

NIS-Elements Offline Deconvolution

Manual

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1. About the Application

1.1. Offline Deconvolution Concept

Offline Deconvolution (OD) is an application for performing multiple deconvolution computations in a batch. Deconvolving of multiple and large data-sets always takes very long time. The basic idea of OD 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.

OD 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 the tray area of the application taskbar.

Note

OD produces floating point images.

1.2. About LIM Deconvolution Module

1.2.1. Introduction

Lets answer couple of basic questions before we start using the Deconvolution module:

Q: What is deconvolution for?

A: Every microscope optical path (or optics in general) makes the resulting images blurry. Deconvolution is for de-blurring images captured by a digital camera. With NIS-Elements it can be applied to 2D images or 3D volume data.

Q: Which deconvolution method is best for my image sample?

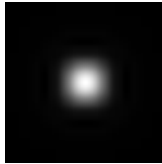
A: Each method of the deconvolution menu is optimized for different purposes. To choose the right method you have to know especially:

- Does the image contain 3D volume data (Z stack) or is it a standard 2D image?
- Do I need a high-quality result?
- Do I know the exact parameters of the optical path - do I know the "point spread function" (PSF)?

Please see: **[1.2.2 Choosing the Deconvolution Method]**.

Q: What is PSF?

A: Every optical path can be described by a so called “point spread function (PSF)” which specifies how a single point source will look like when captured by a camera. The PSF for each channel is usually represented by a grayscale image. A 2D PSF can look like this:



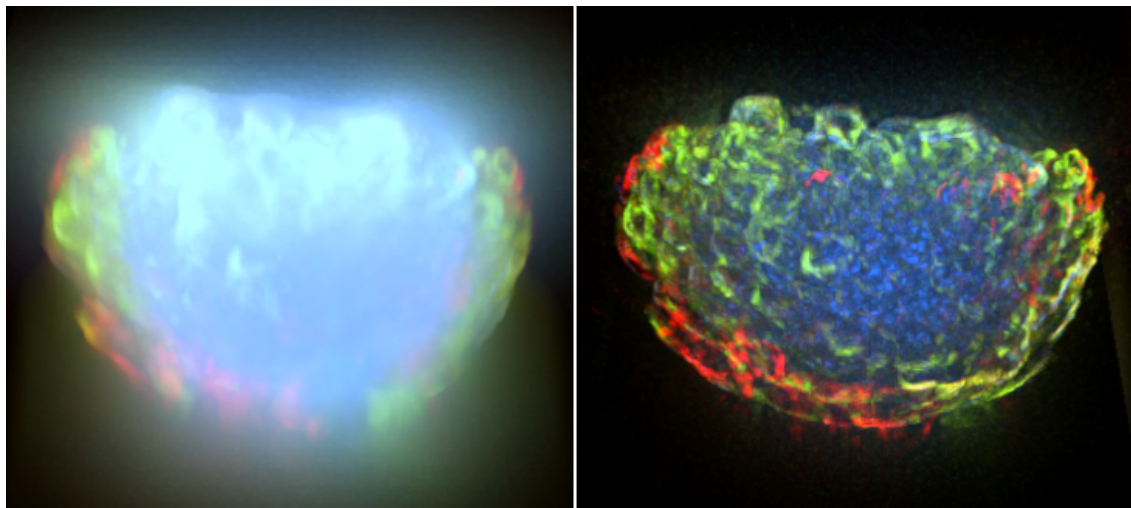
Please see: **[1.2.4 Determining the Point Spread Function (PSF)].**

Q: Can I run the deconvolution module on my PC?

A: It is important to realize that deconvolution methods demand appropriate computer performance. It is required to have a workstation equipped with a 64bit operating system and at least 4GB of RAM, the faster CPU, the better. The need of RAM depends on the size of the processed files, 8GB is a standard, 16GB highly recommended.

1.2.2. Choosing the Deconvolution Method

3D volume data before and after deconvolution



There are different ways to sort deconvolution methods, either by the image data to be processed (2D/3D) or by the method of PSF determination (blind, non-blind), or by expected quality of the result (fast). There are several commands available within the [Deconvolution] pull-down menu. Read the following overview and decide which command will best suit your requirements:

Deconvolution Commands

3D Deconvolution

This command is designed to work strictly with volume data-sets i.e. ND2 files containing Z-stack.

2D Deconvolution

Standard 2D single-frame images or ND2-files (timelapse, multipoint, Z-stack...) can be processed by this command.

