Tutorial for AlgoRIM Interface

Example of use with our dataset

1. First of all, start the download of AlgoRIM. If this is your first time installation this operation may take several minutes. Don't forget to add a shorcut to your desktop.
2. On your computer, create a folder named 'AlgoRIM_Dataset' and a subfolder named 'RAW_DATA' in it, in order to be able to fill them with our dataset given as an example on this GitHub page.
3. Our dataset is on the 'data' folder at the top of the Github page.
4. Download PSF files in 'AlgoRIM_Dataset' folder and ROI1_SUM_image_43_800.tif in 'RAW_DATA' folder.
5. Once your installation is complete, open AlgoRIM interface.
6. *Images from:* Click on 'Other'.
7. *Input data:* Raw data folder is selected.
8. *Select:* Select 'RAW_DATA' folder.
9. *PSF emission:* Select PSF256_520nm.tif.
10. *PSF excitation:* Select PSF256_405nm.tif.
11. *Image expansion factor:* 2
12. *PSF expansion factor:* 1
13. *Regularization parameter:* 0
14. *Number of iterations:* 16
15. *Pre-filtering parameter:* 0.008. You can click on the 'Adjust' button to adjust it.
16. You can click on 'Launch preview' button. Use the 'STOP' button if you don't want to wait until the end of the iterations.
17. Click on 'Launch' button.
18. This will create a reconstruction folder in your 'RAW_DATA' folder, with the reconstructed image in it.

How to use AlgoRIM interface

1. Specify where your data comes from

Allow the interface to be able to identify and differentiate your channels.

- If you select **Other**: No prerequisite on the name of the channels but they will not be differentiated during reconstruction. You can create a subfolder for each channel and reconstructed them separately.
- **Inscoper**: channel 0 is identify with 'c0' in the image name and channel 1 with 'c1'
- **Abbelight**: the image name begins with 'ROI2' or 'ROI1', depending on the channel

2. Input data

Select the type of the input data folder

- **Raw data folder:** Launch reconstruction on one folder containning .tif files.
- **Global folder:** Launch several reconstructions in a row by selecting a folder containing several projects. It is based on Inscoper's raw image backup mode: global_folder/sub_folder/images/RAW_DATA/. If you want you launch preview, you need to select precisely the raw data folder you want to launch preview on.

3. Select folder

- This folder must contain one or more stack of n raw speckle.
- This folder must contain images in .tif format and square.

4. Adjust parameters

Over-sampling factor:

Raw images are usually recorded with a pixel-size about lambda/(4xNA), with lambda the wavelength of the emitted light and NA the numerical aperture of the objective. The super-resolved reconstruction should be discretized with a smaller pixel size. The over-sampling factor is the ratio between the pixel size of the raw images and the pixel size of the reconstructed (super-resolved) image, e.g., 2 will produce an output/reconstructed image with a pixel size that is half the native pixel size of the raw data.

Remark: the reconstruction code is based on Fast Fourier Transforms, which are faster if the number of pixels along any direction is a power of 2 (128, 256, 512, etc.). Of course this is not mandatory. Images must be square.

Collection PSF:

PSF of the objective with respect to the collected light (emitted by the fluorophores). This PSF can be estimated experimentally or generated theoretically with a free software like 'PSF generator'.

The PSF file should be provided in .tif format and the maximum of the PSF should be at the center of the image. Beware that this image should be provided with a pixel size corresponding to that of the super-resolved/reconstructed image.

Excitation PSF:

PSF of the objective with respect to the illlumination light. This PSF corresponds to the autocorrelation of the speckle; it can be generated with a free software like 'PSF generator'. Note that this PSF is not affected by any aberration.

The PSF file should be provided in .tif format and the maximum of the PSF should be at the center of the image. This image should be provided with a pixel size corresponding to that of the super-resolved/reconstructed image.

Number of iterations:

Number of iterative updates in the variance matching algorithm. The early stopping of the iterative scheme is producing a Tikhonov-like regularization of the reconstruction. You can adjust this setting with the preview mode showing the progression of the reconstructed image over the course of the algorithm.

Pre-filtering parameter:

Each raw image is deconvolved using a Wiener filtering. You can adjust this parameter with the adjust button in the preview mode.

5. Launch preview

This mode allows you to preview the reconstruction result on the first raw image. If you want to interrupt it, please use the stop button.

6. Launch reconstruction

This mode starts the reconstruction and saves your results in a folder named according to your settings.