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**NIS-Elements** Version 3.0

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## **User's Guide**

**Laboratory Imaging**



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**(AR)** - Chapters and paragraphs marked by this symbol describe features available only in the Advanced Research version of NIS-Elements.

# NIS-Elements Installation

## Quick Start

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- Insert the installation DVD in the DVD-ROM drive. A splash screen automatically appears.
- Install the selected NIS-Elements software version, additional modules, and device drivers.
- Plug the provided HASP key into the USB port of your PC.
- Run NIS-Elements

## The DVD-ROM Content

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The DVD-ROM contains the NIS-Elements software itself, Windows system drivers for all supported cameras, drivers and utilities for HASP, documentation in PDF format, a sample image database, and sample image sequences.

## The Installation Process

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Note that you have to possess the administrator rights to your computer to be able to install NIS-Elements successfully. When the installation DVD is inserted a splash screen appears.



Select the software package to be installed. Select the one you have got the license for and which is properly coded in the HASP hardlock key. A welcome dialog box appears. Click [Next] to continue.

## Step 1

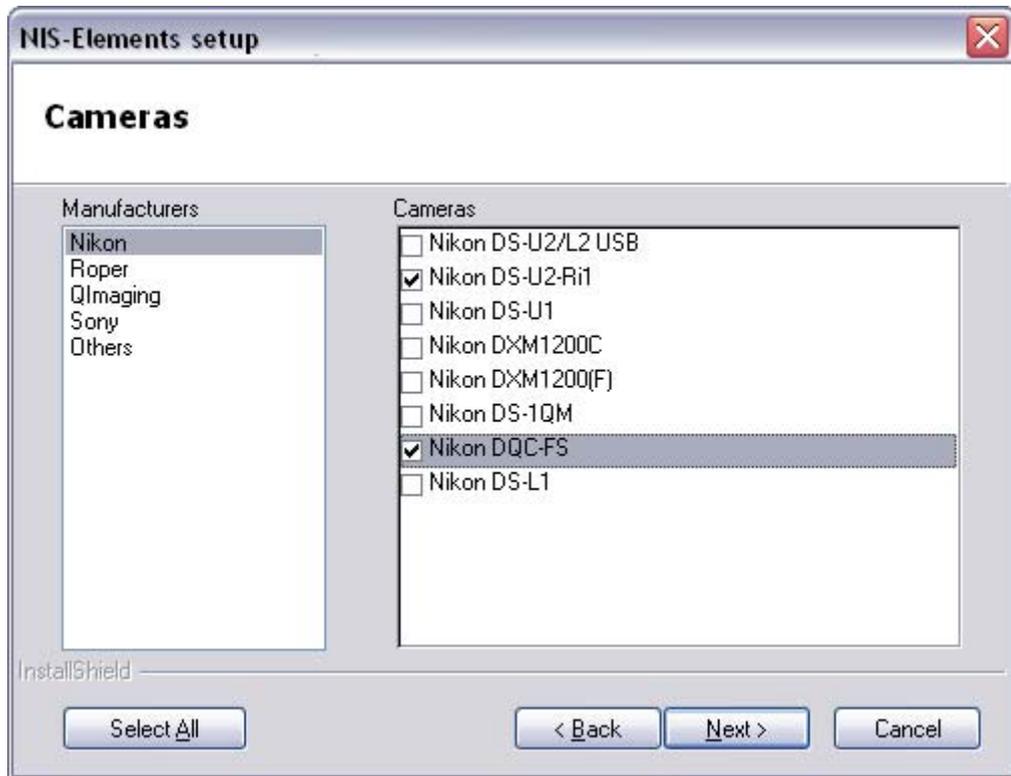
---



Define the folder where NIS-Elements should be installed. We recommend to use the predefined directory. If you want to change the directory anyway, press the [Browse] button and select a new one. Otherwise click [Next].

## Step 2

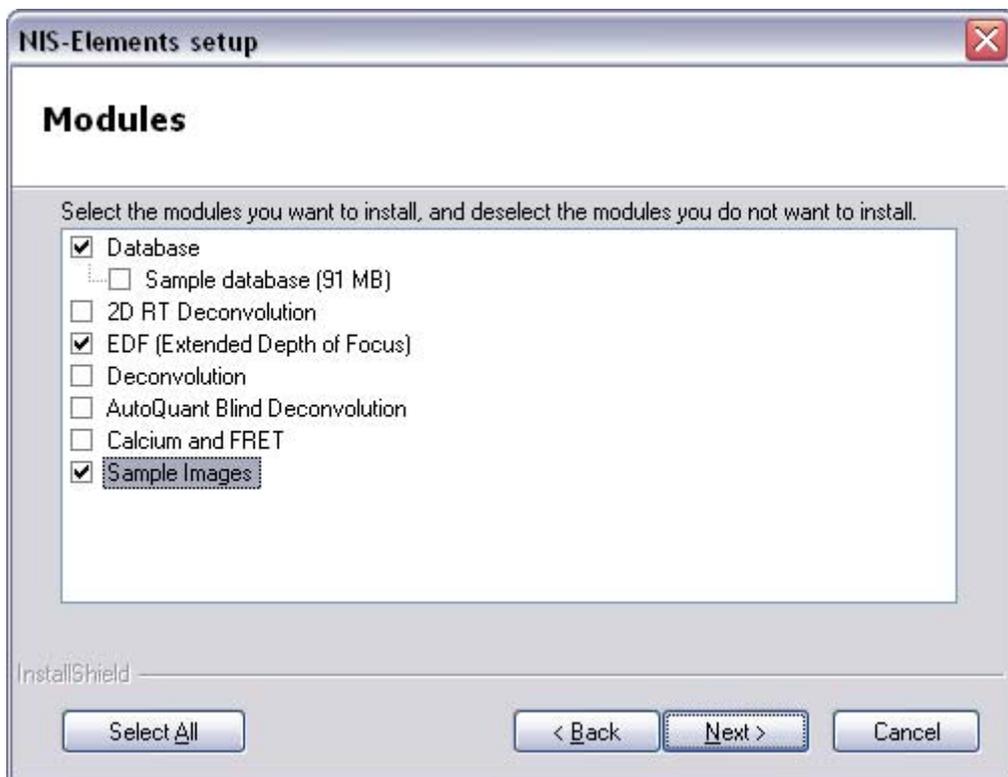
---



Now select the cameras you want to use with NIS-Elements.

### Step 3

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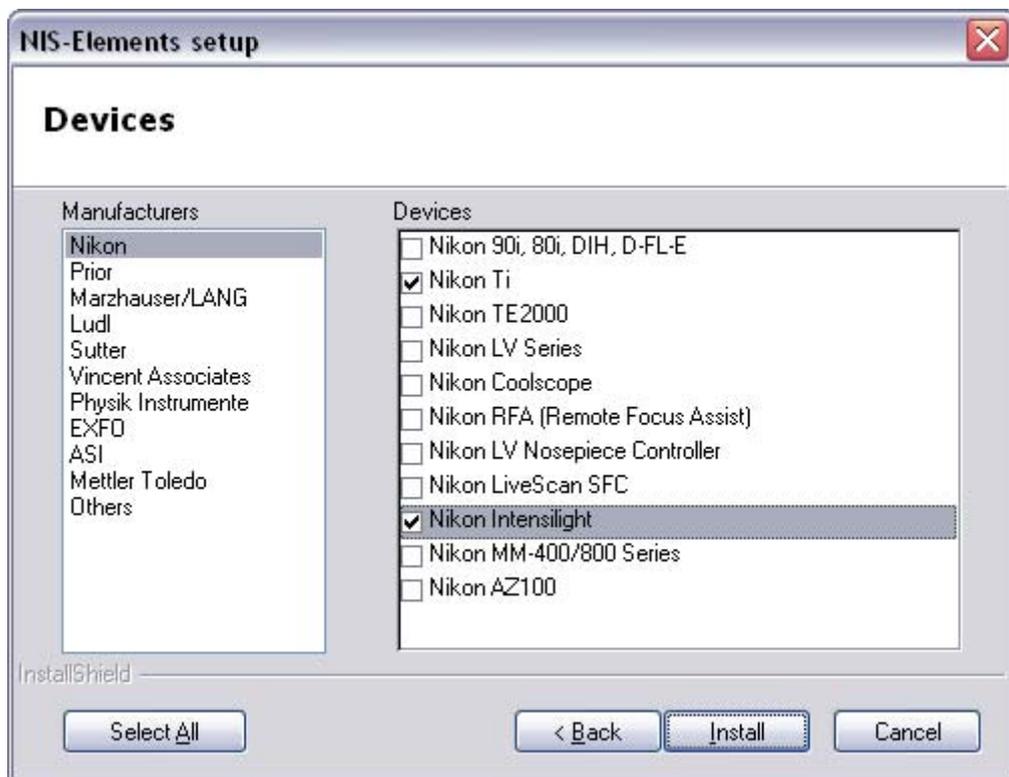


If your licence contains some additional modules besides the NIS-Elements base software, please select them in this window.

**Note:**

*Any module selected will be installed along with NIS-Elements automatically. However, you might not be licensed to use it. The module will run after you get the corresponding code registered in your HASP key.*

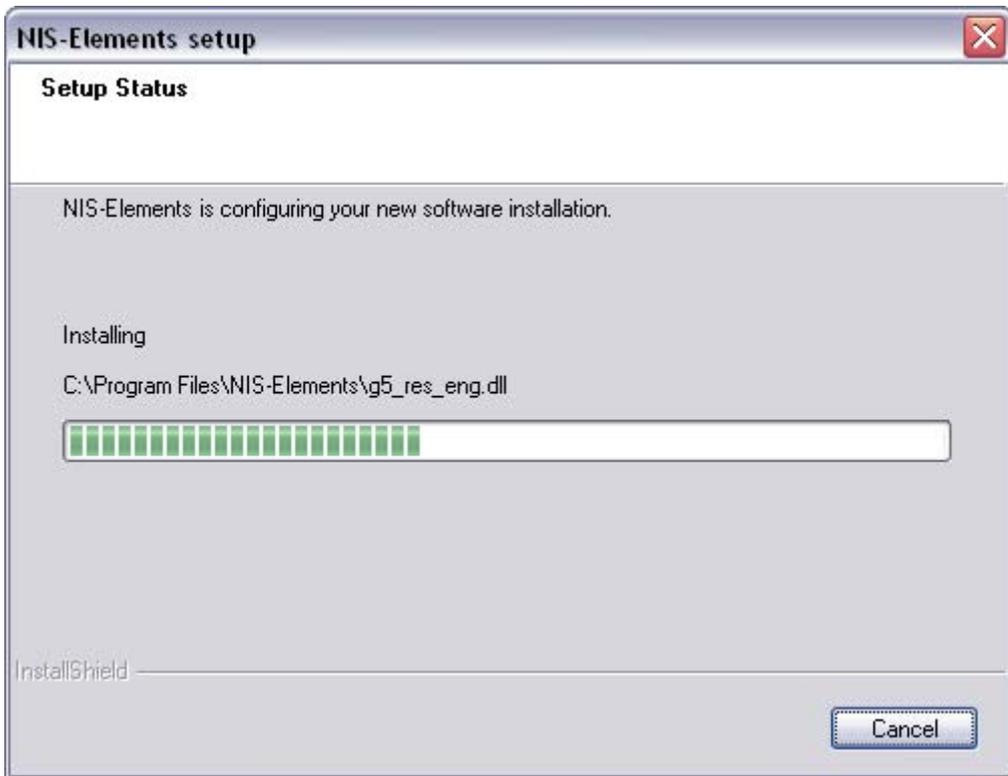
## Step 4



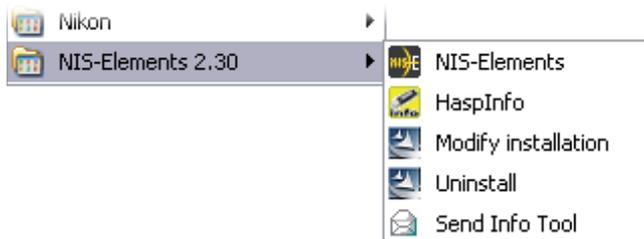
If the licence contains some device controllers listed in this window, please select them. Finish the installation by clicking the [Install] button.

## Step 5

---



The setup creates a new program group containing the following items: [NIS-Elements] application shortcut, the HASP key information shortcut, the [Modify Installation] shortcut (for adding hardware drivers, modules, etc.), the [Uninstall] procedure, and the Send Info Tool. A shortcut to NIS-Elements is created on the desktop too. These changes affect all user profiles of your local Windows operating system.



### **Note:**

*Clicking the [Uninstall] command deletes all installed files from disk, and removes the NIS-Elements program group from the Start menu as well as it removes the desktop icon.*

## **Additional Module/Device Installation**

---

You may need to install a device or an additional module after the NIS-Elements main system installation.

- Go to [Start menu > Programs > NIS-Elements] program group.
- Select the [Modify Installation] command.
- Follow the install shield instructions. Check the checkboxes by the items you would like to add.
- Finish the installation.

## **Sample Database Installation**

---

If you chose to install the Sample Database, a new subdirectory "Databases" is created inside the NIS-Elements installation directory (e.g. C:\Program files\NIS-Elements\Databases\...). The "Sample\_Database.mdb" file is copied to there along with database images (stored in subdirectories). An administrator username/password to access this database is set to:

- Username: "sa"
- Password: "sa"

## **The software Copy Protection**

---

The NIS-Elements software is delivered with a HASP hardware key.



The HASP key contains information about the software licence and allows users to run the corresponding software. Please connect the USB HASP after NIS-Elements installation. The utility called "HASPinfo" is installed to the NIS-Elements directory. It enables the user to view information about the software licence and is accessible via the [Help > HASP Info] menu command.

# Command Line Options

When starting NIS-Elements from the command line (or when editing the desktop shortcut properties) you may take advantage of using the following parameters:

## Syntax and example:

---

executable [switch1] [parameter1] [switch2] [parameter2]

["C:\Program Files\NIS-Elements\NIS AR.exe" -f "C:\Images\starting\_image.jp2"]

This example command runs NIS-Elements and opens the [starting\_image.jp2] at the beginning. By default, only one instance of the application is allowed to run. When the application is running, the command will be applied to this running instance (if the -q switch is not engaged). Some switches do not have parameters.

## Switches:

---

### **-?**

Displays a help screen with the following content.

### **-c [Command]**

The application runs an internal NIS-Elements command.

### **-f [Filename]**

The application tries to open an image pointed to by [Filename].

### **-g [Grabber name]**

The image grabber (driver) of the specified Grabber name will be used. No dialog box is displayed. As the name of the grabber, you can use the name which appears in the startup driver selection dialog, or the name of the driver class. These are listed in Windows registry (HKEY\_CURRENT\_USER):

"Software\Laboratory Imaging\Platform\Platform.INI\CLxGenericProviderGrabber"

### **-gn**

No grabber driver will be loaded.

### **-h [HW Unit Name]**

The specified HW Unit will be used. Two HW Units (e.g. two DS-U2 cameras) can be run by one driver. The available HW Units are listed in the [Acquire > Select Camera] dialog window. When using this option you must also use the [-c] switch. If you do not, the application will use the one selected the last time.

### **-i [Config Name]**

The application creates a registry entry in "Laboratory Imaging\Platform\Config Name" to load

from and save its configuration to.

**-l [Language]**

The application will run in the specified language. Us codes for the names of countries according to the ISO 3166-1 standard.

**-m [Macro File]**

Application will execute the macro file (\*.mac) pointed to by [Macro File].

**-p [Command]**

The application window will be placed according to the parameter:

When [Command] is:

- [left] - the window is placed on the left monitor.
- [right] - the window is placed on the right monitor.
- [top] - the window is placed on the top monitor.
- [bottom] - the window is placed on the bottom monitor.
- [monitor N] - the window is placed on the Nth monitor.
- [rectangle (x0,y0,x1,y1)] - the window is placed in the specified rectangle (in workspace coordinates). The coordinates must be in parenthesis without any spaces.

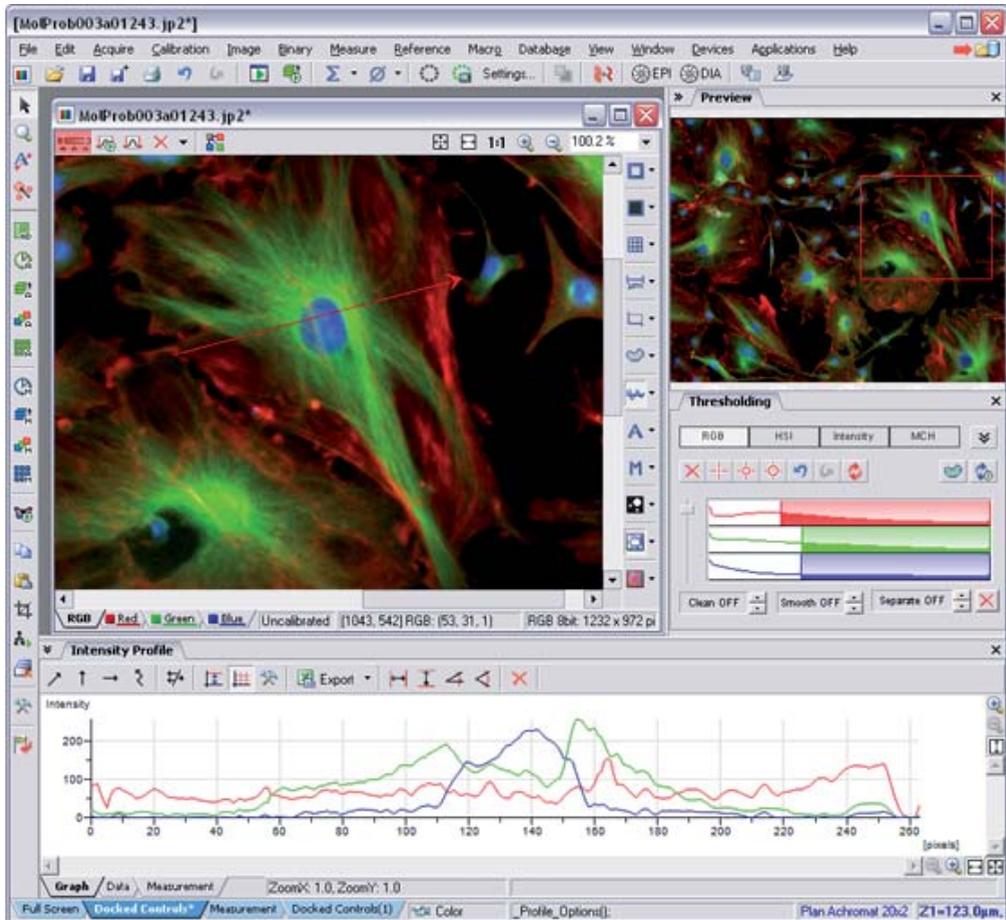
**-g**

A new instance of the application is created.

# NIS-Elements Screen

The following items appear on the screen:

- **The menu bar** containing pull-down menus with sets of commands.
- **Main toolbars (left and top)**
- **Document toolbars (right and top)** which enable to customize the view of each opened image individually. See their description in the [ Document structure ] chapter.
- **Docked control windows** - see more description of docking windows in the [ Modifying Layout ] chapter.
- **The status bar** displaying useful information.



## Horizontal toolbar

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-  [File > Open] invokes the open dialog window.
-  [File > Save] saves changes in the current document.
-  [File > Open/Save Next > Save Next] saves the current image as a new one according to the [File > Open/Save Next > Save Next Options].
-  [File > Print] invokes the [Print] dialog window with a print preview.
-  [Edit > Undo] moves one step back within the history of performed commands .
-  [Edit > Redo] moves one step forward within the history of performed commands.
-  [Acquire > Live Fast] displays the live signal from camera.
-  [Z Stack Live] displays the live signal from camera while moving the active Z drive according to the [View > Acquisition Controls > Z Series Setup] window settings.
-  [Integrate] turns the frames integration ON. Number of frames to be merged can be selected from a pull-down menu.
-  [Average] turns the frames averaging ON. Number of frames to count the average from can be selected from a pull-down menu.
-  [RAM buffer ON] - please see the Capturing to ring buffer chapter.
-  [Capture to RAM buffer] - please see the Capturing to ring buffer chapter.
-  [Acquire > Background Correction > Background Correction ON] applies the background correction, if it was defined.
-  [Calibration > Optical Configurations] displays the optical configurations manager.
-  [Calibration > New Optical Configuration] runs the optical configuration wizard for creating a new optical configuration.

## Vertical toolbar

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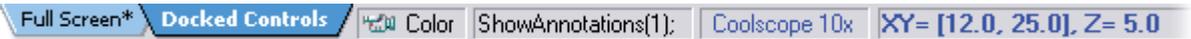
-  [Pointing Tool] is a default cursor which enables to move the image when zoomed in.
-  [Magnifier Glass] is an interactive magnifier tool for examining image details.
-  [View > Analysis Controls > Annotations and Measurement] opens a control window with annotations and interactive measurement tools.
-  [View > Analysis Controls > Binary Toolbar] opens a control window with binary layer modification tools.
-  [Applications > Define/Run Experiment] opens the ND2 capturing dialog window.

-  [Acquire > Capture Time Lapse] automatically/manually opens the ND2 capturing dialog window.
-  [Acquire > Capture Z Series] automatically/manually opens the ND2 capturing dialog window.
-  [Acquire > Capture Multichannel] automatically/manually opens the ND2 capturing dialog window.
-  [Acquire > Capture Multipoint] automatically/manually opens the ND2 capturing dialog window.
-  [Edit > Copy] copies the current image to memory.
-  [Edit > Paste As New Image] creates a new document and inserts the image from memory.
-  [Image > Crop] is a tool for cropping images.
-  [Macro > Run Macro] runs the active macro.
-  [Window > Close All] closes all opened documents.
-  [Edit > General Options] displays the general options dialog window.
-  [View > Customize Toolbar > Setup] enables to add/remove buttons to the left toolbar.

## Status bar

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The status bar at the bottom shows the following information (from left to right): available views, a type of selected camera, the last performed command, the active objective name, and current coordinates of stage and Z-drive (if connected).



# Modifying Layout

Having a well organized application layout can help you make the work with NIS-Elements very effective. The live image, captured images, various control windows (Camera Settings, Measurement, Histogram, LUTs, etc.) can be arranged within the application window. The control windows can be floating or docked inside one of the four docking panes. Connecting of a secondary monitor is also supported. User commands may be added to the main menu, and some tool bar buttons may be added/removed.

## Layout Manager

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A layout in the context of NIS-Elements is a set of options describing the arrangement of control windows, toolbars, and the menu items. The application can operate in two modes: supporting layouts or not. To switch the layouts mode ON/OFF, reach the [Edit > General Options] command and go for the [Appearance] section. There is a check box that enables/disables the mode. Once all layouts are defined, you can make them read-only by selecting another check box.

Enable Layouts

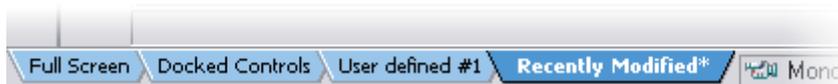
Read Only Layouts

Blue tabs representing active layouts appear in the bottom left corner if layouts are enabled. The [Full Screen] and [Docked Controls] layouts are placed there by default. Other layouts can be added and managed via the Layout Manager.

If the "Read Only Layouts" box is checked, no layout changes are saved during work or on exit.

## To Create a New Layout

---



- Modify the current layout to suit your concept of work.
- The asterisk appears next to the layout name (to indicate it has been modified).
- Right click the layout tab and select the [Save Current Layout As] command.
- Write the new layout name and confirm it by OK.
- A new tab appears and the layout is saved to the list of layouts.

*If you do not need to create a new layout but would like to save the changes made, just right click the current tab (asterisk-signed) and select the [Save] command from the menu.*

## To Reload Previous Layout Settings

---

Unwanted changes of the layout may be made. Mostly, it can be fixed by the [Reload] command.

- Right click the asterisk-signed (recently modified but not saved) tab and choose the [Reload] command.
- The application restores the last saved state of the layout.

## Layout Setup

---

Right click any layout tab and select the [Layout Manager] command. The Layout Manager appears.

## List of Layouts

---

The list of currently available layouts is placed on the left side of the layout manager. Each layout may contain information about the controls, tool bars, menu, and commands to be performed when switching between layouts. Check the nearby boxes to select the type of information to be included. The check marks left of the layout names indicates the layout visibility (displayed/hidden).

 The [New] button adds a new layout to the list of layouts.

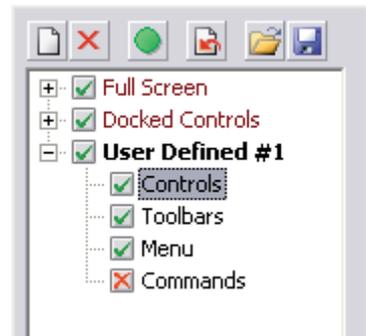
 The [Remove] button deletes the selected layout. The first two layouts cannot be deleted.

 The [Activate] button makes the selected layout active (current)

 The [Load Default] button has the same functionality as the Reload command described above. Except when applied to the Full Screen and the Docked Controls layouts where it loads the original settings - so they look just the same as after the program installation.

 The [Import] button enables to load a previously saved set of layouts from a XML file.

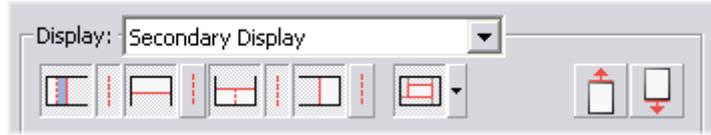
 The settings of all layouts can be saved to an external XML file. Use the [Export] button and define the destination file name.



Controls, Toolbars, Menu, and Commands of each layout settings may be modified in the right part of the Layout Manager. Just select the appropriate name:

## Controls

The layout of docking panes and the control windows arrangement can be modified in this section of the dialog window. There are the following tools and a preview of the layout available.



If you use two monitors, select which screen to set up (primary display, secondary display) in the [Display] pull-down menu.



These icons turn the docking panes ON/OFF. The currently selected docking pane is highlighted by blue color. The splitting is indicated by the dashed line.



Each docking pane can be divided into halves by a splitting line. This button turns the the splitting ON/OFF.



If a two-monitor workstation is installed, this icon button allows you to select the content of the secondary image area of NIS-Elements. The pull-down menu contains the following options:

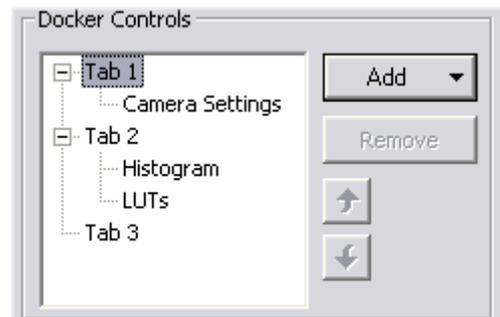
- [Docking View - Horizontal Alignment] - the docked control windows will be arranged one below each other.
- [Docking View - Vertical Alignment] - the docked control windows will be arranged on overlay.
- [Live Window] - places the Live image to the secondary screen, and forces all non live windows to open in the primary screen.
- [Non Live Windows] - opens all document windows except the live image on the secondary screen.



These buttons perform the [Shrink] and [Expand] commands on the selected docking pane. A docking pane can be selected by clicking into the preview window.

### **Arranging the Control Windows**

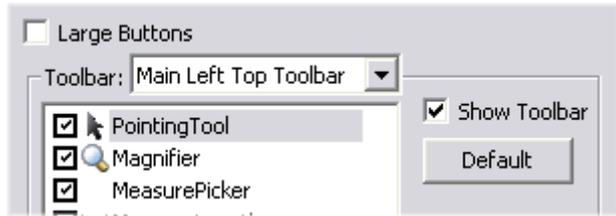
- Select one of the docking panes by clicking into the layout preview above the [Docking Controls] section.
- Select the tab you would like to add a control window to.
- Click the [Add] button and select the control window to be added from the pull-down menu.
- The control windows can be moved up and down within one tab by the arrow buttons.
- The [Remove] button deletes the selected control window from the docking pane.



*There is always one empty tab at the end of the list. When you add a control window to it, another empty one will be created. It does not appear in the application screen.*

## Toolbars

- Choose the one of the toolbars from the [Toolbar] pull-down menu.
- Any button of the chosen toolbar may be hidden by deselecting the nearby check box.
- The [Large Buttons] option switch between two sizes of icons. The setting is shared by all toolbars.
- The selected toolbar may be hidden by deselecting the [Show toolbar] check box.
- The [Default] button discards changes and sets the default setting of the selected toolbar.



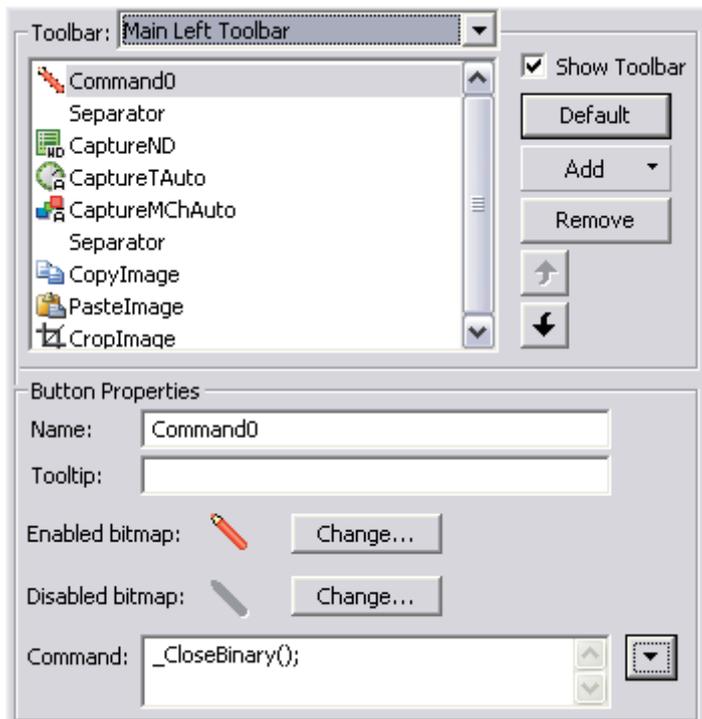
### Main Left Toolbar

Being the most utilized toolbar of all, custom user buttons can be added to it. You can define your own buttons which run application functions or execute macros. Select the [Main Left Toolbar] from the pull-down menu.