Note

Frame-by-frame deconvolution is performed in this case. Z-stack ND2 file can be processed by this deconvolution method as well, but the result will not be as good as you get from the 3D method.

2D Fast Deconvolution

Standard 2D single-frame images or ND2-files (timelapse, multipoint, Z-stack...) where fast performance of the system is preferred to quality should be processed by this command, otherwise, use the **[2D Deconvolution]** command.

Note

Frame-by-frame deconvolution is performed in this case. Z-stack ND2 file can be processed by this deconvolution method as well, but the result will not be as good as you get from the standard 2D or 3D method.

Deconvolution methods

It is possible to choose between the following deconvolution methods. Each method uses a different algorithm to achieve the same goal (see **[1.2.3 Algorithms]**).

IBD - Iterative Blind

This deconvolution method estimates the shape of the PSF from the parameters specified within the deconvolution dialog window. However, if the known PSF is used, it further improves the final result. Concerning RAM usage, it is the most demanding of all the methods.

Advantages

- It produces top quality results.
- It makes very precise estimation of the PSF shape.
- It enhances even the smallest image details.

Disadvantages

- The strength of this method may amplify image noise.
- In some cases, the method might diverge and give unsatisfactory results.

Landweber

Landweber method is the second least demanding method right after Fast deconvolution.

Advantages

- Sometimes it brings better results than Blind deconvolution (depending on the sample).

Disadvantages

- Some samples don't bring satisfactory results with Landweber deconvolution method.

Non-Blind

Using non-blind deconvolution assumes we know the PSF exactly, so the system does not have to estimate it. This type of deconvolution usually leads to reliable results. Concerning RAM usage, it requires about 50% of RAM compared to the Blind deconvolution. See **[1.2.4 Determining the Point Spread Function (PSF)]**.

Advantages

- It is a stable method which produces good results.
- It is faster than blind deconvolution.
- It reduces image noise efficiently.
- It produces decent results even if you do not import PSF.

Disadvantages

- The results are worse than from the Blind deconvolution.
- Some image details may remain blurry.

Fast

This method is optimized for speed.

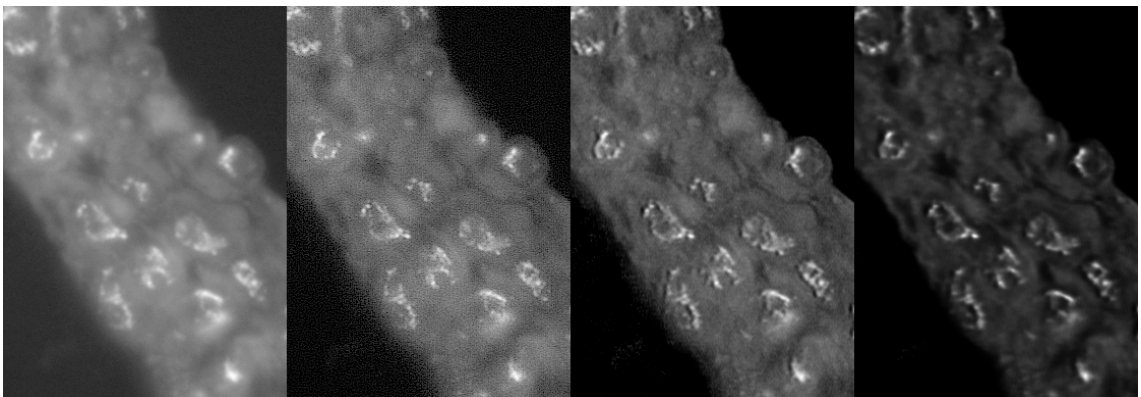
Advantages

- It is super fast
- Despite its swiftness, it can produce good results.

