NIS-Elements™ Batch Deconvolution Tool

User's Guide

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1. Introduction

Batch Deconvolution Tool (BDT) is an application for performing multiple deconvolution computations in a batch. Deconvolving of multiple and large data-sets always takes very long. The basic idea of BDT is to have a simple and safe application which enables the user to create a job-list and, when the batch is run, to process all the jobs on background without any additional user interaction.

BDT provides a solid safety mechanism which guarantees that the jobs will resume even if some unexpected problem occurs (e.g. if some source images are corrupted or if the application will be forced to terminate suddenly - caused by a computer restart due to electricity blackout etc.).

The Application also provides an easy way to compare results of different deconvolution methods. Also, the user sees the input parameters of the deconvolution job for each deconvolved image.

Presence of the core scheduler and its status (running or stopped) is indicated via an icon in tray area of taskbar.

Note

BDT is available in both 32 and 64-bit version. However, it is strongly recommended to install the 64-bit version on a 64-bit operating system. The reason is that the amount of memory available for deconvolution is limited on 32-bit operating systems.

1.1. Deconvolution

The imperfection of a microscope optical path causes convolution of the original image signal. The convolution makes the resulting image blurry. Deconvolution is a mathematical algorithm for repairing the blurred parts of the image. Every optical path can be described by so called "point spread function (PSF)" which specifies how a single point of the image will look like when captured by a camera (after convolution). The PSF is defined by the following parameters:

- Numerical aperture of the objective
- Refractive Index of the immersion medium used
- Microscope modality
- Pinhole size (confocal microscopes)
- Objective magnification (confocal microscopes)
- Objective calibration
- Z-step
- Spherical aberration
- Emission wavelength

The better we know the PSF parameters, the better the de-convolution results will be. There are the following deconvolution methods available in the deconvolution module all of which can be applied to either 2D or 3D (including Z-dimension) image documents:

1.1.1. Deconvolution (Standard)

It is assumed that the input PSF parameters are known accurately. Stable, widely used and well known Lucy-Richardson algorithm is used in this method. The problem which we try to solve can be described by the following equation:

C = A * B +N

We know the captured image (C) and the PSF (B). We look for the true image (A) and we know there it is some additional noise (N) present. The **[*]** operator stands for convolution. So the equation can be rewritten as:

$$C(x,y) = (\sum_{a=-\inf}^{\inf} \sum_{b=-\inf}^{\inf} A(a-x, b-y)B(a,b)) + N(x,y)$$

Lucy-Richarson (LR) algorithm belongs to the class of so-called Expectation Maximization(EM) algorithms where we assume the noise(N) has a Poisson distribution. We don't need any other characteristics of N. This algorithm estimates A from given B and C iteratively. The algorithm always converges to the result but not so quickly. Because of this, we need to let the algorithm do at least 10 iterations. The number of iterations can be set within the dialog window.

Resources

- http://www.adass.org/adass/proceedings/adass97/prukschm.html
- http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.55.3469

1.1.2. Blind Deconvolution

This method uses the accelerated "Iterative Blind Deconvolution (IBD)" algorithm, that is widely know in the academic world. It is called "blind" because PSF is not known exactly. However, at least rough estimation of the PSF parameters is needed. The algorithm improves the PSF/source image estimations iteratively during the deconvolution process. The problem can be described by the following equation

C = A * B +N

We know the captured image (C), and we look for the true image (A), PSF (B) and additional noise (N). The "*" operator stands for convolution. The algorithm then works like this:

- It takes the first estimation of B (B1 - from given parameters) and a spectral characteristic of noise (N1) computed from C and estimates the true image (A1).

    C = A1 * B1+ N1

- In the second step, it uses the C, A1, and N1 values to re-estimate the PSF(B2)

    C = A1 * B2+ N1

In other words this is a sequence of standard deconvolution operations where we reestimate - for odd steps: A_n from C, A_{n-1}; for even steps: A_n from C, B_n.

This re-estimations continue until the defined number of iterations is reached or until the estimated values stop changing. The quality of results of the blind deconvolution depends on the number of iterations. The determination of this number depends on visual inspection of the resulting image.

Resources

- http://www.opticsinfobase.org/abstract.cfm?URI=ol-13-7-547
- http://portal.acm.org/citation.cfm?id=1132228

1.1.3. Fast (Wiener) Deconvolution

It is assumed that the input PSF parameters are known accurately. Compared to the two previous iterative methods, this algorithm takes a fraction of the computing time. The Wiener filter gives us explicit solution of the following equation:

C = A * B + N

If we know spectral characteristic of original image(A) and additive noise(N). B means the PSF and C is observed image.

The result is reached by direct division in Fourier spectrum using the Wiener inverse filter that can be obtained as:

$$FBInv(u,v) = \frac{FB^*(u,v)}{|FB(u,v)|^2 + \frac{PS_N(u,v)}{PS_A(u,v)}}$$

where FBInv is a fourier transform of inverse filter, FB^* is a complex conjugate of fourier transform of PSF, FB is fourier transform of PSF, PS_N is power spectrum of noise, PS_A is power spectrum of original image (can be substituted by power spectrum of observed image).

When we obtain FBInv the reconstructed image can be obtained as follows:

A' = C*BInv

Resources

- http://en.wikipedia.org/wiki/Wiener_deconvolution

2. Installation and Startup

Installing the BDT tool

Note

BDT is available in both 32 and 64-bit version. However, it is strongly recommended to install the 64-bit version on a 64-bit operating system. The reason is that the amount of memory available for deconvolution is limited on 32-bit operating systems.

