User Manual

INTRODUCTION

CANDLE (A new Collaborative Approach for eNhanced Denoising under Low-light Excitation) is a denoising filter proposed for the processing of 3D laser scanning multiphoton microscopy images. The principle of the of the filter is described in:

P. Coupé, M. Munz, J. V. Manjon, E. Ruthazer, D. L. Collins. A CANDLE for a deeper in-vivo insight. *Medical Image Analysis*. 2012

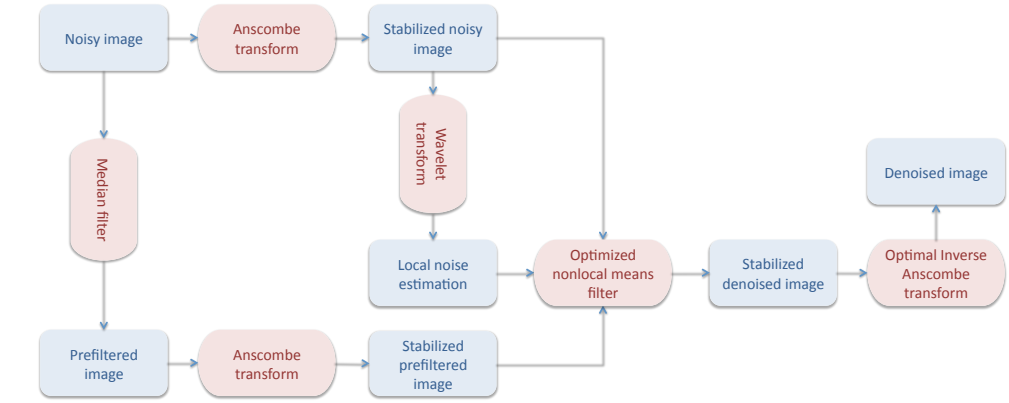


Figure 1: Overall workflow of the proposed method.

COMPONENTS

CANDLE is based on several components described in the following references:

Optimized nonlocal means

Coupe, P., Yger, P., Prima, S., Hellier, P., Kervrann, C., Barillot, C. . An optimized blockwise nonlocal means denoising filter for 3-D magnetic resonance images. *IEEE Trans Med Imaging* 27, 425-441, 2008

Optimal Inverse Anscombe transform

Makitalo, M., Foi, A. . Optimal inversion of the Anscombe transformation in low-count Poisson image denoising. *IEEE Trans Image Process* 20, 99-109, 2011.

Original code can be downloaded here:
http://www.cs.tut.fi/~foi/invansc/#ref_software

Wavelet transform

The used 3D wavelet transform is the freely available library of Brooklyn University:
http://eeweb.poly.edu/iselesni/WaveletSoftware/

