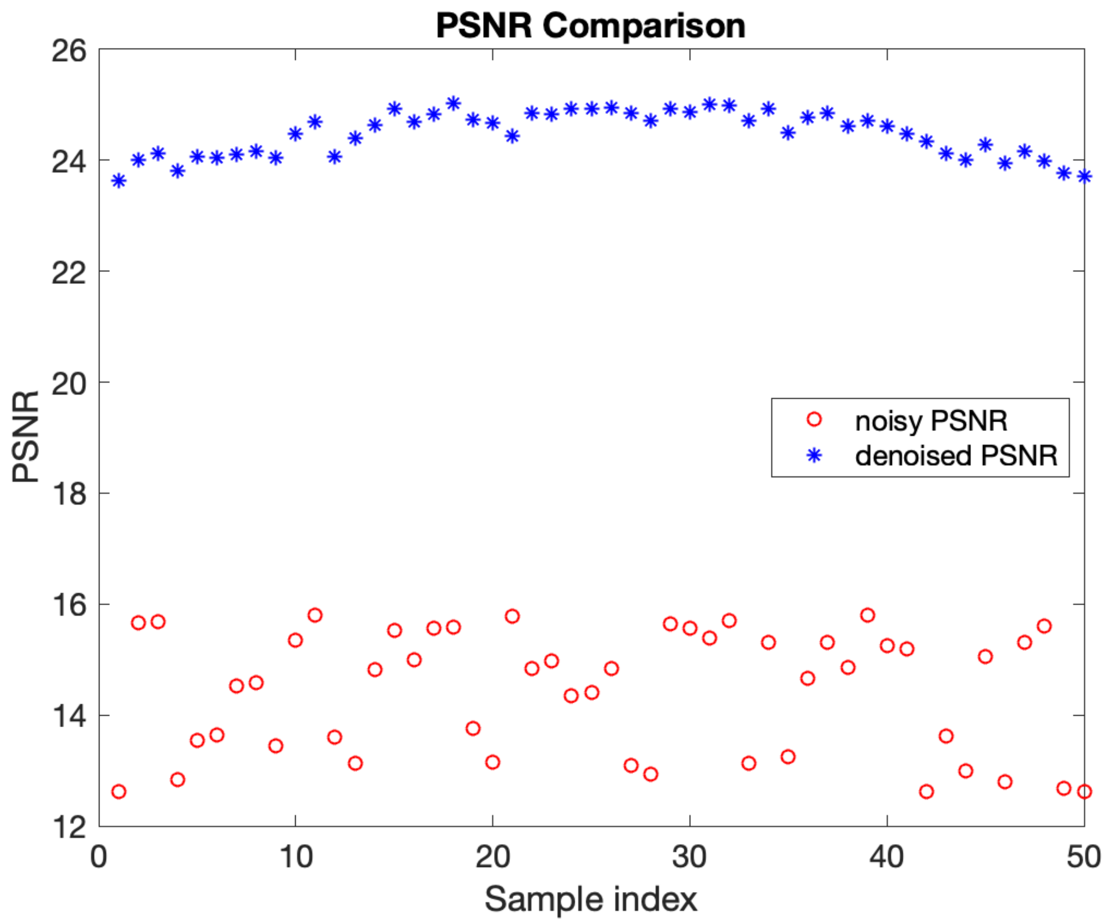


Figure: PSNR of the lifetime images in the same FOV

- Average (over 50 image) input PSNR = 14.4259 dB, denoised PSNR = 24.4810 dB with average difference of 10.0552 dB

Summary of existing methods

- **Lifetime noise type is not same as intensity noise, but we used the same machine learning intensity denoising model to denoise the lifetime.**
- In the preprocess step: Image registration is required since the stage movement is more
- Without image registration, denoising performance is minimal
- Registration method: imregister with "rigid" method in MATLAB
- Clearly, the denoising results with image registration shows there is an improvement of 9dB in PSNR between with and without denoising.

- Artifacts: see the denoised lifetime images here

Suggestions

- Use the BPAE cells instead of mouse kidney (clear indication of improvement in lifetime)
- Instead of denoise lifetime directly, denoise the G and S images and construct the lifetime using the following equation $\tau = \frac{S}{G*(2*\pi*f_{mod})}$ where f_{mod} is the modulation frequency (which is 80MHz in our experiment)

- Questions:
  - How to show **G and S images have the Poisson dominated noise**?
  - What is the improvement in the denoised lifetime value compared to the old experiment?
  - What are the limits of G and S used in the machine learning inference?

- We used G limits are: -0.1 to 1.1 and S limits are: -0.1 to 0.6, clipping the the G and S images and pass through the machine learning inference model. and reconstructed back the lifetime from 0 to 10nsec range.