Lets say that we very often use the [Binary > Close] command. It is useful to add a button providing a shortcut to it to the toolbar. Press the [Add] button, and choose [Command] from the context menu:



A new command (Command0) is added to the buttons list. Now, attach the NIS-Elements command: Open the pull-down menu on the right side of the [Command] edit box and click on the [Command List]. Choose the command from the list (e.g.: `_CloseBinary`) and confirm the selection by [OK]. It is possible to create a sequence of commands, by repeating the procedure.



If you are not satisfied with the default icon, you can change it by pressing the [Change] button. A dialog window for selecting the icon appears. You can select NIS-Elements icons by default, or load some others from files containing icons (ico, dll, ...). You can define icon for the command in a disabled state, too.

It is handy to define a tooltip (a text that appears when placing a mouse cursor over the icon) for your command. Simply write the text into the [Tooltip] box. You can change the position of the command in toolbar by using the arrows located under the [Add] and [Remove] buttons.

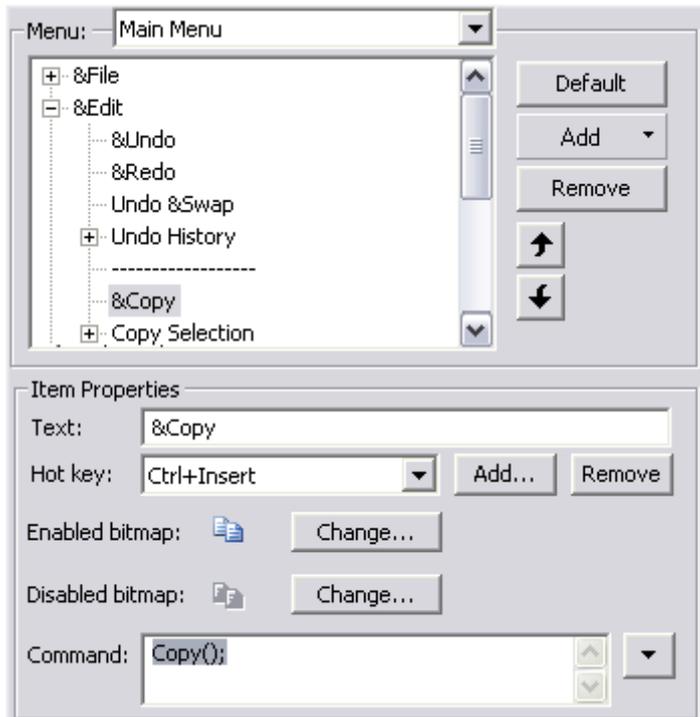
## Menu

The Main Menu and the Picture Context Menu may be modified. Items of the Picture Context Menu (the menu which appears after you right click to an image) can be hidden by unchecking check boxes similarly to most of the tool bars.

### Modifying the Main Menu

- Select the [Main Menu] in the topmost pull-down menu.
- Any item may be added to the main menu - separator, Menu Command, a sub-menu (Menu Popup), and even a new menu (Main Menu Popup) - by the [Add] button.
- Select the existing menu item you would like to place the new item under.
- Click the [Add] button and select the item type from the pull-down menu.
- Edit the [Item Properties].

*The [Default] button discards all changes and loads the main menu original configuration. The [Remove] button deletes the selected item. The arrow buttons move the selected item up/down.*



### Item Properties

- [Text] - This is the text which appears in the pull-down menu. You may place the "&" sign before any letter. Such letter will be considered a shortcut key when browsing the menu.
- [Hot key] - one or more hot key shortcuts may be assigned to the command. Just press the [Add] button and press the key combination. The [Remove] button removes the selected hot key.
- [Enabled/Disabled bitmap, Command] - These fields serve for assigning a bitmap image and a NIS-Elements function to the menu command. It works in the same way as when modifying the Main Left Toolbar (described above).

## Commands

---

These settings enable to run a command or a macro right before and after you switch to the layout. Insert the commands similarly as it is described above.

## Docking Panes without Using the Layout Manager

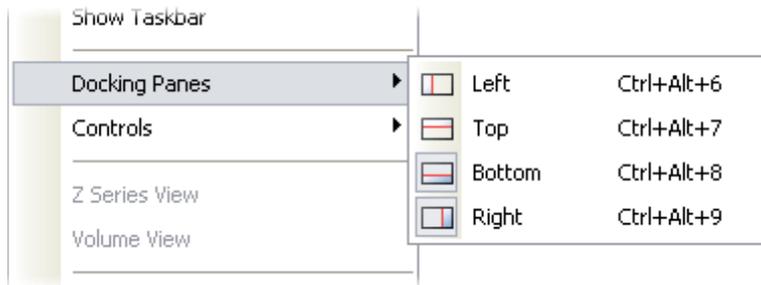
---

Docking panes are empty square spaces inside the application window, where you can place ("dock") the control windows. They can help to keep the screen "tidy". There is one docking pane available on each side (Top, Right, Bottom, Left) and can be further divided into halves.

## To Display a Docking Pane

---

- Go to the [View > Docking Panes] submenu and select the pane you would like to display.
- The docking pane appears, either empty or with some window(s) docked.
- Repeat this task to display more panes.



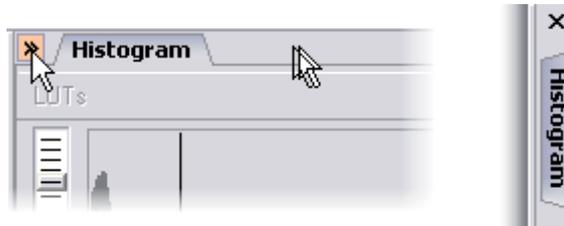
*The same submenu can be also displayed by right-clicking into the empty application screen.*

- To divide a docking pane, right click inside the dockig pane and select the [Split Docker] command.

## To Hide a Docking Pane

---

Either click the arrows in the top left corner, or double click (or Ctrl + click) the empty pane (not the docked control window). The pane minimizes to a stripe by the edge of the application. It can be recalled to its original size by double clicking it.



If you would like to close the pane completely, minimize it and press the cross button, or you can right click the pane and unselect the [Docking View] option.

## To Shrink/Expand Docking Panes

---

Having more docking panes opened, a situation where there is not enough room for the control windows can occur. In that case, the Shrink and Expand commands shall be used.

- Right click the pane you would like to shrink/expand.
- A context menu appears.
- Select the Expand/Shrink command.

*The commands Shrink/Expand Top, Right, Bottom, Left are available. When one of the panes shrinks, the neighbouring pane expands to the emptied corner and vice-versa.*



## Control windows

---

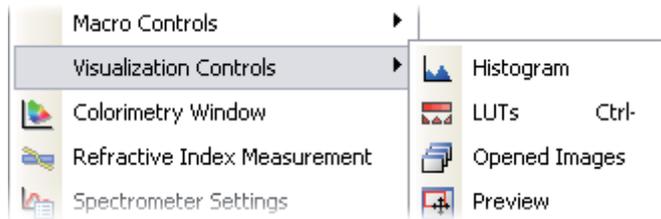
The following control windows can be displayed within the application screen. They can be floating, or docked inside the horizontal or vertical docking panes:

*\* - this control window is optional. An additional software module or a correct device connected is required in order to display it.*

## To Display a Control Window

---

- Go to the [View] menu and select the desired control window. The controls are sorted to four groups: Acquisition, Analysis, Macro, Visualisation controls.
- The control window appears floating on the screen.
- To dock (and undock) it, double click its caption.



*It is also possible to display a control window docked. Right click inside a docking pane and select the one of your choice from the context menu. If the window is already opened somewhere (e.g. in the opposite pane or floating), it closes and moves to the new destination.*

## To Close a Control Window

---

- Click the "cross" button on the right side of the active window caption.
- If the window is docked, you can also right click its caption and uncheck the appropriate window.
- The control window closes.

## Tab or Caption Style?

---

More control windows can be docked in the same place while only the front one is being visible. There are two styles of displaying these windows, Tab and Caption.

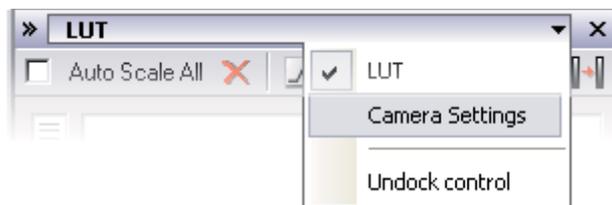
### Tab Style

- Besides the current control window, the other window tabs are visible.
- Switching the windows is a one click operation.



### Caption Style

- All docked windows except the current one are hidden.
- The switching and undocking of windows is done via the menu that appears when you click the arrow button.



# Organizer

Apart from the main application mode used for capturing and image analysis, NIS-Elements provides a special [Organizer] mode.

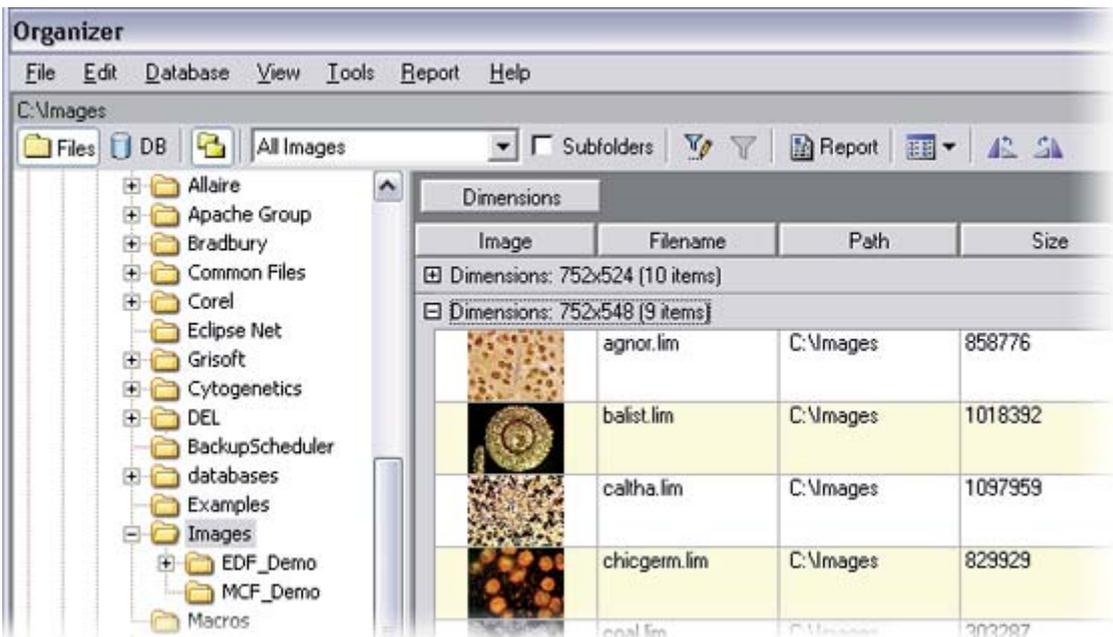
Organizer was designed to ease the work with image files and databases. To activate organizer, click the [Organizer] button located in the top right corner of the application window or press [F10].



The screen is primarily divided into two identical panes, each pane switchable between two modes: the [Files] mode and the [Database] mode. To switch from one pane to the other one use the [View > Next Pane] command. To copy files between the panes, simply drag the images from one side to the other side.

-  **Files** - This button switches the pane to show a directory tree and images from the selected folder (and optionally its subfolders). It is called the [Files view].
-  **Database** - This button switches the pane to show the database structure and lists images from the currently selected database table. This is called the [Database view].

## Features Available in the Files View

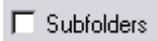




This button toggles the display of the directory tree. You can switch it off to get additional space to display images.



In this pull-down menu it is possible to set the file type to be displayed. You can select a particular extension or show [All Images].



If this check-box is selected all images from the included subfolders are displayed.

## Features Available in the Database View

image	Mineral	State	Locality
		CZ	Mrakotin
	adamina	Mexico	Dur
	anabergit, lavend...	CZ	Zalesi u Javornika
	anabergit, erytrin	CZ	Zalesi u Javornika
	analcim	CZ	Repiste
	analcim	CZ	Repiste



This button displays the detailed information about the selected image. You can switch it off to get additional space to display images.



This pull-down menu displays the active connection point and enables to switch between connected databases.



This pull-down menu selects the active database table.

## Image Filter

---

Both views (Files and Database) have the option to define a filter. It enables you to display only images which fit the defined conditions (one or two).



This button activates the filter.



Pressing this button invokes the filter setup dialog box.

## Basic mode

---

This mode enables you to view files that match one defined condition.

## Advanced mode

---

This mode enables you to define two conditions with a relationship defined between them. Either select [OR] to display files matching at least one condition, or select [AND] to display files which match both conditions.

The screenshot shows a 'Filter' dialog box with the following configuration:

- Left Column:**
  - Search in field: Capturing
  - Field type: Text
  - Condition type: Values (selected)
  - Values: DS-5M
  - Occurrence: Anywhere (selected)
- Right Column:**
  - Search in field: Filedate
  - Field type: Date
  - Condition type: Range (selected)
  - From: 22.11.2004
  - To: 24.11.2004
- Buttons:** '<- Basic', 'AND', 'OR', 'OK', 'Cancel'

First, select the field, where NIS-Elements should search for a given expression. When using a filter in the [Files] view, there are fields from file properties listed. In the Database view, the listbox shows the names of fields from the currently selected database table.

If the selected field is of a numerical type (e.g. Size, Calibration, File date etc.) you can specify, whether you want to find the exact value or a value in a given range. This is selected by the [Condition type] radio button.

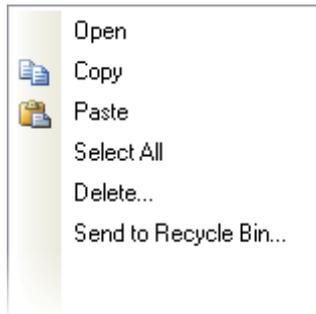
If the field type is [Text], the [Occurrence] setting determines the way of evaluating the expression:

- [Anywhere] - If a given sequence of characters is found anywhere in the sequence of characters in the field, the system will evaluate it as a match. Example: you have entered "set" to the [Values] field. The filter will select records with the following field values: **set** , re **set** , **set** tings, pre **set** , etc.
- [Exact] - If the given sequence of characters exactly equals to the content of the field, it is evaluated as a match. Example: if "set" is entered, fields containing the **set** value will match only.
- [Start] - If the entered string is found at the beginning of a field, it is displayed by filter. Example: If "set" is entered, fields containing **set** , **set** tings, **set** up are selected.
- [All letter strings] - It is possible to search for more expressions. These should be entered separated by commas. If you want to enter an expression with a space, insert it into quotes. If this option is selected, only records in whose fields all of those expressions appear (anywhere) are selected.
- [Any letter string] - This option is for entering multiple expressions as above, but this time every field with an occurrence of at least one from the given expressions is matched.

## Operations with images

---

- To open an image from the [Files] view, double click its thumbnail. NIS-Elements will display it in the main window.
- To select multiple images, either click on the first and the last image holding the [Shift] key (continuous group selection) or click individual image names with the [Ctrl] key down.
- You can copy one or more images from one folder to another by "drag and drop".
- This quick method can be used for inserting images to a database too. Simply drag the image from a folder and drop it onto the pane, where the database table is opened.
- To delete selected images press the [Delete] key.

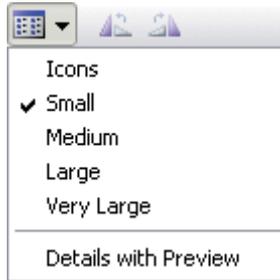


All those operations and some other can be invoked also from the context menu, which appears each time you right click on the image thumbnail:

## Thumbnail displaying options

---

You can adjust the way images are shown in the organizer. Press the [Show thumbnail view with...] button of the main tool bar and select the size of displayed image thumbnails. Selecting the [Details with preview] option will display one image below the other with all available information about it at the side.

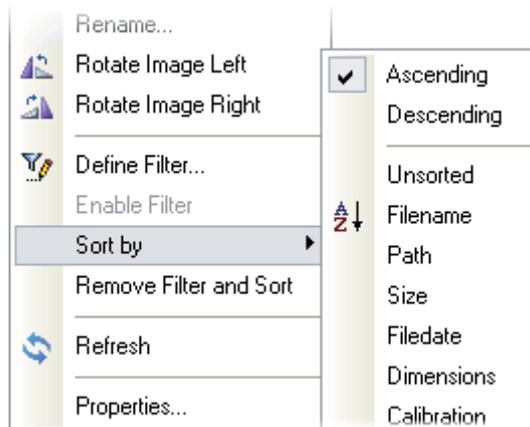


There is a possibility to rotate images from within the organizer. It affects not only the image thumbnails, but the image data too. Press the corresponding buttons.

## Ordering of images

---

To order displayed images, right click anywhere in the pane - a context menu will appear. Move to the [Sort by] submenu which offers several ordering criteria. If the ordering is turned ON already, the icon is displayed on the left side of the applied criterion.



## Grouping of images

---

To arrange the view of images efficiently, you can use the capability of grouping of images. Drag the column name bar to the grouping bar (right above the column name bars). All files with matching field values of the selected column will be grouped together. This can be undone by dragging the column caption back. See the example on the Files View picture above (the Dimensions column is grouped).

## Adjusting the Organizer layout

---

The pane size is adjustable. To resize it move the mouse cursor to the dividing in line the middle. The cursor becomes an arrow with two tips. Press the mouse button and drag it left/right to the new position.



Resizes the panes to achieve the same size for both of them.



Resizes the pane to its maximal/minimal size (one pane is then displayed on the whole screen).

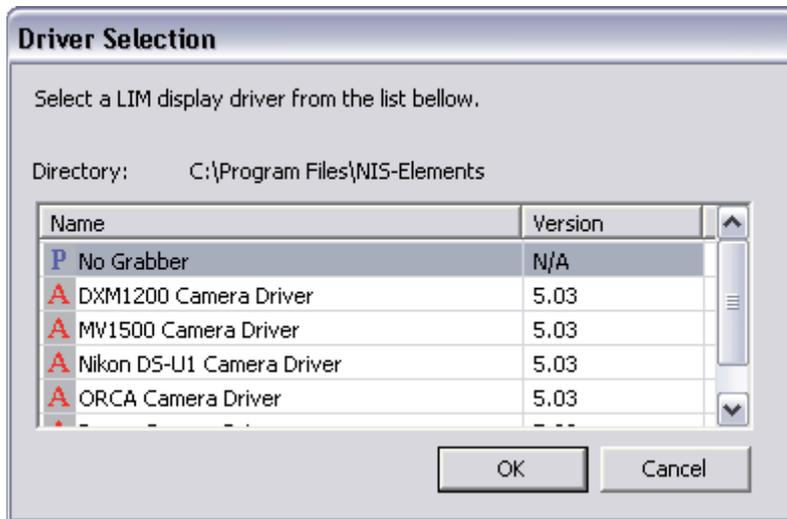
# Cameras

This chapter is dedicated particularly to users running the active version of NIS-Elements - equipped with a camera. Let's assume the camera works properly, is connected to the system with proper system drivers installed and running (if required by camera).

## Selecting the driver

---

You will be asked to select the camera driver everytime you launch NIS-Elements. You can change the driver later, using the [Acquire > Select Driver] command. Choose the driver that matches your camera:



## Selecting the camera

---

Color cameras can be used in a monochromatic mode. The actual camera type (color/mono) can be selected by the [Acquire > Select Camera] command. Confirm the choice with [OK].

## Setting up the camera

---

Exposure time, camera resolution, and other camera-specific features are adjustable from the [Camera Settings] window. To invoke it, use the [Acquire > Camera Settings] command.

## Live signal from camera

---



This button opens a new document window with the Live-Fast image from camera. New buttons for controlling the camera appear in the document window to control the camera.

## Live formats

---

A camera format is a set of attributes of a video signal such as: resolution, bit depth, frame rate etc. The live signal is a stream of image data, coming from the camera to your computer in real time. NIS-Elements provides two different camera smodes: [Fast] and [Quality], each serving a different purpose. The format of both modes can be set within the [Camera Settings] control window.

### **Live - Fast**



This tool bar button runs the [Fast] live mode. It is optimized to give as many frames per second as possible in low resolution. This mode increases gain and uses short exposure times.

### **Capture - Quality**



This tool bar button runs the [Quality] live mode. It produces pictures in high resolution, but the frame-rate is low. This mode sets the gain as low as possible and extends the exposure time. This mode is applied every time the [Capture] button is pressed.

## Capturing Images

---

Although it is possible to perform some procedures directly on the live image, other image operations require [captured] or [frozen] images. If you try to perform such operation when the live image is active, NIS-Elements automatically freezes the image. Let's see the difference between the [Capture] and the [Freeze] commands:



This is the [Capture] button. When pressed, the camera exposure runs till the end, and the next frame is captured and displayed on the screen. In fact, it is the first frame with the complete exposure after you have pressed the [Capture] button.



The [Freeze] button interrupts the camera exposure, and displays the very last complete frame.

*If you are using the [Fast] mode and press [Capture], NIS-Elements will automatically switch to the quality mode to capture the image. When the image is captured, it is opened on the screen as a new document.*

# Configuring Attached Hardware

Typically, laboratory computer image analysis systems consist of a computer, a camera, and a microscope equipped with all necessary accessories (objectives, filters, shutters, light, rotary changers, etc). Most of the mentioned microscopic hardware is often motorized and therefore can be controlled by NIS-Elements. Thanks to the NIS-Elements concept, it is possible to integrate single settings of all these devices into one compact set called an [Optical Configuration]. It is recommended to create several optical configurations containing particular devices settings. Then a single click can completely change the hardware configuration.

## Creating New Optical Configuration

---

Please check that all the devices (microscopes, cameras, etc.) that you want to associate with the new optical configuration are properly attached to the system and working. Insert a calibration slide to the microscope.

## Start the Optical Configuration Wizard

---

Choose the [ Calibration > New Optical Configuration ] command from the Calibration menu. The Optical Configuration Wizard appears and the camera is automatically set to Live mode.



- Type the name of the optical configuration. We recommend to use short descriptive names, e.g. the name of the associated objective.
- Select whether to associate an objective, a camera or a microscope.

- Use the [ Camera Setup ] button to adjust the camera settings.
- You can start the illumination correction process by pressing the [ Capture Correction Image ] button.
- Adjust the Microscope settings using the [Microscope Pad] and select which parts of the microscope shall be included in the configuration by checking them in the [Options] dialog box.
- If there is more than one shutter and you would like to associate a shutter with the optical configuration, select which one is the [Active Shutter] from the pull-down menu.

Press the [Finish] button (if the Objective was not selected) or the [Next] button to continue...

## Selecting Objective

---



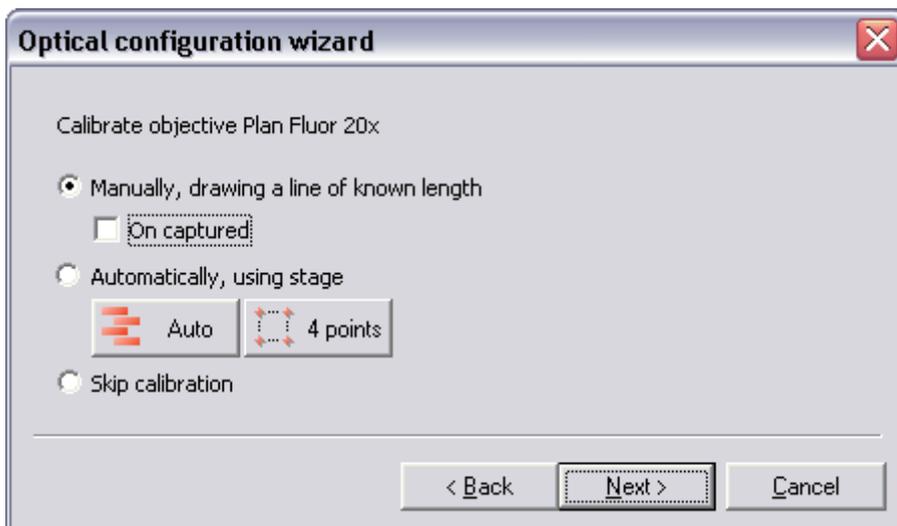
Now, please select one of the following alternatives:

- Select one of the already defined objectives from the pull-down menu or insert one of the predefined objectives via the [Insert] button.
- Or, you can create a new objective by filling its name to the [Create new objective] edit box.

Press the [Next] button to continue...

## Objective Calibration

All digitized images consist of a number of rectangular elements called pixels. A calibration assigns real size to one pixel, so that correct and realistic measurements can be performed. It is necessary to create a calibration for each objective because it is bound to the objective magnification. It is possible to assign names to objective calibrations and to store them.



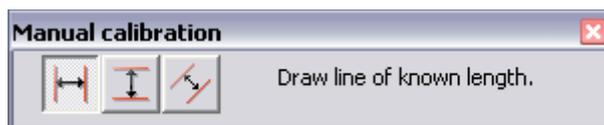
Select one of the following alternatives:

- The [Manual] calibration lets you draw a line into a picture and define its real length (see below). The "On Captured" check box allows to decide whether live or captured image is used for the calibration.
- Having a motorized microscope, there are the [Auto] and [4 points] alternatives available.
- Skip the calibration, if the selected objective (selected in the previous step) has been calibrated already.

Press the [Next] button to continue...

### **Manual Calibration**

This step is dedicated to the manual calibration.



- The distance is defined by placing lines (Horizontal, Vertical, Parallel) to the image. Choose the orientation of the lines, then click into the image to place the first line. Place the second line in the intended position by another click. You can modify the line position while holding the mouse button, not later.
- When "Parallel lines" are selected, at first, define the beginning of the first line. The next click defines its orientation and length. When satisfied, finish the first line creation by

right-click. The second line can be placed by another click to the image, this time only to adjust the distance from the first line. The process is completed by right-click.

The following dialog box appears...



- Now enter the distance between the two lines and select correct units.

Press the [OK] button to continue...

### **Automatic Calibration (the Auto method)**

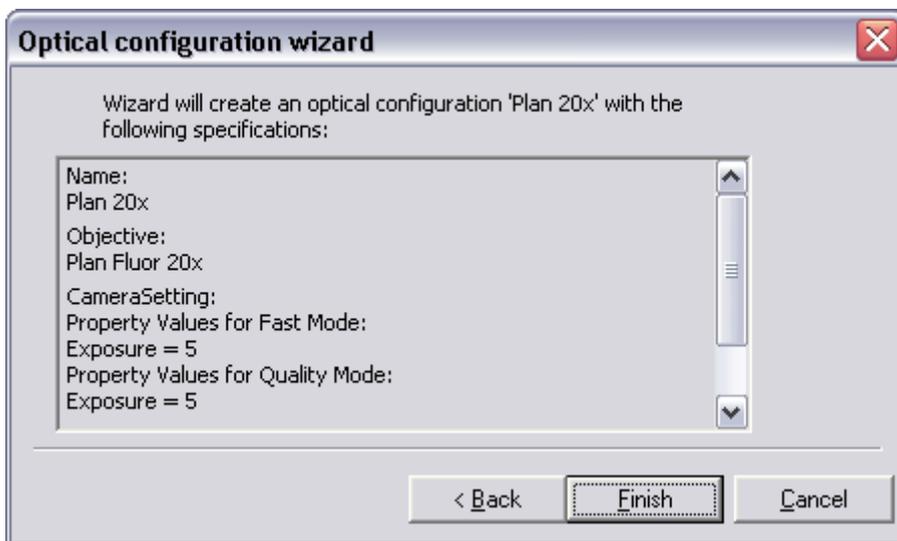
This method is fully automatic. NIS-Elements moves the motorized stage, acquires two images, and calculates the calibration from the shift of the images.

### **Automatic Calibration (4 points method)**

The system draws four points on the screen (subsequently) and asks user to move some significant part of the specimen to match the points. After all four steps are completed, the calibration is calculated from the moves of the stage.

## Optical Configuration Completion

---



The last status window informs you about the parameters of the optical configuration. Click [Finish] to complete the process.

- You can create more calibrations by repeating the above procedures.
- The calibrations are saved to registry immediately and can be exported to an XML file ([Calibration > Optical Configurations > Backup]).
- The selected calibration is maintained. It is applied during any image input from the camera.
- The selected calibration remains unchanged even if an image containing a (different) calibration would be loaded. The different calibration is applied to the actions regarding the loaded image only.
- It is possible to adjust the created calibration. Select the [ Calibration > Optical Configurations ] command.

## Managing Optical Configurations

---

To display the optical configurations manager, run the [Calibration > Optical Configurations] command. You can make the following actions from the dialog window:

- Create, duplicate, rename, delete, and switch between the optical configurations.
- Add, create new, edit, duplicate, delete, and calibrate objectives.
- Import and export optical configurations and the set of objectives to/from an XML file.

## Optical Configurations

---

Once created, the configuration appears in the list and can be shared with other users by changing the [Private] option to [Shared]\*. The following settings of a selected optical configuration can be adjusted:

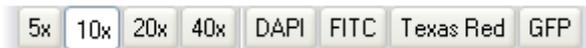
- It can be deleted by pressing the [Remove] button. A confirmation dialog box appears.
- Its name can be changed by the [Rename] button. The configuration name appears in the main toolbar (if the [Show on toolbar] option is checked).
- A copy of the configuration can be made via the [Duplicate] button.
- It can be turned ON by the [Set As Active] button.
- Settings of all configurations can be exported to an external XML file using the [Backup] button.
- The previously exported optical configurations settings can be loaded from an XML file via the [Restore] button.
- The list can be ordered manually via the nearby arrow buttons.
- Each configuration can be arbitrary modified within the [Details] section of the optical configurations window.

\* - Please see the [ User Management ] chapter for details.

## **Details**

The [Details] section enables you to completely modify the selected optical configuration. Select whether to include the objective, camera settings, microscope setting, and one of the shutters from the [Active Shutter] pull-down menu.

Buttons representing optical configurations are placed on the main toolbar by default. You can remove the button of the currently selected configuration by unchecking the [Show on toolbar] box.