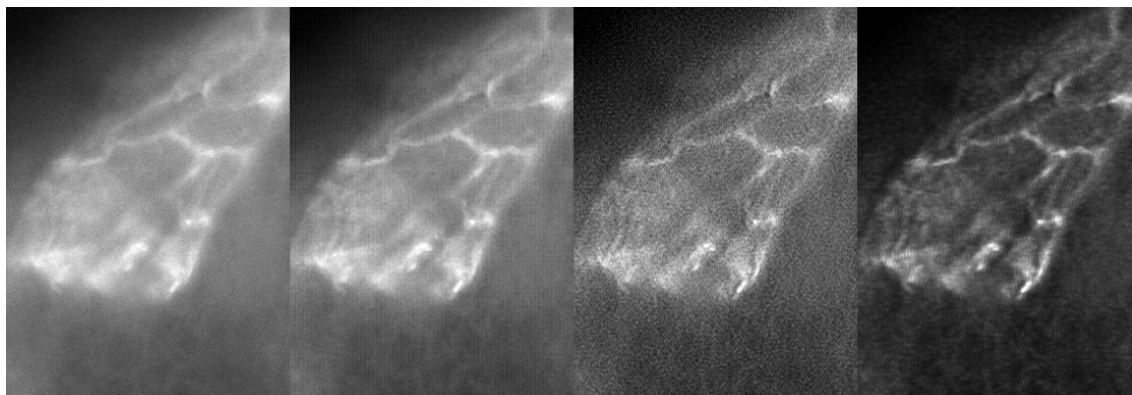
Disadvantages

- The results are not as good as from the other methods.
- It can add unwanted artifacts to the image.

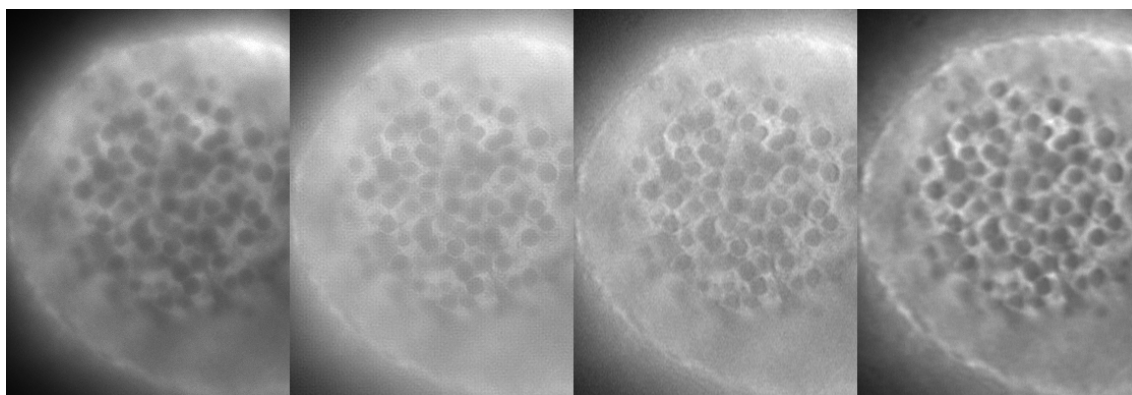
Comparison of the four deconvolution methods. From the left: original image, fast, non-blind, blind.



Comparison of the four deconvolution methods. From the left: original image, fast, non-blind, blind.



Comparison of the four deconvolution methods. From the left: original image, fast, non-blind, blind.



1.2.3. Algorithms

1.2.3.1. Resources

Articles concerning the algorithms were published by IEEE PAMI. Online access to the articles is charged and therefore cannot be shared here. Follow the links below to gain some more information.

Non-blind deconvolution

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

Blind deconvolution

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

Fast Deconvolution

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

1.2.4. Determining the Point Spread Function (PSF)

There are two ways to determine the Point Spread Function:

Capturing image of a nano-bead

This way is the best way to determine the real PSF of your system. Even two equal microscope systems can produce different point spread functions.

- Insert a size-standard (a nano-bead) instead of the specimen. Size-standards are available commercially. The size of the bead should be as small as possible - i.e. ideally match the size of one subpixel (smaller than a pixel) in the resulting image.

Note

The same nano-bead can be used with different objectives (20x, 40x, etc.) as long as the sub-pixel condition is satisfied.

- Capture an image (or a z-stack) of a single nano-bead. If it is difficult to set just one bead into the field of view, you can crop the image after the acquisition. Make sure the background of the image is black (0). It is likely that the image of the bead will be blurry - this is the actual PSF of your microscope. If you plan to deconvolve multichannel images, you should capture a nano-bead with multi emission wavelength.
- Save the image to a file. For 3D deconvolution, the image must be a Z-stack ND2 file, for 2D deconvolution, the image must be in TIFF format.
- Use these images within the deconvolution dialog windows after pressing the [Import PSF] button.

Caution

It is important that ALL(!!!) capturing settings used during PSF acquisition (for example Z-step, objective, scan zoom settings for confocal, etc...) match the settings used for capturing the specimen. Deconvolution results where a non-matching PSF was used can not be satisfactory!

