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**SECTION MICROTECHNIQUE**

ÉCOLE POLYTECHNIQUE
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# 3D DECONVOLUTION IN DYNAMIC MICROSCOPY

## USER MANUAL

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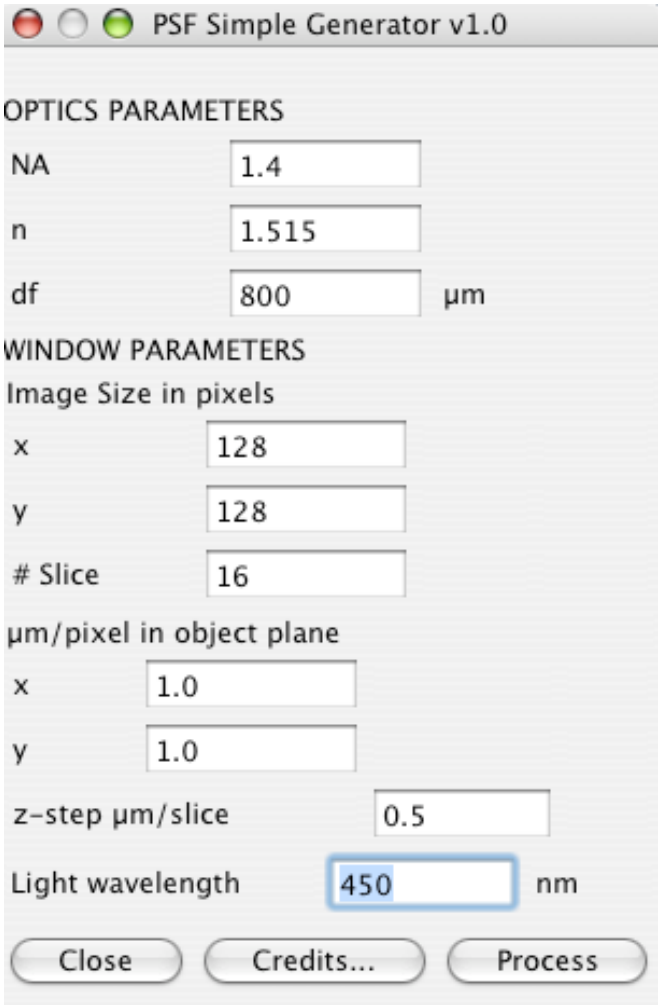
# I. INSTALLATION

ImageJ installation and the plugin installation procedure are described on http://bigwww.epfl.ch/demo/deconvolution3D/

# II. PSF GENERATOR

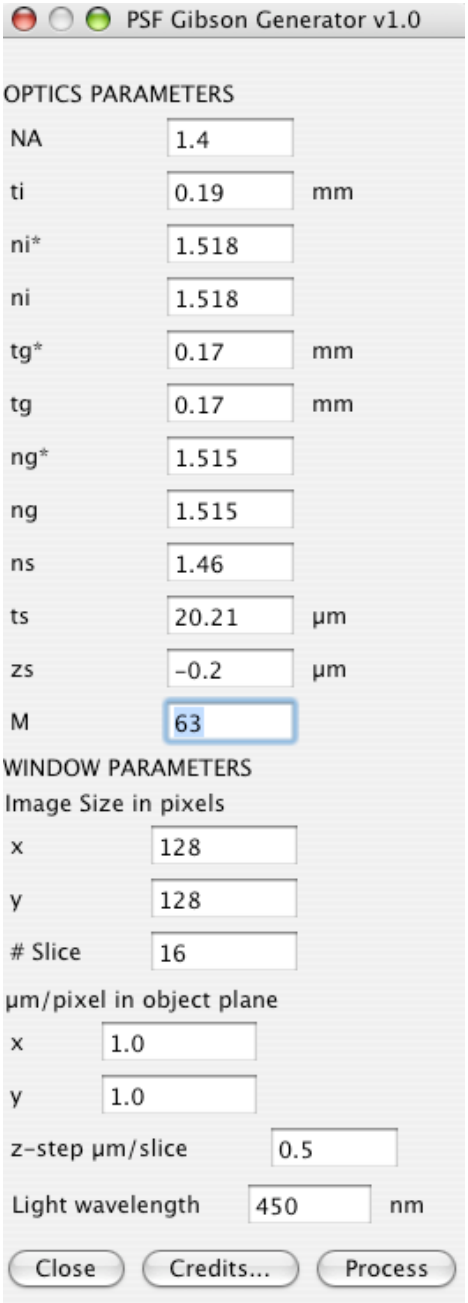
The plugins “PSF Simple” and “PSF Gibson” are used for simulating the behavior of a transmitted brightfield optical microscope. They may be launched from the plugin menu.