INSTALLATION

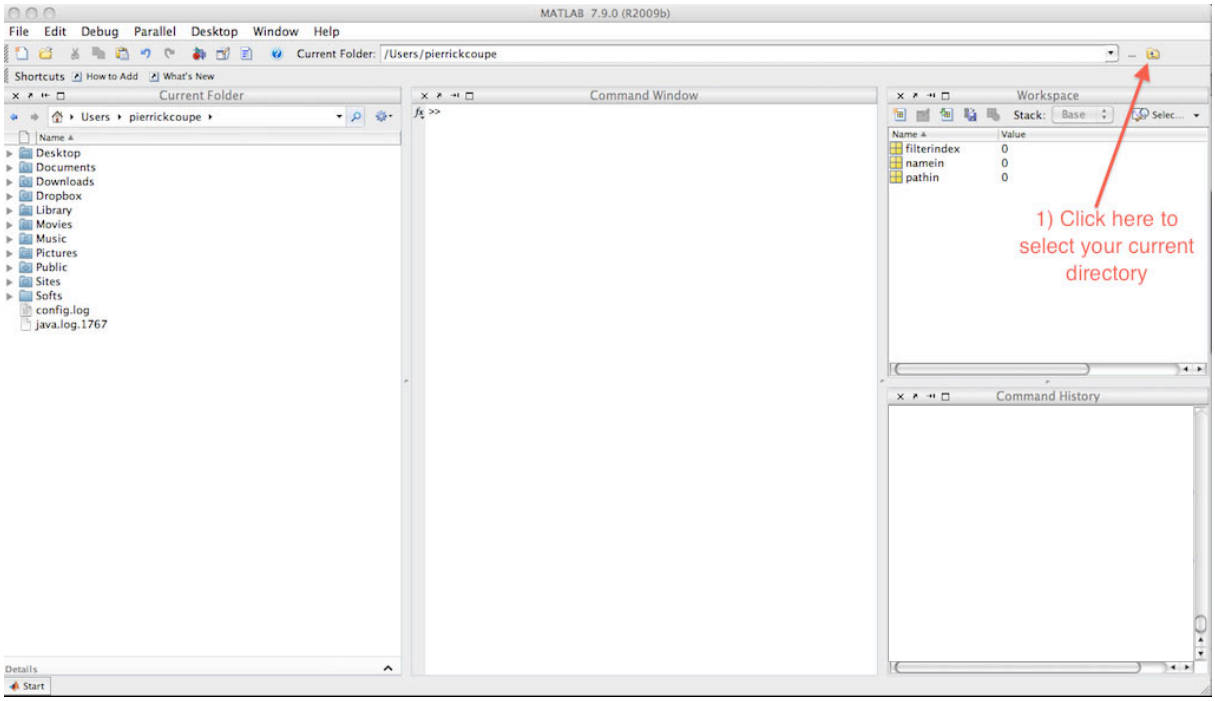
Requirement

CANDLE software is a package based on Matlab© software and thus Matlab© is required for its utilization. The decision to use Matlab© was motivated by the intention to provide multi-platform software requiring minimal user interaction during installation.

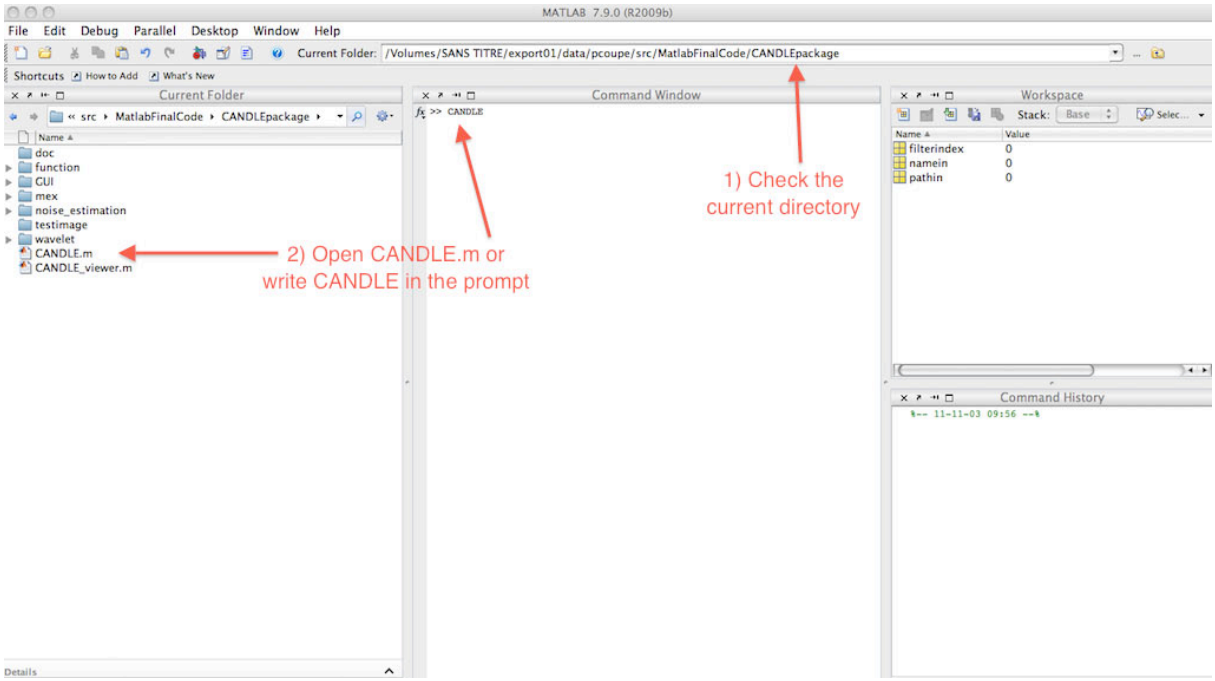
The provided Matlab© package is dedicated to Linux (64 bits), Windows (32/64 bits) and MAC (64bits). In fact, based on MEX code, the core of the algorithm has been precompiled for these platforms. A test image is provided to test your architecture compatibility. Please, do not hesitate to contact us if you need binaries for another architecture. Furthermore, we decided to provide multithreaded implementation, by default the number of threads is 8.