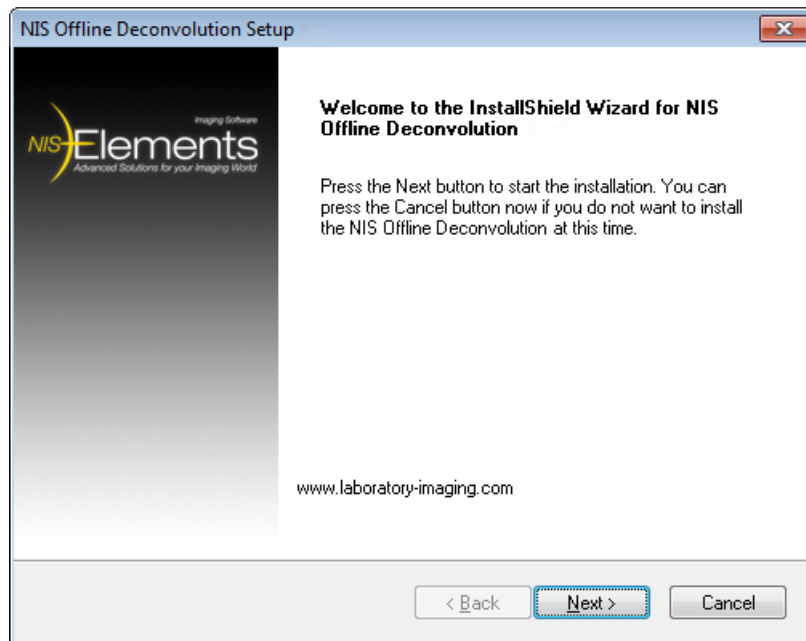
Calculating PSF from Properties of the Optical Path

Set these parameters within the deconvolution dialog window. The PSF image will be created and used upon starting of the command. You can also use the [Export PSF] button to save the estimated PSF to an ND2/TIFF file. Such file can be used to load the PSF later for other images.

2. Installation and Startup

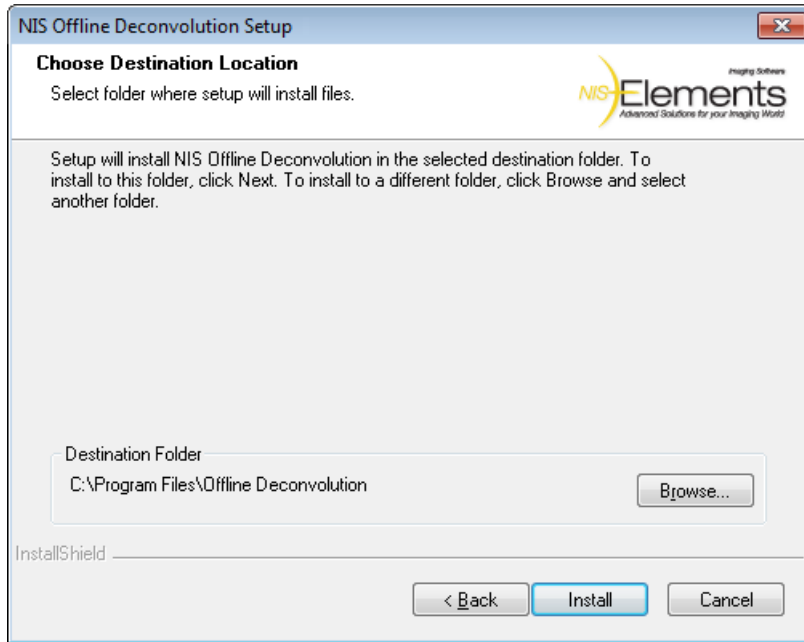
Installing the OD

- 1) Run the installation [exe] file. The following window appears:



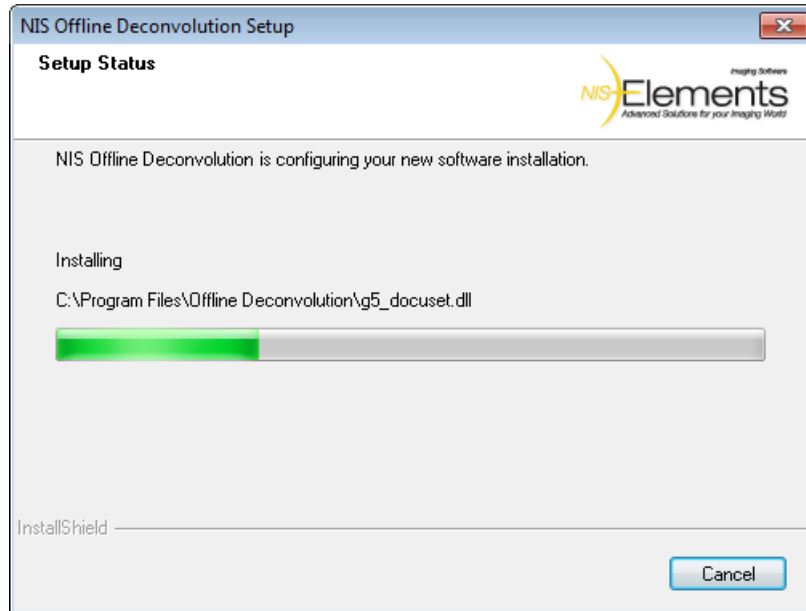
Click [Next].

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

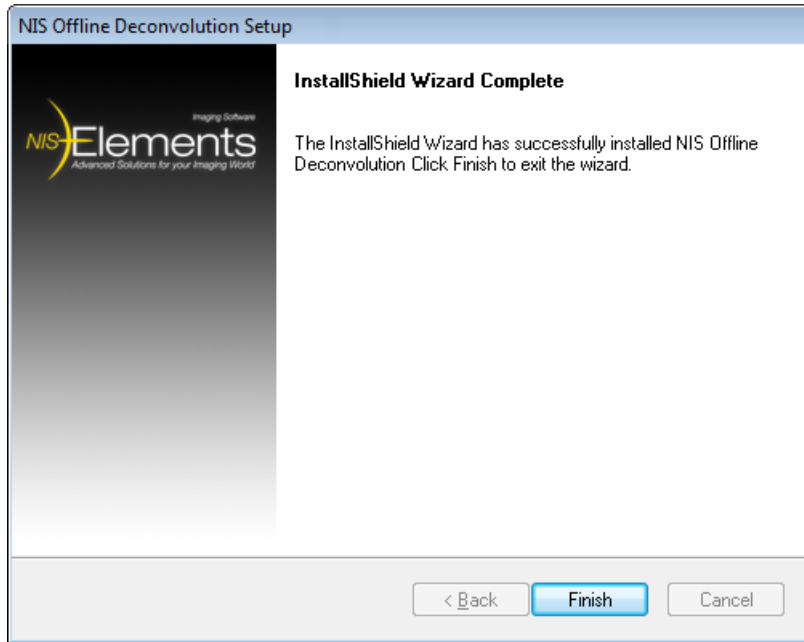


Click [Install].

- 3) The files needed to run the application will be copied to the destination folder.

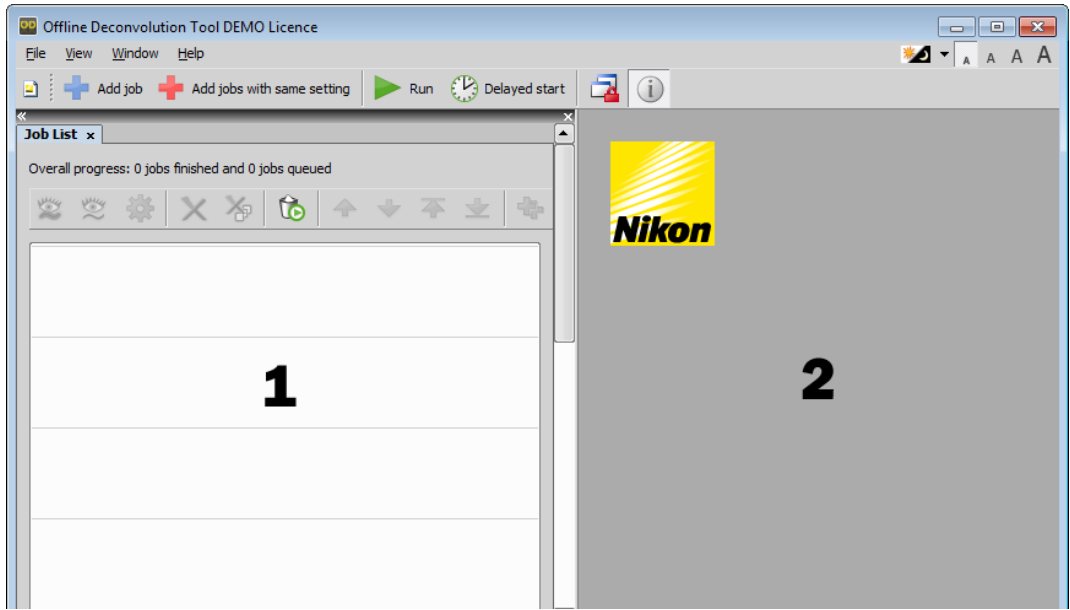


- 4) The installation process is finished. Click [Finish] to confirm it.