Lifetime denoising – Option3

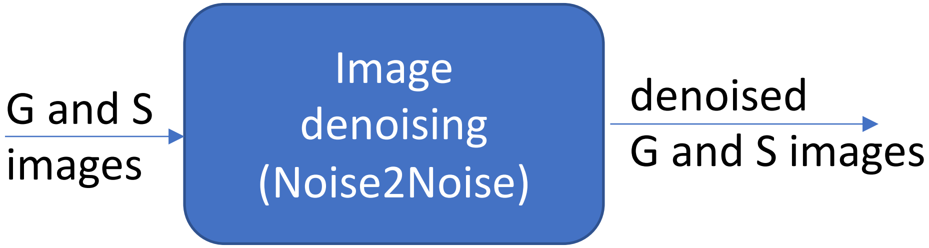
- Use the BPAE cells instead of mouse kidney (clear indication of improvement in lifetime)
- Instead of denoise lifetime directly, denoise the G and S images and construct the lifetime using the following equation $\tau = \frac{S}{G*(2*\pi*f_{mod})}$ where f_{mod} is the modulation frequency (which is 80MHz in our experiment)
- Questions: How to show **G and S images have the Poisson dominated noise**?

  Hint: In the JOSA paper, we define the lifetime (τ) as the ratio of two variables U and V which has mean of μ_1 and μ_2 with variance of σ_1 and σ_2. So can we assume U and V are Gaussian distribution? -> NO, for any unknown distribution, we just consider the statistics of 1^{st} and 2^{nd} order moments.

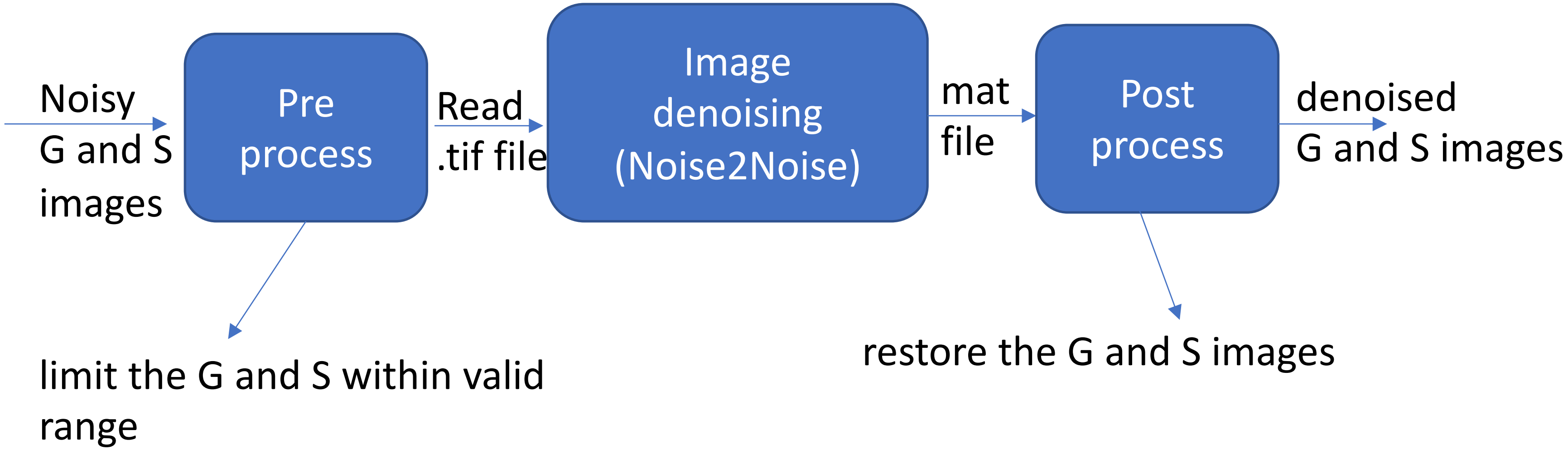
  - What are the limits of G and S used in the machine learning inference?
  - What is the improvement in the denoised lifetime value compared to the old experiment?
- We used G limits are: -0.1 to 1.1 and S limits are: -0.1 to 0.6, clipping the the G and S images and pass through the machine learning inference model and reconstructed back the lifetime from 0 to 10nsec range.