Procedure

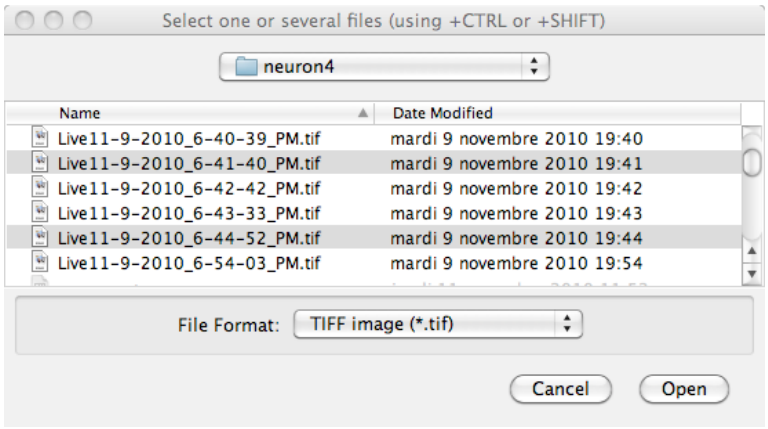
1. Download the package
2. Extract the files from .zip archive
3. Open Matlab
4. Select the directory of the CANDLEpackage as current directory



5. Run the script CANDLE.m (by opening it and pressing 'F5' (or by pressing ▶), by writing 'CANDLE' in the prompt).



6. Use the browser of the GUI to select the input 3D tiff image(s). By using +ctrl or +shift you can select several images to process. The browser is configured to select .tiff files only.