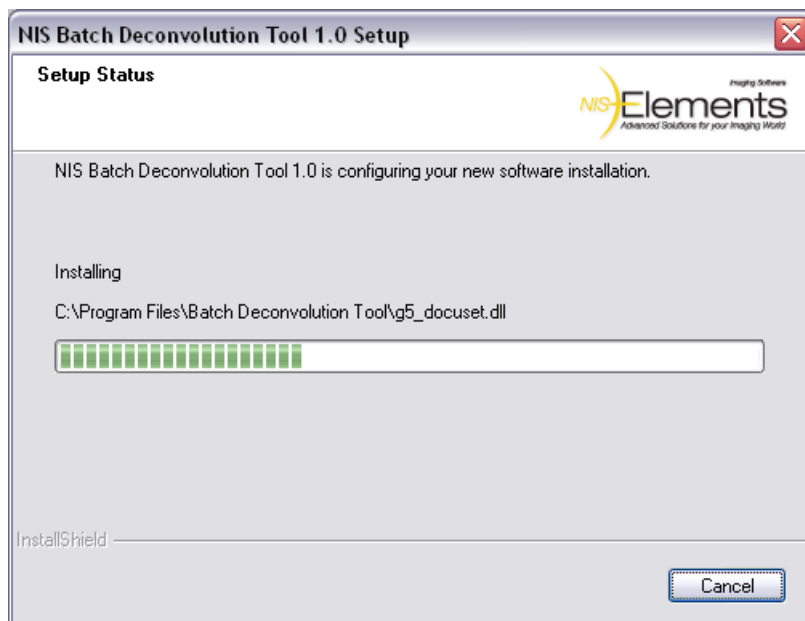| 1. Run the installation **[exe]** file. The following window appears:
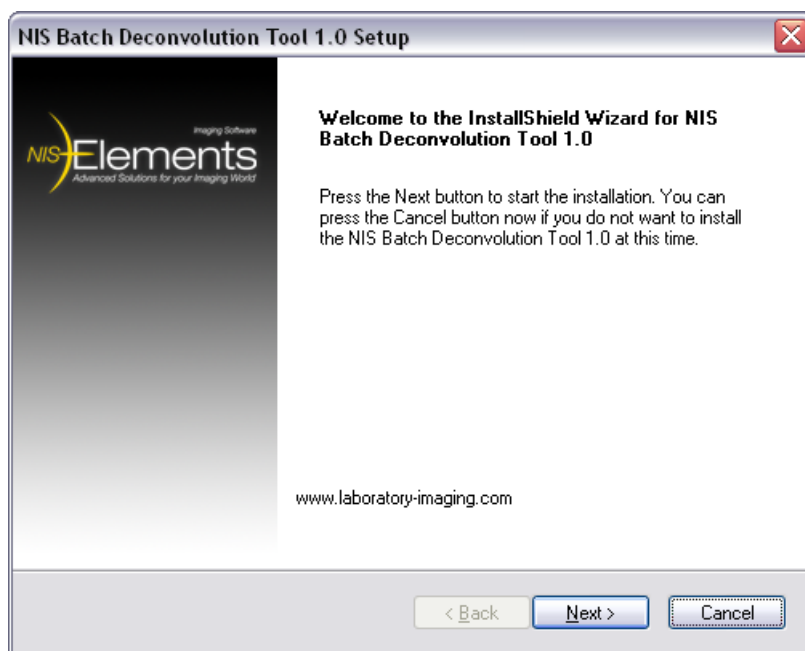
Click **[Next]**.

| **2.**   Select the destination folder where the application shall be installed. It is recommended to leave the default folder.
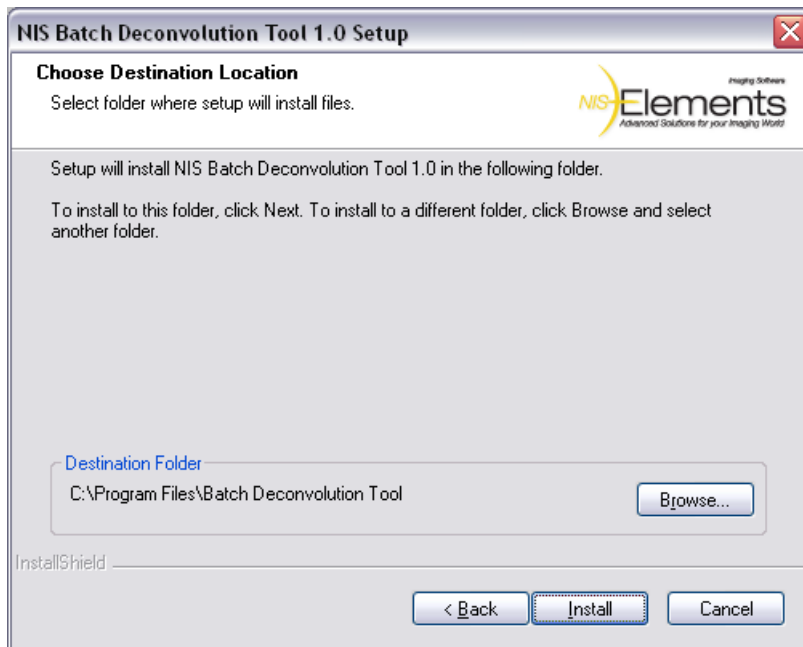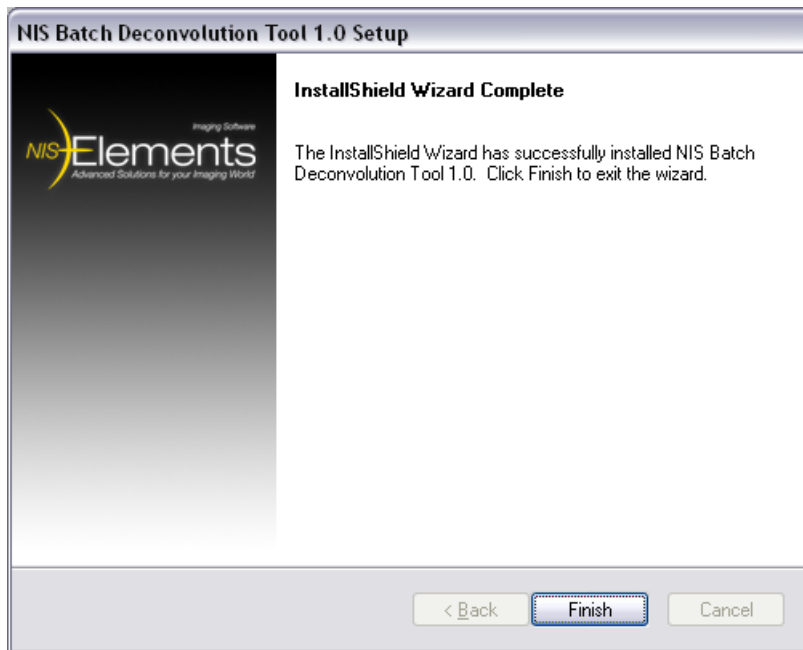