Lifetime denoising: Option3

- High-level block diagram of lifetime denoising is here



- Low-level block diagram of lifetime denoising is here $\tau\_denosied = \frac{S\_denoised}{G\_denoised * (2 * \pi * f_{mod})}$

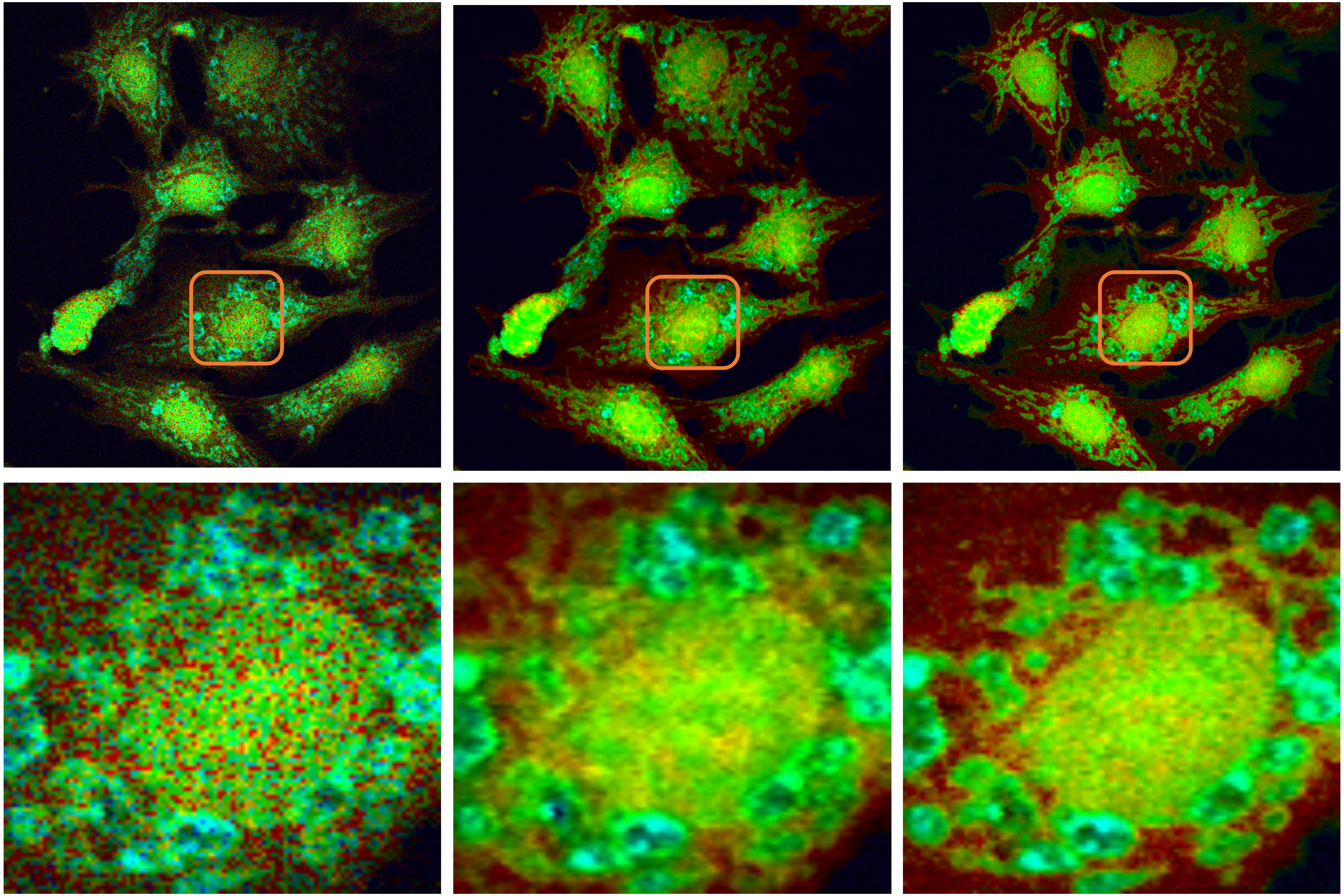


Results: Noisy and denoised images (HSV) (with registration) From G and S denoising