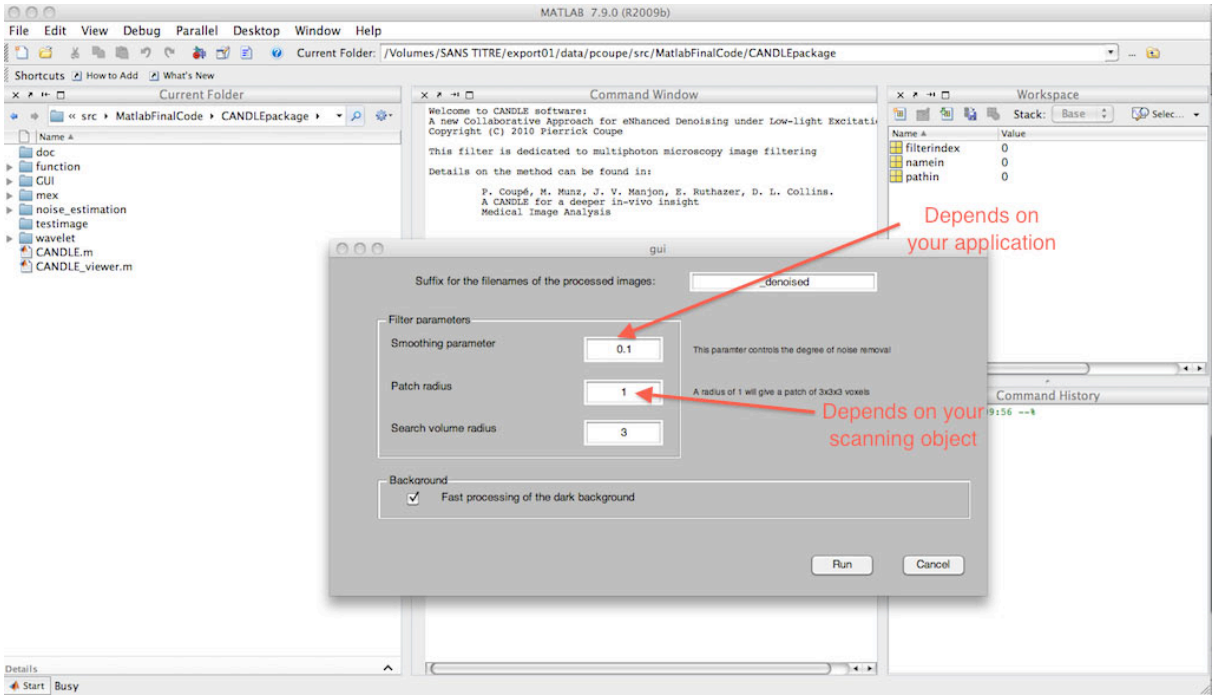


Moreover, you can try the example image (in the “testimage” direction) provided in the package in order to verify your architecture compatibility.

7. Use the GUI to select and tune the different filtering parameters. These parameters will be discussed further in the next section.

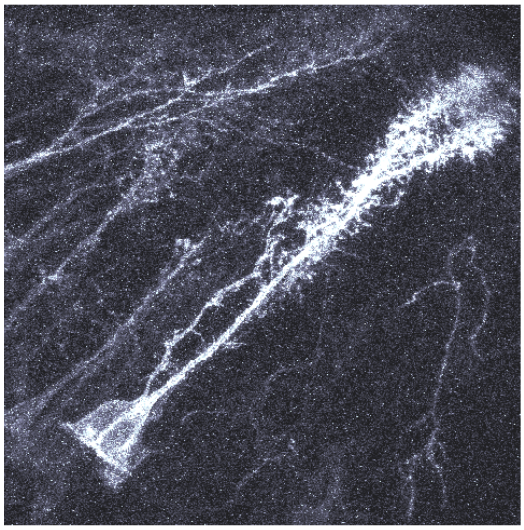
- First, select the suffix of the output file names. When you are processing several 3D images, each output file will have input name “myImage.tiff” with the chosen suffix (e.g., “myImage_denoised.tiff”).
- Second, select your smoothing parameter (beta). This parameter controls the amount of denoising that you want to apply. In practice, values between 0.1 and 0.4 are fine for visualization while higher values might be useful for segmentation or registration purposes.
- Third, select the patch radius. For large view of fine structures a radius of 1 voxel (i.e., patch of 3x3x3 voxels) is recommended while for limited field of view of large structures a radius of 2 voxels is preferred (i.e., patch of 5x5x5 voxels).
- The radius of the search volume is also a user-controllable option. However, we do not recommend increasing it, as it will produce much longer computational time with minimal denoising improvement.

- Finally, uncheck “fast processing of the dark background” if you want to carefully denoise the background. CANDLE assumes that the background is black and the object white during background extraction. Fast processing is a time-saving measure that minimizes computational effort in regions dominated by background. It should be used in cases where the object of interest is restricted to a subfield of an otherwise empty background.

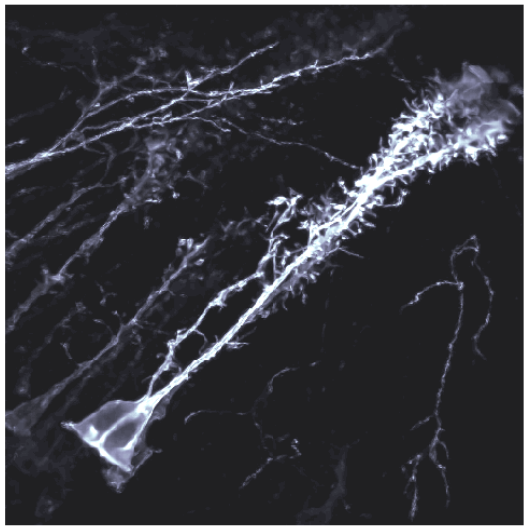


- Check the results on the created figures: one figure displaying the central slice for each sub-stack and one figure diplaying the MIP of the entire stack. The denoised.tiff file is automatically saved in the same directory as your input file.