## **Objectives and Calibrations**

---

- All images captured while a calibrated optical configuration has been selected are calibrated automatically.
- If you need to change image calibration or you want to calibrate the image acquired via scanner or transferred from a digital camera, use the [ Calibration > Recalibrate Document ] command.
- If there is no calibrated optical configuration, the simplest way to create it is to invoke the [ Calibration > Quick Calibration ] command. It creates a new optical configuration containing a calibrated objective.
- Calibration of the currently selected (active) objective can be modified via the [ Calibration > Recalibrate Objective ] command. The calibration procedure is described above.
- The active objective name is displayed on the application status bar. Double click it to invoke the optical configurations settings dialog window.



## Objectives

---

A list of often used objectives can be created within the Optical Configurations dialog window. Any objective can be associated with one or more optical configurations. Use the following buttons to manage the objectives:

- [Insert] - click the button and select one of the predefined objectives from the pull-down menu (it is ordered by the objectives magnification).
- Use the [New] button to create a custom objective. A wizard will lead you through the calibration process.
- The [Edit] button displays a dialog window where it is possible to adjust the objective properties, calibration, etc. The name, working distance, numerical aperture, and the refractive index values of the predefined objectives cannot be changed.
- If zoom is used, the objective calibration must be re-counted accordingly. Use the [Duplicate] button to make a copy of an objective and define the zoom factor in the dialog window which opens.
- The [Remove] button deletes the selected objective.
- The [Recalibrate] button starts the calibration of the selected objective. Please see [Objective Calibration] above.
- The [Backup] button enables you to export the complete list of objectives to an XML file. The standard [Save As] dialog window appears.
- The [Restore] button enables you to import a complete list of objectives from an external XML file previously created by the [Backup] command.
- The [Current unit] button invokes a pull-down menu where units for the whole application can be selected.
- The [Create config. from objective] uses the currently selected objective to create a new optical configuration. Just fill in the configuration name when asked.

## Units

---

NIS-Elements supports the following units: pixels, nanometers, micrometers, millimeters, centimeters, decimeters, meters, kilometers, inches, and mils. If the image is uncalibrated, pixels are the only units available. In case of a calibrated image, it is possible to select other units. All values (e.g. measured length/area) are then displayed in the selected units. There are two ways how to select the desired units:

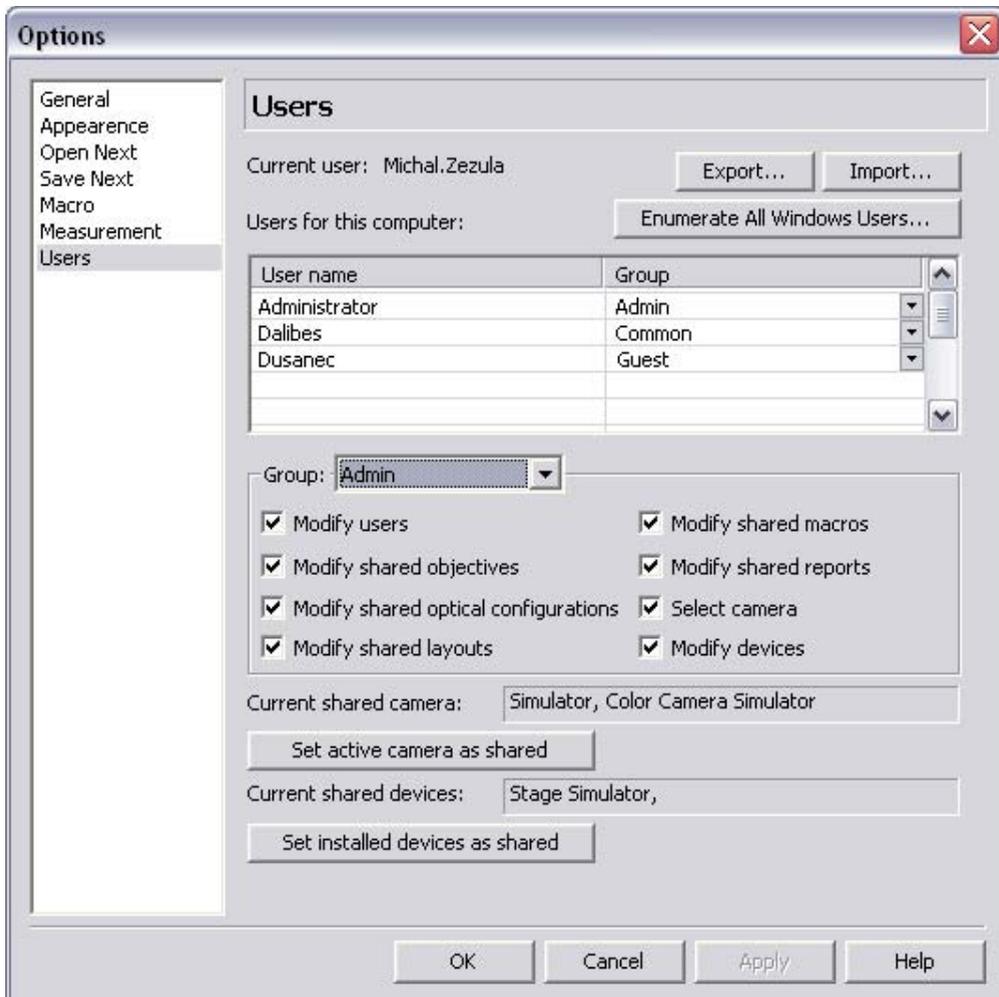
- Right click the status bar of the document window where the calibration is displayed. Select the units and precision of their display.
- Or, click the [Current unit] button located in the Optical configurations dialog window and select the units.

# User Management

NIS-Elements user rights management utilizes MS Windows user accounts. Different user rights to some NIS-Elements features can be assigned to three user groups:

- Admin
- Common
- Guest

Run the [Edit > General Options] command and select [Users] in the left column, the following dialog window appears:



Lets explain the user rights on the example of layouts: Layout settings can be stored in two places (storages): private or shared. When you create a new layout, it is set as [private] automatically and saved to your windows account profile. Nobody else can ever use or change the private layout. However, you can decide to share the layout with other users and set it [shared] (within the layout manager). All users can utilize the shared items, but only members of a group with permission to modify them can do so.

### **Export/Import**

These buttons enable anyone to save/load complete settings of users and user groups to/from an external XML file. Standard [Open] and [Save As] dialog windows appear. Having the data exported may be useful when copying the settings to other computers.

### **Enumerate All Windows Users**

This button starts copying MS Windows accounts to NIS-Elements. The accounts are assigned to user groups (admin, common, guest) according to their Windows permission settings.

## **Group Settings**

---

Select the group you would like to modify permissions of from the [Group] pull-down menu. You have to have the [Modify users] permission to do that.

### **Modify users**

Users can modify permissions of all groups in this dialog window, and apply the [Set active camera as shared] and the [Set installed devices as shared] buttons.

### **Modify shared objectives**

This option enables user to delete, recalibrate, etc. objectives within the [Calibration > Optical Configurations] dialog window.

### **Modify shared optical configurations**

This option enables user to modify existing optical configurations in the [Calibration > Optical Configurations] dialog window.

### **Modify shared layouts**

This option enables user to modify shared layouts in [View > Layout > Layout Manager] dialog window.

### **Modify shared macros**

This option enables user to manage shared macros via the [Macro > Manage Shared Macros] command.

### **Modify shared reports**

This option enables user to manage shared reports via the [File > Manage Shared Reports] command.

### **Select camera**

Users with this permission can select any camera driver from the [Acquire > Select Driver] dialog window. Otherwise they are forced to use the current shared camera (below).

### **Modify devices**

Users with this permission can add/remove/modify devices within the [Devices > Manage Devices] dialog window.

### **Set active camera as shared**

This button sets the currently selected camera as default for all users without the [Select camera] permission.

### **Set installed devices as shared**

This option sets all devices currently listed in the [Devices > Manage Devices] dialog window as shared, which means that other user will be able to use them. If there was a list of shared devices before, it is overwritten by the new list.

# Images Capturing

Having all hardware accessories set up and NIS-Elements installed, you can start capturing images. The easiest way is to capture a single image. There are also several other ways of acquiring images, some of them specialized needing a specific hardware. They are described in the subsequent chapters.

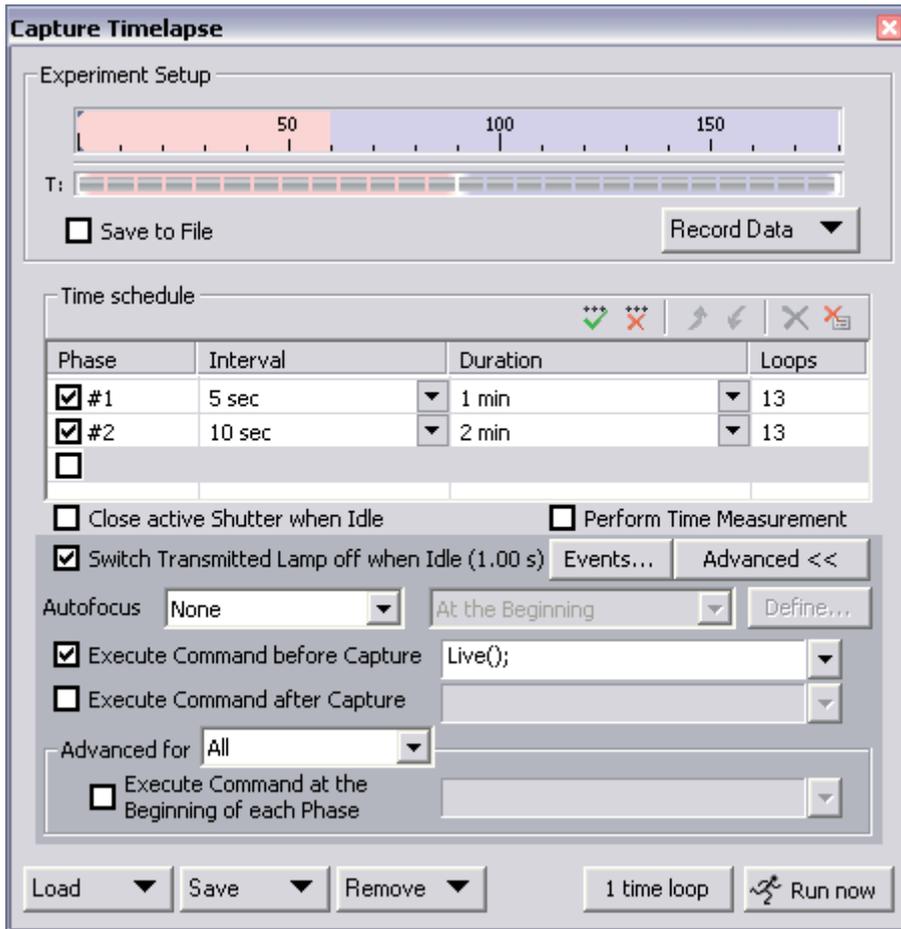
## Single Image Capture

---

- Turn the connected camera and other devices ON and start NIS-Elements. Select the corresponding camera driver.
- When the application is started display the Camera Settings control window - [View > Acquisition Controls > Camera Settings].
- Switch camera to live mode - [Acquire > Live Fast] - and adjust the settings to get the live image clear.
- Focus the objective on the scene you would like to capture the image of.
- Switch to the [Live - Quality] mode and adjust its settings, especially the [Exposure time] and [Format]. These settings will be used every time on image capture.
- Switch back to the [Live - Fast] mode. It is the usual mode for smooth work because less of the hardware resources are engaged.
- Capture the image by pressing the capture button on the horizontal document toolbar, by invoking the [Acquire > Capture] command, or using the [Ctrl & -] shortcut.
- A new image is opened and named "Captured" automatically.

# Time Lapse Acquisition

Detailed studying of long-lasting processes is enabled by the time lapse acquisition mode of NIS-Elements. The possible experiment duration is almost limitless. Invoke the [Acquire > Capture Time Lapse] command to set the experiment up:



## Time Schedule

[The Time schedule] table enables you to define consecutive time phases where duration, interval between single images, and number of images of the phase can be adjusted for each phase. The Interval, Duration, and Loops settings are bound together, so you just need to set two of these parameters. The remaining parameter is calculated automatically. Only the checked time phases will be captured.

## Auto Focus

---

Automatic focusing can be used during experiment. You can select the autofocus method that best meets your needs from the pull-down menu. The next pull-down menu determines whether the focusing shall be performed at the beginning of the whole experiment or at the beginning of each time-phase. The [Define] button shows a dialog window where you can define parameters of the selected focusing method.

## Special Options

---

The shutter can be closed between the acquisitions. Just check the [Close active Shutter...] box.

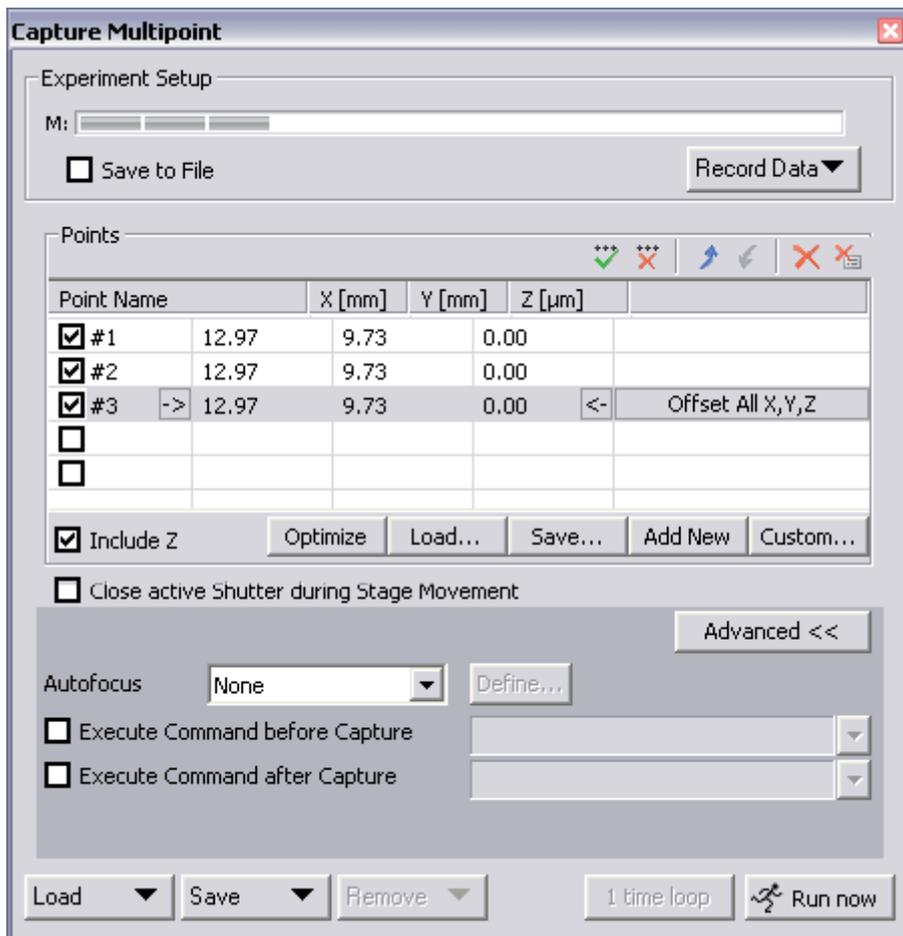
The [Advanced for] combo box enables to run a command (or a macro) at the beginning of each loop. You can define the command (or the macro) for all loops of one dimension or set it individually for each loop.

You have an option to run a command\* (or a macro) before and after the each image acquisition. It is possible to type the command directly, or press the rightmost button to show the context menu, where you can select a command from the [Command List], or search for macro files on a disk ([Run Macro]).

*\* - The `Live();` command can be used often. However, in some exceptional circumstances the `Live();` command may not work properly. In such case, please, use the `LiveNoMsgLoop();` command instead.*

## Multipoint acquisition

This feature is available when a motorized XY(Z) stage is present in the system and connected properly. An arbitrary array of XY(Z) positions can be defined to be scanned during a multipoint capture experiment. The Z position can be included optionally. The defined array can be saved (and loaded later) to an XML file by the [Save] ([Load]) button. Invoke the [Acquire > Capture Multipoint] command.



### To define a multipoint array:

- Move the stage to the first point (via joystick or the "Move" command).
- Press the [Add New] button. The new line containing current coordinates appears in the list.

- Move to the next position and repeat the steps until you have all the intended points defined.

## To change a single Z coordinate:

---

- Click inside the line you would like to change.
- Move the Z drive to the new position.
- Click the [**<-**] button.

*The XY coordinates of one point cannot be adjusted (unless you delete one and add a new point).*

## Offset All

---

This button appears next to the currently selected point. It can shift the XY coordinates of all points in the same way:

- Select one point of the list. (The stage moves to its coordinates automatically)
- Move the XY(Z) stage to a new position (define the offset).
- Press the [Offset All] button.
- The coordinates of all points are overwritten (The same shift as you made with the stage is added/subtracted).

## Special Options

---

If the [Optimize] button is pressed, the system will re-order the defined points in order to minimize the XY stage trajectory.

A grid of points aimed to be used for scanning well-plates can be defined using the [Custom] button.

The shutter can be closed between the acquisitions. Just check the [Close active Shutter...] box.

Automatic focusing can be used during the experiment. You can select the autofocus method that best meets your needs from the pull-down menu. The [Define] button shows a dialog window where you can define parameters of the selected focusing method (depending on your stage equipment and hardware drivers).

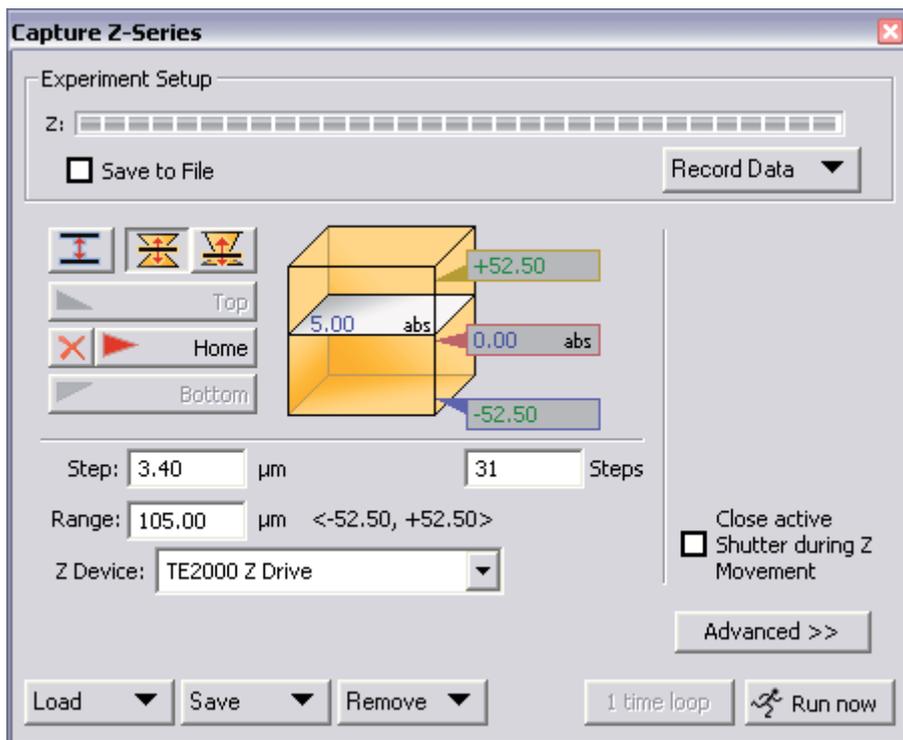
The [Advanced for] combo box enables to run a command (or a macro) at the beginning of each loop. You can define the command (or the macro) for all loops of one dimension or set it individually for each loop.

You have an option to run a command\* (or a macro) before and after the each image acquisition. It is possible to type the command directly, or press the rightmost button to show the context menu, where you can select a command from the [Command List], or search for macro files on a disk ([Run Macro]).

*\* - The `Live()` command can be used often. However, in some exceptional circumstances the `Live()` command may not work properly. In such case, please, use the `LiveNoMsgLoop()` command instead.*

# Z Series Acquisition

Capturing images from different focal planes of the specimen can be performed using the [Acquire > Capture Z Series > Capture Automatically] command.



This dialog serves for setting the method of capturing in Z Series. There are the following ways of defining the Z drive range:

## Top/Bottom Position, Step



Press this button, the cube turns blue. The range is defined by the top and the bottom position:

- Run the Live camera signal, select the [Z device] (in case you have more than one connected) from the pull-down menu.
- Set the Z range: move the Z drive to the top position and press the [Top] button, move it to the bottom position and press the [Bottom] button.
- Define the [Step] size in  $\mu\text{m}$  or the number of [steps] to be captured.

## Home Position, Range

---

Press one of the following buttons, the cube turns yellow. The range is defined by the home-position and the scanning range:



**Symmetric mode** Define the [Range] by inserting a value in  $\mu\text{m}$ .



**Asymmetric mode** The range is specified by two values, [Below] - the distance below home position, and [Above] - the distance above home position.

- Run the Live camera signal, select the [Z device] (in case you have more than one connected) from the pull-down menu.
- Move the Z drive to the position which you would like the Z drive to move around. Press the [Home] button to define the home position.
- Specify the scanning [Range].
- Define the [Step] size in  $\mu\text{m}$  or the number of [steps] to be captured.

## Special Options

---

The [Reset] button discards the Top, Home, and Bottom positions settings.

The shutter can be closed between the acquisitions. Just check the [Close active Shutter...] box.

Automatic focusing can be used during the experiment. You can select the autofocus method that best meets your needs from the pull-down menu. The [Define] button shows a dialog window where you can define parameters of the selected focusing method (depending on your stage equipment and hardware drivers).

The [Advanced for] combo box enables to run a command (or a macro) at the beginning of each loop. You can define the command (or the macro) for all loops of one dimension or set it individually for each loop.

You have an option to run a command\* (or a macro) before and after the each image acquisition. It is possible to type the command directly, or press the rightmost button to show the context menu, where you can select a command from the [Command List], or search for macro files on a disk ([Run Macro]).

*\* - The `Live()`; command can be used often. However, in some exceptional circumstances the `Live()`; command may not work properly. In such case, please, use the `LiveNoMsgLoop()`; command instead.*

# Large Image acquisition

There is a special option in the ND2 acquisition window, to capture a Large Image. A motorized XY stage is required to have this option enabled. When set to work, it captures several frames within a defined area and stitches them together, so you get an extra large image. The area can be defined in two ways:

## Setting Array of Frames

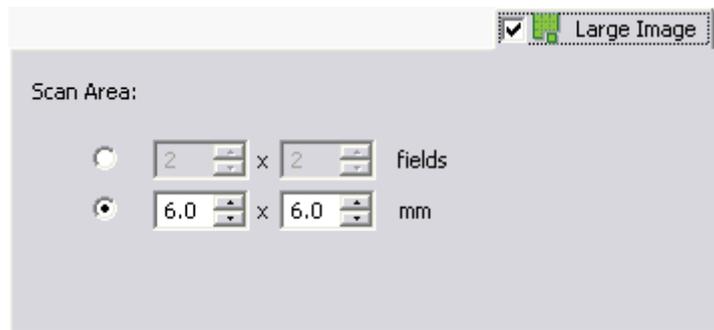
---

Check the first radio button and define the number of rows and columns of the scanning grid.

## Set the Area Size

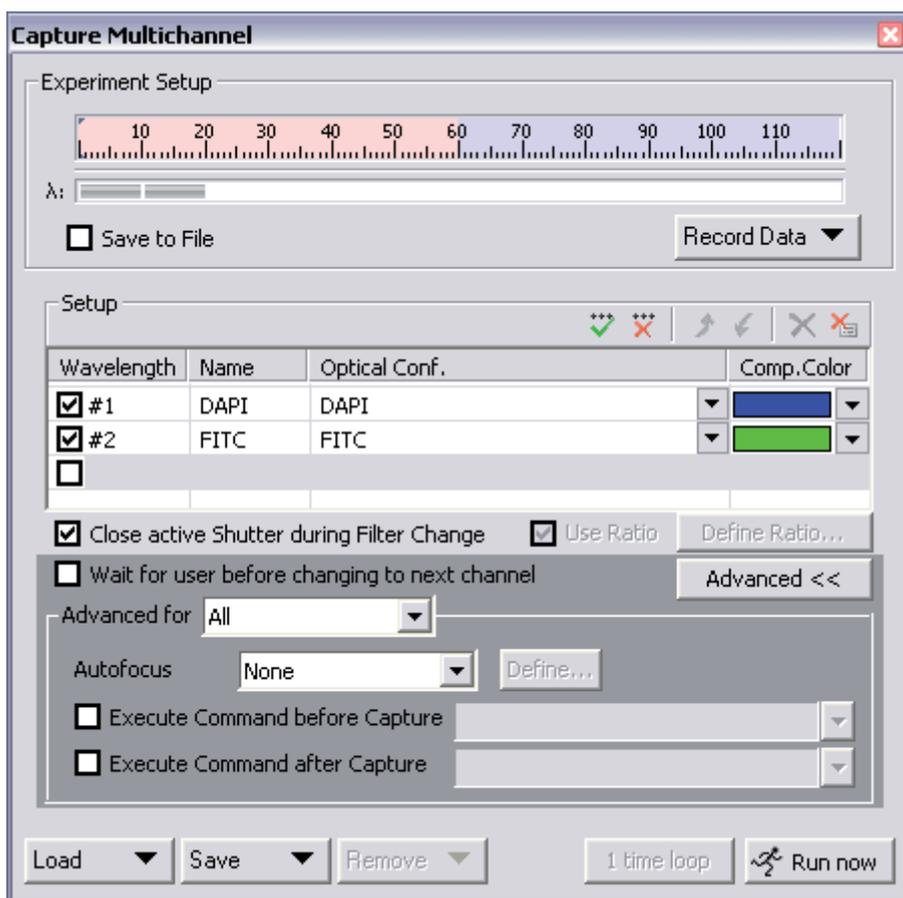
---

Check the second radio button and define the size of the scanned area in millimeters.



# Fluorescence acquisition

Fluorescence (multi-channel) pictures can be acquired using the [Acquire > Capture Multichannel Image > Capture Manually/Automatically] commands. The Multichannel setup should be adjusted first. Run the [Acquire > Capture Multichannel Image > Multichannel Setup] command:



## Channels Setup

This table defines the number and the type of captured channels. Set a descriptive name of each channel and select the optical configuration that is to be used for its acquisition. If no suitable configuration exists, you can create a new one by selecting the [<define new>] option from the Optical Configuration pull-down menu. Comp. color specifies the color tone, in which the channel will be displayed.

## **Advanced ND2 Acquisition**

If there are T and Z dimensions engaged in the experiment, another two columns called T Phase and Z Phase appear in the table.

[T Phase] - the number of captured channels can be reduced by selecting [First] or [n-th] - the channel is included in the first or every n-th time phase only.

[Z Phase] - the number of captured channels can be reduced by selecting [Home] - the channel will be captured within the home position of each Z serie.

*The phase settings can be combined in any way, but at least one channel must be included in every frame (the [All] setting in both columns).*

## **Manual Filter Change**

The [Wait for user before changing to next channel] option enables you to switch the wavelength filters manually during the multichannel experiment. Use this option if there is no automatic filter changer available.

## **Special Options**

The shutter can be closed between the acquisitions. Just check the [Close active Shutter...] box.

Automatic focusing can be used during the experiment. You can select the autofocus method that best meets your needs from the pull-down menu. The [Define] button shows a dialog window where you can define parameters of the selected focusing method (depending on your stage equipment and hardware drivers).

The [Advanced for] combo box enables to run a command (or a macro) at the beginning of each loop. You can define the command (or the macro) for all loops of one dimension or set it individually for each loop.

You have an option to run a command\* (or a macro) before and after the each image acquisition. It is possible to type the command directly, or press the rightmost button to show the context menu, where you can select a command from the [Command List], or search for macro files on a disk ([Run Macro]).

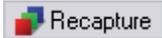
*\* - The `Live()`; command can be used often. However, in some exceptional circumstances the `Live()`; command may not work properly. In such case, please, use the `LiveNoMsgLoop()`; command instead.*

## Manual Capture

---



The [Acquire > Capture Multichannel Image > Capture Manually] command opens a new document window with the live image and the [Capture] button. After pressing the [Capture] button one channel is acquired. The number of channels depends on the Multichannel Setup.



After all channels are acquired, the [Recapture] button replaces the [Capture] button. It enables you to capture the multichannel document again. All previously obtained multichannel data will be lost. When a single channel is selected, it can be recaptured separately.

# Capturing to Ring Buffer (AR)

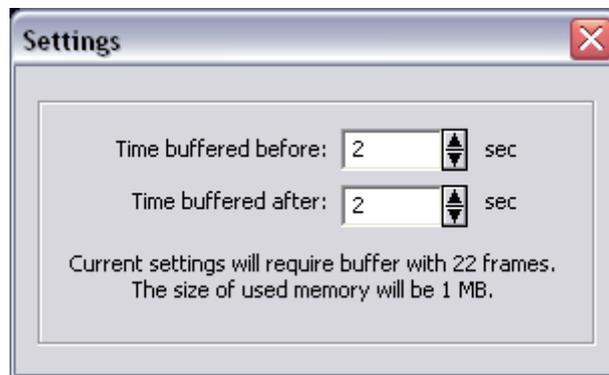
The Capturing-to-RAM technique enables user to record sequences displaying very quick actions, lasting tens of milliseconds. The quality and number of frames in a sequence depends on hardware capabilities of the computer system.

## Set the ring buffer size

---

The technique uses a ring buffer to store temporary data. The ring buffer is a part of virtual memory that is being constantly (and repeatedly) filled with the live image data. Depending on how fast the action to be captured is, you should set the ring buffer size in milliseconds.

- Click on the [Settings] command next to the RAM capture buttons.
- Set the [Time buffered before/after] values. These represent the time before and after you click the RAM Capture button. The whole time interval will be included in the sequence.
- Confirm it by OK.



## Turn the buffer ON

---

- Click on the leftmost button of the RAM capture tool bar to enable recording of Live image to RAM.
- The ring buffer functionality is activated.

*When the Camera is switched to Live mode, the left-side ring icon begins to indicate the buffer activity. If the RAM Capture button is pressed then, the captured sequence contains frames from the whole time interval before and after the button press. If you press the button while in the Frozen mode or without the time buffer ON, only the [Time buffered after] sequence is grabbed.*

## **Capture a sequence**

---

- Press the [RAM Capture] button.
- A new document window containing the captured sequence opens.