Noisy input | Denoised output | Target

Full frame

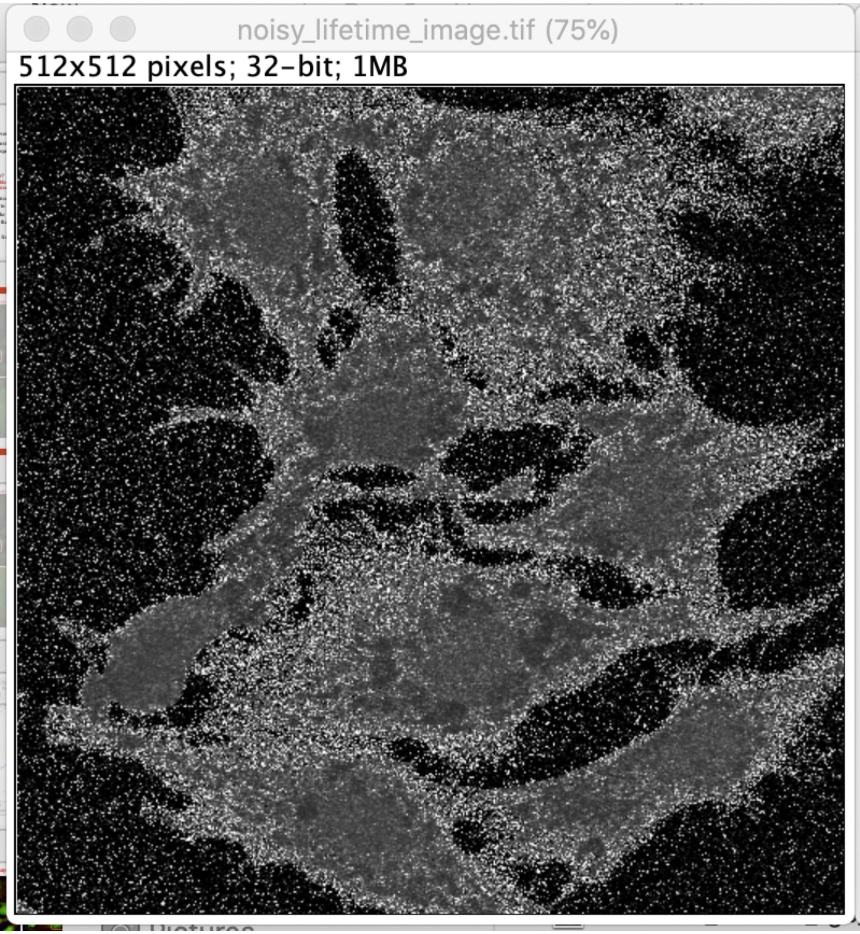
ROI (100x100) at (position 225,300)