MIP of Input image



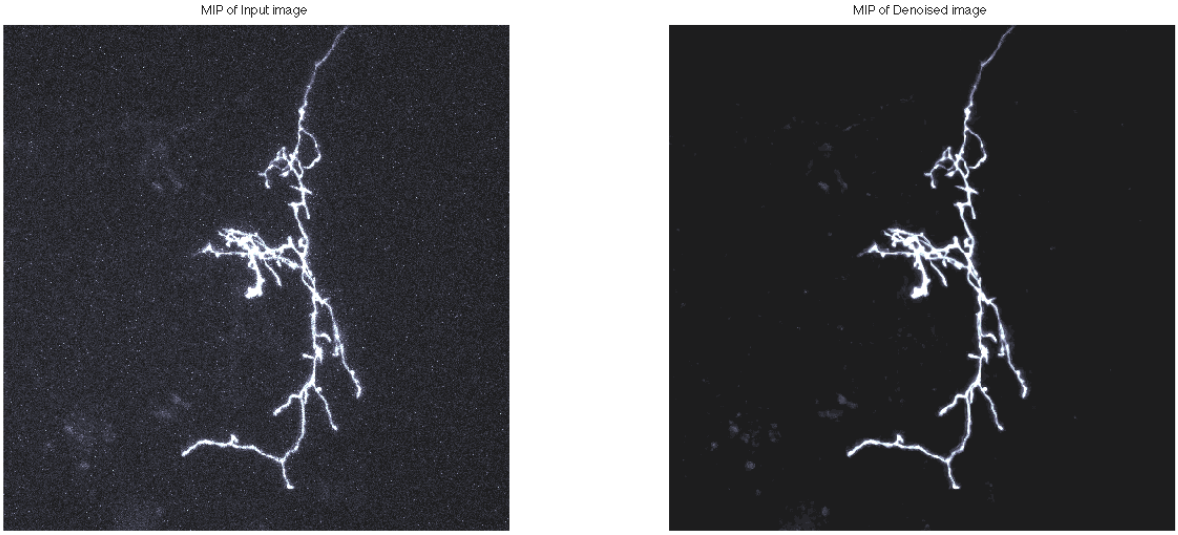
MIP of Denoised image



PARAMTER SETTING

The choice of filtering parameters can have a significant effect on denoising quality. In this section, we provide some examples of settings according to the image type as well as some intuitive rules to help tune parameters. However, your denoising step should be mainly driven by the application that you want to use: visualization, automatic segmentation…. So, adapt the following suggestions according to the context.

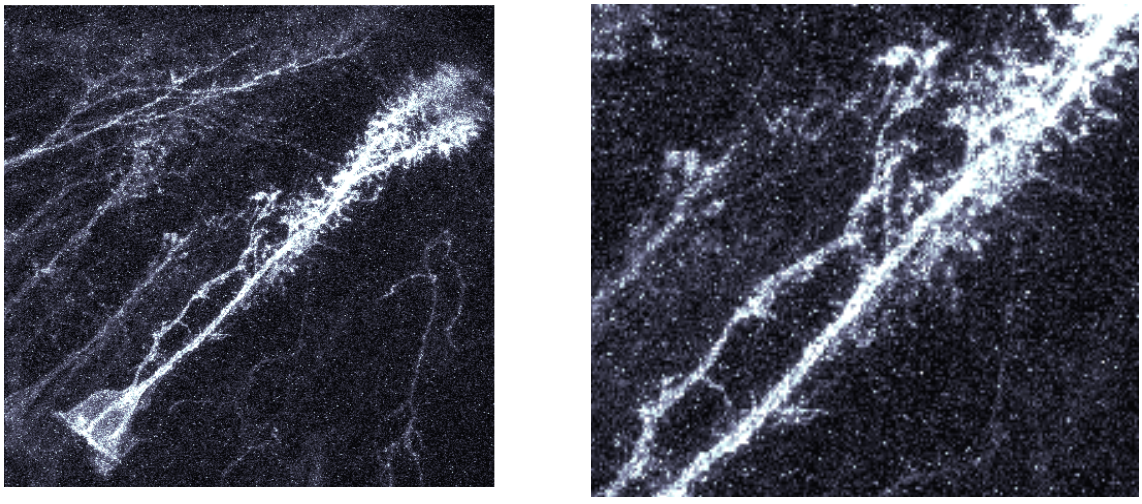
Global view of simple structure with low level of noise