Click **[Install]**.

| **3.**   The files needed to run the application will be copied to the destination folder.
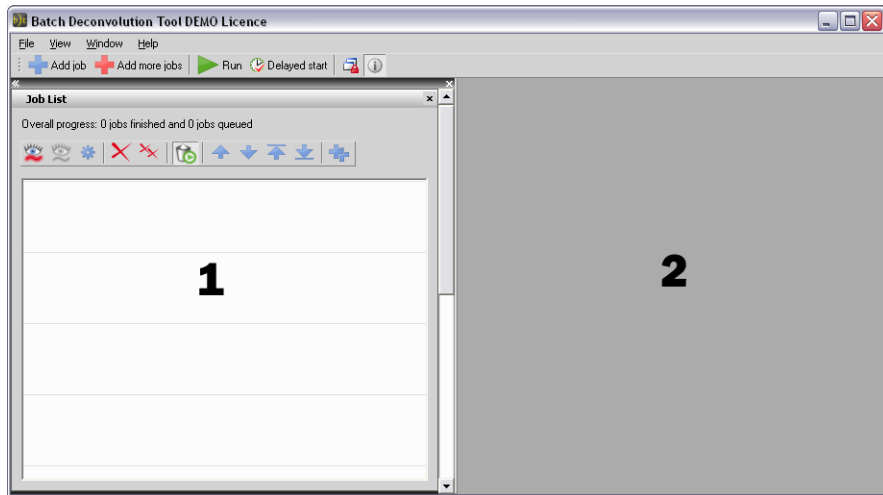

| **4.**   The installation process is finished. Click **[Finish]** to confirm it.

Starting BDT

| **1.** Run **[Start > Programs > NIS Batch Deconvolution Tool x.x > Batch Deconvolution Tool]**. Or - a quick way - double click the batch deconvolution tool tray icon.

| **2.**   The application window appears. It is composed of two main parts: the **[Job List]** control window, and the image area.
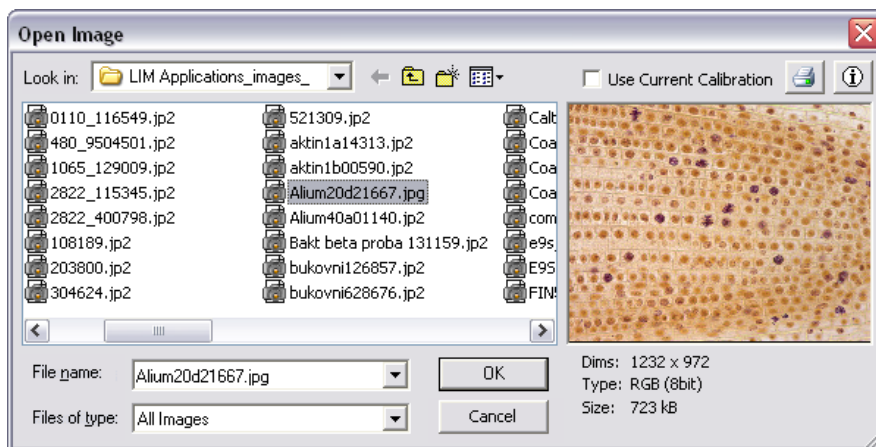

1. The job list control window
2. The image area

3. Scheduling Jobs

There are several ways of how to edit the job list.
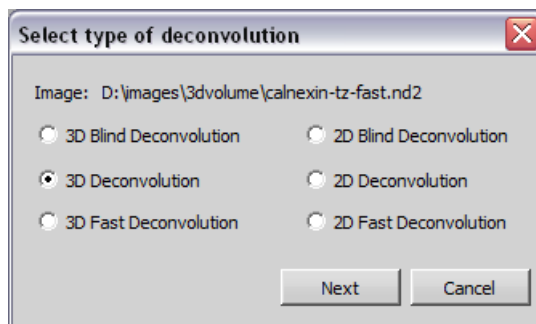
Adding job(s) to the job list

| **1.**   Click the **[Add job]** ➕ button. A standard **[Open]** window appears.

| **2.**   Select one of more files to be processed. One job will be created for each image. Click **[OK]**.

| **3.**   The following window appears where the deconvolution type type must be selected.

Select the deconvolution you would like to perform on the **[Image]** and click **[Next]**.

> ### Note
>
> *Some of the options may be disabled depending on the format of the image (containing Z-series or not) and on the licence coded in your HASP key.*

| **4.**   A window appears where you shall define the deconvolution options. See Deconvolution Settings. Confirm the settings by the **[OK]** button.

| **5.**   A new job will appear in the job list.

> ### Note
>
> *If you selected more than one file to be added to the job list, the described procedure will be repeated until the deconvolution options for every image are defined.*

Performing equal deconvolution on several files

If you would like to apply matching deconvolution setups on several files, it is very easy.

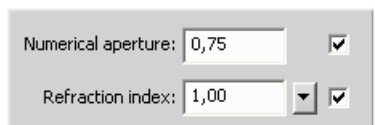| **1.**   Press the **[Add more jobs]** ✚ button. Select the files to be deconvolved from the **[Open Image]** window. Confirm it by **[OK]**.

> ### Note
>
> *If a single file is selected, just one deconvolution job will be added.*

| **2.**   Select the type of deconvolution to be performed. Click **[Next]**.

| **3.** Define the deconvolution settings. A check-box appears next to some deconvolution parameters:

De-select the check-box if you want to load the corresponding parameter from each document meta-data. When the box is left selected, the same value will be used for all the documents.

Note

Before de-selecting any of the check-box, make sure the meta-data are available in all the documents.

| **4.** As many jobs as how many files you selected will be added to the job list.

Deconvolution Settings

- [2D Blind Deconvolution Settings]
- [3D Blind Deconvolution Settings]
- [2D Deconvolution Settings]
- [3D Deconvolution Settings]
- [2D Fast Deconvolution Settings]
- [3D Fast Deconvolution Settings]

Job List Options

Select a job from the list and you can perform the following operations.

View original
Displays the original image (before deconvolution).

View deconvolved
Displays the image after deconvolution.

Deconvolution properties
Displays the deconvolution settings window. If you modify some of the settings of a finished job and press **[OK]** you will be prompt to restart the job with the new settings.