# AVI Acquisition

AVI acquisition is an easy way of recording actions in real time. It produces \*.avi video files, which can be played by any standard video player, so you do not need NIS-Elements to view the results.

## Capturing AVI movie

---

- Display the AVI Acquisition control window the [View > Acquisition Controls > AVI Acquisition] command.
- Adjust the advanced settings, especially define the file name and the destination folder.
- Click the [Record] button. The live image appears inside the application window and is being recorded according to the settings.
- Stop recording by the [Stop] button, or wait until the time defined in the [Duration] field passes.
- When stopped, the AVI file remains opened in a new document window.

## Settings

---

Press the [Advanced] button to view the whole control window and set the following options:

### Live

Choose the format of the camera. The Live-Fast or Capture-Quality mode settings will be used while capturing the AVI movie.

### Rate

The number of frames per second of the movie can be defined. Possible values range from 1 to 50 fps. There is also the [Max Fps] option. When selected, the system captures every frame sent from a camera. If the frame rate is beyond hardware capabilities of your system, some frames will be omitted.

### Duration

Several predefined lengths of the movie may be selected (60 min, 10 min, 1 min, 10 s) or the [Infinite] option is available. When selected, the movie will be captured until the [Stop] button is pressed or the hard drive is filled to capacity.



## **File - the path and the name**

Define the path to a directory and the file name of the AVI file. If you capture more than one movie without changing the file name setting, the other files will be numbered automatically.

## **File - Codec**

It is common to encode (compress) movies in order to save some disk capacity. This can be done using various types of codecs. Press the [Open] button on the right and a dialog window appears. Select one of the codecs available in the system or use the [Default] option. Remember that the codec you choose must be available for the AVI playback.

*Video codec is a software tool which can [cod]e and [dec]ode video files. We recommend to use one of the following codecs (FPS - maximum camera framerate which can be handled, size - size of a reference 10s video, platform availability):*

- *Cinepack: FPS-1.1; size-1.2 MB; availability: Windows XP + Mac*
- *Intel Indeo: FPS-5.9; size-1.7 MB; availability: Windows XP*
- *Microsoft YUV: FPS-14.3; size-172 MB; availability: Windows XP*

## **Size**

The size is determined by the resolution of the connected camera. However, the size may be adjusted by the following options:

- Original, 1/2 Original, 1/4 Original - sizes counted from the applied camera resolution
- NTSC TV, PAL TV - standard TV resolutions
- Free Size - an arbitrary [Width] and [Height] in pixels may be defined.

No matter what size is selected, the video always displays the whole scene. If the defined size has different aspect ratio than the live image, the empty space is filled with black.

## **Maintain aspect ratio of**

If the [Free Size] option is selected, the aspect ratio may be defined. One of the width and height dimensions are then counted automatically.

# NIS-Elements Document Structure

## Image Layers

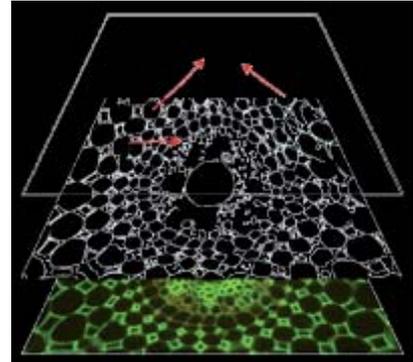
---

Each NIS-Elements document can consist of the following image layers, serving different purposes:

**Annotation layer** - In this layer, graphical objects and texts can be stored in a vector format. The results of interactive and automatic measurements, arbitrary text notes and other annotations are stored there.

**Binary layer** - Pixels in this layer can be found in two states only (i.e. Black/White). A binary image is usually result of thresholding. It is used mainly for performing automatic measurements of the thresholded objects. More than one binary layer can be created for one image.

**Color layer** - Contains image data. When you open an image from disk, it is loaded to this layer. It can handle images with the depth of up to 16 bits per color component. The dimensions of this layer determine the view of the other layers.



*When saving an image, only the JPEG2000 and ND2 file format can handle all image layers and are capable of saving them. The other image formats will save the content of the color layer only.*

## Document types in NIS-Elements

---

### RGB documents

Images acquired by a color camera typically consist of three components that represent red, green and blue channel intensities. Pixel values for each component range from 0 to 65535 (in 16 bit depth). You can display a single color channel using the tabs located in the bottom-left corner of a document window, or an arbitrary combination of them can be selected while holding the [Ctrl] key down.



### Multi-channel documents

These documents usually arise from fluorescence microscopy. Instead of 3 color components (R, G, B), multichannel images can be composed of many user-defineable color planes. When a multichannel image is opened, the channel panes in the bottom-left corner are not the standard ones (Red, Green, Blue), but are named differently according to the channel colors.

*If there is a document that contains more than 8 components, the tabs are replaced by the wavelength dimension, similarly to how the dimension loops of ND2 documents are displayed.*

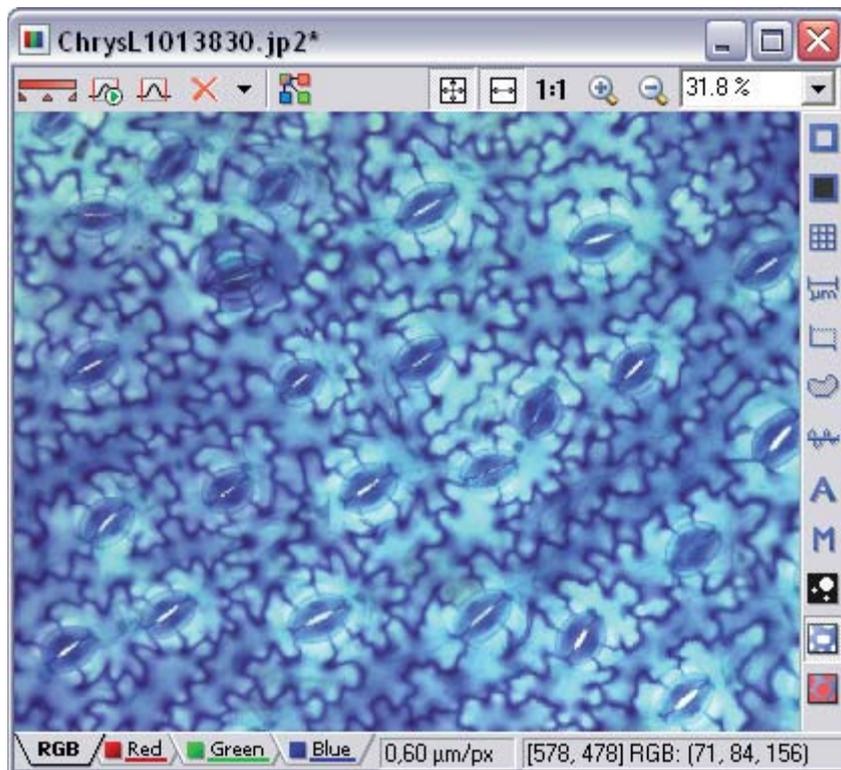
### **ND2 (N dimensions) documents**

The ND2 document is a set of images. There are four types of ND documents depending on the method of acquisition: [T] for timelapse acquisition, [XY] for multipoint acquisition, [Z] for Z-series (slices) acquisition and [Lambda] for acquisition of a defined wavelength (fluorescence imaging). All these methods can be combined together.

### **Document window**

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Since NIS-Elements supports multiple windows, all controls affecting the view of the image (layers, channels, etc.) are located in every image window. Therefore it is possible to control each opened image individually.



## LUTs

---



[Enable LUTs] button applies LUTs to the image.



[Keep Auto Scale LUTs] button applies the AutoScale command to the image continuously.



[Auto Scale] button performs automatic setting of LUTs .



[Reset LUTs] button discards the LUTs settings.

## Split components

---



[Split Components] button turns ON a special view, where color channels of the image are tiled.

## Zoom controls

---



[Fit to screen] button adjusts zoom to view the whole image within the NIS-Elements screen. It corresponds to the [ View > Zoom > Fit to screen ] command.



[Best Fit] button adjusts zoom to fit the NIS-Elements image window in one direction but to fill the screen. Corresponds to the [ View > Zoom > Best Fit ] command.



[1:1 Zoom] button adjust zoom so that one pixel of the image matches one pixel of monitor. It corresponds to the [ View > Zoom > 1:1 Zoom ] command.



[Zoom In] Increases magnification of the image. It corresponds to the [ View > Zoom > Zoom In ] command.



[Zoom Out] Decreases magnification of the image. It corresponds to the [ View > Zoom > Zoom Out ] command.

## General controls

---



[Show Probe] button activates the probe. The probe affects histograms, auto exposure and auto white balance functions.



[Show Background Probe] button activates the background probe. Some commands (Image > Background > Subtract Background) uses the BG probe data as reference.



[Show Grid] button displays the grid for rough measurements.



[Show Scale] button displays the image scale.



[Show Frame] button displays and applies the measurement frame.



[Show Measurement ROI] button displays the Measurement Region Of Interest.



[Show Profile] button displays the [View > Analysis Controls > Intensity Profile] control window. It allows you to specify a linear section in the image of which the pixel intensities graph will be created.

*Right click the icons to invoke a context menu where properties of each tool can be modified.*

## Status bar

---

The status bar at the bottom of the application window displays the following information (from left to right): image calibration, pixel coordinates of the mouse cursor along with channel intensities, color mode, bit depth, and picture size in pixels.

0.34  $\mu\text{m}/\text{px}$

[990, 997] RGB: (111, 111, 144)

RGB 8bit, 1280 x 1024 pixels

## Working with Vector Objects

---

There are two types of vector objects which can be placed over the image: annotation and measurement objects.

### Annotation Objects

---



This button toggles viewing of the annotation objects (text notes, arrows, etc.). Right-clicking the button displays a context menu with the following options:

- [Clear All Objects] will delete annotation and measurement objects.
- [Select Annotation Objects] will select all objects that are not the measurement ones.
- [Select All Objects] and [Deselect] will select/deselect all annotation and measurement objects.

### Measurement Objects

---



This button toggles viewing of the measurement objects of the interactive and automatic measurements. Right click it to display a context menu with the following options. The [Clear All Objects], [Select All Objects], and [Deselect] options work the same as described above. There are two different options:

- [Remove Automatic Measurement objects] will remove only objects created by automatic measurement.
- [Select Interactive Measurement objects] will select all measurement objects created by hand.

## Working with Binary Layer

---



This button turns on the view of a binary image. It can be edited by hand using the [ Binary > Binary editor ] command, or by pressing the [Tab] key.



The binary layer can be displayed together with the color layer using the overlay mode. A number of binary layers can be created for one color image. Manage them using the [View > Visualization Controls > Binary Layers] control window.



This button switches to view of a color image.

# ND2 files

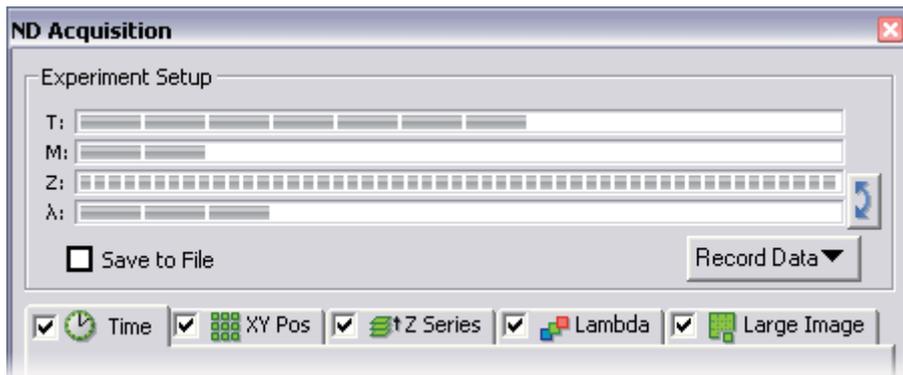
A special document type consisting of one or more image sequences is a so-called ND (n-dimensional) document. N-dimensional experiments combine up to 5 acquisition types together: Z series, time-lapse, fluorescence (Lambda), multipoint, and large image. The resulting image sequence (\*.nd2 file) includes information about the experiment structure and its progress.

## ND2 Acquisition

---

All the four dimensions\* can be combined together creating a 6-dimensional ND document (dimensions: X, Y, Z, multipoint, time, wavelength).

- Run the [Applications > Define/Run Experiment] command.
- The following control window appears.



- Select one or more dimensions (tabs) which should be included in the resulting ND2 file. Above the tabs, there is a preview of how the nd2 file structure will look like.
- Set the parameters of each dimension acquisition. \*\*
- Select whether to save the ND document directly to a file or just to keep it in memory to be saved later. The path and the file name shall be defined if saving to a file is preferred.
- Click the [Run now] button to start the acquisition.

\* - Two of the four available dimensions can be combined in the Basic Research package.

\*\* - Please see the *Timelapse-*, *Multipoint-*, *Z series-*, *Fluorescence-*, and *Large Image Capturing* chapters for further details.

## Save to File

---

An image can be captured and saved manually after the experiment is finished, or it can be saved to harddisk "on the fly":

- Check the [Save to File] box. The dialog window reveals an extra section.
- Insert the [Path] or use the [Browse] button to define the destination folder.
- Specify the [Filename].

## Record Data

---

The nd2 file contains various [Recorded Data] which carry e.g. acquisition times of single frames. Some of them can be recorded optionally:

- Press the [Recorded Data] button. A pull-down menu appears.
- The data to be recorded are check-marked.
- To view the recorded data of an opened ND2 file, invoke the [File > Image Properties] command.

## Browsing ND2 document

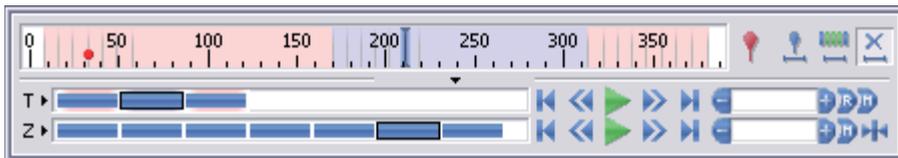
---

When an ND2 file is opened, its structure is pictured at the bottom of the document window. There is a time line with all captured images indicated by gray markers. The blue-highlighted marker indicates the currently observed image. Below the time line, there is one row of blue rectangles for each dimension. Blue rectangles stand for single phases of each experiment dimension. In case the multichannel dimension is included, the channels are indicated by the color tabs at the very bottom of the document window.

## ND Control Bar

---

Browse the ND document by clicking inside the time line - single time phases are highlighted by the alternating red and blue background. You can also display a frame by selecting the corresponding blue rectangle. There are other ways how to observe the ND document using the following buttons:



## Insert User Event



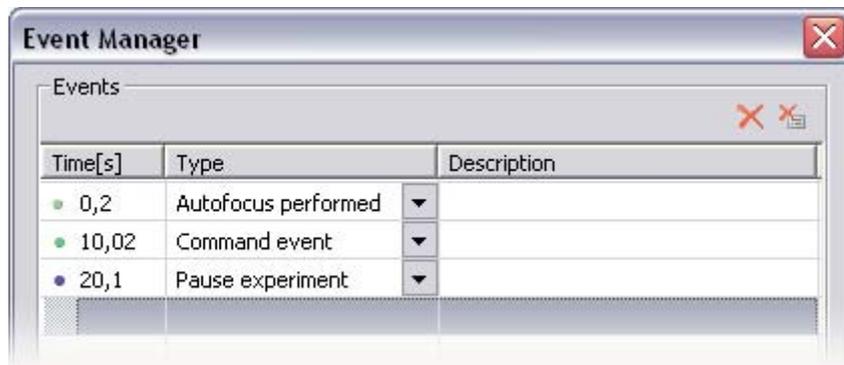
This button inserts a marker to the current frame. User events can be pinned to any of the frames of the document in order to highlight a remarkable scene. There are number of event types. Some of them are inserted automatically (marked with \*) while the ND experiment is running, others must be defined by user:

- Autofocus performed\*
- Command event\*
- Pause experiment\*
- Resume experiment\*
- Cancel experiment\*
- Acquisition zero time\*
- Next phase\*
- Experiment paused for refocusing
- Macro event
- Event from macro
- Stimulation
- User event 1, 2, 3, 4

Once there is one or more events within the time line, you can manage them via a context menu. Right click the event marker, a menu appears with the following options:

- Remove All Events
- Event Playing Range
- Event Manager
- Remove Event
- Edit Event

When you open the [Event Manager], all events within the ND document may be edited through the following window. The time can be put in, and the event type selected from a combo box.



### **User Event Selection**



This button does a selection of frames of the ND file according to the [Event Playing Range] settings. Right click an event marker and pick the [Event Playing Range] command from the context menu.

A simple dialog window appears. Define the number of frames/seconds which will be selected around each event. It is specified by a single value (absolute selection size) or by the before/after values (asymmetric range).

## **Range Selection**



Clicking this button selects a user defined range of frames. The selection is done by mouse. A range can be selected by mouse while holding the [Shift] key down. Single frames can be added/removed by mouse while holding the [Ctrl] key down similarly as when using Windows Explorer.

ND2 files may contain extremely large number of frames. It may be helpful to omit some of the frames from the selection. Right click the [Range Selection] button and a context menu appears. It enables you to select every 2nd, 5th, 10th, 20th, 50th, 100th frame. The [Select All Frames] option selects the whole range of frames as if you selected the first and the last time phase with the [Shift] key down, but preserves the [Select Every...] setting.

## **Disable Selection**



Press this button to deselect all frames of the ND document.

## **Playing options**



[Play Sequence] displays the selected/all images of the browsed dimension.



[Stop Playing] stops playing the sequence at the current frame.



[Previous Position] displays the previous frame of the browsed dimension.



[Next Position] displays the next image of the browsed dimension.



[Increase Playing Speed] - the speed ranges from 0.5 FPS to 500 FPS.



[Decrease Playing Speed] - the speed ranges from 0.5 FPS to 500 FPS.



[Real Time Playing Speed] sets the playing speed to real-time (as the document was captured).



[Maximum Playing Speed for Every Frame] sets the playing speed to maximum while the display of every frame is guaranteed (when the speed is set to maximum by the + button, some frames are usually omitted when playing the sequence depending on your graphic card).



[Home Position] displays the frame of the Z dimension that was set as "home" during the acquisition.

## Observing ND2 Data Sets

---

There are several views that can display the ND2 data sets in different ways. When you switch the view, a new document window opens. Some views are available for some dimensions only. If a view is available for two or three dimensions of the ND document, a pull-down menu appears in the top document tool bar. There you can select the dimension to be displayed.

### **Main View**



When you open an ND2 file, it opens in this view.

### **Maximum/Minimum Intensity Projection View**



*Available for Z, T.* These projections analyse all frames of one dimension and pick pixels with the maximum/minimum intensity values. These pixels are used in the resulting image.

### **Slices View**



*Available for Z, T.* This view displays orthogonal XY, XZ, and YZ projections of the image sequence.

### **Volume View**



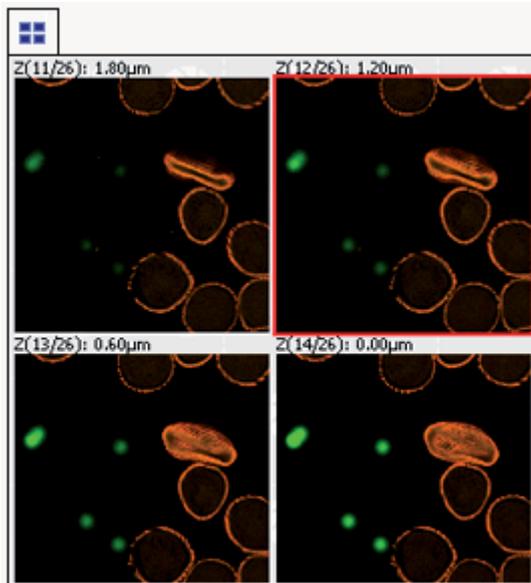
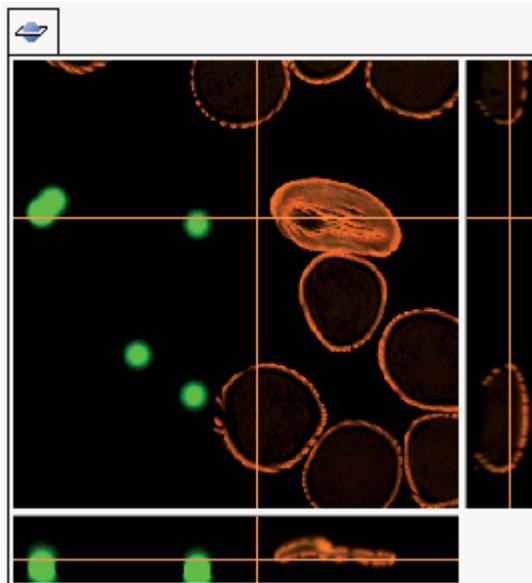
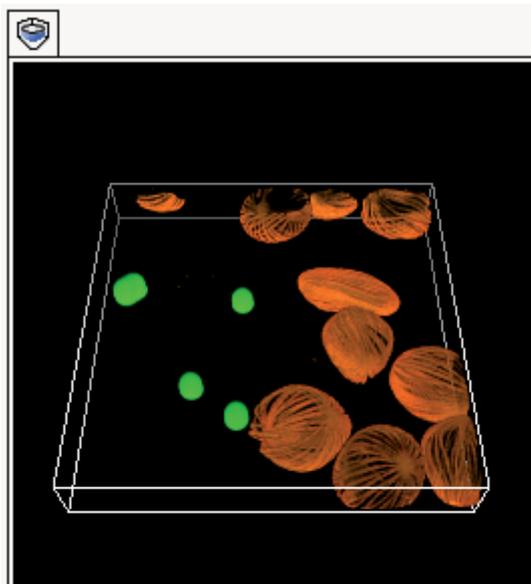
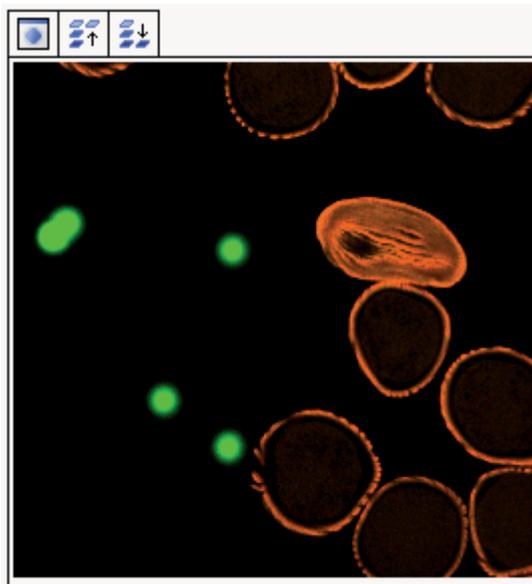
*Available for Z.* This view creates a 3D model of the acquired object.

### **Tiled View**



*Available for Z, T, and Multipoint.* This view displays frames of the selected dimension arranged one next to other.

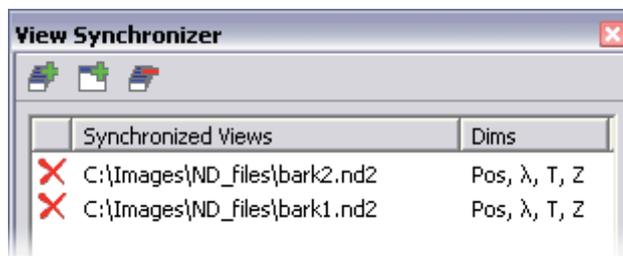
**Examples:**



## Synchronizer

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Synchronizer enables to compare (run and view) two or more N-Dimensional documents. It automatically synchronizes view of all documents added.



### **To add ND documents to Synchronizer**

- Open at least two ND documents you would like to observe.
- Use the [Add All] button (the first one) to add them to the synchronizer. *In case there are more documents opened, use the [Add Current View] button (the second one) instead. The ND2 file being added must be active.*
- The names of selected documents appear in the View Synchronizer window.

### **To observe synchronized documents**

All documents added to Synchronizer are automatically being synchronized.

- Set one of the selected documents active.
- Any action regarding the view (Z position, displayed frame, etc.) is performed on the other documents as well as on the active one.

### **To remove a document from View Synchronizer**

- To remove one document from the synchronizer, click the check box next to its name.
- To empty the whole synchronizer, click the [Remove All Views] button (the third one).

## Processing of ND2 Documents

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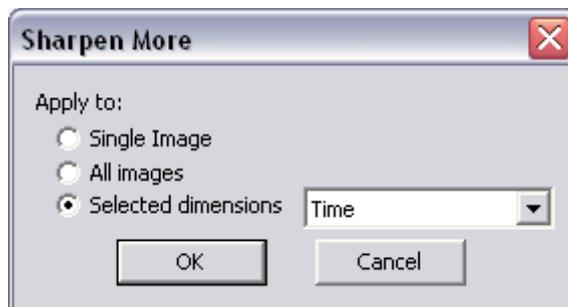
Most of the image processing and binary image processing commands can be applied to one image, all images, or selected dimension of the ND2 document. When you use such command with ND2 file, the following options are added to its dialog window:

### **Single Image**

The operation is performed on the current frame of the ND2 document.

### **All Images**

The operation is performed on all frames of the current ND2 document.



### **Selected Dimension**

The operation is performed on all frames of the selected dimension.

*E.g. When you have a document containing T and Z dimensions. Selecting the Z-Series dimension performs the operation on all Z frames of the current T dimension.*

# Working with Documents

It is possible to work with multiple image documents opened at the same time. The Multi-Document Window Environment ensures that every document is opened to a single window that contains the most used controls affecting the image appearance (Layers, LUTs, Zoom etc.).

## Opening Saved Files

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NIS-Elements offers several ways to open an image file:

### Using the Open dialog-box

---

To invoke the Open dialog box, you have the following options:

- Select the [File > Open] command...
- Press [Ctrl + F12] keys simultaneously...
- Press the [Open] button located at the beginning of the application main toolbar...

### Using Organizer

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An image can be opened by double clicking its filename within the Organizer layout. Please see the [ Organizer ] chapter for more details.



### Using the Recent files list

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You can quickly access the last opened images using the [File > Recent files] submenu. The number of the files listed in the submenu can be adjusted in the [Edit > General Options] window.



## Using the Open next/previous/first/last commands

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These commands enable you to continuously open the next/previous images from a particular directory or a database table. This is useful when editing multiple files placed in one directory. The commands are located in the [File > Open/Save Next] submenu. You can define sorting, filtering and other features in the [ File > Open/Save Next > Open Next ] settings dialog box.

## Straight from Windows explorer

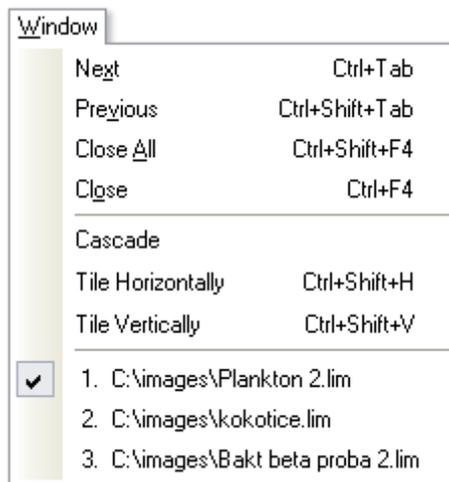
---

During installation, NIS-Elements creates file associations to files that are considered a native format for storing images (JPEG2000, ND2). The JPEG2000 (JP2) and ND2 image files can be then opened in NIS-Elements just by double clicking their names in Windows Explorer.

## Switching between loaded documents

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The commands for managing the opened images are grouped in the [Window] menu. The presently opened files are listed in its bottom part. The currently displayed image is indicated by a check mark. To change the current image, select it from the list or use the [Next] or the [Previous] command (represented by [Ctrl + Tab] and [Ctrl + Shift + Tab] shortcuts). Another possibility is to arrange document windows automatically using the [Tile horizontally] or [Tile vertically] commands. This will change the size and position of the opened documents and they will be aligned ordered in the selected direction.



## Closing documents

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- The currently displayed image can be quickly closed by pressing the cross button, located in the top-right corner of the document window.
- The image can be also closed by invoking the [Window > Close] command or by pressing the [Ctrl+F4] shortcut.
- If you want to close all images, use the [Window > Close all] or the [Ctrl+Shift+F4] shortcut.
- If you try to close an image that has been changed, NIS-Elements will display a confirmation dialog box, offering to save the changes.

## **Image Formats**

---

NIS-Elements supports the following number of standard file formats. In addition, NIS-Elements uses its own image file formats to fulfil specific application requirements.

### **JPEG2000 Format (JP2)**

An advanced format with optional compression rates. Image calibration, text descriptions, and other meta-data can be saved together with the image in this format.

### **ND2 Format (ND2)**

This is the special format for storing sequences of images acquired during ND experiments. It contains various information about the hardware settings and the experiment conditions and settings.

### **Joint Photo Expert Group Format (JFF, JPG, JTF)**

Standard JPEG files (JPEG File Interchange Format, Progressive JPEG, JPEG Tagged Interchange Format) used in many image processing applications.

### **Tagged Image File Format (TIFF)**

This format can save the same amount of meta-data as JPEG2000. TIFF files are larger than JPEG2000 files but are loaded faster. TIFF files have several ways to store image data, therefore there are many versions of TIFF. NIS-Elements supports the most common TIFF modalities.

### **CompuServe Graphic Interchange Format (GIF)**

This is a file format commonly used on the Internet. It uses a lossless compression and stores images in 8-bit color scheme. GIF supports single-color transparency and animation. GIF does not support layers or alpha channels.

### **Portable Network Graphics Format (PNG)**

This is a replacement for the GIF format. It is a full-featured (non-LZW) compressed format intended for a widespread use without any legal restraints. NIS-Elements does not support the interlaced version of this format.

### **Windows Bitmap (BMP)**

This is the standard Windows file format. This format does not include additional image description information such as author, sample, subject or calibration.

### **LIM Format (LIM)**

Developed for the needs of laboratory image analysis package. Nowadays, all its features (and more) are provided by the JPEG 2000 format.