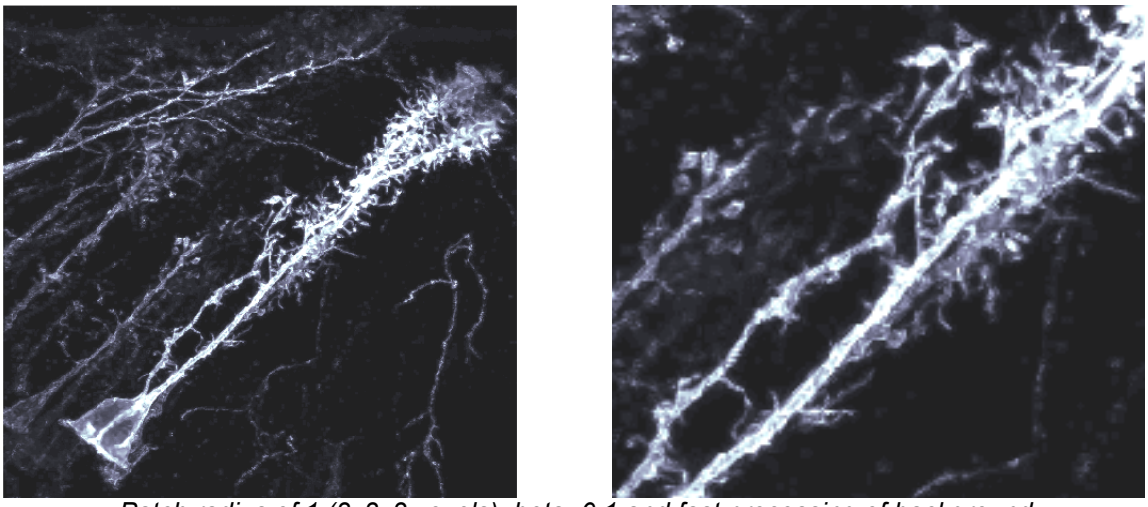
Patch radius of 1 (3x3x3 voxels), beta=0.1 and fast processing of background.

For this type of image, we suggest to use small patch size (3x3x3) adapted to finest structure and a low beta (smoothing parameter) value around 0.1 since noise is not really pronounced. Using small patch size enables to drastically reduce the computational time. Moreover, since no structure appears in background, fast background processing is recommended.

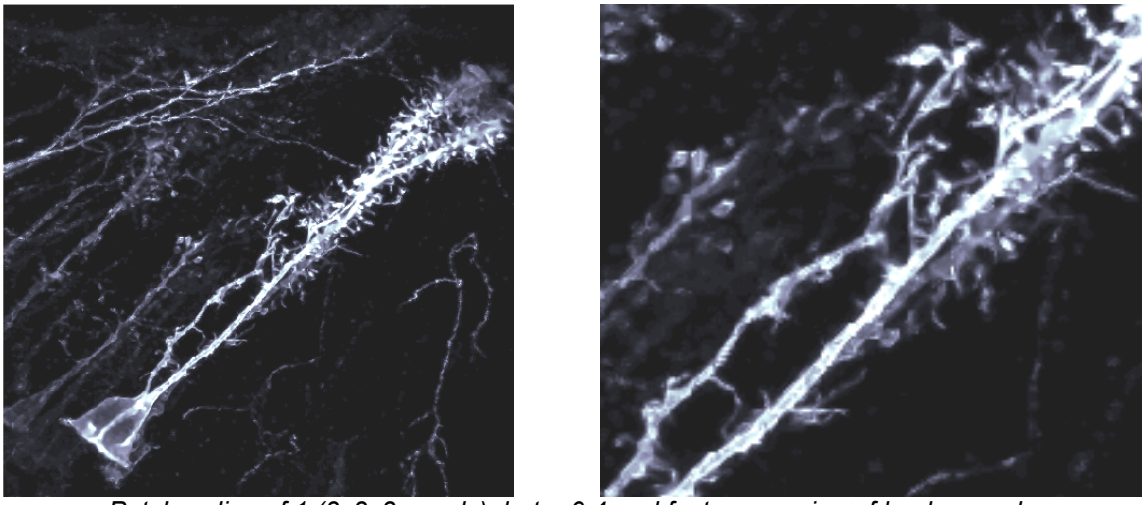
Global view of several structures with middle level of noise