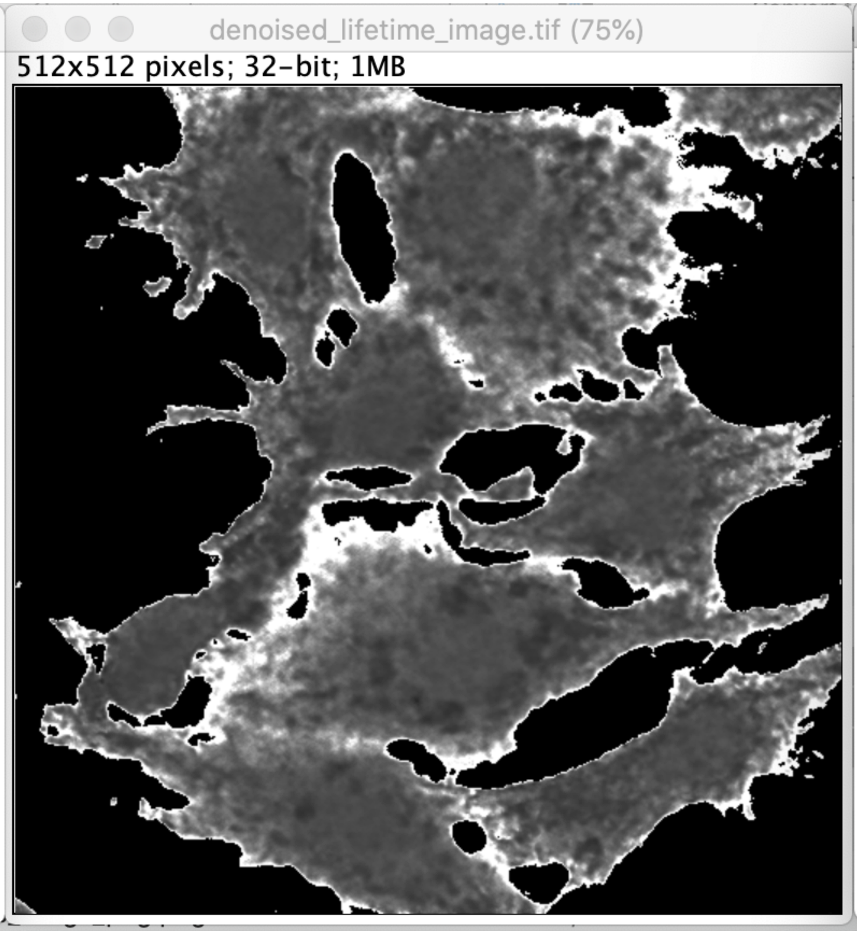


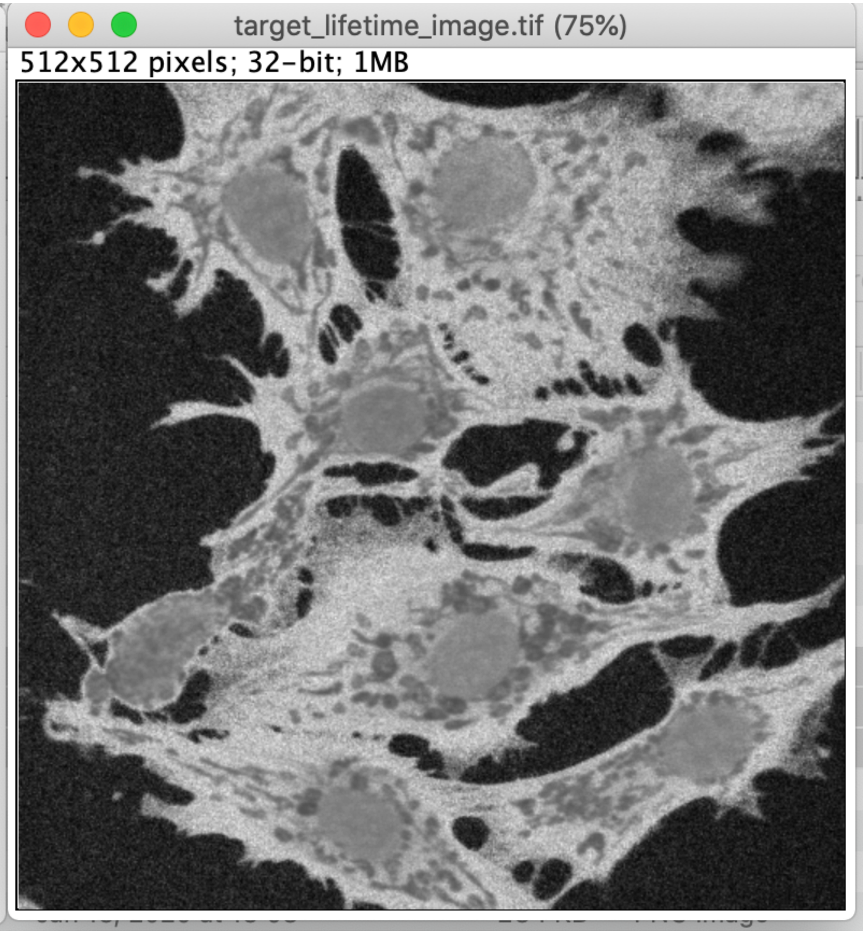
Adjusted brightness in noisy intensity image