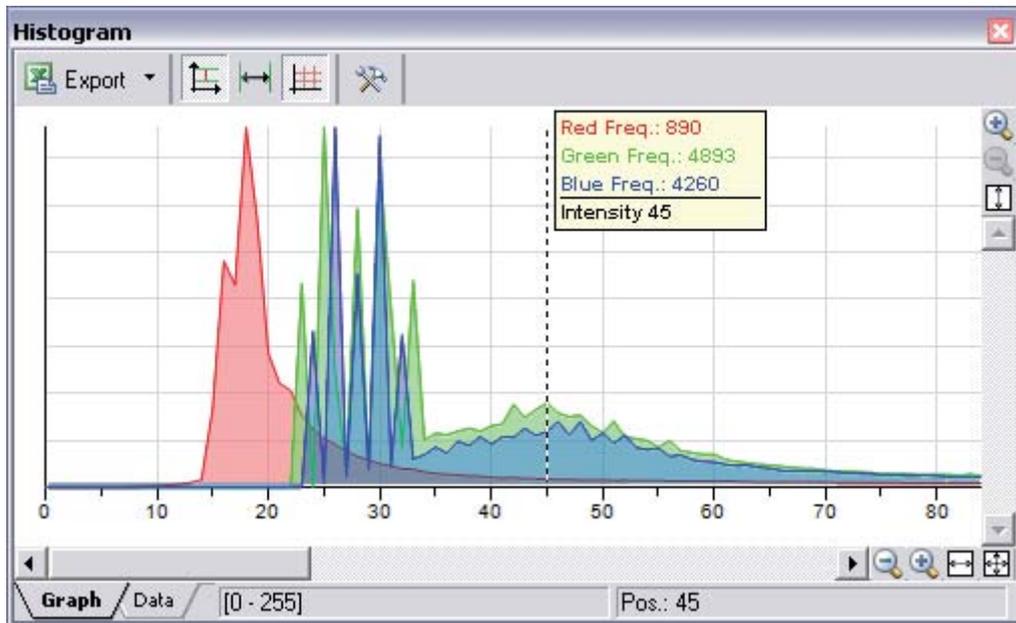
### **ICS/IDS image sequence**

ICS/IDS sequences are generated by some microscopes and consist of two files: the ICS file with information about the sequence; the IDS file containing the image data. The ICS file must be stored in the same directory together with the IDS file.

# Histogram and Look Up Tables

## Histogram Window

A histogram is a graphical display of tabulated frequencies. In NIS-Elements, it displays frequencies of pixels with a certain intensity value. The intensity values range from 0 (black) to 255 or 65535 (white) depending on the image bit depth. A separate graph is created for each color channel. Run the [View > Visualization Controls > Histogram] command:



## Histogram tools

Source data of the histogram can be viewed by switching the tabs in the bottom-left corner of the control window.

### Export



The source data or the histogram image can be exported to an external file. Click the [Export] arrow button to select a destination from a pull-down menu.

*Please, see the [Exporting Results](#) chapter for further details. The source data table will appear if you click the [Data] tab at the bottom of the control window.*



The [Export ND histogram] button enables to export histogram data of all frames of the current ND document. The destination selected in the [Export] pull-down menu is maintained.

### **Graph appearance**

The histogram can be zoomed in and out using the zoom buttons on sides of the window, or autoscale buttons can be used:



[Auto Scale Vertical] zooms in the graph of each channel separately. When this function is ON, the histogram is not proportional.



[Auto Scale Horizontal] zooms the graph so that the marginal zero frequencies, if there are some, are excluded from display.

The graph appearance can be modified via the following buttons:



[Show Grid] toggles display of the grid in the background.



[Options] displays the Histogram options dialog window where the following properties can be set:

- **Background/Axes Color** - the colors can be selected from a pull-down menu.
- **Pen Width** - width of the histogram line can be set to 1, 2, or 3 pixels.
- **Fill Graph Area** - The area below the histogram line can be filled with the channel color.
- **Graph Area Opacity** - select the opacity of the Graph Area fill in [%].
- **Vertical/Horizontal AutoScale, Show Grid** - these options equals the corresponding buttons of the histogram window.
- **Interpolation method** - select the way of drawing the graph line. The [Linear] (smooth) and [Quick] (precise) options are available.
- **AntiAlias** - smoothes the edges of the graph line.
- **Horizontal axis always visible** - if checked, the axis does not leave the graph area while zooming in the graph.

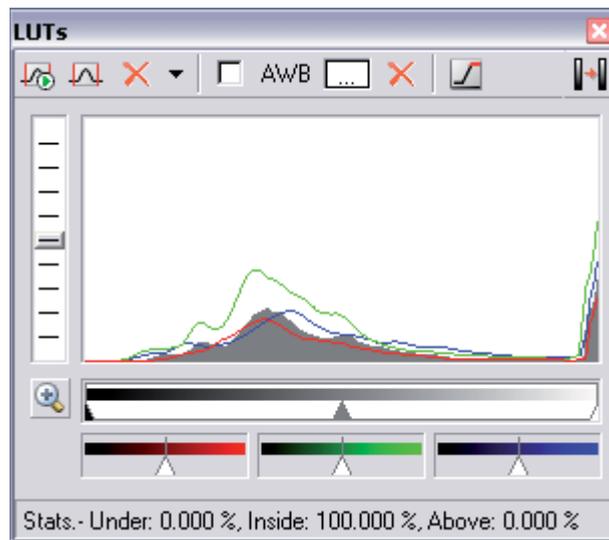
## LUTs

---

A look-up table represents a useful tool for color modifications. It takes a value, maps it to a location in a table, and replaces the incoming value with the contents of the table entry. There are 3 modes of LUTs, depending on the image you are working with. Different controls will be available when applying LUTs to monochromatic, RGB, or multichannel image. When LUTs are activated the LUTs button in the top-left corner of the document window is highlighted red.

### LUTs on RGB images

---



The main part of LUTs window is occupied by a histogram of the current image. There are 3 separate curves for each RGB component, and one gray filled curve for the whole image. You can adjust the histogram view by moving the slider in the left part of the window.

The black and white triangular sliders define thresholds. All the pixels with values smaller than the black slider indicates (left of the slider) will be displayed as pure black. All values to the right of the white slider (all pixels with higher values) will be displayed as white. The remaining color shades will be composed of the pixels with values between the two sliders with defineable gamma parameter. Gamma is adjustable by moving the gray slider.

 This button extends the sliders and displays the histogram as if LUTs was applied. The range of pixel values currently displayed becomes indicated in the top right corner.

Three color bars with sliders (displayed underneath the histogram) are representing RGB components. The slider movement affects the brightness of each component.

## **Auto scale**



The [Auto Scale] button sets the white slider parameter automatically in order to enhance the image reasonably. If you select the [Use Black Level] option from the pull-down menu, the black slider will be affected too. If you select the [Find Best Gamma] option from the same menu, the Gamma slider position will be optimized as well.

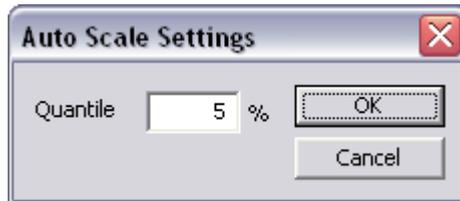


It is possible to apply the auto scale procedure only once by pressing the [Auto Scale] button, or to run it permanently (on the live image) by selecting the [Keep Auto Scale] button. When you turn the Keep Auto Scale off, the settings remain as if the [Auto Scale] button was pressed once.

If you want to discard all LUTs settings, press the red cross button, located next to the [Auto Scale] button.

## **Settings**

Press the arrow next to the [Auto Scale] button, a pull-down menu will appear. Invoke the [Settings] command.



[Quantile (0-10%)] - this value determines how many of all pixels of the picture are left outside the sliders when LUT is applied.

## **AWB**

The AWB (Auto White Balance) mode adjusts the image to get the color neutral white. Similarly as the LUTs auto scale, it can be used once, or permanently on the live image by selecting the [Keep Auto White Balance] checkbox left of the [AWB] button. When you uncheck the box, the settings remains as if the [AWB] button was pressed once.

If you know which undertone your white has, you can select this color by the color picker that appears after pressing the [...] button on the right of the [AWB] button. And again, all changes are discarded using the red cross button.



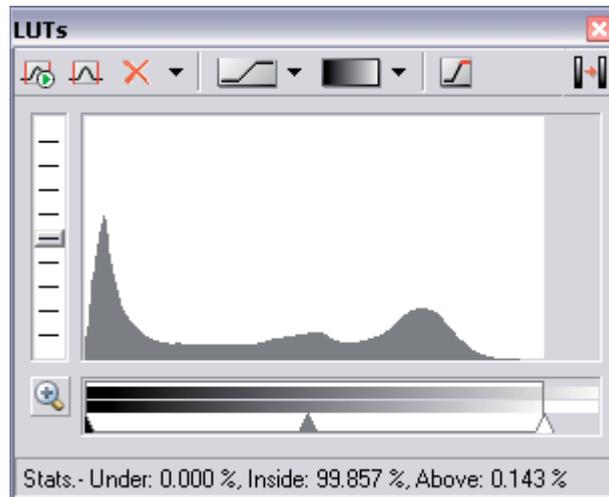
The color overexposed button. When this button is activated, all pixels values of which reach maximum will be color-highlighted.



Press this button to apply the LUTs settings to the image data - the original image will be overwritten. Until you press this button, no changes are made to the image data.

## LUTs on Monochromatic images

---



All features mentioned above are also valid for monochromatic images. When adjusting a monochromatic image, you don't have the AWB function available and only one bar with sliders for the mono channel appears.

### **Mapping Mode**

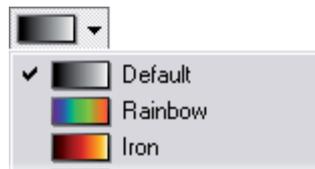
This pop-up menu is used for picking the mode of mapping. You can select from [Contrast] or [Window] modes.



- [Contrast] - all pixels with values higher than the white slider will be set to white.
- [Window] - all pixels with value higher than the white slider will be set to black.

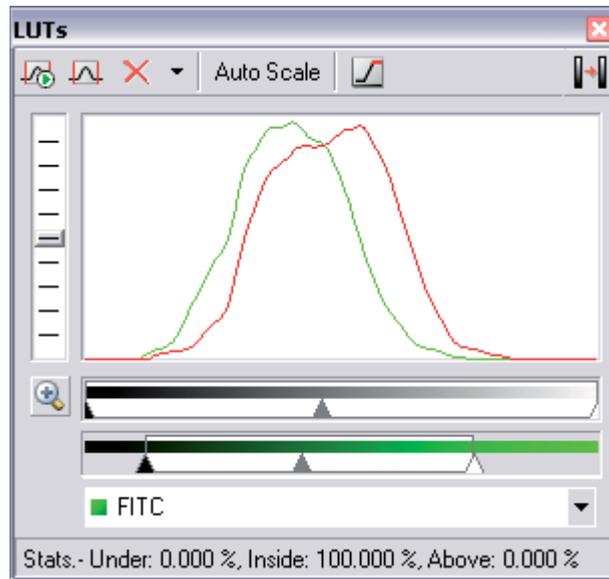
### **Color Schemes**

This button indicates the selected color table and displays a pull-down menu, where you can select the color scheme in which you want to display your image. Try a few of the schemes to see which one highlights most of the details you want to see.



## LUTs on Multichannel images

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In case of multichannel image the histogram shows different color curves - each color for one color channel. To set LUTs for a single channel, this channel must be selected in the pull-down menu at the bottom of the LUTs window.

### **Auto Scale**

A single channel can be adjusted automatically using this [Auto scale] button.

*Look-up tables are useful for equalizing images. For example, if the image is very dark (which usually happens with quantitative cameras), you can restrict the view to display just the low pixel intensities. Hidden details then become more apparent.*

# Thresholding

Specifying correct threshold limits is a crucial procedure of the automated image analysis. The point is to determine, which pixels will and which will not be included in the binary layer, and thereby distinguish analyzed objects from background. Thresholding can be performed in the following modes:

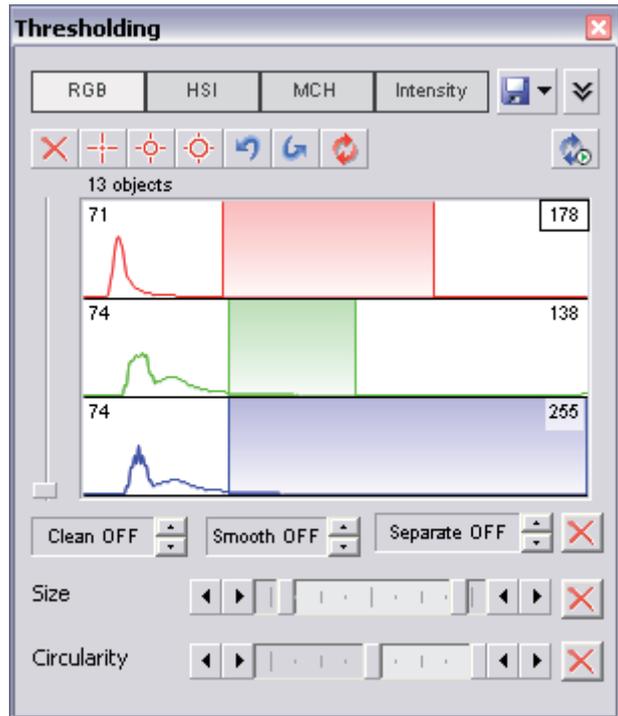
Run the [View > Analysis Controls > Thresholding] command.

## RGB Mode

The [RGB] thresholding mode is available only if an RGB image is opened. There are two ways how to define the threshold limits, by choosing reference points within the image, or by defining the limit values for each color channel.

To threshold the image use the following tools:

-  This button invokes a pull-down menu which enables to load/save the current threshold settings from/to an external file (\*.threshold).
-  This button displays/hides the [Size] and [Circularity] restriction bars.
-  Single point threshold tool. Select it and click inside the image to define the threshold. The threshold ranges will be adjusted so the selected pixel will fit inside.
-  3 point circle tool picks threshold from the radius of 3 pixels.
-  6 point circle tool picks threshold from the radius of 6 pixels.
-  This button erases the threshold settings (no binary objects are created).
-  Undo - reverses the previous threshold operation.
-  Redo - returns the threshold as it had been before the [Undo] button was used.
-  Rethreshold once - after you press this button, the current binary layer will be re-created according to the current threshold settings.\*





Keep updating the binary layer - If turned ON, the binary layer is updated during some user actions.\*\*



Threshold ND image - when an ND2 file is opened, this button appears in the tool bar. The thresholding is performed on a single frame by default. Press this button to perform it on all frames of the ND2 document.

\* - *The binary layer could have been modified e.g. by the [Binary > Clean] command or other commands; all these modifications will be discarded.*

\*\* - *When a new image is opened, the binary layer is created automatically. Or, when thresholding inside the Measurement ROI and the ROI is moved, the new ROI area is thresholded.*

## Threshold adjustments

---

Thresholding parameters can be adjusted very precisely in the following way:

- Threshold limits of each channel can be adjusted by rewriting the values in the top-left/top-right corner of each channel histogram.
- Threshold range of each channel (the colored stripe) can be shifted by mouse. Place the cursor in the middle of the range (a circle appears) and drag it left or right.
- Threshold limits of each channel can be adjusted by mouse. Place the cursor on the edge of the range and drag it left or right.

The two last behaviours can be further modified by pressing additional keys:

- **Move the threshold using the right mouse button** - the binary layer in the image is not updated continuously, but after the mouse button is released. Such operation is quicker.
- **Hold [Shift] while moving the threshold limits** - the threshold adjustment is performed on all channels together (RGB images only).
- **Hold [Ctrl] while moving the threshold limits** - both the low and the high threshold limits move in opposite directions.

## Binary operations

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Three basic operations can be performed on the binary layer before it is displayed on screen. To turn the operation on, click on the up arrow button in order to define the number of its iterations:

- Clean - removes small objects from binary image.
- Smooth - smoothes the binary image contours.
- Separate - separates objects using mathematical morphology methods.

## Restrictions

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The amount of objects that appear within the binary layer can be restricted according to their [Size] and [Circularity] features. The restrictions can be defined by moving the sliders to the center of the bar. The actual limits appear in a tooltip when moving the sliders.

- Size - defines the minimum and the maximum size of the objects to be displayed.
- Circularity - is a coefficient which defines how much the object is similar to circle. The range is from 0 to 1.

## HSI Mode

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The [HSI] thresholding mode is available only if an RGB image is opened. The thresholding procedure works the same way as in the RGB mode except that the pixel values are displayed in the the HSI (Hue, Saturation, Intensity) color space.

The only difference from the RGB mode is that the [Saturation] and [Intensity] channels can be switched OFF. Therefore you can threshold over the H, HS, and HI channels only. Switch the channel off by unchecking the box in the top-left corner.

## Intensity Mode

---

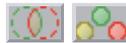
The thresholding procedure works the same way as in the RGB mode except that it is performed on the intensity pixel values.

## MCH Mode

---

The [MCH] mode is not available when working with a monochromatic (single channel) image. This mode is dedicated to perform threshold on multichannel images, but can be applied to RGB images as well.

- There can be number of channels, therefore the channel histograms are minimized. To expand a histogram, click the appropriate color stripe.
- The binary layer(s) can be displayed in two modes. Each channel can create its own binary layer, or one layer can be created as an intersection of all channels binaries. The following button indicates one of the two modes:



# Binary Editor

The binary layer, as a result of thresholding, can be modified by hand using the binary layer editor. It is a built-in application providing various drawing tools and morphology commands. Go for the [Binary > Binary Editor] command or press the [Tab] key. New controls appear on the application toolbars:

## Controls (horizontal tool bar)

---

-  Reverses the changes made to the binary image. [U]
-  Clears the binary image (fills the entire image with the "background" color). [R]
-  Loads the image previously saved by the [Save] button. [L]
-  Temporarily saves the current binary image. It can be loaded anytime before the binary editor is closed by the [Load] button. [S]
-  Displays this help page.
-  Stores the changes and quits the editor. [ESC] or [TAB]

*The other buttons of the vertical tool bar are simplified versions of mathematical morphology functions. Please see the [Mathematical Morphology Basics](#) chapter for more details.*

-  Dilate
-  Erode
-  Close
-  Open
-  Separate Objects
-  Clean
-  Fill Holes

## Drawing tools (vertical tool bar)

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The binary image can be modified using various drawing tools. Although the way of use of some tools differs, there are some general principles:

- Make sure you are in the right drawing mode (drawing background/foreground)
- Any object that has not been completed yet can be canceled by pressing [Esc].
- The polygon-like shapes are drawn by clicks of the left mouse button. The right button finishes the shape.
- The auto-drawing tools (threshold, auto detect) have a changeable parameter. It can be modified by +/- keys or by mouse wheel.
- The scene can be magnified by the UP/DOWN arrows when mouse wheel serves another purposes.
- The right mouse button drags the image when magnified.
- A line width can be set in the upper left corner.
- Hints are displayed below the horizontal tool bar.

## Drawing tools

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Switches between the foreground and the background editing mode. [Ctrl+SPACE]



The Hand tool. Serves for moving the image when magnified. [Ctrl+W]



Bezier hollow tool. The object is defined by placing points on its perimeter. The lines connecting those points can vary from straight lines to bezier curves. (Use +,- keys to adjust them). To finish creation press the right mouse button. [Ctrl+F11]



Bezier fill tool. It equals the Bezier hollow tool, but the resulting object is filled. [Ctrl+F12]



Draws a filled polygon. While holding the left mouse button down, you are in the free hand mode. When you release it, each click defines a corner of the polygon. The polygon is enclosed and filled by pressing the right mouse button. [F4]



Draws a polygon. It equals the Filled polygon tool, but the resulting object is not filled. [F3]



Draws a filled circle. Click to determine the center and define the perimeter holding the left mouse button down. [F8]



Draws a circle. Click to determine the center and define the perimeter holding the left mouse button down. [F7]



Draws a filled circle defined by three points placed on the perimeter. [Ctrl + F8]



Draws a circle defined by three points placed on the perimeter. [Ctrl + F7]



Draws a filled rectangle. [F10]

-  Draws a rectangle. [F9]
-  'Draws a filled moveable circle/ellipse. If you grab the ellipse near the center, you can move it. If you grab it near the border, the nearest semi-axis is being modified. When holding down either the SHIFT or the CTRL key, both semi-axes change equally (forming a circle). [F12]
-  Draws an ellipse. [F11]
-  Drawing by hand. [F1]
-  Draws a straight line. [F2]
-  Draws a polyline. [F5]
-  The Auto Detect filled tool. Detect hollows using threshold techniques. Click to the image to place the probe, the detected area is drawn. You can adjust the thresholding range by using the mouse wheel or by pressing the +, - keys. [Ctrl+B]
-  Threshold tool. Click into the image to place the probe (or more of them) to define the initial color level for thresholding. Pres +,- keys or use the mouse wheel to change the thresholding range. [J]
-  Fill an enclosed shape. [F6]
-  Area of interest. All objects outside the selected region will be erased.
-  Text tool. Displays dialog box for defining text parameters.
-  [Commands] tool. Displays a pop up menu offering user some additional commands:

## Commands

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-  The Auto Detect hollow tool. Detect hollows using threshold techniques. Click to the image to place the probe, the hollow is drawn. You can adjust the thresholding range by using the mouse button or by pressing the +, - keys.
-  Draws rectangularly crossed lines.
-  Draws a rose. Click to the image to define the center, than drag the mouse to set the length of it's arms.
-  Places a marker to the image. Simply click into image...
-  Selects binary objects.



The Connect tool. Draws a line(s) from the place you've clicked to the nearest object(s).



Displays a grid. Visible only when using magnification 400% and higher.



Hides the binary image and displays the just the color image.



Inverts the binary image. Foreground becomes background and vice versa.

*The last two buttons perform simplified versions of mathematical morphology functions. Please see the [Mathematical Morphology Basics](#) chapter for more details.*



Smooth



Contour

*Tools are either applied in a "draw" (foreground) or "erase" (background) mode. Some tools (like threshold or bezier) have editable parameters. Tips how to use the tools are displayed in the vertical toolbar.*

## Multiple Binary Layers

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An arbitrary number of binary layers can be created within one document. Click this [Create New Binary Layer] button to add a new binary layer. The binary layer that you are currently editing can be selected in the nearby pull-down menu. Binary layers can be managed from the [View > Visualization Controls > Binary Layers] control window.

# Measurement

Measurement is an advanced and complex image analysis technique. It starts with calibration of the NIS-Elements system and finishes with a quantitative evaluation of the image.

## Calibration

---

Calibration is of crucial importance to measurement. A calibration assigns real size to one pixel, so that correct and realistic measurements can be performed and the objects of different images could be compared to each other. A correct calibration has to be made before measurement begins. See the *Configuring Attached Hardware* chapter which explains how to calibrate the NIS-Elements system.

## Rough Measurement

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Quick and approximate measurements can be performed utilizing graticules. They behave like adjustable floating rulers. User can simply align a graticule with the measured object and read the distance value (e.g. the diameter). To activate the graticule, press the [Graticules] button placed in the top-right corner of the document window.

## Select Graticule Type

---

The type of the graticule ruler is indicated by a picture on the [Graticules] button. To change the graticule type, click on the graticules button with the right mouse button and select the appropriate item from the context menu:

- Rectangular Grid
- Circle
- Simple Circle
- Cross
- Industrial Cross
- Simple Cross
- Vertical Ruler
- Horizontal Ruler
- Graticule Mask

## Define Graticule Properties

---

Right click the [Graticules] button and select the [Graticules properties] command from the pull-down menu. A dialog window appears where display parameters of all graticules (shape, color, line width, density of lines) can be adjusted.

### Density

The density value - the closest distance between two line intersections of the graticule - can be

set, or you can let NIS-Elements adjust it automatically according to the current zoom factor. The units selection depends on the image calibration (calibrated/uncalibrated).

## Measure the Image

The graticule measurement offers the following options:

- The graticule can be moved by mouse arbitrary.
- The graticule position can be reset by using the [Move Graticules to Center] command from the context menu.
- A binary layer can be created from the current graticule using the [Graticules -> Binary] command.
- A [Graticule Mask] can be created from the current binary layer using the [Binary -> Graticules] command.
- A [Graticule Mask] can be stored/loaded to/from an external file via the [Save Graticule Mask As] and [Open Graticule Mask] commands.
- A new image containing graticules can be created by the [Edit > Create Full View Snapshot] command.

## Manual Measurement

Length, area, angles, taxonomy, counts, circle radius, and ellipse semiaxes can be measured manually over an image. The results are being recorded to a simple statistics table, which can be exported to a file or clipboard. Also, the data can be presented as a graph.

- Run the [View > Analysis Controls > Annotations and Measurements] command. The manual measurement control window appears.
- Select a tool corresponding to the feature you are going to measure. There are several tools for measuring each feature.
- Measure the objects in the image using mouse.
- Select where to export results in the [Export] pull-down menu.
- Export the results using the [Export] button.

The screenshot shows the 'Annotations and Measurements' window. It has a toolbar with icons for various measurement tools. Below the toolbar, there are sections for 'Measure length:', 'area:', 'radius:', 'semiaxes:', and 'angle:'. At the bottom, there is a table with two columns: 'Source' and 'Length'.

	Source	Length
1	F:\Images\crystal.jp2	366,49
2	F:\Images\crystal.jp2	238,11

### **Example:**



Measurement of a crystal: Select the *Horizontal parallel lines* tool. Place the first line on the top edge of the crystal by clicking into the image. The position of the line can be adjusted while you hold the left mouse button down. After you release it, the line is positioned. Repeat this to place the second line on the bottom edge of the crystal. When finished, an arrow is drawn between the lines, and the result of the measurement is attached. A record with the measurement type and measured value is added to the results table.

*Please see more about manual measurement tools in the Command Reference of the help.*

## **Automated Measurement**

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Automated measurement is the very powerful of image analysis features. In combination with user macros, NIS-Elements can become semi- or fully-automatic image analysis tool. There are several key procedures which a proper automated measurement should involve:

- Optical system calibration. Please see the [Optical Configurations] chapter for more details.
- Image capture. Please see the [Capturing Images] chapter for more details.
- Defining threshold, creating the binary layer. Please see the [Thresholding] chapter for more details.
- Performing the automated measurement.
- Results presentation.

## **Image Layers Involved in Measurement.**

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Automated measurements are performed on the two image layers:

- **Binary layer** - is typically used for shape and size measurements (area, perimeter, surface fraction).
- **Color layer** - intensity or hue measurements are carried out on the color layer. The area covered with the binary layer is used as the source data.

## **Object/Field Measurement**

---

Generally, NIS-Elements distinguishes two types of automated measurements: object and field.

### **Object measurement**

Groups of neighbouring pixels of the binary layer are called objects. Object measurements provide user with specific object data such as Length, Area, CentreX/Y (the X,Y position of the central pixel of an object), etc.

## **Field Measurement**

Field measurements produce information regarding the whole measurement frame area such as Area Fraction, Mean Brightness, Density Variation, etc.

*Please see the complete list of measurement features in the [ Measurement Features ] chapter.*

## **Restricting the Area of Measurement**

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The area of measurement can be restricted by the measurement frame or by a user defined region of interest (ROI).

### **Region of Interest**

ROI is a user defined area of the image. It can be modified using the commands of the pull-down menu which appears when you right-click the [Turn Measurement ROI On/Off] button in the top-right corner of the document window tool bar. Or, it can be edited in the [Measure > ROI Editor]. Turn the ROI ON via the [Measure > Use ROI] command.

### **Measurement Frame**

The measurement frame is a resizable rectangular frame which serves for delimiting the accepted area for measurement. Turn the measurement frame ON via the [Measure > Use Measurement Frame] command.

*The binary objects which touch edges of ROI or the measurement frame can be treated differently (excluded from the measurement or included). This behaviour can be specified within the [Edit > General Options > Measurement] dialog window.*

## **Statistics and Data Presentation**

---

NIS-Elements calculates basic statistics - mean value, standard deviation and distribution of all measured features. The features to be measured can be selected in the [Measure > Object Features] and the [Measure > Field & ROI Features] dialog windows. Results of all automated measurements are presented and can be exported from the [View > Analysis Controls > Automated Measurement Results] control window.

# Measurement Features

The following list describes all the features that can be measured within NIS-Elements Measurements. Suitable type of measurement (object/field/interactive) and the required image layer (binary, color/binary) are included in the description.

## a

a - coordinate in the CIE-LAB color system

<b>Measurement Type</b>	object, field
<b>Image Type</b>	color

## AcqTime

Calling the Grab Sequence command, the AcqTime is set to zero at the beginning. Then, NIS-Elements assigns the AcqTime to every newly grabbed image file header, which denotes time elapsed from the beginning of the grabbing. If the Grab Sequence command is not called, than the AcqTime is the time elapsed since the start of NIS-Elements.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	binary

## Area

Area is a principal size criterion. In a non-calibrated system, it expresses the number of pixels; in a calibrated one, it expresses the real area.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	binary

### **AreaFraction**

AreaFraction is the ratio of the segmented image area and the MeasuredArea. It has a strong stereological interpretation: in the case of isotropic uniform random sections it is equal to the volume fraction.

AreaFraction = Area/MeasuredArea

<b>Measurement Type</b>	field
<b>Image Type</b>	binary

### **b**

b - coordinate in the CIE-LAB color system

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color

### **Blue**

Blue value is the the mean of blue component pixel values. It is the usual statistical mean of intensity values of the blue component.