## *A. PSF Simple Model*




The simplified model does not take into account the non-stationarity of the PSF in the space. Thus, generated PSF has the focal plane as symmetry plane and optical axis as symmetry axis.
This model is to be used when the knowledge about the parameters is not precise, and uses only 4 parameters that are generally known.

## *B. PSF Gibson's Model*




Contrary to the simplified model, Gibson's model takes into account the non-stationarity of the PSF. The focal plane may not be a symmetry plane anymore.
Hence the PSF should be chosen in order to give optimum results at the middle of the stack.

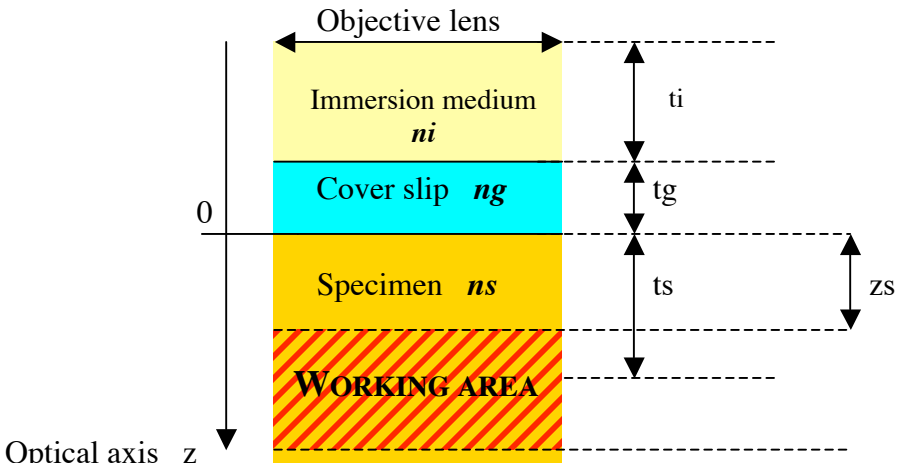
## *Parameters*

### *Optics parameters*

| Label | Detail | Model | |
|---|---|---|---|
| | | Simplified | Gibson |
| NA | Numerical aperture | X | X |
| n | Refractive index of immersion medium | X | |
| df | Focal length of objective lens | X | |
| ti | See schemes | | X |
| ni* | " | | X |
| ni | " | | X |
| tg* | " | | X |
| tg | " | | X |
| ng* | " | | X |
| ng | " | | X |
| ns | " | | X |
| ts | " | | X |
| zs | " | | X |
| M | Magnification | | X |

*Label:* Name in the "PSF" plugin.
*Detail:* Optical meaning of the label.
*Model:* A cross shows that this parameter is used for the model.

### *Scheme of Gibson's parameters*



| Parameters | Meaning |
|---|---|
| ti | Width of immersion index |
| ni | Refractive index of immersion index |
| tg | Width of cover slip |
| ng | Refractive index of cover slip |
| ts | Depth of the region of interest in the specimen |
| zs | Depth to which starts the recording (may be negative) |

Index with * are the designed (perfect) parameters while with no star means actual. Often it is considered that actual parameters are perfect.

*Window parameters*

| Parameters | | Meaning |
|---|---|---|
| Image Size in pixels | x | Dimensions x and y of the PSF in pixels |
| | y | |
| | z | Number of slices |
| $\mu$m/pixel in Object Plane | x | Size of a CCD sensor in the object plane (real size/M) |
| | y | |
| | z | Step between two consecutive images |
| Light wavelength | | Wavelength of the emitted light |

NOTE:

- The focal plane (or its closest plane) is at the top of the stack. The remainder is constructed cyclically.
- It is recommended to save the created PSF. Creating a PSF may be time consuming.

*Conditions on parameters*

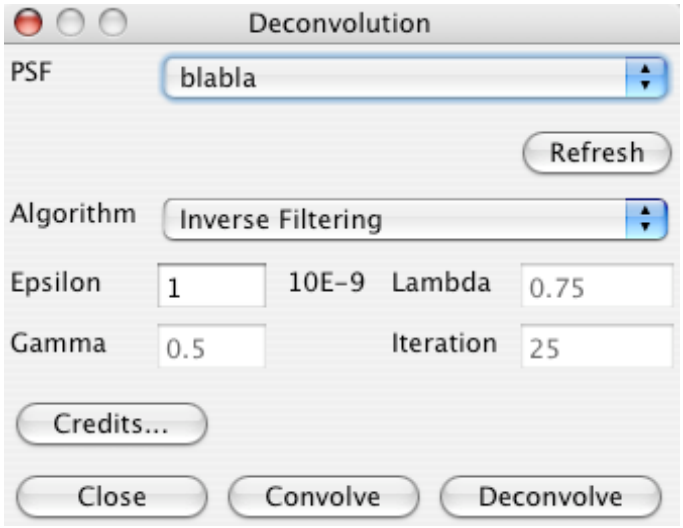
- Simplified model
  - x and y sizes of the image (in pixels) have to be dyadic numbers ($2^n$).
- Gibson's model
  - x and y sizes of the image (in pixels) have to be even numbers.