Results: Noisy and denoised images (with registration) From G and S denoising

Noisy input | Denoised output | Target | range of tau: 0 to 10nsec

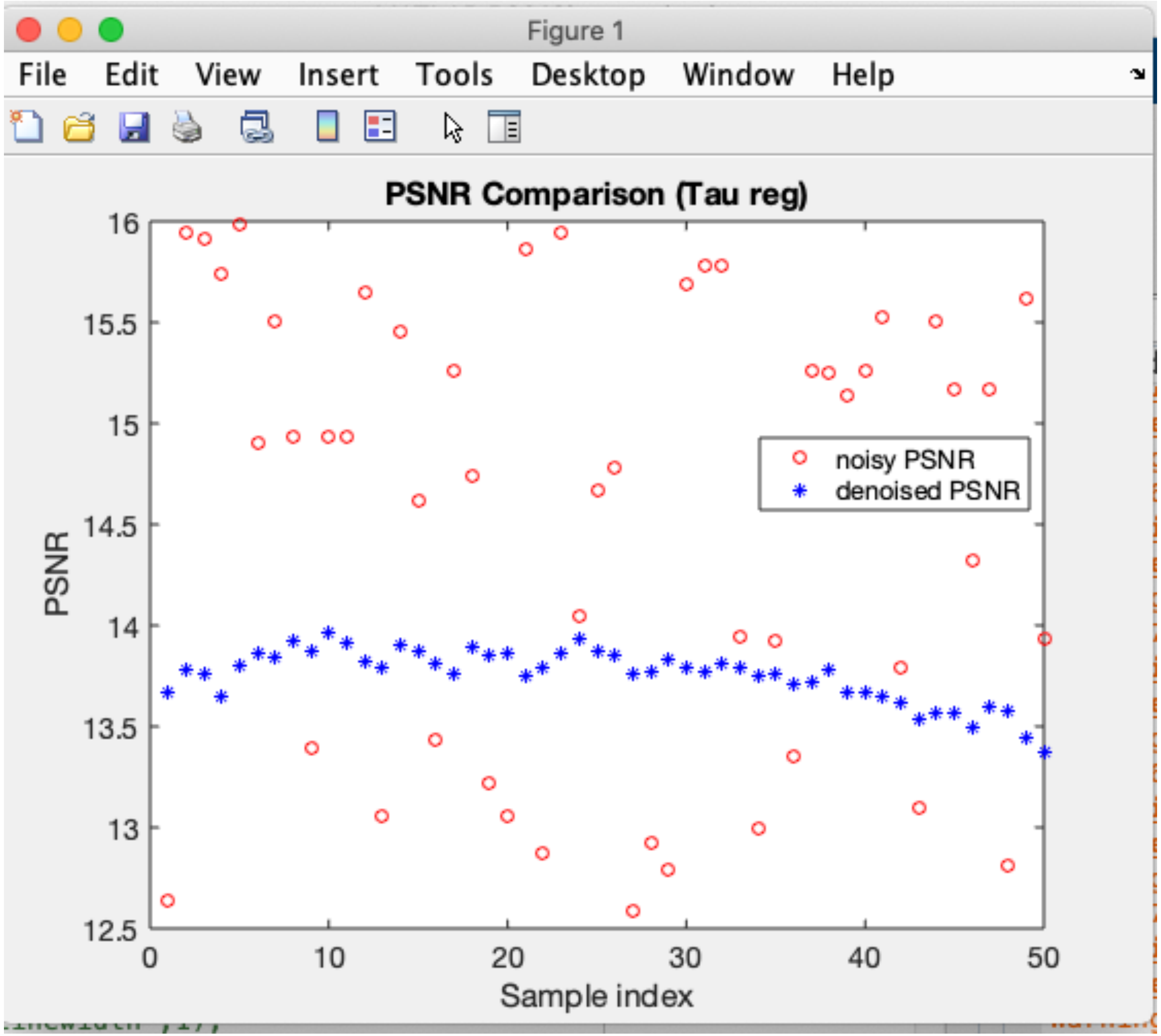






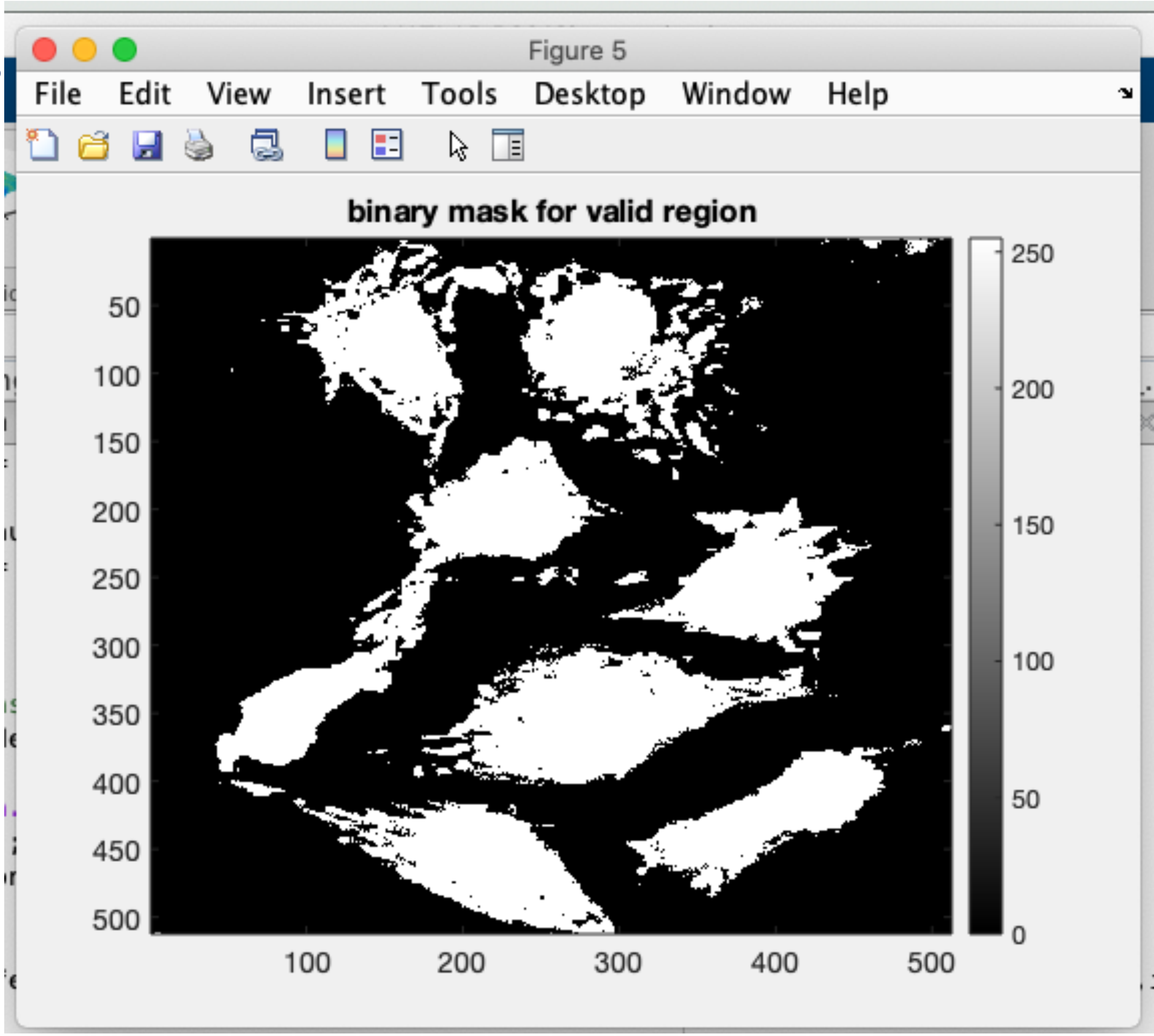
Results: Noisy and denoised images (HSV) (with registration) From G and S denoising