<b>Measurement Type</b>	interactive
<b>Image Type</b>	color

### **BoundsLeft, BoundsRight, BoundsTop, BoundsBottom**

Theses are distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle. The units of calibration are used if the image is calibrated. Otherwise, the coordinate value is in pixels.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **BoundsAbsLeft, BoundsAbsRight, BoundsAbsTop, BoundsAbsBottom**

These are absolute distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle within a motorized stage range. This feature can be measured only when the motorized stage is connected.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **BoundsPxLeft, BoundsPxRight, BoundsPxTop, BoundsPxBottom**

These are distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle. Unlike the BoundsLeft,... distance, this value is always in pixels.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **BrightVariation**

The BrightVariation is derived from brightness values. It is the usual standard deviation of brightness values. It is calibration dependent. For more details about bright variation see also the Densitometric and brightness measurements section in the Measurement chapter.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	color/binary

### **CentreX**

CentreX is the x co-ordinate of the center of gravity. The units of calibration are used if the image is calibrated. Otherwise, the coordinate value is in pixels.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **CentreY**

CentreY is the y co-ordinate of the center of gravity. The units of calibration are used if the image is calibrated. Otherwise, the coordinate value is in pixels.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **CentreXabs**

CentreXabs is the absolute x co-ordinate of the center of gravity within a motorized stage range. This feature can be measured only when the motorized stage is connected.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **CentreYabs**

CentreYabs is the absolute y co-ordinate of the center of gravity within a motorized stage range. This feature can be measured only when the motorized stage is connected.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **CentreXpx**

CentreXpx is the x co-ordinate of the center of gravity. Unlike the CentreX coordinate, this value is always in pixels.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **CentreYpx**

CentreYpx is the y co-ordinate of the center of gravity. Unlike the CentreX coordinate, this value is always in pixels.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **Circularity**

Circularity equals to 1 only for circles; all other shapes are characterized by circularity smaller than 1. It is a derived shape measure, calculated from the area and perimeter. This feature is useful for examining shape characteristics.

$$\text{Circularity} = 4 * \pi * \text{Area} / \text{Perimeter}^2$$

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **Class**

NIS-Elements recognizes 12 classes (1-12). If you have selected class feature (function Object Features or Field Features), system automatically asks you to specify the class via dialog box. In field or scan objects measurements, the application asks for a class before measurement is performed on the current image. In single object measurement, NIS-Elements asks for a class for every measured object. In the case you want to pass by, there is a possibility to run the SetClass function.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	binary

### **DensityVariation**

DensityVariation is derived from density values. It is a usual standard deviation of density values. For more details about density features see also the Densitometric and brightness measurements section of the Measurement chapter.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	color/binary

### **Elongation**

Elongation is determined as a ratio of MaxFeret and MinFeret features. This feature is useful for shape characteristics.

$$\text{Elongation} = \text{MaxFeret}/\text{MinFeret}$$

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **EqDiameter**

The equivalent diameter is a size feature derived from the area. It determines the diameter of a circle with the same area as the measured object:

$$\text{Eqdia} = \sqrt{4 * \text{Area} / \pi}$$

<b>Measurement Type</b>	object, interactive
<b>Image Type</b>	binary

### **ExPurity**

This feature specifies the amount of white in the measured color, if the measured color can be composed of white and a pure spectral color (see below).

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color

### **FillArea**

In case an object does not contain holes then the FillArea is equivalent to the Area. If an object contains holes, FillArea remains the same while Area is reduced by the area of the holes.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **FillRatio**

FillRatio is the ratio of Area and FillArea:

$$\text{FillRatio} = \text{Area}/\text{FillArea}$$

If an object does not contain holes the FillRatio is equal 1. If an object contains holes, FillRatio is less than 1. This feature can distinguish objects with and without holes.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

### **IntensityVariation**

IntensityVariation is derived from an intensity histogram. It is an usual standard deviation of intensity values. This feature describes the inner structure of an object or a field.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	color

### **Green**

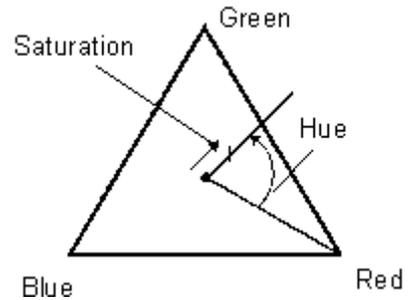
Green value is the mean of green component pixel values. It is the usual statistical mean of intensity values of green component.

<b>Measurement Type</b>	interactive
<b>Image Type</b>	color

### **HueTypical**

HueTypical is the hue value with maximum frequency in a hue value histogram. This feature describes the most frequent hue (color) in an object or field.

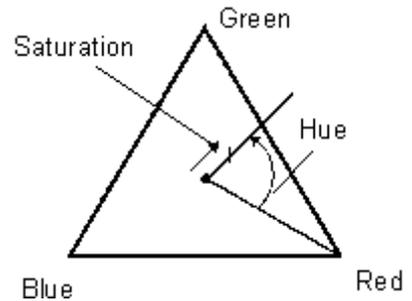
<b>Measurement Type</b>	object, field
<b>Image Type</b>	color/binary



### **HueVariation**

HueVariation is the usual standard deviation of hue values. This feature describes hue (color) distribution of inner structure of an object or field.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	color/binary



### **SumBrightness**

Sum Brightness is defined as the sum of brightness in every pixel of the object.

<b>Measurement Type</b>	object, interactive
<b>Image Type</b>	color/gray

### **SumDensity**

SumDensity is the sum of individual optical densities (O.D.) of each pixel in the area being measured. This feature describes, for instance, the amount of a substance in biological sections. Optical density is evaluated according to the following formula:

$$\text{O.D.} = -\log((\text{PixelIntensityValue} + 0.5)/62.5)$$

<b>Measurement Type</b>	object, field, interactive
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<b>Image Type</b>	color/binary
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### **SumIntensity**

Sum Intensity is defined as the sum of intensity in every pixel of the object.

<b>Measurement Type</b>	object
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<b>Image Type</b>	color/gray
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### **L**

L - coordinate in the CIE-LAB color system

<b>Measurement Type</b>	object, field, interactive
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<b>Image Type</b>	color
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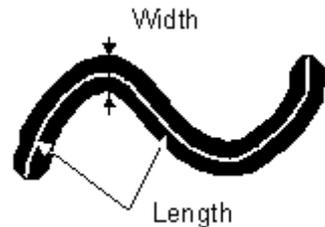
### **Length**

Length is a derived feature appropriate for elongated or thin structures. As based on the rod model, it is useful for calculating length of medial axis of thin rods.

$$\text{Length} = (\text{Perimeter} + \sqrt{(\text{Perimeter}^2 - 16 * \text{Area})}) / 4$$

<b>Measurement Type</b>	object, interactive
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<b>Image Type</b>	binary
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### LineLength

Line length is defined as the length of the object with elongated shape.

<b>Measurement Type</b>	object
<b>Image Type</b>	color/gray

### Luminance

Luminance is defined as a radiant power weighted by a spectral sensitivity that has characteristics of human vision.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color

### MaxFeret

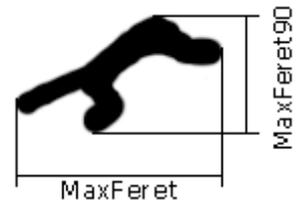
The MaxFeret is the maximal value of the set of Feret's diameters. Generally (for convex objects), Feret's diameter at angle  $\alpha$  equals the projected length of object at angle  $\alpha$ ,  $\alpha \in (0,180)$ ; NIS-Elements calculates Feret's diameter for  $\alpha=0, 10, 20, 30, \dots, 180$ .

<b>Measurement Type</b>	object, interactive
<b>Image Type</b>	binary

### MaxFeret90

The MaxFeret90 is a length projected across the MaxFeret diameter.

<b>Measurement Type</b>	object, interactive
<b>Image Type</b>	binary



### **MaxIntensity**

MaxIntensity value is derived from the intensity histogram. It is the maximal of intensity pixel values.

<b>Measurement Type</b>	interactive
<b>Image Type</b>	color/gray

### **MeanBlue**

MeanBlue value is the mean of blue component pixel values. It is the usual statistical mean of intensity values of blue component.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color/binary

### **MeanBrightness**

MeanBrightness value is the usual statistical mean of brightness values of pixels. NIS-Elements uses brightness calibration curve for evaluation of this parameter.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color/binary

### **MeanDensity**

MeanDensity value is the usual statistical mean of density values of pixels. NIS-Elements uses density calibration curve for evaluation of this parameter.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color/binary

### **MeanIntensity**

MeanIntensity value is derived from the intensity histogram. It is the usual statistical mean of intensity values of pixels.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color/binary

### **MeanGreen**

MeanGreen value is the mean of green component pixel values. It is the usual statistical mean of intensity values of green component.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color/binary

### **MeanChord**

MeanChord is the mean value of secants in the 0, 45, 90 and 135 degrees directions. It is a derived feature and is calculated from the Area and mean projection according to the following formula.

$$\text{MeanChord} = 4 * \text{Area} / (\text{Pr}_0 + \text{Pr}_{45} + \text{Pr}_{90} + \text{Pr}_{135})$$

<b>Measurement Type</b>	object, field
<b>Image Type</b>	binary

### **MeanRed**

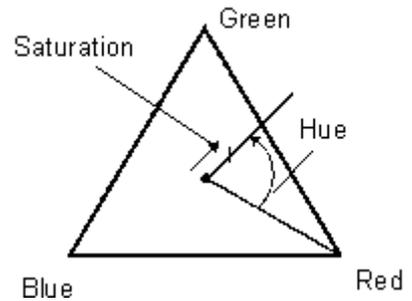
MeanRed value is the mean of Red component pixel values. It is the usual statistical mean of intensity values of red component.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color/binary

### **MeanSaturation**

MeanSaturation is a statistical mean of saturation values of pixels.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	color/binary



### **MeasuredArea**

MeasuredArea value is the area of the measurement frame or of a mask within the measurement frame, if the mask status is on.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	binary

### **MinFerret**

The MinFerret value is the minimal value of the set of Feret's diameters. Generally (for convex objects), Feret's diameter at angle  $\alpha$  equals the projected length of object at angle  $\alpha$ ,  $\alpha \in (0,180)$ ; NIS-Elements calculates Feret's diameter for  $\alpha=0,10,20, 30, \dots, 180$ .

<b>Measurement Type</b>	object, interactive
<b>Image Type</b>	binary

### **MinIntensity**

MinIntensity value is derived from the intensity histogram. It is the minimum of intensity values of pixels.

<b>Measurement Type</b>	interactive
<b>Image Type</b>	color/gray

## NumberObjects

NumberObjects value returns the number of objects in the measurement frame. Exclusion rules for counting objects are taken into account.

<b>Measurement Type</b>	field
<b>Image Type</b>	binary

## Orientation

Orientation is the angle at which Feret's diameters have their maximum. The diameters are calculated with 5 degrees angle increment.

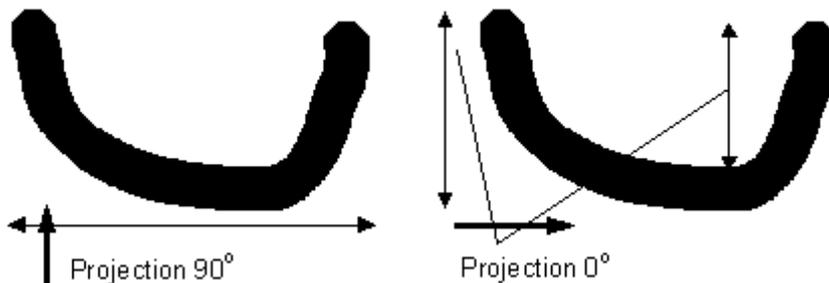
<b>Measurement Type</b>	object
<b>Image Type</b>	binary

## Perimeter

Perimeter is the total boundary measure. It includes both the outer and inner boundary (if there are holes inside an object). The perimeter is calculated from four projections in the directions 0, 45, 90 and 135 degrees using Crofton's formula

$$\text{Perimeter} = \pi * (\text{Pr}_0 + \text{Pr}_{45} + \text{Pr}_{90} + \text{Pr}_{135}) / 4$$

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	binary



## **Red**

Red is the mean of red component pixel values. It is the usual statistical mean of intensity values of red component.

<b>Measurement Type</b>	interactive
<b>Image Type</b>	color

## **StartX**

StartX is the x co-ordinate of a pixel on the object boundary.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

## **StartY**

StartY is the y co-ordinate of a pixel on the object boundary.

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

## **StgPosX**

StgPosX feature is the x co-ordinate of the absolute position of the measured field. It is available only for systems equipped with a stage.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	binary

### **StgPosY**

StgPosY feature is the y co-ordinate of the absolute position of the measured field. It is available only for systems equipped with a stage.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	binary

### **SurfVolumeRatio**

SurfVolumeRatio is a feature with a strong stereological interpretation: if you measure on fields that are sampled systematically and independently of the content of the sections, then the feature is an unbiased estimator of the surface area of objects (inner structure) per volume of the whole sample.

$\text{SurfVolumeRatio} = (4/\pi) * \text{Perimeter} / \text{MeasuredArea}$

<b>Measurement Type</b>	field
<b>Image Type</b>	binary

### **Time**

Time assigns the time to a field (object) after a measurement has been performed. See the SetReferenceTime function help.

<b>Measurement Type</b>	object, field
<b>Image Type</b>	color/binary

### **u**

u - coordinate in the CIE-uv color system.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color

## v

v - coordinate in the CIE-uv color system.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color

## VolumeEqCylinder

This parameter is based on the rod model. Length is interpreted as height and Width as the base diameter of a cylinder. Bases are considered to be spherical.

$$\text{VolumeEqCylinder} = (\pi d^2) * (l-d) / 4 + \pi d^3 / 6,$$

where  $l = \max(\text{MaxFerret}, \text{Length})$ ,  $d = \min(\text{MinFerret}, \text{Width})$ .

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

## VolumeEqSphere

Supposing a profile was created as an intersection of a ball and a section that contains the center of the ball. VolumeEqSphere is the volume of the ball.

$$\text{VolumeEqSphere} = \pi * \text{Eqdia}^3 / 6$$

<b>Measurement Type</b>	object
<b>Image Type</b>	binary

## WaveLen

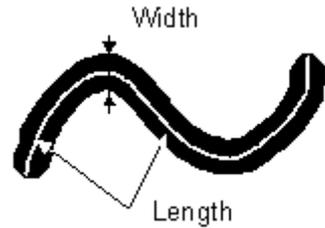
Dominant wavelength. This feature is defined as a wavelength of the pure spectral color that is, together with white, the measured color composed of. The color in the purple must be composed of more than one pure spectral colors therefore they have no dominant wavelength.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color

## **Width**

Width is a derived feature appropriate for elongated or thin structures. It is based on the rod model and is calculated according to the following formula:

$$\text{Width} = \text{Area}/\text{Length}$$



<b>Measurement Type</b>	object
<b>Image Type</b>	binary

## **x**

x - coordinate in the CIE-xy color system.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color

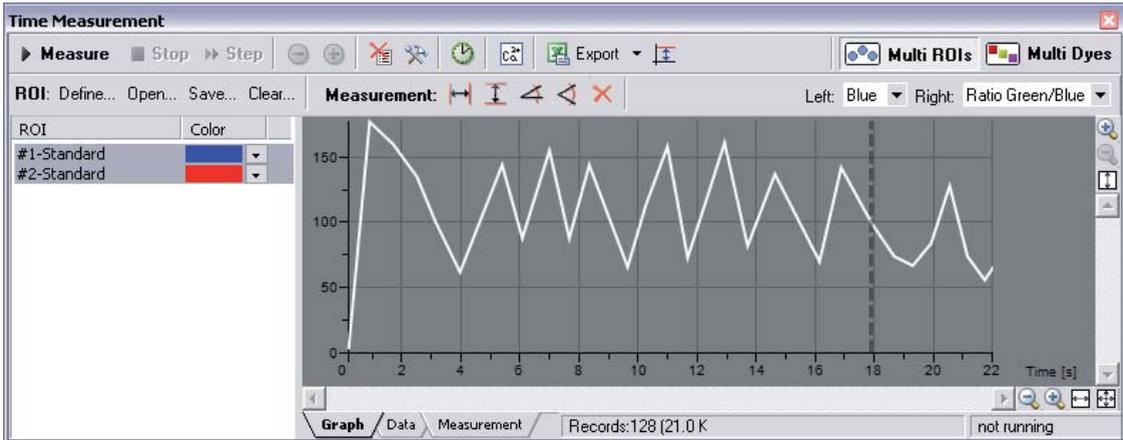
## **y**

y - coordinate in the CIE-xy color system.

<b>Measurement Type</b>	object, field, interactive
<b>Image Type</b>	color

# Time Measurement

The time measurement tool enables to record average pixel intensities within ROI during a time interval. The measurement can be performed on Live camera signal. Before the measurement is started, a region of interest (ROI) shall be defined/enabled. Run the [View > Analysis Controls > Time Measurement] command to open the Time Measurement control window.



- Display the [View > Analysis Controls > Time Measurement] control window.
- Enable the Measurement ROI by the [Measure > Use ROI] command. Time measurement will be performed under the Measurement ROI area.
- Run the [Acquire > Live Fast] command to display the camera signal.
- Press the [Measure] button. It changes to [Pause].
- Press the [Stop] button to pause/finish the measurement.

*If you run an ND2 experiment and the [Run during ND acquisition] button is pressed at the same time, time measurement will begin automatically right as the experiment starts. The measurement results can be exported via the [Export] button, please, see the [Exporting Results] chapter.*

## ROI Definition

To define areas where the measurements will be performed:

- Run the [Acquire > Live Fast] command to display the camera signal.
- Enable ROI by the [Measure > Use ROI] command.
- To modify ROI, right-click the ROI button on the right side of the document window and use one of the [Draw...] commands from the context menu.
- Or, use the [Define] button placed in the time measurement window toolbar to display the ROI definition bar.
- Or, ROI can be modified using the [Measure > ROI Editor]. When finished, quit the ROI editor by the [Tab] key.

## Measurement Options

---



Click the [Options] button. A dialog appears where you can specify the time measurement details:

The screenshot shows a dialog box titled "Time measurement options" with a close button (X) in the top right corner. The dialog is organized into several sections:

- User events:** A button labeled "Define hot keys..." and a text field showing "User hotkeys: none, none, none, none".
- Timing:** Two radio buttons. The first is "Measure every:" with a value of 3 and a unit of "frame". The second is "Measure every:" with a value of 300 and a unit of "ms". To the right, "Timing on ND files" has a "Measure every:" value of 1 and a unit of "frame".
- Graph & Data:** A section with various settings:
  - Background: a white dropdown menu.
  - Pen width: a dropdown menu showing "2 px".
  - Vertical axes always visible: checked checkbox.
  - Horizontal axis always visible: checked checkbox.
  - Vertical AutoScale: unchecked checkbox.
  - Axes color: a black color selection dropdown.
  - Show grid: checked checkbox.
  - AntiAlias: checked checkbox.
  - Display graph points (if possible): unchecked checkbox.
  - Use mouse wheel to zoom in/out axes: a text label.
  - Interpolation method: a dropdown menu showing "Linear".
  - Data decimals: a dropdown menu showing "2".
  - Second data line pen: a sub-section with:
    - Width: a dropdown menu showing "2 px".
    - Color: a green color selection dropdown.
    - Style: a dropdown menu showing "Dash".

At the bottom of the dialog are "OK" and "Cancel" buttons.

## User events

---

*Arbitrary time of some user events can be indicated in the data table or the graph by a marker.*

- [Define Hot Keys] - Press this button to define up to 4 hot keys. Select a shortcut key combination and insert a user text. A marker and the user text will be inserted each time the shortcut is used during the time measurement.

## Timing

---

A time interval shall be set to determine how often one partial measurement will be performed.

- The [Measure every N frame] section defines after how many (N) frames one measurement is performed.
- The [Measure every N ms] section defines directly the time interval in milliseconds.

*This setting can be modified during a time measurement process by pressing the [+] and [-] buttons. These buttons increase/decrease the speed of recording the time measurement data.*

## Graph & Data

---

Some of the graph and data table properties can be modified:

### **Display Properties**

The colors and the line widths of the graph can be selected from a pull-down menu.

### **Vertical/Horizontal axes always visible**

If checked, the axes do not leave the graph area while zooming in the graph.

### **Vertical AutoScale**

This option optimizes the displayed graph area. If the measured intensities do not reach maximum values, the top part of the graph is hidden.

### **Show grid**

The grid in the background may be or may be not displayed by selecting this option.

### **AntiAlias**

Turning this option on will make the graph line edges look smooth.

### **Display graph points (if possible)**

Small dots indicating the actual data values position can be displayed on the graph line. The points appear only if the distance between them is big enough for them to be recognized (they usually appear when you zoom in the graph).

### **Interpolation method**

The profile line shape may be smoothed out by using an interpolation method. Select one of the three available: Quick (basic), Linear (smoother), Bicubic (really smooth).

### **Data decimals**

Select the precision of the results in the data table.

## Multi ROIs, Multi Dyes

---

The [Multi ROIs] and [Multi Dyes] buttons swap between two measurement modes:

- **Multi ROIs** - One or more continuous areas can be defined within a measurement ROI. In this mode, data for each of the areas are available.
- **Multi Dyes** - In this mode, data for each color channel (averaged from the whole measurement ROI) are available.

# Object Count

If you run the [View > Analysis Controls > Object Count], the following control window appears. It combines important image analysis tools, which enable users to threshold, measure the image, and export the measured data to a file in a smooth way. Object count can be performed even on Live image.



## Thresholding the image

Threshold limits have to be defined by choosing reference points in the image. Select one of the following tools:



**1 point threshold tool.**



**3 points circle threshold tool.**



**6 points circle threshold tool.**

Click inside the image to define typical object areas. The system will detect similar parts of the image and highlight it by color edges and fill. The threshold limits are indicated in the histogram and can be further modified by dragging the limit lines by mouse.

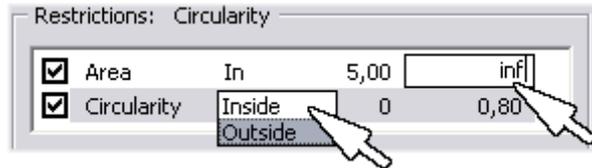
*Please, refer to the Thresholding chapter for further details.*

## Applying measurement restrictions

The number of objects that are included in the results table can be restricted by defining some limiting features. The objects that do not fit these limits will not be taken into account.

- Right click to the restrictions field to select one or more of the following measurement features: Area , EqDiameter , Perimeter , Length , Width , MaxFerret , MinFerret , Circularity , Elongation , MeanIntensity , SumIntensity

- Select the restriction feature you would like to define.
- Move sliders below the histogram to set the limits. The limit values are indicated next to the feature name, and can be modified directly by double clicking the indicated value\*.
- Decide whether the defined interval will be excluded or included from/in results. This is done by setting the **Inside/Outside** value next to the feature name.
- The nearby checkbox indicates whether the restriction is applied or not. If applied, the histogram below is color, otherwise it is gray.



\* - infinitude can be defined by entering "oo" or "inf".

### Reset

The [Reset] button discards restriction settings of all features.

### Bins

This is a number of columns in the histogram.

## Working with measured data

---

The following results appears in the results table:

- **Total Area** is the area of the whole image in px<sup>2</sup> or **calibration units** <sup>2</sup>.
- **Area Fraction** is the ratio between Total Area and the area of the thresholded objects in %.
- **Number of objects** counts the thresholded objects.
- **Table of results** - every thresholded object is measured and the results are inserted to this table. Select the columns to display/hide from the context menu that appears when you right-click one of the column names.
- **#** - number of selected objects is displayed in the box next to the buttons. One or more objects can be selected by mouse while holding the [Ctrl] or the [Shift] key.

The following tools can be used:



**Delete selected objects** removes the selected objects. The objects can be selected by mouse holding the **Ctrl** key down.



**Invert selection** deselects the selected objects and vice versa.



**Generate binary** converts the current OC layer to binary layer (the previous binary layer is overwritten).



**Count objects in ND image** - when an ND2 file is opened, this button can be used. The thresholding, measurement, and restrictions is applied to a single frame by default. Pressing this button to apply it to all frames of the ND2 document and export the results to a file or clipboard according to the current export options.



**Save/Load Object Count configuration** invokes a pull-down menu which enables to load/save the current object count settings from/to an external file (\*.counting).



**Keep updating count** refreshes the measured results each time the OC layer changes.

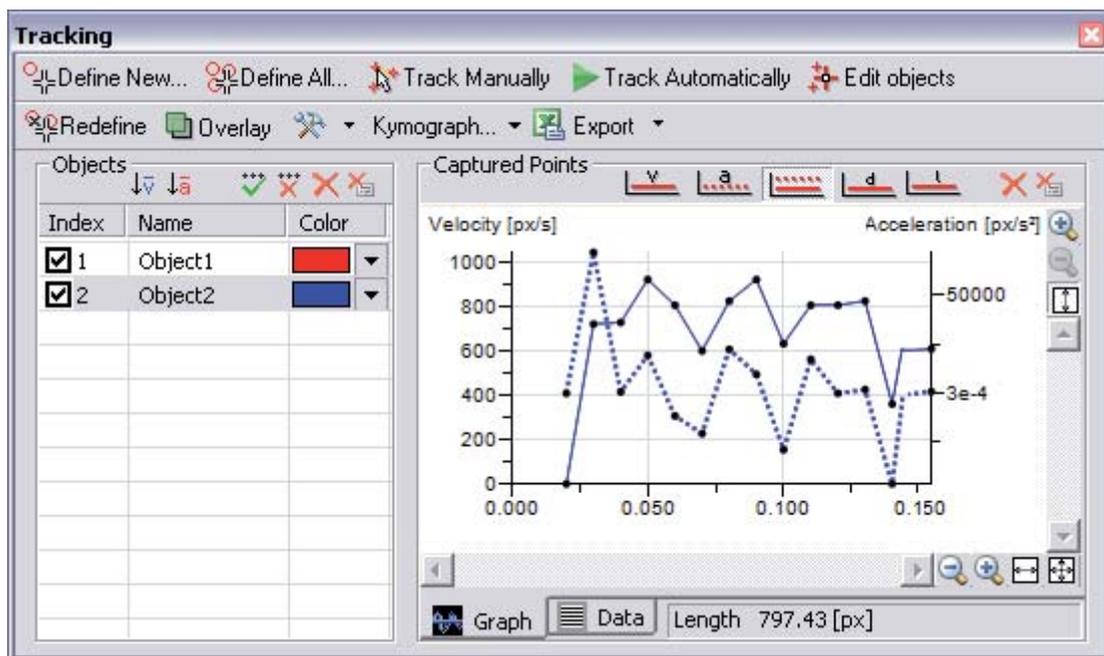
## Data Export

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The measured data can be exported to clipboard or a file using the [Export] button. Please refer to the [ Exporting Results ] chapter for further details.

# Tracking (AR)

Moving objects (e.g. living cells) captured by time lapse acquisition can be tracked automatically or manually using the [Tracking] control window. Various indicators of the object movements such as current position, velocity, acceleration, etc. can be measured. The output is a table or a graph. Run the [View > Analysis Controls > Tracking] command to display the control window:



## Automatic Tracking

---

Define the objects to be tracked. Sometimes, images of the image sequence (ND2 file) may need to be enhanced, so the differences between objects and the background would become obvious.

### One by One Definition

---



Select the frame from which you would like to track an object. Zoom in the image, click the [Define New] button, and mark the object by mouse:

- Click to the middle of the object and drag the cursor to its edge.
- An auto-detection algorithm will determine the object area according to the pixel intensity values in the image.
- When the red line which indicates the computer-estimated object edges is around the object edges, release the mouse button.
- Define as many objects as you would like to, then click the [Finish] button.

### Overall Definition

---



Select the [Define All] button. The definition method is the same as described above, except that the auto-detection algorithm tries to detect all objects in the image similar to the one you are just defining. Therefore it is enough to define one object only.

## Tracking

---

If you finished defining objects, run the automatic tracking procedure:

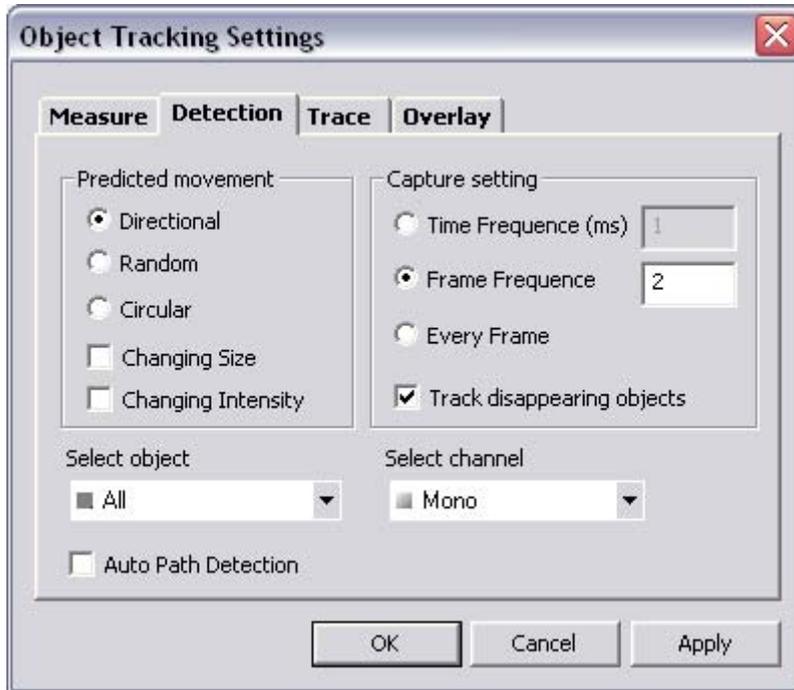
- Select the first frame of the image sequence.
- Press the [Track Automatically] button.

## Tracking Options

---



Click the [Settings] button, the following options dialog window appears. Select the [Detection] tab.



### **Predicted movement**

The tracking algorithm can be optimized for a certain type of movement. In the left half of the window, select whether the objects are moving directly, randomly, or moving in circles, changing size and intensity, or not. These settings may be assigned to a single object or to all of them - select the object (Object1, Object2, ..., All) from the bottom pull-down menu.

### **Auto Path Detection**

If you redefined or edited an object, only the frames that you were working with are actually affected. However, you can apply the performed changes to all subsequent frames by turning the [Auto Path Detection] option ON. The workflow should look like this:

- Turn the [Auto Path Detection] ON.
- Edit an object in one of the above described ways.
- The trace from the edited frame to the end is re-traced automatically.

### **Capture setting**

The tracking does not need to be performed on every frame of the image sequence e.g. if the objects move very slowly, or for other reasons. Select the tracking frequency on the right side of the dialog window and select which color channels will the measurement be performed on from

the bottom pull-down menu. A single channel or [All] channels are the options.

### **Track disappearing objects**

Objects may disappear or seem to disappear from the image during the acquisition. This option tells the system to try to find the trail of such lost objects. If this option is disabled, the object track will be finished where the object has been last detected.

### **Manual Tracking**

---

- Define the objects to be tracked (follow the procedure described in the Automatic Tracking portion of this chapter). Lets assume that you have a 10-frames image sequence and you defined objects [1], [2], and [3] to be tracked.
- Press the [Track manually] button.
- The objects [2] and [3] disappear momentarily, and the 2nd image from the image sequence is displayed automatically. The position of object [1] in the 1st image frame is indicated with white color. You are about to define its position in the 2nd frame.
- Click to the center of the object to define the position of it in the frame 2.
- Object [2] appears highlighted in white. Define its position in the 2nd frame by another click.
- This way you define the 2nd trajectory point of object [3], too.
- Then, the 3rd image frame of the sequence is displayed waiting for the third trajectory point of object [1] to be defined, etc.