Remove single job
Removes the job from the list.

Remove all jobs
Clears the Job List.

Remove finished jobs after restart
When pressed, all finished jobs (finished successfully or failed) will be automatically removed from the job list.

Move up/down
Move the job up or down within the job list. The jobs will be processed from top one by one.

Move to top/bottom
Move the job to the top or to the bottom of the job list. The jobs will be processed from top one by one.

Duplicate job
This button opens the **[Deconvolution properties]** window and enables you to to adjust them. When you confirm the changes by **[OK]**, a new job will be created using the same file.

## Images Options

Overlay Info
When this button is pressed, job information is displayed over opened images involved in the job list.

Synchronization
It enables to compare (run and view) two or more ND2 documents. It automatically synchronizes the view of all ND2 documents opened within the image area.

4. Running the Batch

Once you have scheduled all the deconvolution jobs to be performed you can run the batch. The jobs will be processed from top. Finished jobs are highlighted by green color, the job being processed is highlighted purple and the jobs ready to be processed are left white.
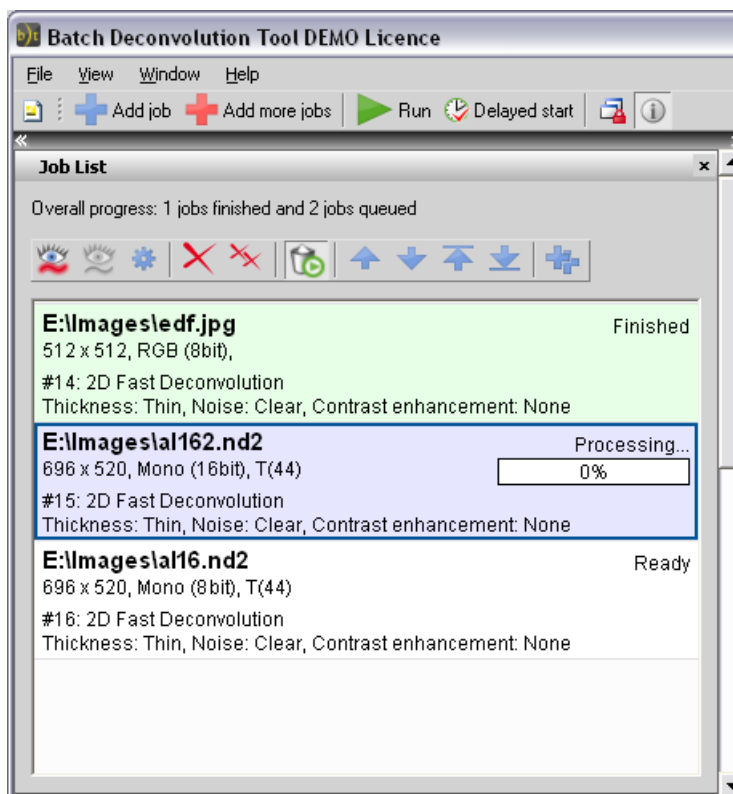
Immediate start

Press the **[Run]** ▶ button placed in the main toolbar.

Delayed start

Processing large image sequences uses the computer CPU to full capacity and consequently slows down other processing. The start of the job list can be postponed to any later time. Press the **[Auto startup settings]** button, the following window appears:
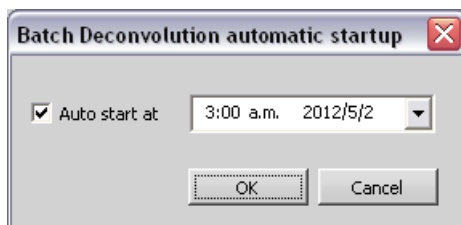
Check the box to engage the automatic (delayed) startup and edit the time and date the process shall be run. Confirm the settings by **[OK]**. A check-mark appears over the **[Auto startup settings]** button to indicate that the automatic start is planned.

5. Deconvolution Settings

5.1. 2D Blind Deconvolution Settings
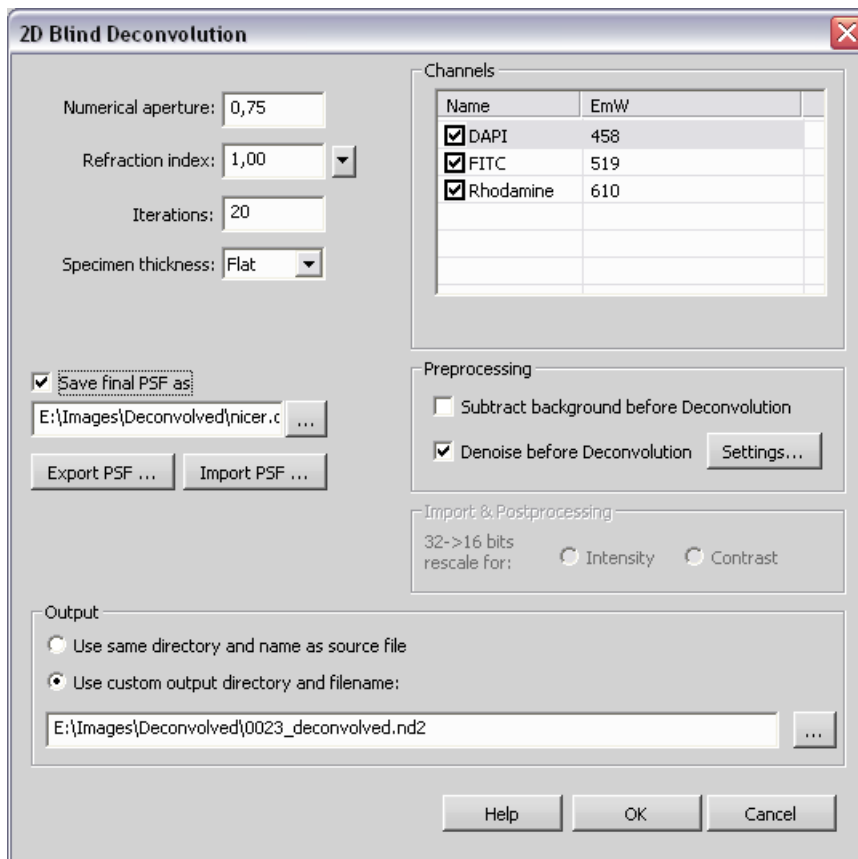
See also [Deconvolution].