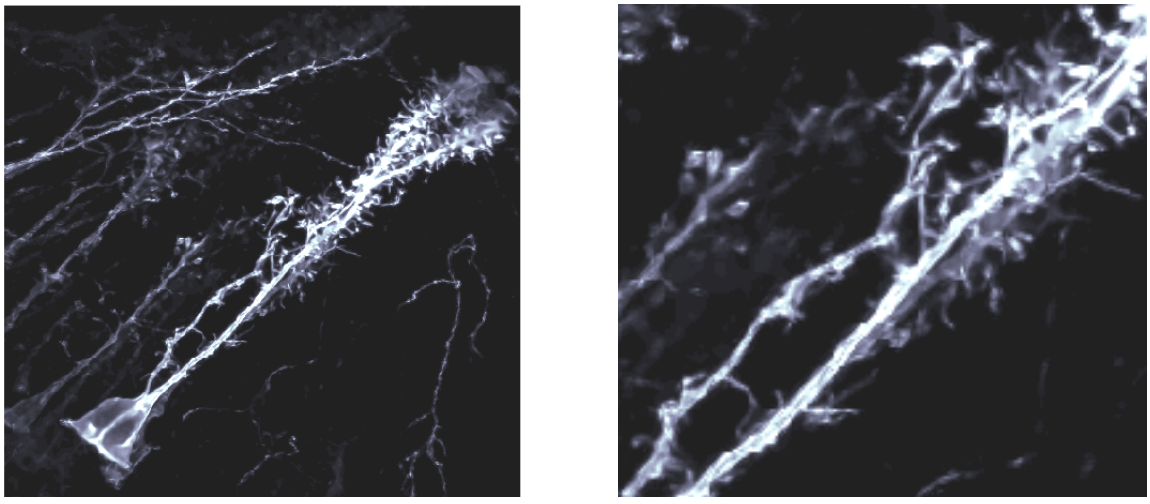
In case of more noisy images, the value of beta can be increased. In the following, we present several settings and we discuss about advantages and limitation of each of them.



Patch radius of 1 (3x3x3 voxels), beta=0.1 and fast processing of background.



Patch radius of 1 (3x3x3 voxels), beta=0.4 and fast processing of background.



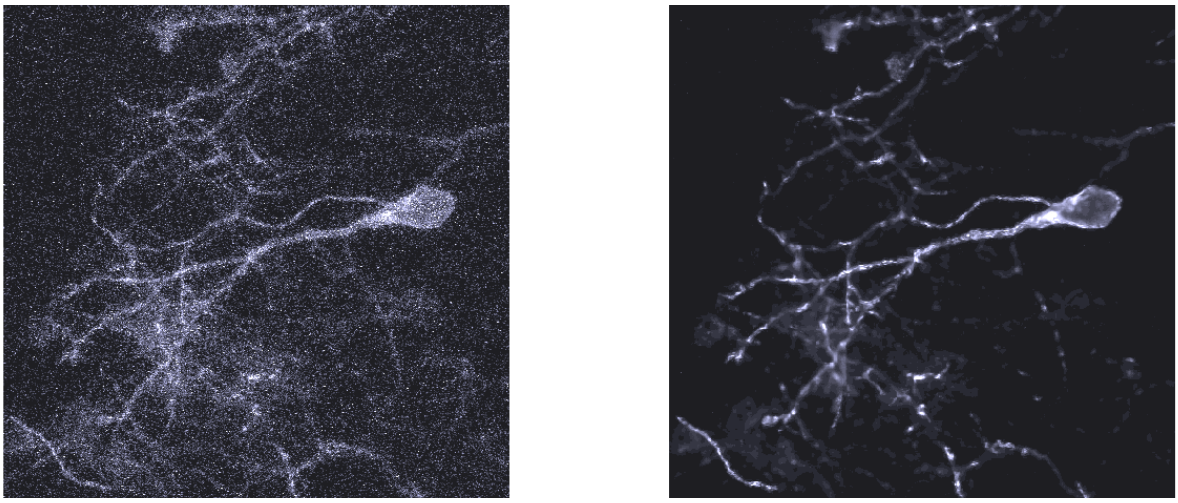
Patch radius of 2 (5x5x5 voxels), beta=0.4 and fast processing of background.