### **Object Redefinition**

---

Some objects, especially if they are living cells, can change their characteristics - fade, change size, etc - during the movement. In such cases, the automatic tracking algorithm may get "confused" and loose the track for a while. This can be manually corrected by the following tools:

### **Redefine**

---

When an object changes its characteristics so that the system becomes unable to track it properly, use the [Redefine] button:

- Select the frame of the sequence where the tracking algorithm got lost for the first time.
- Select the object in the [Objects] table next to the graph.
- Click the [Redefine] button and define the object (the object definition is described above).
- Features such as area and intensity characteristic of the object change when redefined.

### **Edit Objects**

---

The editing mode works similarly as when redefining an object, but does not change the object characteristics, only the trajectory is changed.

- Switch to the editing mode by pressing the [Edit Objects] button and display object traces by the [Show Trace] button.
- Click the point you would like to modify and drag it by mouse to the right position.

## Visualization

---

The following tools help to visualize the tracking process:

### Overlay

---

You might want to see the whole image sequence in one image. The [Overlay] button can display an additional layer over the current image. To adjust its properties, invoke the [Settings] button and select the [Overlay] tab in the dialog window. Three types of overlay image can be selected:

- [Maximum Intensity Projection] - pixel values with the same XY coordinates are compared throughout the image sequence and only the pixels with the highest intensity value are displayed in the layer.
- [Minimum Intensity Projection] - pixel values with the minimum intensities of the whole image sequence are displayed in the image.
- [Sequence Intensity Projection] - this is a special overlay, which dyes the trajectories by a color scale (blue-red). The movement direction and the object velocity become obvious.

[Color] and [Transparency] of the layer can be adjusted as well.

### Show Trace

---

- Press the [Show Trace] command from the [Settings] pull-down menu to display colored trajectories of all tracked objects in the document. The color can be changed within the table of objects.
- Select to [Show Points/Show Names] from the same pull-down menu. Positions of the objects in each measured frame will be indicated by a cross and a numbered name (Object1, ...).

Click the [Settings] button and select the [Trace] tab to adjust the trace properties:

#### Line Properties

Select one of the radio buttons which offers to "paint" the trace **1/** [by objects] - each object trace has different color; **2/** [by velocity/time] - a random color gradient may be defined in order to indicate the object velocity or the time of its occurrence at a certain place.

#### Tail Properties

If the [Show tails] option is checked, colored tails appear in the image following each of the traced objects. The length of a tail indicates the current object velocity. Number of segments that the tail is composed from and the tail start width can be adjusted here.

#### Cross Properties

The cross appearance (height and line width) of the [Show Points] markers is adjustable here.

## **Kymograph**

---

A kymograph gives a graphical representation of spatial position over time. In NIS-Elements, it displays pixel intensities changes under a defined linear section over time. A trajectory of a tracked object, or a user-drawn line can be used as the kymograph line.

### **Create Kymograph by Line**

- Select the [Create Kymograph by Line] command from the [Kymograph] pull-down menu. The mouse cursor changes.
- Draw a line in the image. Finalize it with right-click.
- A new document called "Kymograph" is created.

### **Create Kymograph on Selected Object**

- Select one of the objects in the objects table the trajectory of which you want to use for the kymograph line.
- Select the [Create Kymograph on Selected Objects] command from the [Kymograph] pull-down menu.
- A new document called "Kymograph" is created.

## **Measurement**

---

A data set of object features is gathered during the tracking procedure. Common features such as object area or object position are measured as well as movement-descriptive features such as acceleration and velocity. Their complete list is to be found in the [Settings] dialog box. To display it, click the [Settings] button and switch to the [Measure] tab. The check boxes indicate features that are going to be included in the [Data] table. Display the [Data] table by clicking the tab at the bottom of the control window (next to the [Graph] tab).

## **Define Reference Point**

---

There are three movement-descriptive features which can be measured in relation to a reference point (if it has been defined). These are the Reference Length, Reference Velocity, Reference Acceleration. If an object was moving in circles around the reference point, the features will be constant/zero.

- Click the [Define] button. The dialog window disappears.
- Place the Reference point to the image by mouse. The dialog window reappears.

## **Export**

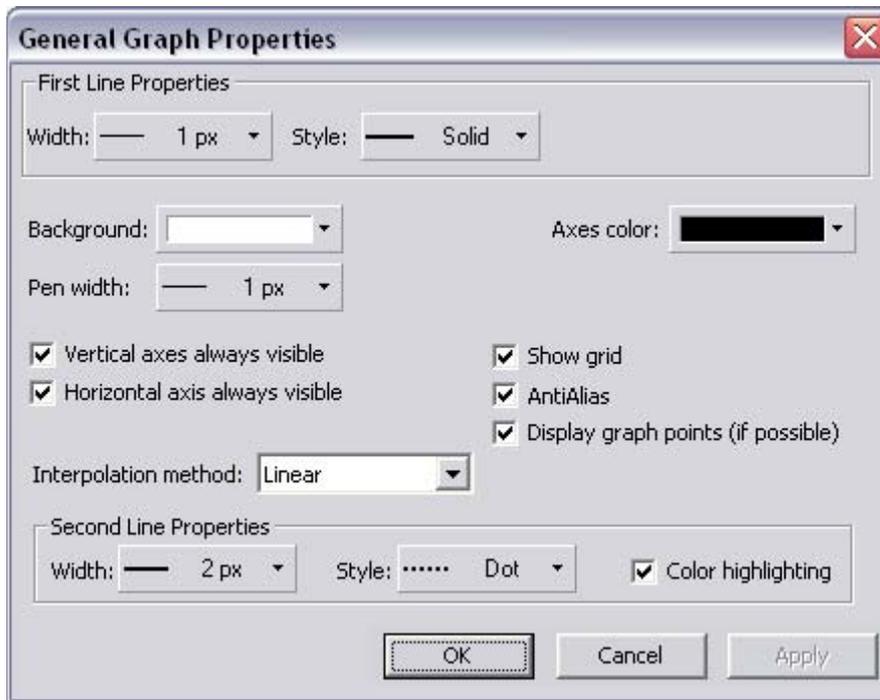
---

The measured data or the graph can be exported to an external file using the [Export] button. Please refer to the [Exporting Results](#) chapter for further details.

## Graph Properties

---

The appearance and behaviour of the graph can be modified in the [General Graph Properties] dialog window. Right-click inside the graph area and select the [Options] command from the context menu.



### **First/Second Line Properties**

These settings (line width and style) are applied to the velocity and acceleration graph lines. The [Color Highlighting] option ensures that the two lines will differ in color.

### **Vertical/Horizontal axes always visible**

If checked, the axes do not leave the graph area while zooming in the graph.

### **Show grid**

The grid in the background may be or may be not displayed by selecting this option.

### **AntiAlias**

Turning this option on will make the graph line edges look smooth.

### **Display graph points (if possible)**

Small dots indicating the actual data values position can be displayed on the graph line. The points appear only if the distance between them is big enough for them to be recognized (they usually appear when you zoom in the graph).

### **Interpolation method**

The profile line shape may be smoothed out by using an interpolation method. Select one of the three available: Quick (basic), Linear (smoother), Bicubic (really smooth).

# Mathematical Morphology Basics

The binary image as a result of thresholding often needs to be modified before any measurements are performed. Edges of the objects can be smoothed, holes in the objects filled etc. by using the mathematical morphology commands. "Image Analysis and Mathematical Morphology" by J. Serra (Academic Press, London, 1982) was used as a reference publication for the following overview.

The basic processes of mathematical morphology are: erosion, dilation, open, close and homotopic transformations.



**Erosion** - After performing erosion, the objects shrink. Marginal pixels of the objects are subtracted. If an object or a narrow shape is thinner than the border to be subtracted, they disappear from the image.



**Dilation** - After performing dilation, the objects enlarge. Pixels are added around the objects. If the distance between two objects is shorter than the thickness of two borders to be added, these objects merge together. If a hole is smaller than the thickness of two borders, it disappears from the image.



**Open** - Open is erosion followed by dilation so the size of the objects is not significantly affected. Contours are smoothed, small objects are suppressed and gently connected, particles are disconnected.



**Close** - Close is dilation followed by erosion so the size of objects is not significantly affected. Contours are smoothed, small holes and small depressions are suppressed. Closely neighbouring objects may be connected together.

## Homotopic transformations

Transformations that preserve topological relations between objects and holes inside them Using a homotopic transformation, an object with 5 holes will be transformed to another object with 5 holes. Two objects without any holes will become also two objects without holes, but likely with a different shape and size. Opening, Closing, Erosion and Dilation are not homotopic transformations. Typical homotopic transformations in NIS-Elements are: Skeletonize, Homotopic Marking and Thickening.

There are other very important transformations of binary images implemented in NIS-Elements binary editor:



**Clean** - This transformation is also called geodesic opening. The image is eroded first so small objects disappear. Then, the remaining eroded objects are reconstructed to their original size and shape. The advantage of this algorithm is that small objects disappear but the rest of the image is not affected.



**Fill Holes** - Fills the holes inside objects. This transformation is handy when objects have a rich inner structure with intensities typical for background. Then, only boundaries are detected correctly, but after applying the [Fill Holes] transformation, objects become homogenous.



**Contour** - This transformation converts an object into its contours.



**Smooth** - Smooth transformation affects sharp edges of the objects and smooths them.



**Morpho separate objects** This transformation detects standalone objects that are connected together and detaches them.

**Note:**

Applying the above mentioned transformations in computerized image analysis brings some limitations due to digitization. In digitized images, information is known only at grid verticals, and only a limiting cut-out of the real scene is available. This is the reason why the most frequent terms in binary image processing are: structuring elements, connectivity, grids, problem with border areas.



When speaking about binary image processing, a binary image is considered as a set of pixels where values 1 represent objects (white areas) and values 0 represent background (black areas). The NIS-Elements system uses a square grid. In this type of grid two possible connectivities can be used, the 8-connectivity and the 4-connectivity. The difference between the 8 and 4 connectivities is shown in the example picture. If the 8-connectivity is considered, the two pixels represent one object. If the 4-connectivity is considered, there are two single point objects in the picture. NIS-Elements works with the 8-connectivity model, so all points neighboring by the corner belong to one object.

**Implementation in NIS-Elements**

---

Erosion, dilation, opening and closing are defined by kernel(matrix/structuring element) and number of iterations. There are the following kernels used in NIS-Elements:



The bright pixel in the center or near the center of the kernel represents its midpoint.

**Erosion**

---

Lets assume the 1 and 0 values represent object(1) and background(0) pixels. You can imagine an erosion by a kernel as the following algorithm:

Move the midpoint of the kernel to every point of the image. Each time, look at the neighborhood and make the following decision:

- If there are object(1) pixels in all the positions of the kernel, set the midpoint to object(1).
- If there is at least one background(0) pixel in the neighborhood, set the midpoint to background(0).

The second parameter, the Number of iterations defines how many times the erosion is to be applied.

## Dilation

---

You can imagine a dilation by a kernel as the following algorithm:

Move the midpoint of the kernel to every point of the image. Each time, look at the neighborhood and make the following decision:

- If there is at least one object(1) pixel in any position of the kernel, set the midpoint to object(1).
- If there are background(0) pixels in all the positions of the kernel, set the midpoint to background(0).

## Open and Close

---

Open is performed by eroding the image and then applying a dilation to the eroded image. On the contrary, Closing is performed as a dilation followed by erosion.

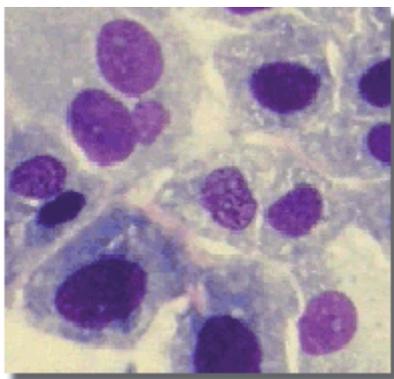
### **Note:**

*If the midpoint is not in the center, applying erosions or dilations by odd number of iterations causes image to shift by 1 pixel. Normally, the total image shift would be determined by the Number of Iterations (in pixels). NIS-Elements eliminates this shift: it changes the position of the midpoint 1 pixel down-rightwards within the kernel for even operations. For opening and closing it is possible to eliminate this shift totally. However, if you run the erode or dilate processes again and again using the kernel with even dimensions and odd number of iterations, then the shift becomes significant.*

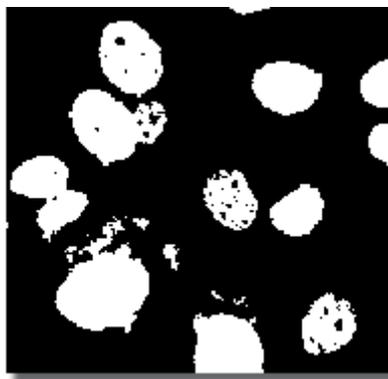
## Examples

---

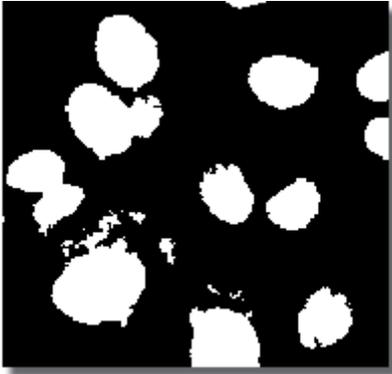
Original image



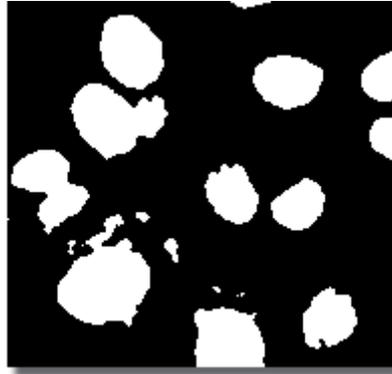
Threshold();



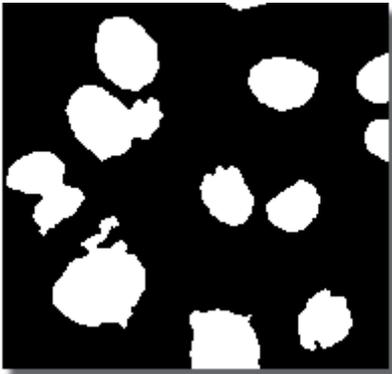
CloseHoles();



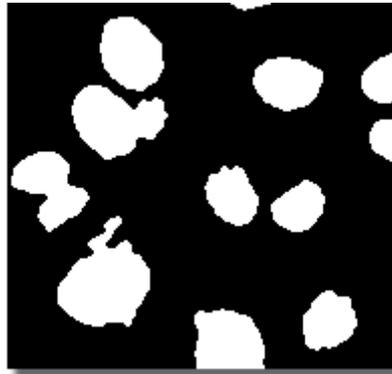
SmoothBinary();



CleanBinary(2,4);



OpenBinary(1,4); CloseBinary(1,4);



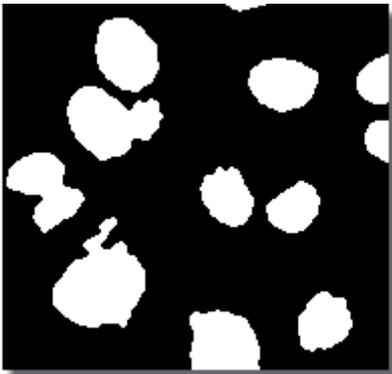
OpenBinary(2,4);



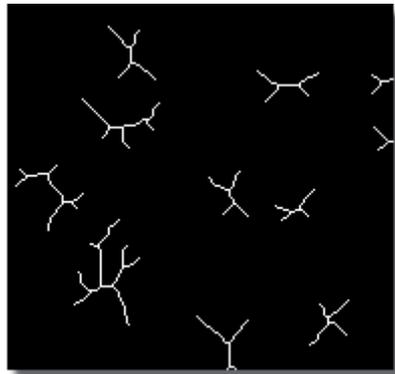
MorphoSeparateObjects(4,1);



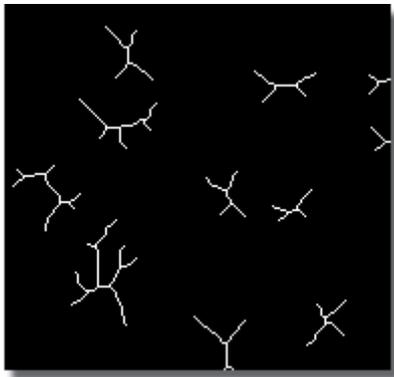
Source



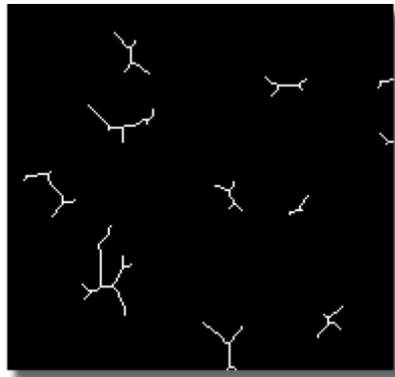
Skeleton



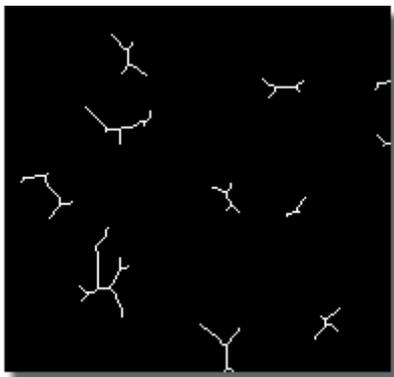
Source



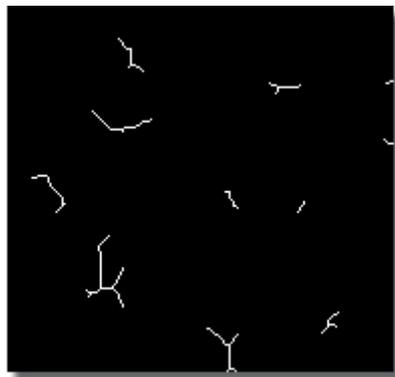
Pruning 3-times



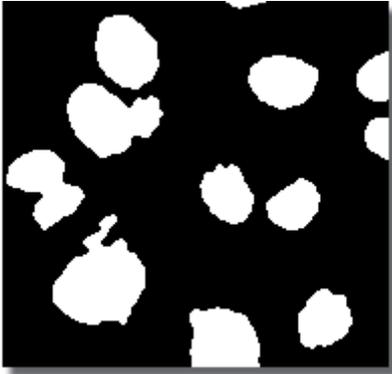
Source



Pruning 2-times



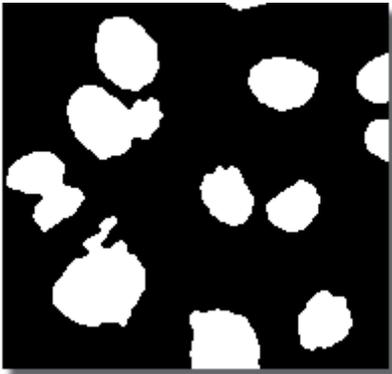
Source



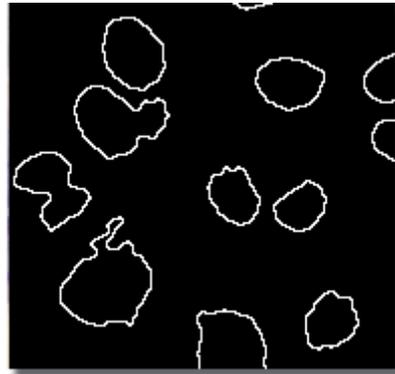
Convex Hull



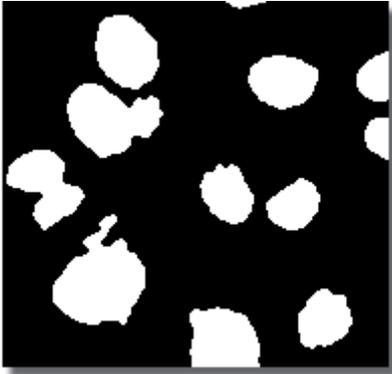
Source



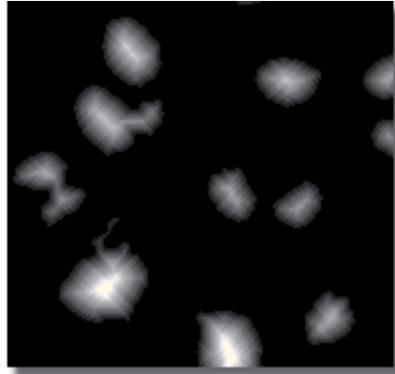
Contour



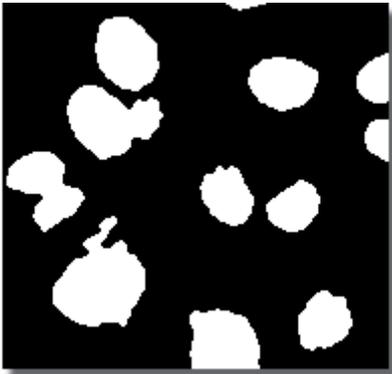
Source



Distance Function



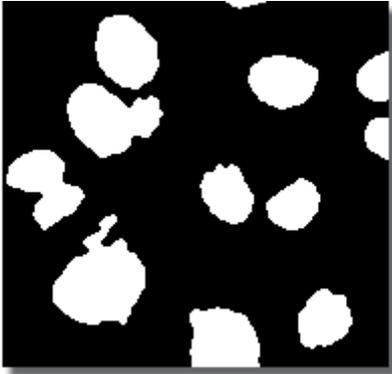
Source



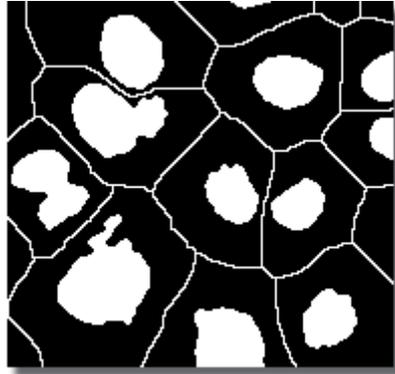
Ultimate Erosion



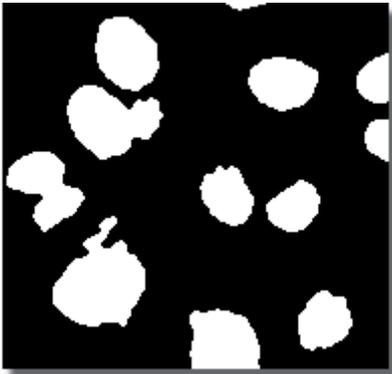
Source



Zones of Influence + Source



Source



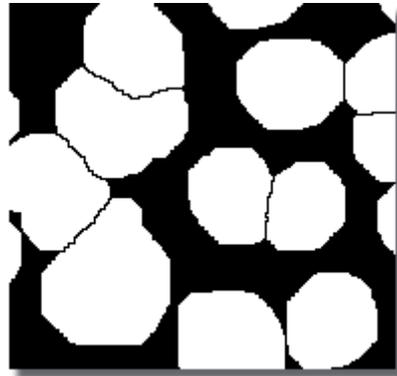
Homotopic Marking



Source



Thickening 9-times



# User Macros

A macro - an executable sequence of commands - can make the work very effective. NIS-Elements provides a C-like programming language utilizing its internal set of commands. The sequence of commands can be created either by recording the performed actions, by writing the commands inside the macro editor, or by modifying the command history (the history is recorded automatically during the work). The created macro can be saved to an external (\*.mac) file for later use.

## Recording a Macro

---

The fastest way to create a macro is to record it.

- Start the NIS-Elements macro recorder by selecting the [Macro > Record] command.
- Perform the series of actions you would like to record.
- Finish the macro creation by the [Macro > Stop Recorder] command.
- It is recommended to check the macro in the macro editor before saving. Run the [Macro > Edit] command to display it.
- Save the macro to a file via the [Macro > Save As] command.

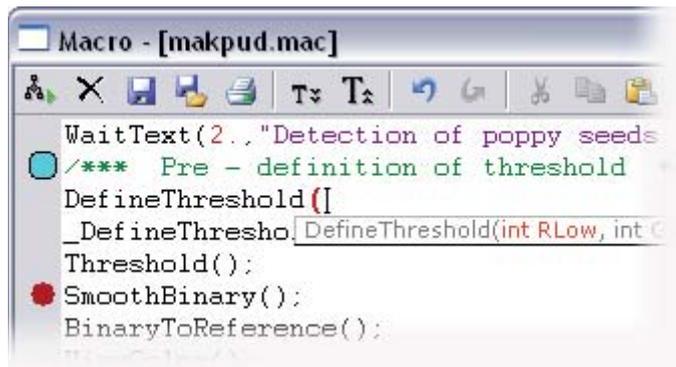
## Writing/Editing a Macro

---

A macro can be written by hand in the built-in macro editor. Run the [Macro > Edit] command to display it.

The macro editor provides:

- Direct output to a printer.
- Undo/Redo functionality.
- Command insertion from the list of available commands.
- Interactive command names list. Press [Ctrl+Space] to display a simplified list of all commands while typing the macro.
- Syntax hints appear as you type a command name. Parameter types and names appear.
- Bookmarks can be placed to the code so you can easily roll to the important parts of the macro.
- Breakpoints can be placed to the code. A breakpoint forces the macro to stop the execution at a certain point so you can check variables state, and inspect the macro "per partes".
- Syntax highlighting.
- Help on commands with detailed description.



```
Macro - [makpud.mac]
WaitText(2., "Detection of poppy seeds
/*** Pre - definition of threshold
DefineThreshold([
_DefineThresho DefineThreshold(int RLow, int C
Threshold();
SmoothBinary();
BinaryToReference();
```

## Creating a Macro from History

---

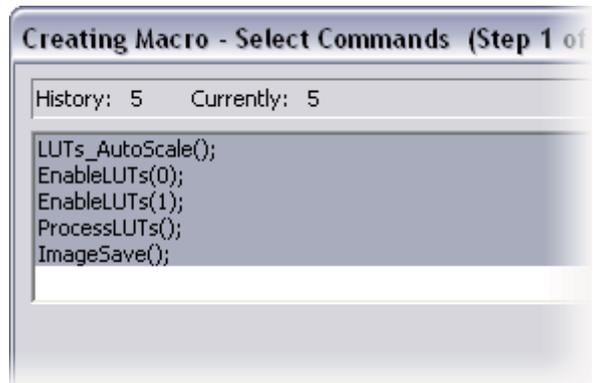
You can create a macro using the list of recently performed commands. Run the [Macro > History] command to display the list and push the [Create Macro] button. The "Create Macro" wizard appears. You can also call this wizard from within the macro editor by pushing the [Insert Commands from History] button.

### 1. Creating Macro - Select Commands

---

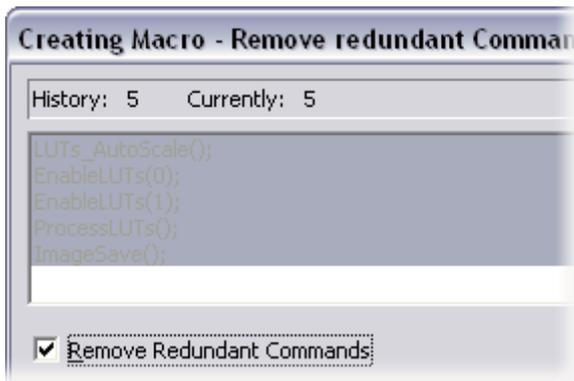
Pick single commands holding the [Ctrl] key, or select a range holding the [Shift] key down. The selected commands will be used in the macro.

- [History] - number of commands performed in the current NIS-Elements session.
- [Currently] - number of currently selected commands.



### 2. Creating Macro - Remove Redundant Commands

---

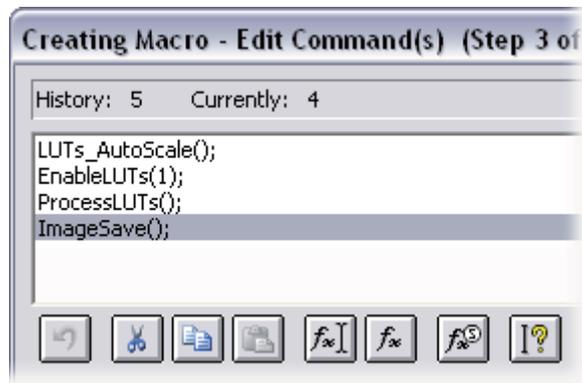


[Remove redundant commands] - the sequence of commands that make no image changes is automatically removed. Usually, it concerns commands that toggles the image between two states and were used several times in a row. It can be for example the ShowAnnotations(), ShowProbe(), or EnableLUTs() command. No functionality is lost when only the last command is kept.

### 3. Creating Macro - Edit Single Command

---

-  Reverses changes that you made within this dialog window such as cutting, copying, pasting or inserting commands.
-  Removes the selected command(s) and copies it to clipboard.
-  Copies the selected command(s) to clipboard.
-  Inserts the command(s) from clipboard before the currently selected command.
-  Opens the list of commands and enables you to edit the parameters of the selected command (or even change the command).
-  Inserts a command from the list of all commands. Select the command you want to paste and press the [OK] button.
-  Inserts a special command. The dialog window that appears includes the most frequently performed actions, such as Run Macro from File, Pause Macro, etc. Select the action you would like to insert and press the [Insert] button.
-  Displays the help page of the selected command.



### 4. Creating Macro - Saving As Macro

---

The last window of the wizard is a standard [Save As] dialog window. Select the destination folder and the macro name and confirm it by the [OK] button. Or, the macro can be saved as [Untitled] which is NIS-Elements default macro (it is always loaded in the list of macros). Press the [Untitled] button (the [File Name] path is filled automatically) and the [OK] button.

#### Saving a Macro

---

When you create a macro by recording, you can save it using the [Save or the Save As item in the Macro menu. To save a macro during editing it, press the Save button in the macro editor.

#### Running a Macro

---

NIS-Elements provides several ways to run a macro.