PSNR check?



Select the mask area using ROIs

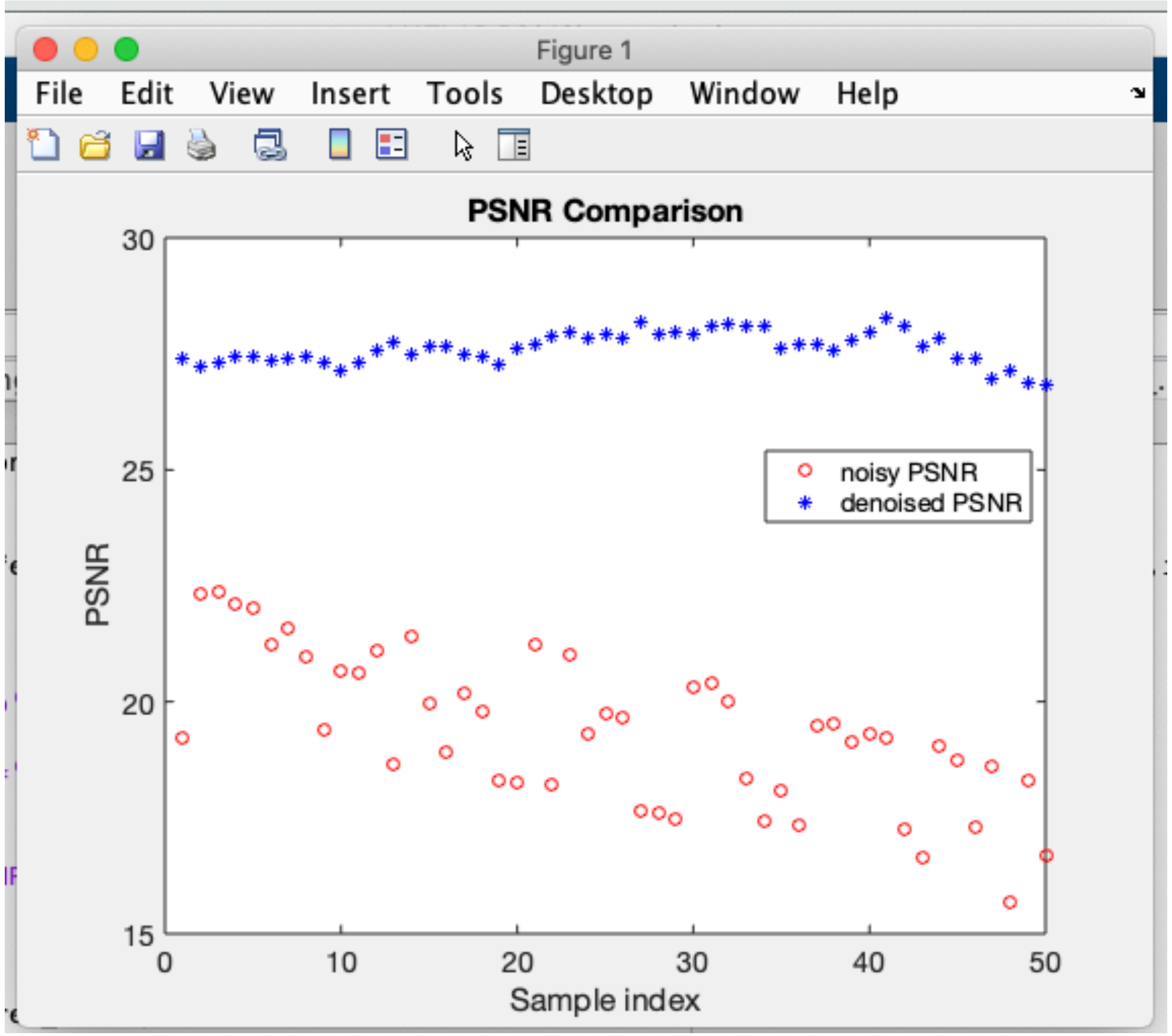
- The area marked in blue circle is the noise region in the phasor diagram
- get the mask of this noisy region and remaining image is the valid pixels
- Export the segments and save this image as “noisy-region” where pixel value is zero. otherwise it is valid region with pixel value of 255.
- Now compute the metric (PSNR) for the selected region only