### *C. How to use*

Choose a model, then type your own parameters. Press “Process” to create the PSF, “Close” to quit or “Credits…” to see the credits.

# III. DECONVOLUTION PLUGIN

## *A. Screenshot*




## *B. How to use*

Once you have created your PSF:

1. Open the image to deconvolve or convolve,
2. Ensure that the generated PSF is selected as the PSF.
3. Choose an algorithm.
4. Enter the corresponding constant.
5. Press the desired action.

NOTE:

- Epsilon has to be small and is used to perform a stable division in the Fourier-Space. Default value is recommended.
- Lambda is used for Constraint Inverse Deconvolution. For noise free signal, lambda should be small (0.1). For a very noisy signal, lambda should be large (0.8).
- Gamma is used to ensure the convergence of Van Cittert's algorithm. Using a PSF generated above, the condition is: Gamma < 1/#slice. If you use a measured PSF, you should enter a lower Gamma (may be 0.8 / #Slice).

- Iteration is the number of iterations proceeds during Van Cittert's algorithm. Larger is #slice, larger should be Iteration in order to obtain good results.
- Click "Refresh" to check new images and actualize the list.
- THE X AND Y DIMENSIONS OF THE STACK TO BE DECONVOLVED MUST BE DYADIC!!!! ELSE THE ALGORITHMS DO NOT WORK. THIS IS DUE TO THE FAST FOURIER TRANSFORM. NOTE THAT IF THE NUMBER OF SLICE IS ALSO A DYADIC NUMBER THEN THE ALGORITHMS ARE MUCH FASTER.

### *C. Memory and time*

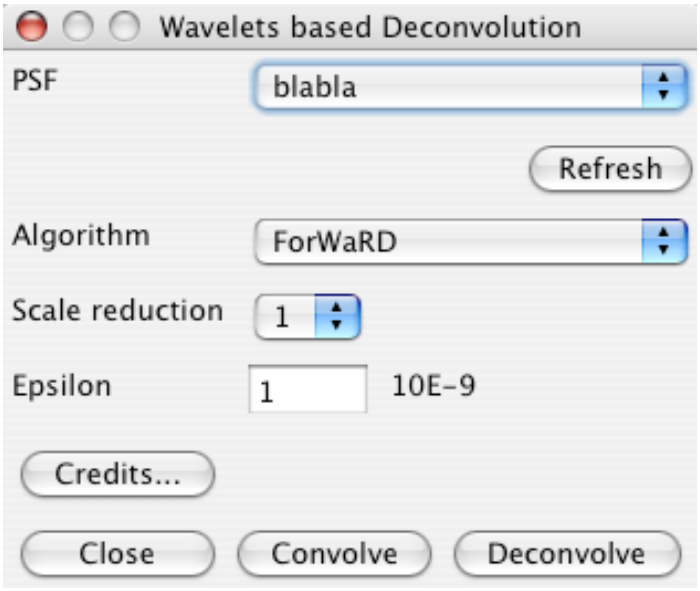
Depending on the size of the stack of image, the time and the memory needed for the process can be large.
To be able to run the plugin on large images, more memory should be given to ImageJ. Please set the parameter "Maximum Memory" (Edit -> Options -> Memory…) to at least 640 MB. With this setting, you are able to deal with images up to 64MB (512 x 512 pixels, 64 slices, 32 bits). Setting memory at 1.3 GB allows us to deal with much larger stacks (1024x1024x102 32 bits gray scale).

Operations may be very time consuming. For example, with an input stack (1024x1024x20  32 bits gray scale 80 MB):

| Algorithm | Time |
|---|---|
| Constraint Inverse | 55 min |
| Iterative deconvolution 40 iterations | 1 h 45 min |
| ForWaRD | 4 h 30 min |

# IV. ODELETTES PLUGIN

## *A. Screenshot*




## *B. How to use*

The principle was described in section III. B.
Scale reduction is the number of wavelet transform applied on the stack of image.
NOTE:

- For all the dimensions in pixels, make sure that dim / (scale reduction +1) is still integer.

  For example: input stack 10*10*16
  The highest Scale reduction that can be used is 1.

- Others notes are the same.