Starting OD

- 1)** Run [Start > Programs > NIS Offline Deconvolution... > Offline Deconvolution]. Or - a quick way - double click the Offline Deconvolution tray icon.
- 2)** The main application window appears. It is composed of two main parts: the [Job List] control window, and the image area.



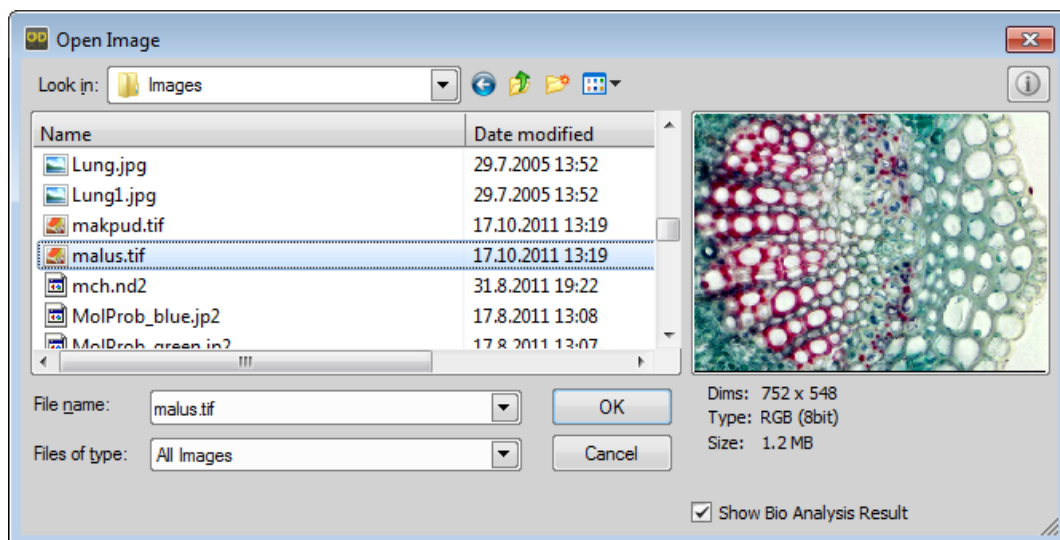
1. The job list control window
2. The image area

3. Scheduling Jobs

There are several ways of how to edit the job list.

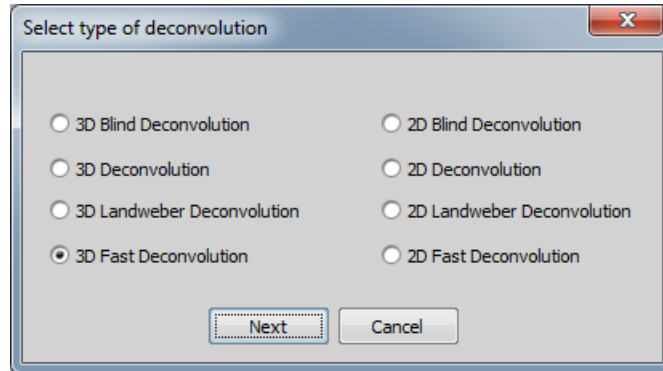
Adding job(s) to the job list

- 1) Click the [Add job] + button. A standard [Open] window appears.



- 2) Select one or more files to be processed. One job will be created for each image. Click [OK].
- 3) The following window appears where the deconvolution 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.

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

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 with same setting]  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 and click [Next].
- 3) Define the deconvolution settings. A check-box appears next to some deconvolution parameters:



The image shows a dialog box with two rows of settings. The first row is labeled 'Numerical aperture:' and has a text input field containing '1.30' and a checked checkbox to its right. The second row is labeled 'Refraction index:' and has a dropdown menu containing '1.51' and a checked checkbox to its right.

De-select the check-box if you want to load the corresponding parameter from each image meta-data. When the box is kept selected, the specified value will be used for all the images.