Dialog Box Options

Numerical Aperture

Enter the numerical aperture of objective. In case document contains this information, press this ⊞ button and the

aperture is filled automatically.

Refraction Index

Enter the refraction index of an immersion medium. In case document contains this information, press this [icon] button and the refraction index is filled automatically. There are some predefined refraction indexes of different media in the pull down menu.

Iterations

Specify the number of iterations.

Specimen Thickness

Select the relevant specimen thickness: Flat, Thin, Normal, or Thick.

Save final PSF as

To save the final Point Spread Function (estimated by the algorithm),check this option. Define the location to which the function should be saved.

Export PSF

Enables to export image of the current Point Spread Function.

Import PSF

Enables to import the Point Spread Function.

Channels

Check all the channels in the list to activate the selected channels for deconvolution. You can edit the emission wavelength of channel.

Subtract background before deconvolution

If checked, the application estimates the average background value and subtracts it from the image before deconvolution

Denoise before Deconvolution

Click the **[Settings]** button to display the a new window where the following parameters can be adjusted.

Preserve Edges

Define which edges (how significant) to preserve. The options are **[Weak]**, **[Medium]**, **[Strong]** (distinctive).

Level of Details

Set how big the noise to be removed is (approximately). The range is from 1 to 8 pixels.

Denoising Power

Set the strength of the denoising. The higher the value, the stronger the resulting image will be affected. Select the values from 1 to 10.

Adjust the settings and click **[OK]** to confirm the changes. Select the check-box in order to engage the de-noising.

32 -> 16 bits rescale for:

32 bit values are used for computing the 3D-deconvolved image and also some file types (ICS) can be saved as 32-bit. Either when opening a 32-bit ICS image sequence or when a 3D deconvolution is finishing, the 32 bit data must be converted to 16bits in order to be displayed correctly. By this option you determine the way of the conversion:

- **[Intensity]** - preserves the mean intensity of the image

- **[Contrast]** - stretches the histogram in order to fit the 16bit range (similarly as a common auto-contrast)

Output

Select where to save the resulting (de-convolved) document. Select **[Use same directory as source file]** and the file will be saved to the directory of the original document using its name and a "-deconvolved" postfix. Or, select **[Use custom output directory and filename]**, and specify any destination path and filename.

5.2. 3D Blind Deconvolution Settings

See also [Deconvolution].
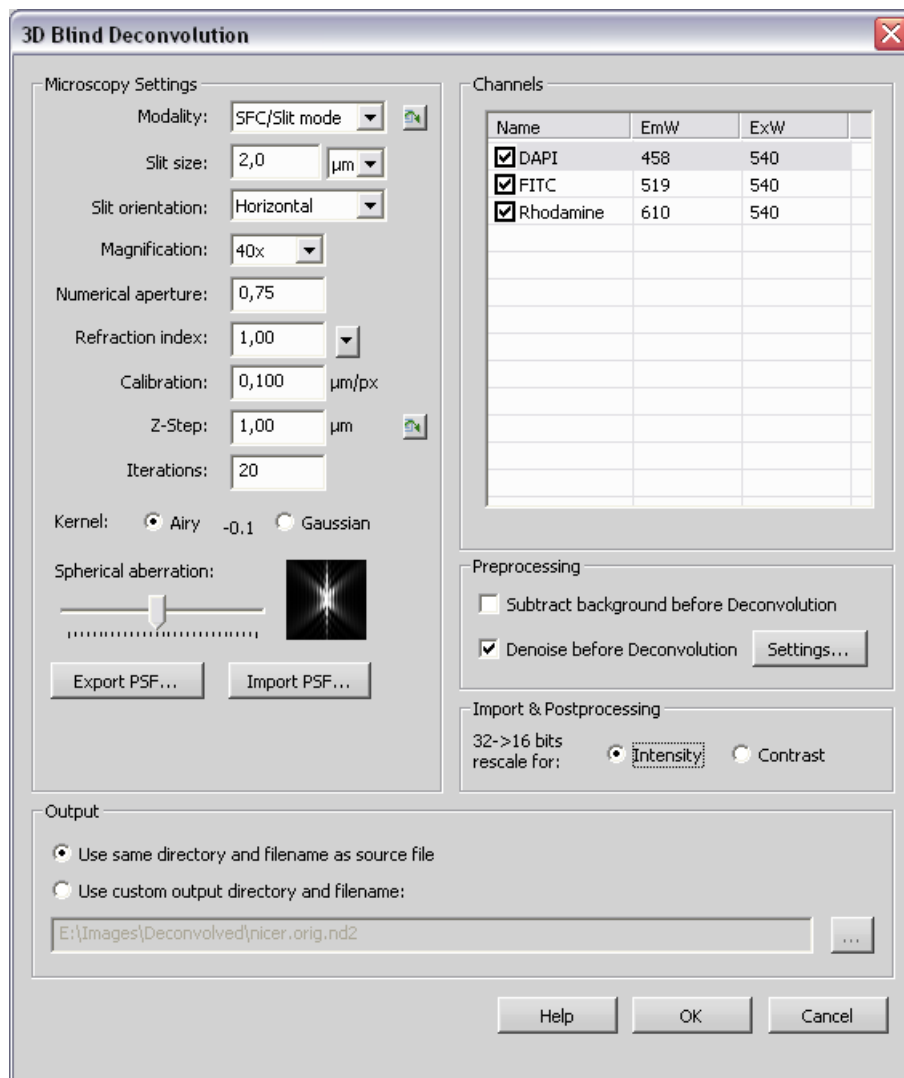
Dialog Box Options

Modality
Choose the deconvolution modality: Wide-field or Confocal. In case document contains this information, press this

🔲 button and the modality is chosen automatically.

Pinhole size
Set the pinhole size value and choose the proper units.

Magnification
Specify the objective magnification.

Numerical Aperture
Enter the numerical aperture of objective. In case document contains this information, press this 🔲 button and the aperture is filled automatically.

Refraction Index
Enter the refraction index of an immersion medium. In case document contains this information, press this 🔲 button and the refraction index is filled automatically. There are some predefined refraction indexes of different media in the pull down menu.

Calibration
Enter the calibration. In case document contains this information, press this 🔲 button and the calibration is filled automatically.