The first setting is based on small patch (3x3x3) and low beta value (0.1). By doing that, the denoising is really protective. All the small details are preserved. However, some remaining noise is visible and the visual continuity of the structures is not fully restored. This setting may be suitable for visual inspection of the smallest structures of the image. For automatic segmentation, this setting will not produce the best results.

The second setting is based on small patch (3x3x3) and middle beta value (0.4). By increasing beta, the remaining noise is less visible. The small patch radius preserves the finest details but does not fully restore the structure continuity.

The third setting is based on big patch (5x5x5) and middle beta value (0.4). By increasing beta, the remaining noise is more suppressed. The big patch radius is less effective at preserving the finest details but fully restores structure continuity. This setting is visually pleasant and can be used for automatic segmentation of the main structure.

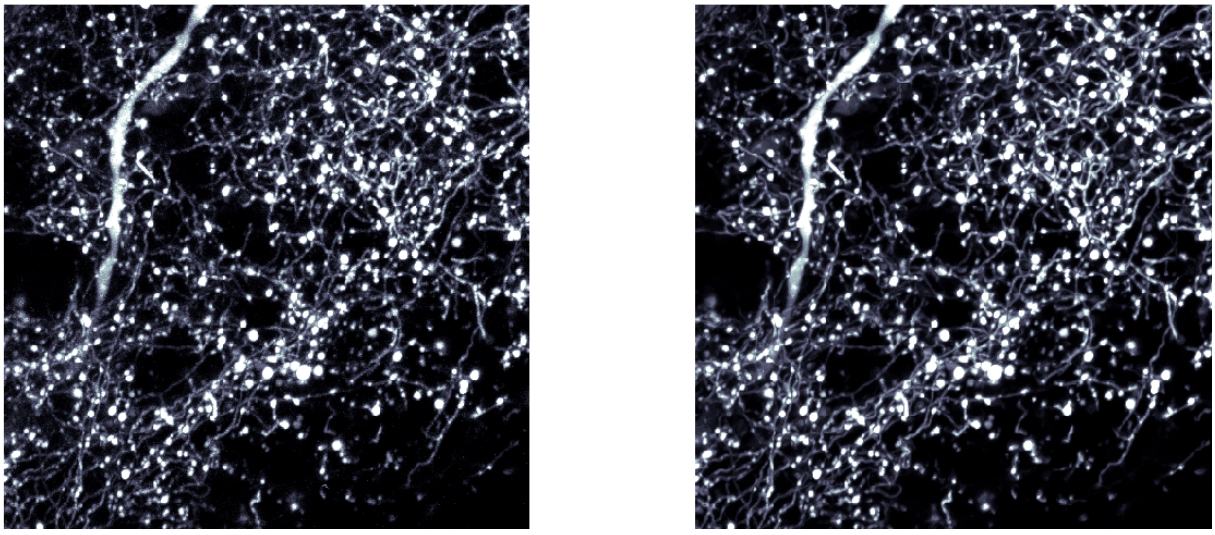
Restricted field of view with high level of noise



Patch radius of 2 (5x5x5 voxels), beta=0.4 and fast processing of background.

In this example, the relative size of structures compared to patch size is large, thus bigger patch sizes are recommended. Moreover, due to high levels of noise, beta=0.4 or even higher is suggested.

Textured image with no background and low level of noise



Patch radius of 1 (3x3x3 voxels), beta=0.1 and NO fast processing of background.

In this last example, there are a lot of small structures, thus we used a small patch size. Moreover, the level of noise is low thus beta=0.1 or lower is recommended. Finally, since there are a lot of details over the entire image, the option of fast processing of background should be deactivated.

SUPPORTED FILES

CANDLE is dedicated to 3D images in the form of a stack of .tiff images. Unfortunately, after processing some information added by the image acquisition software in the file header such as XY-resolution or power can be lost. To solve that you may be able to use ImageJ to edit file image properties based on the original image. In next version of Matlab© this problem should be fixed and thus CANDLE will be able to keep this information.

Multichannel dataset

We recommend splitting each channel into its own file using ImageJ before denoising images with CANDLE. After denoising of each channel separately, ImageJ can then be used to combine the channels.