Note

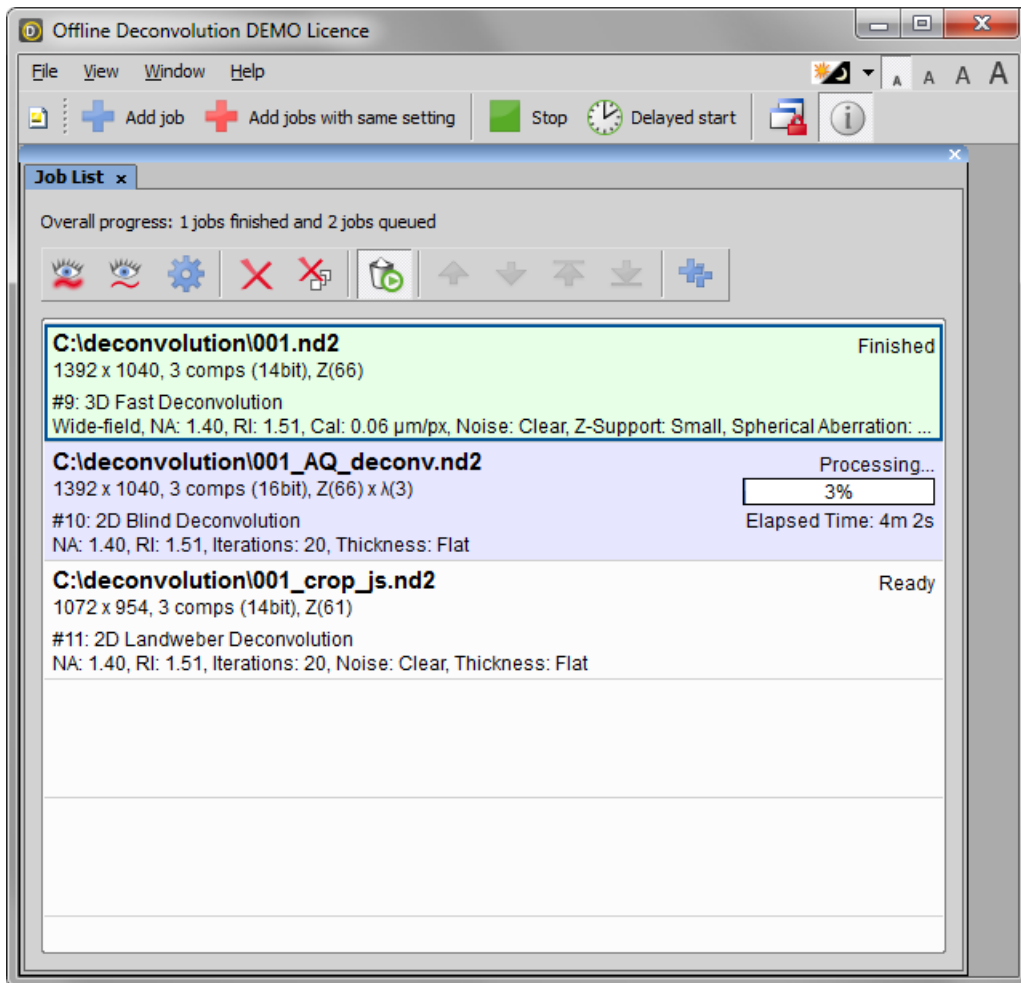
Before de-selecting any of the check-box, make sure the meta-data are available in all the images.

- 4) Each selected file will create its own job that will be added to the job list.

4. Running the Scheduled Jobs

Once you have scheduled all the deconvolution jobs to be performed you can start the processing. The jobs will be processed from the start of the list.

Finished jobs are highlighted by green color, the job being processed is highlighted purple and the jobs ready to be processed are white.



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 prompted to restart the job with the new settings.



Remove single job

Removes the job from the list.



Remove all jobs

Clears the Job List.



Automatically remove all 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 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

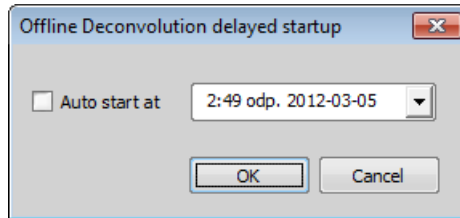
It enables to compare (run and view) two or more ND2 image files. It automatically synchronizes the view of all ND2 images opened within the image area.

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 [Delayed start] ⌚ 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. Specifying the Temporary Files Folder

During offline deconvolution, the application uses a folder on your hard drive to store temporary data. By default, it uses the [Application Data] folder belonging to the current user. Make sure there is enough space on the hard drive where the [Application Data] folder is placed. The lower RAM memory, the higher requirements for the space on the hard drive. This is important for the deconvolution to run smoothly.