Z-Step
Set the step size of the sequence. In case document contains this information, press this 🔲 button and the Z-step value is chosen automatically.

Iterations
Specify the number of iterations.

Kernel
Opt for the kernel to be used. You should try which option will lead to the best results. The **[Gaussian]** option is mathematically simplified kernel.

Spherical aberration
Specify the spherical aberration.

Channels
Check all the channels in the list to activate the selected channels for deconvolution. You can edit the emission wavelength of channel.

Subtract background before deconvolution
If checked, the application estimates the average background value and subtracts it from the image before deconvolution

Denoise before Deconvolution
Click the **[Settings]** button to display the a new window where the following parameters can be adjusted.

Preserve Edges

Define which edges (how significant) to preserve. The options are **[Weak]**, **[Medium]**, **[Strong]** (distinctive).

Level of Details

Set how big the noise to be removed is (approximately). The range is from 1 to 8 pixels.

Denoising Power

Set the strength of the denoising. The higher the value, the stronger the resulting image will be affected. Select the values from 1 to 10.

Adjust the settings and click **[OK]** to confirm the changes. Select the check-box in order to engage the de-noising.

32 -> 16 bits rescale for:

32 bit values are used for computing the 3D-deconvolved image and also some file types (ICS) can be saved as 32-bit. Either when opening a 32-bit ICS image sequence or when a 3D deconvolution is finishing, the 32 bit data must be converted to 16bits in order to be displayed correctly. By this option you determine the way of the conversion:

- **[Intensity]** - preserves the mean intensity of the image

- **[Contrast]** - stretches the histogram in order to fit the 16bit range (similarly as a common auto-contrast)

Output

Select where to save the resulting (de-convolved) document. Select **[Use same directory as source file]** and the file will be saved to the directory of the original document using its name and a "-deconvolved" postfix. Or, select **[Use custom output directory and filename]**, and specify any destination path and filename.

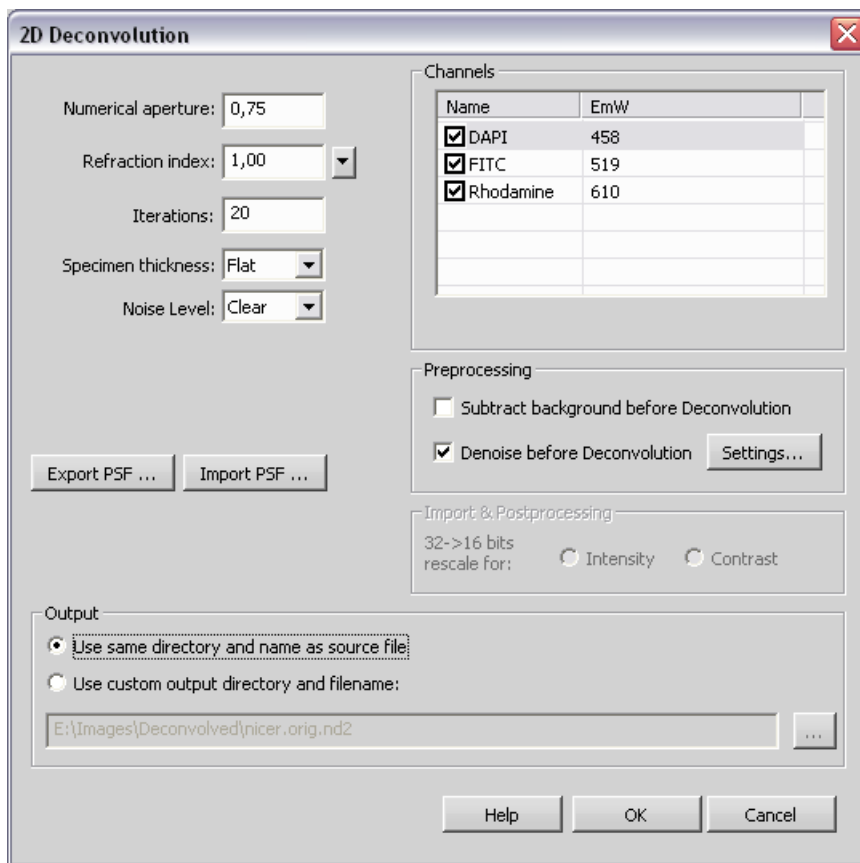## Export PSF

Enables to export image of the current Point Spread Function.

Import PSF

Enables to import the Point Spread Function.

5.3. 2D Deconvolution Settings

See also [Deconvolution].

Dialog Box Options

Numerical Aperture

Enter the numerical aperture of objective. In case document contains this information, press this button and the aperture is filled automatically.

Refraction Index

Enter the refraction index of an immersion medium. In case document contains this information, press this 🖼 button and the refraction index is filled automatically. There are some predefined refraction indexes of different media in the pull down menu.

Iterations

Specify the number of iterations.

Specimen Thickness

Select the relevant specimen thickness: Flat, Thin, Normal, or Thick.

Export PSF

Enables to export image of the current Point Spread Function.

Import PSF

Enables to import the Point Spread Function.

Channels

Check all the channels in the list to activate the selected channels for deconvolution. You can edit the emission wavelength of channel.

Subtract background before deconvolution

If checked, the application estimates the average background value and subtracts it from the image before deconvolution

Denoise before Deconvolution

Click the **[Settings]** button to display the a new window where the following parameters can be adjusted.

Preserve Edges

Define which edges (how significant) to preserve. The options are **[Weak]**, **[Medium]**, **[Strong]** (distinctive).

Level of Details

Set how big the noise to be removed is (approximately). The range is from 1 to 8 pixels.

Denoising Power

Set the strength of the denoising. The higher the value, the stronger the resulting image will be affected. Select the values from 1 to 10.

Adjust the settings and click **[OK]** to confirm the changes. Select the check-box in order to engage the de-noising.

32 -> 16 bits rescale for:

32 bit values are used for computing the 3D-deconvolved image and also some file types (ICS) can be saved as 32-bit. Either when opening a 32-bit ICS image sequence or when a 3D deconvolution is finishing, the 32 bit data must be converted to 16bits in order to be displayed correctly. By this option you determine the way of the conversion:

• **[Intensity]** - preserves the mean intensity of the image

• **[Contrast]** - stretches the histogram in order to fit the 16bit range (similarly as a common auto-contrast)

Output

Select where to save the resulting (de-convolved) document. Select **[Use same directory as source file]** and the file will be saved to the directory of the original document using its name and a "-deconvolved" postfix. Or, select **[Use custom output directory and filename]**, and specify any destination path and filename.