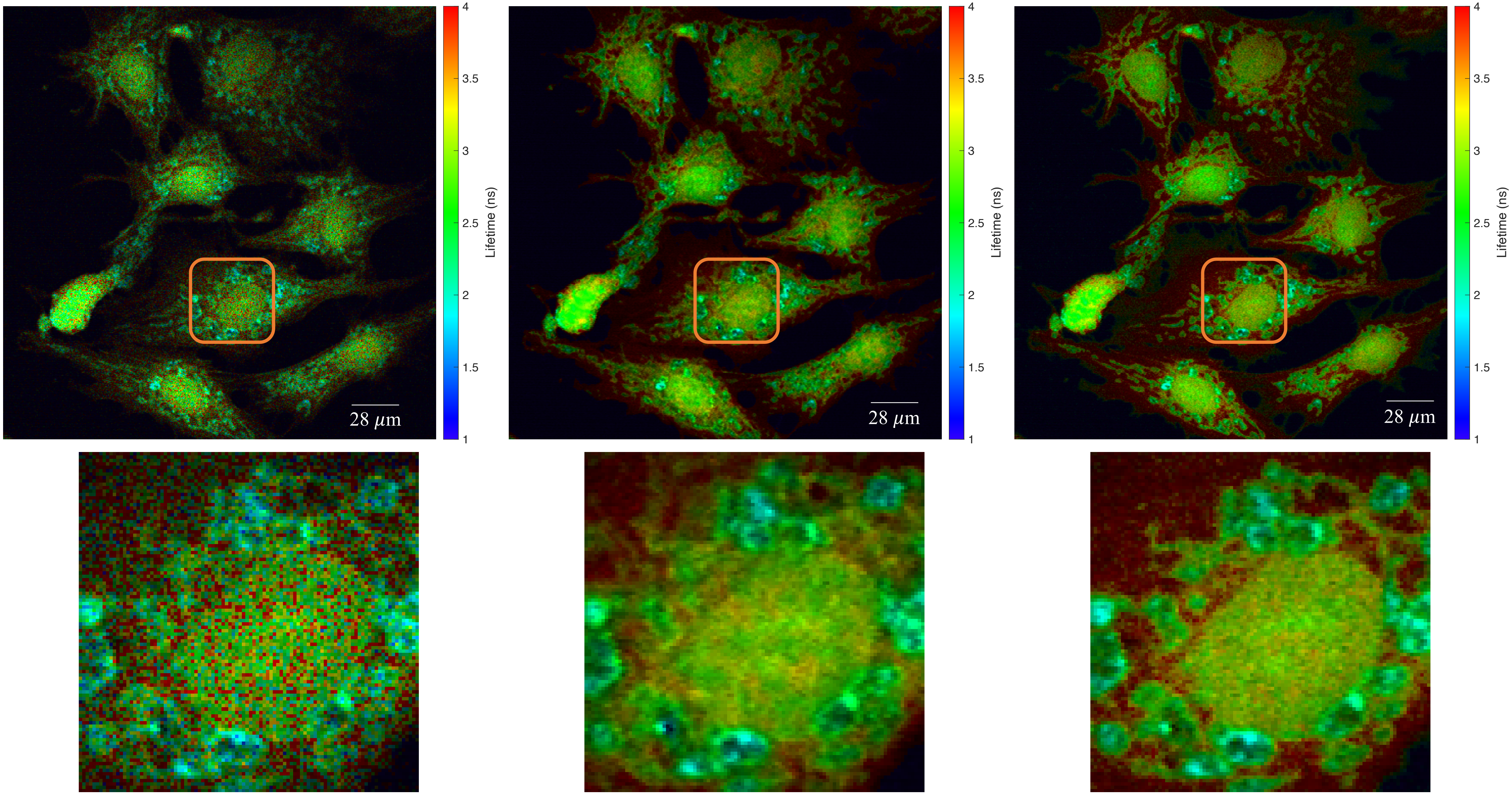


Lifetime denoising (metric on the valid region of pixels in lifetime)

- Average PSNR of noisy input is 19.3551 dB
- Average PSNR of denoised output is 27.6194
- Improvement of **8.26dB** (average over 50 images)

- Clearly, PSNR of ROI is greater than PSNR of full image





(a) (b) (c)