If there is not enough space on the default hard drive, use the [File > Set Folder for Temporary files...] command to select a different directory (placed on another drive with enough free space) where the temporary data will be stored. Having small RAM memory, there should be at least (!) several-times more free space than is the size of the largest image file to be processed.

5.2. Deconvolution Settings Reference

Apply To

Z-series

When processing an ND2 image, the [Apply To] or the [Z-series] option can appear within the window. Select the part of the ND2 image to be processed.

Channels

Image channels produced by your camera are listed within this table. You can decide what channel(s) shall be processed by selecting the check boxes next to the channel names. The emission wavelength value may be edited.

Note

Brightfield channels are omitted automatically.

Calibration (3D)

Enter the image calibration in $\mu\text{m}/\text{px}$

Contrast enhancement

This option specifies whether contrast post-processing shall be performed after deconvolution. Choose its strength: None, Low, Medium, Strong.

Note

If you are not sure about it, you can leave this option disabled by selecting [None]. After the deconvolution is finished, you can use the [Contrast] command instead.

Deconvolution Method

Select which deconvolution method is applied. See **[1.2 About LIM Deconvolution Module]** for further details.

Export PSF

Enables to create and export an image of the Point Spread Function specified by the deconvolution parameters. An ND2 file is created for 3D deconvolution, TIFF file is created for 2D deconvolution. When exporting PSF for blind deconvolution, the PSF is calculated from the parameters set in the dialog window.

Image Noise Level

Specify an estimation of the amount of noise present in the image. Select whether the image can be: clear, normal, or noisy.

Import PSF

Enables to import the Point Spread Function image. If this is done, the deconvolution parameters will be disabled. Click the [Reset PSF] button to make them editable again.

Importing PSF can be of great help especially if you have determined the PSF image by capturing a nano-bead. It is reasonable to import the PSF for fast, blind and non-blind deconvolution either. See **[1.2.4 Determining the Point Spread Function (PSF)]**.

Iterations

The Blind and Non-blind deconvolution are iterative methods. More iterations (repetitions) take more computation time but usually improves the result quality. Specify the number of iterations.

Kernel

Select which kernel (type of the point spread function) is used. You should try which option leads to the best results. The [Gaussian] option is mathematically simplified kernel. In most cases (about 90%) the [Airy] is better. We recommend to test both and select the better result. Experienced users are able to determine the PSF shape from the sample by themselves.

Magnification (3D)

Specify magnification of the objective used to capture the image sequence.

Modality (3D)

To handle the out-of-focus planes correctly, it is important to know how exactly the image sequence has been acquired. Select the microscopy modality: Wide-field, Point scan confocal, etc...

Numerical Aperture

Enter the numerical aperture of objective.

Output

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

Pinhole size (3D)

Depending on the [Modality] setting, set the pinhole/slit size value and choose the proper size units.

Refraction Index

Enter refraction index of the immersion medium used. There are some predefined refraction indexes of different media in the nearby pull down menu.

Reload parameter From ND file

Usually, acquisition-related information are contained within image meta-data. If the current image meta-data contain such information, it is possible to load the values to the window by clicking this button. If there is no such information, the button is not displayed.

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.

Slit size (3D)

See **Pinhole size (3D)**.

Spherical aberration (3D)

This option lets you specify the spherical aberration of the objective. It influences the PSF shape. Move the slider to determine positive or negative spherical aberration. The spherical aberration coefficient can be determined visually:

- 1) Display a captured Z-sequence of a nano-bead in the [Slices View] and check whether the PSF is symmetric in Z as it is displayed in the deconvolution window for spherical aberration = 0.0.

Spherical aberration = 0.0



- 2) If the PSF is asymmetric, the spherical aberration distorts the image and shall be determined. Move the slider in order to make the preview look similar to the nano-bead XZ/YZ view.
- 3) Export the PSF to a file and compare the file with the nano-bead Z-sequence in the [Slices View].
- 4) Repeat these steps until the exported PSF and the captured nano-bead look the same. Write down the spherical aberration coefficient.

If you import PSF, the spherical aberration coefficient becomes disabled.

Subtract background before deconvolution

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

Note

This option works on the sample, not on the PSF.

Specimen Thickness

Estimate thickness of the specimen: Flat, Thin, Normal, or Thick. This parameter influences the PSF shape.

Z-Step (3D)

Set the step size of the sequence.