5.4. 3D Deconvolution Settings

See also [Deconvolution].

Dialog Box Options

Microscopy Settings

Modality
Choose the deconvolution modality: Wide-field or Confocal. In case document contains this information, press this ▦ button and the modality is chosen automatically.

Pinhole size
Set the pinhole size value and choose the proper units.

Magnification
Specify the objective magnification.

Numerical Aperture
Enter the numerical aperture of objective. In case document contains this information, press this ▦ button and the aperture is filled automatically.

Refraction Index
Enter the refraction index of an immersion medium. In case document contains this information, press this ▦ button and the refraction index is filled automatically. There are some predefined refraction indexes of different media in the pull down menu.

Calibration
Enter the calibration. In case document contains this information, press this ▦ button and the calibration is filled automatically.

Z-Step
Set the step size of the sequence. In case document contains this information, press this ▦ button and the Z-step value is chosen automatically.

Iterations
Specify the number of iterations.

Kernel
Opt for the kernel to be used. You should try which option will lead to the best results. The **[Gaussian]** option is mathematically simplified kernel.

Spherical aberrtion
Specify the spherical aberration.

Export PSF
Enables to export image of the current Point Spread Function.

Import PSF
Enables to import the Point Spread Function.

Channels
Check all the channels in the list to activate the selected channels for deconvolution. You can edit the emission wavelength of channel.

Subtract background before deconvolution
If checked, the application estimates the average background value and subtracts it from the image before deconvolution

Denoise before Deconvolution

Click the **[Settings]** button to display the a new window where the following parameters can be adjusted.

Preserve Edges

Define which edges (how significant) to preserve. The options are **[Weak]**, **[Medium]**, **[Strong]** (distinctive).

Level of Details

Set how big the noise to be removed is (approximately). The range is from 1 to 8 pixels.

Denoising Power

Set the strength of the denoising. The higher the value, the stronger the resulting image will be affected. Select the values from 1 to 10.

Adjust the settings and click **[OK]** to confirm the changes. Select the check-box in order to engage the de-noising.

32 -> 16 bits rescale for:

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Output

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32 -> 16 bits rescale for:

32 bit values are used for computing the deconvolved image. When finished, the 32 bit result must be converted back to 16bits in order to display it correctly. By this option you determine the way:

- **[Intensity]** - preserves the mean intensity of the image
- **[Contrast]** - stretches the histogram in order to fit the 16bit range (similarly as a common auto-contrast)

5.5. 2D Fast Deconvolution Settings

See also [Deconvolution].
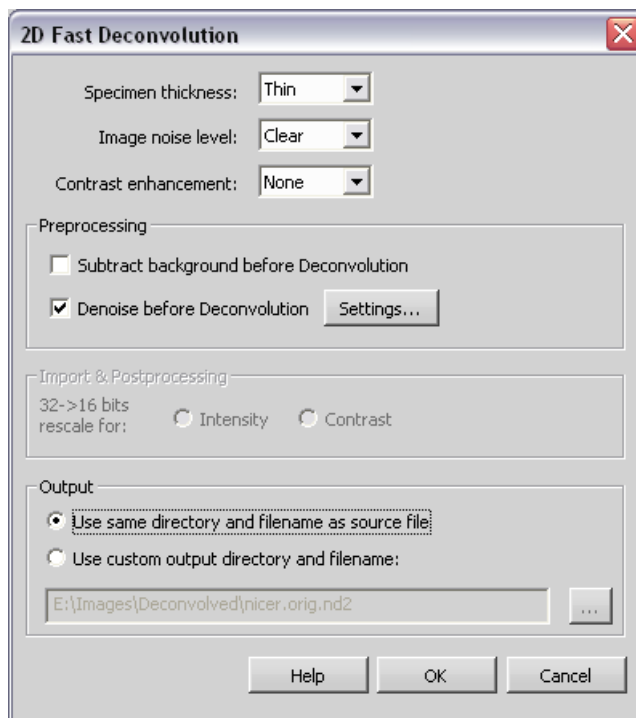
Dialog Box Options

Specimen Thickness
Select the relevant specimen thickness: Flat, Thin, Normal, or Thick.

Image Noise Level
Specify the amount of noise present in the image: Clear, Normal, Noisy

Contrast enhancement
Choose the contrast enhancement strength: None, Low, Medium, Strong.

Subtract background before deconvolution
If checked, the application estimates the average background value and subtracts it from the image before deconvolution

Denoise before Deconvolution

Click the **[Settings]** button to display the a new window where the following parameters can be adjusted.

Preserve Edges

Define which edges (how significant) to preserve. The options are **[Weak]**, **[Medium]**, **[Strong]** (distinctive).

Level of Details

Set how big the noise to be removed is (approximately). The range is from 1 to 8 pixels.

Denoising Power

Set the strength of the denoising. The higher the value, the stronger the resulting image will be affected. Select the values from 1 to 10.

Adjust the settings and click **[OK]** to confirm the changes. Select the check-box in order to engage the de-noising.

32 -> 16 bits rescale for:

32 bit values are used for computing the 3D-deconvolved image and also some file types (ICS) can be saved as 32-bit. Either when opening a 32-bit ICS image sequence or when a 3D deconvolution is finishing, the 32 bit data must be converted to 16bits in order to be displayed correctly. By this option you determine the way of the conversion:
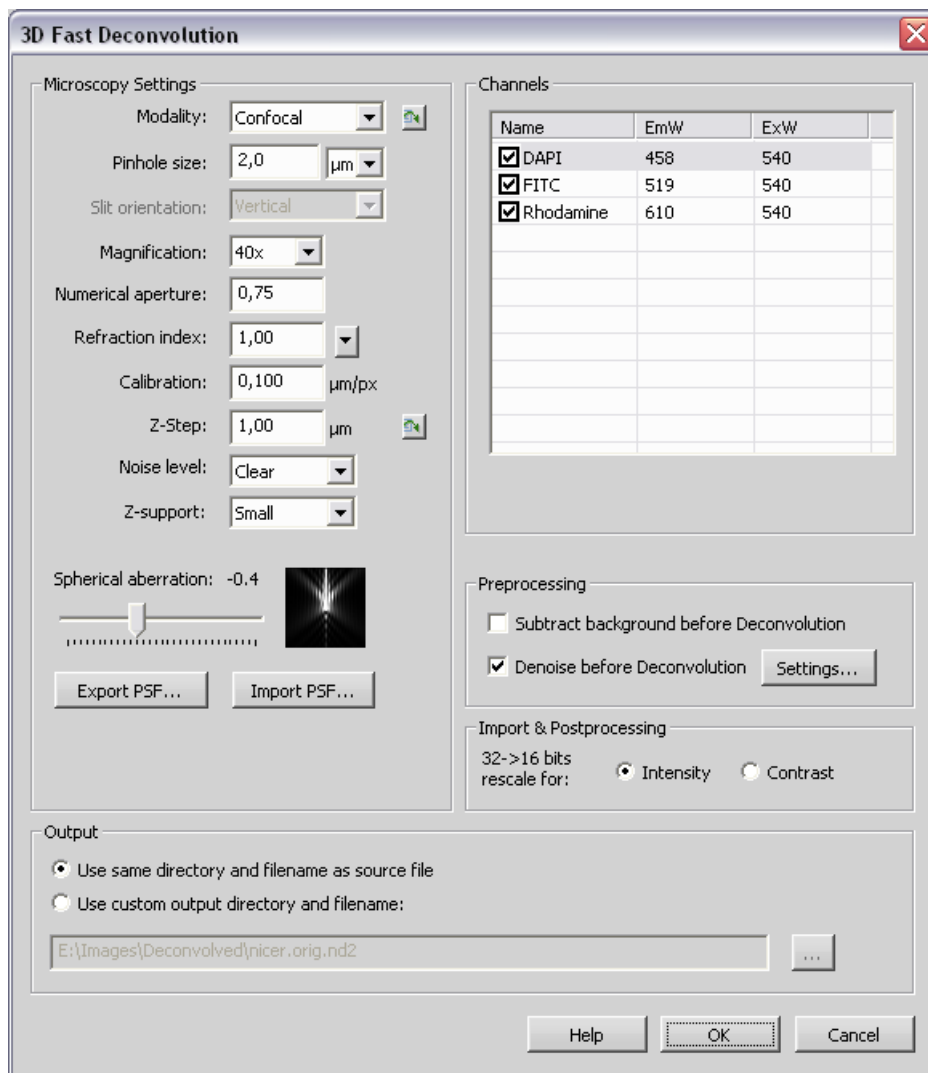
- **[Intensity]** - preserves the mean intensity of the image

- **[Contrast]** - stretches the histogram in order to fit the 16bit range (similarly as a common auto-contrast)

Output

Select where to save the resulting (de-convolved) document. Select **[Use same directory as source file]** and the file will be saved to the directory of the original document using its name and a "-deconvolved" postfix. Or, select **[Use custom output directory and filename]**, and specify any destination path and filename.

5.6. 3D Fast Deconvolution Settings

See also [Deconvolution].

Dialog Box Options

Microscopy Settings

Modality

Choose the deconvolution modality: Wide-field or Confocal. In case document contains this information, press

this button and the modality is chosen automatically.

Pinhole size

Set the pinhole size value and choose the proper units.

Magnification

Specify the objective magnification.

Numerical Aperture

Enter the numerical aperture of objective. In case document contains this information, press this button and the aperture is filled automatically.

Refraction Index

Enter the refraction index of an immersion medium. In case document contains this information, press this button and the refraction index is filled automatically. There are some predefined refraction indexes of different media in the pull down menu.

Calibration

Enter the calibration. In case document contains this information, press this button and the calibration is filled automatically.

Z-Step

Set the step size of the sequence. In case document contains this information, press this button and the Z-step value is chosen automatically.

Noise Level

Specify the amount of noise present in the image: Clear, Normal, Noisy

Z-support

Select the range of Z-series which will be used for calculation of the Point Spread Function. The options are: Small, Medium, Large.

> **Note**
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> *From a definition of a geometrical optics the support of the PSF is infinite. It means that even the infinitely distant z-plane would have influence on the focused z-plane image. However, this does not work in reality. Deconvolution using PSF with infinite support gives us bad results in 3D Fast Deconvolution. Select [small] to tell the algorithm that only close z-planes have influence on the resulting image. If you select [large] you say that even distant z-planes influence the resulting image.*

Spherical aberration

Specify the spherical aberration.

Export PSF

Enables to export image of the current Point Spread Function.

Import PSF

Enables to import the Point Spread Function.

Channels

Check all the channels in the list to activate the selected channels for deconvolution. You can edit the emission wavelength of channel.

Subtract background before deconvolution

If checked, the application estimates the average background value and subtracts it from the image before deconvolution

Denoise before Deconvolution

Click the **[Settings]** button to display the a new window where the following parameters can be adjusted.

Preserve Edges

Define which edges (how significant) to preserve. The options are **[Weak]**, **[Medium]**, **[Strong]** (distinctive).

Level of Details

Set how big the noise to be removed is (approximately). The range is from 1 to 8 pixels.

Denoising Power

Set the strength of the denoising. The higher the value, the stronger the resulting image will be affected. Select the values from 1 to 10.

Adjust the settings and click **[OK]** to confirm the changes. Select the check-box in order to engage the de-noising.

32 -> 16 bits rescale for:

32 bit values are used for computing the 3D-deconvolved image and also some file types (ICS) can be saved as 32-bit. Either when opening a 32-bit ICS image sequence or when a 3D deconvolution is finishing, the 32 bit data must be converted to 16bits in order to be displayed correctly. By this option you determine the way of the conversion:

- **[Intensity]** - preserves the mean intensity of the image

- **[Contrast]** - stretches the histogram in order to fit the 16bit range (similarly as a common auto-contrast)

Output

Select where to save the resulting (de-convolved) document. Select **[Use same directory as source file]** and the file will be saved to the directory of the original document using its name and a "-deconvolved" postfix. Or, select **[Use custom output directory and filename]**, and specify any destination path and filename.

When all the settings are properly set, press the **[Deconvolve]** button to start deconvolution.