- You can run the current macro loaded to NIS-Elements by choosing the [Macro > Run] command or by pressing [F4].
- You can run a macro that is opened by the [Macro > Open] command by pressing [F4].
- You can run a macro directly by pressing a special [hotkey] combination (Ctrl+Alt+1,2,3 ... 9) that you have assigned to it in the [Macro > Options] dialog window.
- You can run a macro at the beginning of the NIS-Elements session, by assigning the

[StartUp] flag to the macro in the the [Macro > Options] dialog window.

- You can run a macro directly by using the [Macro > Run From] command.
- You can run a macro by clicking a tool bar button that you have assigned to it by the [View > Customize Toolbar > Setup] command.

## **Breaking a Macro**

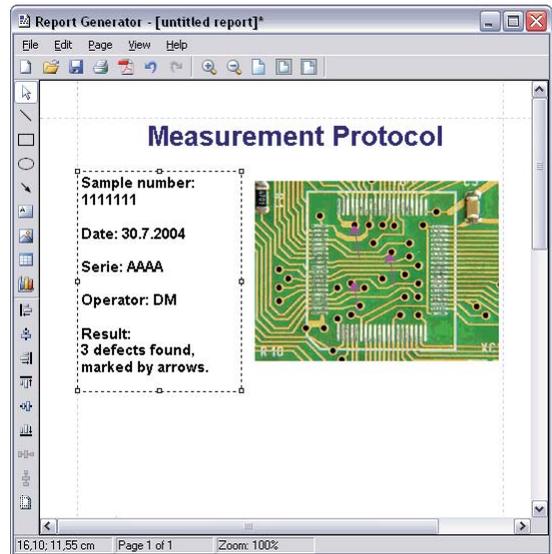
---

The macro execution can be stopped by pressing the [Ctrl+Break] key shortcut.

# Creating Reports

Report Generator enables a user to create customized reports containing images, measured data, user texts, graphics, or database entries. PDF files can be created directly from NIS-Elements. When you invoke the [File > Report > New Blank Report] command, an empty Report Generator appears. It provides the following tools:

-  Draw line...
-  Draw rectangle...
-  Draw ellipse...
-  Draw Arrow...
-  Insert text field...
-  Insert image from file...
-  Insert Table
-  Insert graph
-  Aligns the selected objects to the position indicated by the line (left)
-  Aligns the selected objects to center
-  Distributes the objects regularly in specified direction
-  Determines whether to apply the align commands relatively to the whole page or to selected objects



## Context menu

Once you insert an object, its appearance, behavior, and position can be changed via the context menu which appears on right-click:

- The [Align or Distribute] command enables moving objects within the page or align them to another object.
- The [Resize object] command enables to resize two objects to the same size.
- The [Bring to front] command changes the order of overlapping objects and brings the current object to front.
- The [Send to back] command moves the object to the bottom layer.
- The [Lock] command locks the object disabling any further changes to it until it is unlocked.

- The [Properties] command opens a dialog window where object properties such as color, background color, border size and color, object size, font, aspect ratio, alignment, and shape can be adjusted.

## **Working with Report Objects**

---

When you double-click any of the objects of a report, a dialog window appears where the properties can be adjusted. Some properties are common for all types of objects, and some are special.

### **Common Properties**

---

- The precise position can be determined by defining the XY coordinates of the top-left corner of the object.
- Line and fill colors can be selected in a pull-down menu.
- Line widths can be adjusted within the range of 0,75 to 6,0 pts.
- Precise width and height of the objects can be adjusted.
- Rotation of some objects can be adjusted.

### **Special Object Properties**

---

#### **Ellipse**

- Minor and major axes lengths can be modified.

#### **Arrow**

- The arrow tip shape and size can be modified.

#### **Text Box**

- Format of the text can be adjusted like in a standard text editor.
- The current date and time can be inserted from a pulldown menu.

#### **Image**

- A new picture can be loaded to the image frame via the [Load Picture] button.
- The current image scale is displayed in the properties dialog window. It shows the *current scale/original image dimensions* ratio (the image must be calibrated to use this feature, e.g. scale 1:2 means, that the image will be printed in half of its original size).
- A scale bar can be displayed below the image. The position and width of the scale bar can be defined.

#### **Table**

- Inner and outer borders can be hidden/displayed arbitrary.
- Number of rows/columns can be modified.

#### **Graph**

- The range of displayed values can be limited by defining the min/max values.
- Histogram labels can be edited.

## Aligning Objects

---

You can align two objects to the same horizontal or vertical level.

- Select more objects (e.g. by holding down the CTRL key and clicking with the left mouse button).
- Right-click one of the selected objects and select the [Align or Distribute > Align ...] command from the context menu.
- The objects will be aligned as indicated on the command icons.
- If the [Relative to Page] option is turned ON, the objects will be aligned to the edges/center of the page.

Objects of similar size can be distributed uniformly in the horizontal/vertical direction.

- Select three or more objects.
- Right click one of the objects and select the [Align or Distribute > Distribute...] command from the context menu.
- Distances between the objects will be adjusted to equal.
- If the [Relative to Page] option is turned ON, the marginal objects will be moved to the edges of the page.

Sizes of objects can be unified too.

- Select two or more objects.
- Right-click the "master" object to the size of which you want to resize the other objects.
- Select one of the [Resize Objects] submenu commands.

## Dynamic Data

---

Results of automatic/interactive measurement, graphs, or the current image can be inserted to reports.

- Insert an object which can contain dynamic data (text, picture, table, or graph).
- Right-click the object and select [Insert Dynamic Data/Insert Dynamic Picture] from the context menu.
- A dialog window appears.
- Select one of the available sources, and click [Next].
- Finish the source definition and click [OK]. The data appear on the report page.

### **List of available dynamic data sources:**

- **Data inserted by user** (*available for: image, text, table*) - the system will ask you to type a text or browse for an image to insert during the report creation. When creating a dynamic object of this type, a query text which will be used to prompt you for the data can be defined.
- **System data** (*available for: text, table*) - enables you to insert some general data such as date, the name of the user account currently logged in, the page number, or the page count.
- **Macro** (*available for: text, table*) - enables you to insert expressions, values, or results of the macro which is running simultaneously.
- **Measurement** (*available for: text, table, graph*) - enables you to insert results of

automatic/interactive measurement.

- **Database** (*available for: image, text, table*) - this data source is displayed only when creating a database report template (see below). It enables you to insert a link to any of the database records.

The real purpose of the dynamic data turns up when creating reports using report templates:

## **Working with Report Templates**

---

A report template is a layout of the future report(s) which is ready to be filled with data. The data can be inserted by the user "on demand" or automatically (dynamic data).

### **To Create a Report Template**

---

- Run the [File > Report > New Template] command. An empty Report Generator appears.
- Edit the report template in the same way as a common report.
- Insert the dynamic data where it is appropriate - measurement results to text boxes/tables, the current image to the image field, etc.).
- Save the report template (\*.rtt) via the [File > Save] command.

### **To Insert Dynamic Data to Report Templates**

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- Insert an object which can contain dynamic data (text, picture, table, or graph) to the template.
- Right-click the object and select [Data Source] from the context menu.
- A dialog window appears.
- Click the [Define] button. Another dialog window appears.
- Select one of the available sources, and click [Next].
- Finish the source definition and click [OK]. The data appear in the template.

### **To Create a Report from the Template**

---

There are two ways of creating the report.

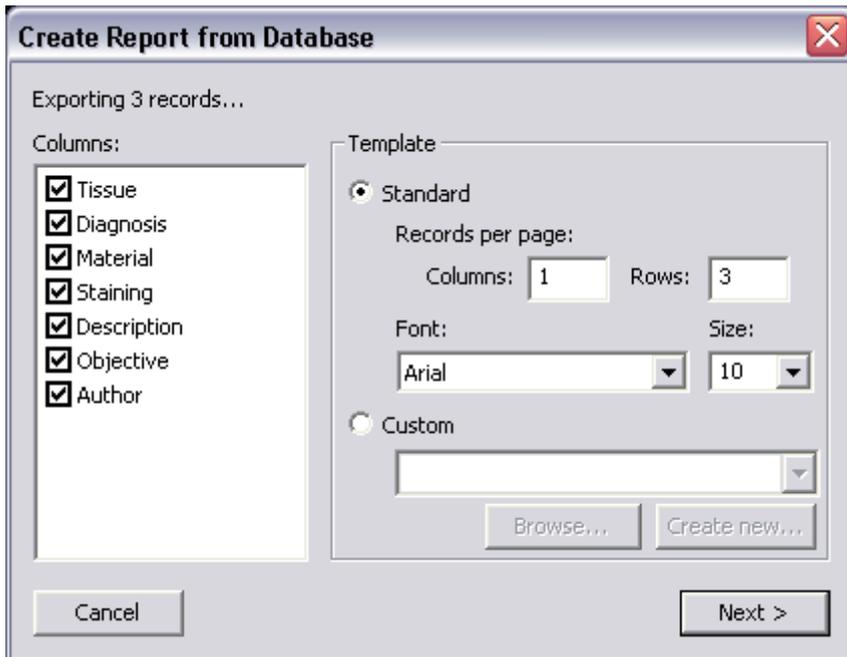
- If a template is opened inside the Report Generator, run the [Report Generator > File > Create Report] command. Otherwise, use the [NIS-Elements > File > Report > New Report from Template] command to open a template from harddisk.
- A report opens and the dynamic data is inserted automatically.
- Save the report, print it, or export the page(s) to PDF using the commands from the [File] menu.

## Creating Reports from Database

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Pictures of a database together with the associated table data, or common images with the associated [Image Info] can be exported straight to a report.

- Switch NIS-Elements to [Organizer] by the [View > Organizer Layout] command.
- Select one or more images. These images will be exported to the report.
- Click the [Report] button on the main toolbar. The following dialog window appears:



- In the [Columns] section, you can select fields, which will be included in the report. If you are exporting the images from database, you can select database fields. If you are exporting images from a directory, this enables you to select the image text description fields (applies only to images with text info: JPEG2000, LIM and TIFF).
- The [Template] portion of the dialog specifies the report template. If you select the [Standard] template, images will be organized in rows and columns. It is possible to set the number of columns and rows.
- If you select the [Custom] report template, then a user report template (\*.rtt) can be opened from the disc and used for the report creation. If there is an existing report template, click on the [Browse] button, otherwise click on the [Create new] button.

### After You Pressed the [Create New] Button

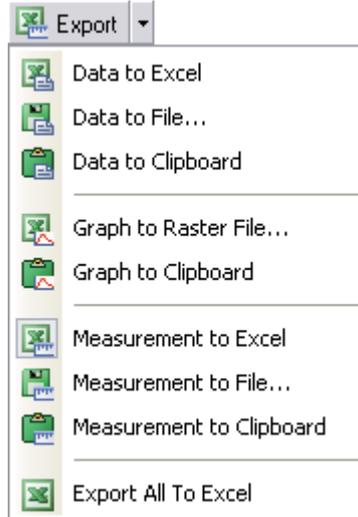
---

- A wizard appears. Select number of columns and rows of the new report template. Click [Next].
- Select the paper size, page orientation, and define margins. Click [OK].

- An untitled report template opens containing a grid spaced according to the [columns/rows] settings.
- Edit the top-left cell of the grid. You can change the text/image boxes position, size, and mapping. During report creation, the other cells of the grid will be filled automatically according to the settings of the first one.
- A custom header or automatic page numbering can be added to the report.

# Exporting Results

Some image analysis data and measurement tasks results can be transferred out of NIS-Elements so they could be used further. This regards for example the intensity profile, histogram, measurements control windows. There is a standard [Export] pull-down menu in some of the control windows which enables the export.



The set of commands of the menu differs according to the control window type. Click the [Export] button to display the pull-down menu. When you select the subject of export and the destination, the pull down menu hides and the icon corresponding to what you selected appears on the [Export] button. The export action is performed after the button is pressed once more.

## **Export Destinations**

---

It is possible to export tabular data or a graph:

### **To MS Excel**

---

Tabular data can be exported to MS Excel. A new XLS sheet opens and the table is copied to it automatically. Sometimes, there is also the [Export (All) To Excel] option, which copies the tabular data and the graph image into the sheet.

### **To a File**

---

Data tables can be exported to an external \*.txt file, graph images to a \*.bmp file. Select the command from the pull-down menu and define the target file name in a standard Save As dialog window, which opens. Confirm the export by the [Save] button.

### **To Clipboard**

---

Data tables and graph images can be exported (copied) to Windows clipboard. Then the data or the image can be inserted into any appropriate application (text editor, spreadsheet processor, graphics editor) typically by the [Paste] command.

### **To Report**

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Tabular data and graph images can be exported to NIS-Elements report. If a report is already opened for editing, the data/graph will be appended to it. Otherwise, a new report will be created and the exported data inserted to it.

### **To Web Browser, To Clipboard as HTML**

---

Some tabular data can be exported as a HTML table. It can be displayed in a default internet browser (the [To Web Browser] option), or the HTML code can be copied to Windows clipboard, ready to be inserted to any HTML editor.

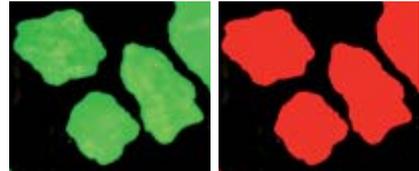
# Tips & Key Shortcuts

## Binary Layer Properties

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When in overlay mode:

- [Insert] switches between predefined overlay colors.
- [Ctrl + Up/Down] increases/decreases the binary layer transparency.



Single binary objects can be erased in the following way:

- Run the [View > Analysis Controls > Binary Toolbar].
- Select the [Delete Object] tool from the toolbar.
- Click inside the objects to be erased.

## Copying Channels by Drag and Drop

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### Single images

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- Drag one of the channel tabs and drop it:
  - To another image, so it will become a multichannel image.
  - Inside the NIS-Elements application window in order to create a new document.
- Even the [All] and [RGB] tab can be copied.
- When extracting a channel from Live image, it does not freeze the camera signal.



### ND2 documents

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- Drag one of the channel tabs and drop it by the [left mouse button]. It behaves the same way as if performed on a single image.
- Drag one of the channel tabs by the [right mouse button] and drop it to the application screen. A context menu appears that enables you to select whether to create the new document from all frames or just a single frame.
- [Two ND2 documents] that have matching structure can be [merged into one] easily. E.g. two single channel Z stacks with the same number of Z positions can be merged into a single two component ND2 Z-stack by dragging one into the other.

- Drag one of the channel tabs of a [single image] and drop it [to an ND2 document]. The channel will be copied to every position of the ND2 file. *This behaviour too takes place when copying channels that do not have matching structure (just the current frame is copied to every position of the target ND2).*

## **Interactive channel selection**

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Two or more channels can be selected to be viewed:

- Click the appropriate channel tabs while holding the [Ctrl] key down.
- If some processing is performed, it is applied to the selected channels only.

## **Shifts on ND2 documents**

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A single color channel or the whole image (all channels) can be shifted using commands of the [Image > Shift] submenu. If an ND2 file is opened, the shift can be applied arbitrary to:

- the current image frame.
- all images of the ND2 document.
- a selected dimension of the ND2 document.

The procedure works equally to other image processing performed on ND2 files:

- Select the image frame and the channels to be shifted.
- Perform the shift using [Ctrl+Shift+Arrows] key shortcuts.
- A standard ND2 image processing dialog window appears.
- Select which part of the ND2 document to apply the shift to, and confirm it by [OK].

## **Live Image, Frozen Image**

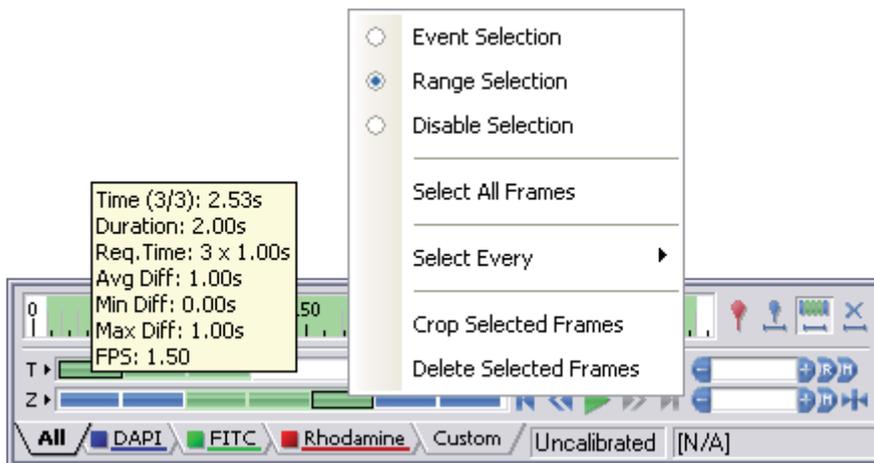
---

- [+] turns the [Live - Fast] camera mode ON.
- [-] freezes the Live image.
- [Ctrl] + [+] turns the [Quality - Capture] camera mode ON.
- [Ctrl] + [-] performs image acquisition in the Quality - Capture mode.

## ND2 documents in detail

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- Make a user selection of the frames by mouse holding [Ctrl] or [Shift] down.
- Right-click the navigation bar to invoke the context menu. A selection can be adjusted, deleted, or the nd2 file can be cropped.
- Detailed info about dimensions will be displayed after you click the leftmost button of the navigation tool bar (T>, Z>, MP>).
- Place the cursor over the navigation tool bar of one of the dimensions. A tooltip which displays statistics of the dimension appears.



## LUTs

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A context menu can be displayed when right clicking the LUTs graph area. The following options can be selected:

- [Draw trend style] - When ON, the LUTs curves will be smoothed to display the data trends rather than represent the actual image data values.
- [Copy LUTs, Paste LUTs] - The current LUTs settings may be copied and inserted to another image.

The position of each triangular slider at the bottom of the LUTs window can be reset by double clicking it. The pixels that reach the maximum value can be highlighted using the LUTs [Color Overexposed] button.

## Modifying Application Appearance

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- All buttons on main tool bars may be enlarged. Go for [View > Layout > Layout Manager], select [Toolbars] in the layout tree, and check the [Large Buttons] option. It affects all layouts.
- The channel tabs at the bottom of image window may be hidden to save some space of the screen. Go for [Edit > General Options > Appearance] and unselect the [Show Channel Tabs] option.

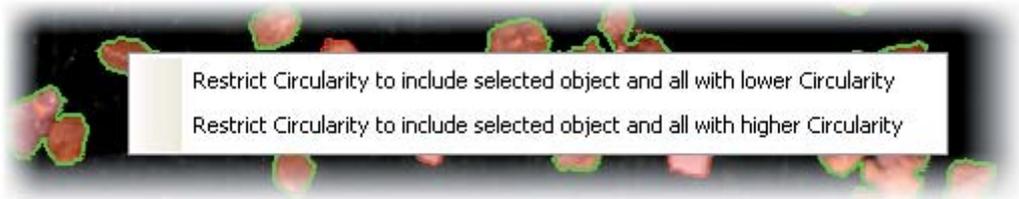
- When an image is displayed in great magnification, scroll bars automatically appear by the sides of the document window. You can hide them by selecting the [Hide Scroll Bars] command from a context menu (right click inside the image).

## Restrictions

---

An easy way to apply restrictions to objects measured via the [Object Count] tool is available:

- Open the Object Count control window, threshold the image to separate objects from background.
- Right click the list of restrictions features and pick all features you would like to use.
- Select the one restr. feature you would like to set (now) by mouse. Lets assume you have selected Circularity.
- Right click the thresholded object that is going to be used as a reference one. A context menu appears.
- Select whether to include the selected object and all others with higher/lower circularity.
- The restriction will be applied and indicated by colors within the image.



## Key Shortcuts

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[Z] - It switches to the [Magnifier Glass] tool. When a detail is being observed with Magnifier Glass and you press the [Z] key again, local contrast enhancement is performed inside the magnified area. This feature may help to increase the contrast of dark parts of images that are no more enhanced by the global LUTs settings.

- When using the Magnifier Glass tool, pressing the [Ctrl] key switches to pointing tool functionality temporarily (so that the image can be dragged by mouse).
- If the [Shift] key is pressed while applying the Magnifier Glass, you can move the magnified part of the image aside.
- When applying the Magnifier Glass to a Z-ND document, pressing the [Ctrl] key changes the mouse wheel functionality. The mouse wheel zooms the image instead of browsing through the Z-sequence.

[X] - It performs the Create View Snapshot command. The snapshot includes even the magnifier glass tool when the X shortcut is pressed while observing a detail.

[`] - Zooms the current image to Fit to Screen mode.

[1] - Zooms the current image to 1:1 mode.

[2] - Zooms the current image to Best Fit mode.

[Up] - Increases zoom of the image.

[Down] - Decreases zoom of the image.

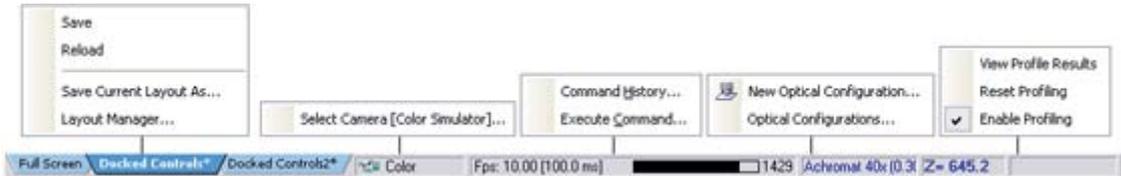
## Context menus

---

Number of context menus can be displayed by clicking the right mouse button on various items throughout the application:

## Main Status Bar

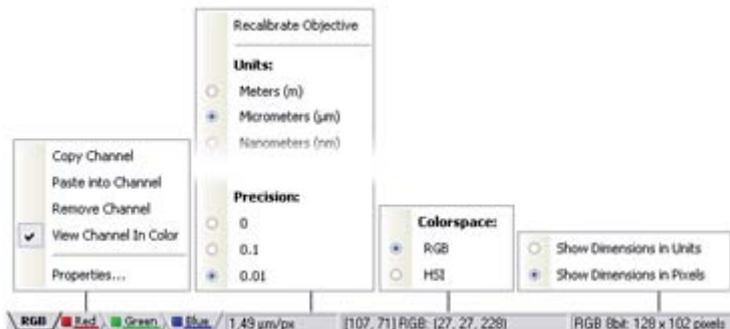
---



- **Layout Tabs** - [Save Current Layout As] and [Layout Manager] commands are available.
- **Selected camera area** - the [Select Camera] command is available.
- **Command History area** - the [Command History] or [Execute Command] dialog windows may be invoked.
- **Current Objective area** - [New Optical Configuration] and [Optical Configurations] commands are available.
- **Profiler area** - [View Profile Results], [Reset Profiling], and [Enable Profiling] commands are available

## Document Status Bar

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- **Channel Tab area** - [Copy Channel], [Paste Channel], [Remove Channel], [View Channel In Color], and [Properties] commands are available.
- **Document Calibration area** - [(Re)calibrate Document] and [Calibrate Using Objective] commands are available when the current image is uncalibrated. [Precision] of the calibration display, [Units] to work with can be set, and the [Recalibrate Document] and the [Set Document] as uncalibrated commands are available when the current image is calibrated.
- **Mouse Position area** - Color space to show the pixel values in can be selected (if an RGB

image is opened).

- **Image Info area** - [Show Dimensions in Units] and [Show Dimensions in Pixel] options are available (when the current document is calibrated).

## Mouse Wheel

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### Zooming

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The mouse wheel serves for zooming images in/out by default. Its behavior differs according to the type of the currently opened document:

- **ND files containing Z** - browses through the Z stack of images.
- **Other document types** - zooms the image in/out.

[ **Ctrl + mouse wheel** ] - zooms in/out every image including the Z-ND files.

### Auto Detection Tools

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Some auto detection tools use the mouse wheel for adjusting the detection parameters. This regards the [Auto Detect] tool of the [View > Analysis Controls > Binary toolbar], and of the [View > Analysis Controls > Annotations and Measurements] control windows.

## Vector Objects

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Vector objects (report objects, annotations, interactive measurement objects) can be copied by "drag and drop" while holding the [Shift] key down.

- Select the object(s) (text, shape, interactive measurement).
- Press [Shift] and drag the object somewhere.
- A copy of the object is placed where you release the mouse button.

### Default Color Settings

---

The default colors of vector objects can be set in the following way (each annotation tool keeps its own color, manual measurement objects share the color settings):

- Draw a vector object.
- Right click the object and select [Properties] from the pull-down menu. A dialog window appears.
- Adjust the color (and other) settings and confirm them with [OK].
- The settings will be used when drawing subsequent objects of the same type.

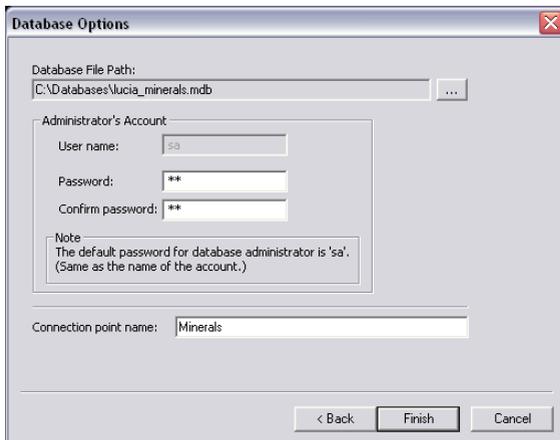
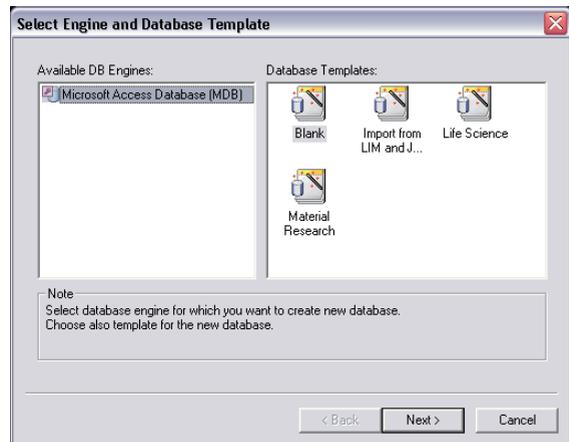
# Database module

NIS-Elements offers a database tool to efficiently manage images along with additional information. After you install the Database module, the [Database] menu appears in the main toolbar.

A database is an organized body of related information. It consists of one or more database tables filled with data. Before you can start filling the tables, you need to create a new empty database. This can be done by selecting the [Create database] command from the Database menu.

## Creating database

Select the Microsoft database engine in the left list and the [Blank] template from the list of templates. Click on the [Next] button.

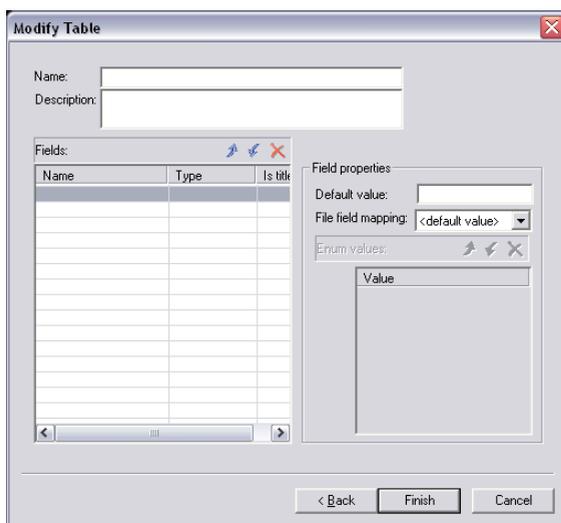
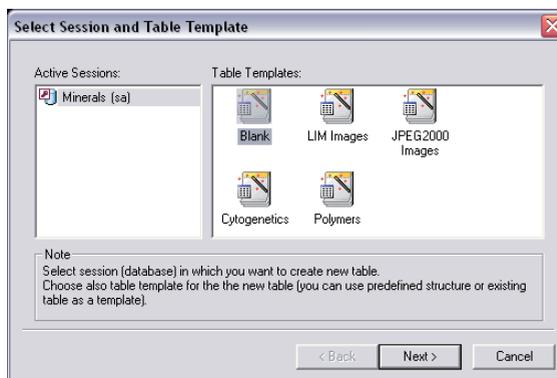


Specify where the database file should be stored on a disk - define the [Database File Path] („C:\Databases\minerals.mdb“ for example) and the database file name. You, as the database creator, are automatically considered as an administrator (the user name and password are „[sa]“ by default). The administrator is a user with all possible rights to the database. Next, define a descriptive name of a connection point, e.g [Test] for the testing database. Click [Finish] and an empty database is created and connected through a connection point (see below).

## Creating tables

A database can contain one or more tables to store your data to. Select the [Create table] command from the Database menu.

Select the session from the left list. In our case, there should be only one: „[Test(sa)]“. It means: the user „sa“ is connected to the database through the „Test“ connection point. It is possible to have one user connected to more databases or more users connected to the same database on one system. On the right side, choose the [Blank] template and click the [Next] button.



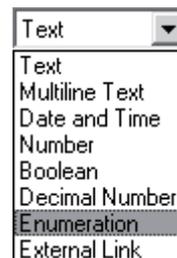
Enter the [Name] of your new Table (e.g. Minerals). The Description editbox isn't necessary to fill, but it might prove helpful to put down a short comment.

Define the table fields. First, click to the [Name] field and fill it in (e.g. Author). Press [Enter] and define the data type of this field. This is done by selecting one option from the combo box, which opens after clicking into the [Type] field. Select the [Text] type, because names are usually short enough to fit in this data type. The [Is title] field determines, which database entry is used as a description of images while viewing the table using the thumbnails view of Organizer. Define all fields you want to include in the database table and click [Finish]. The new table is created.

## Special Field Types

Let's assume that pictures come from a closed group of people. In this case it is useful to set the field type of the „Author“ field to [Enumeration]. The Enumeration value edit box on the right becomes active and you can enter all names of possible authors. When adding a new image to the database later, you can just select the author's name from the combo box instead of having to type it.

Another special field type enables to insert arbitrary external links into database tables. Select the [External Link] option as the field type. Later, you can insert a command, link to a website etc. to these fields.



## **File Field Mapping**

It may be very handy to insert some image properties to the database. Therefore the [File field mapping] can be used to map file properties (such as filename, file date, calibration, author etc.) included in the image file to the table fields. Select the information type from the combo box. If the <default value> is selected, fill in the [Default] value text box and that text will be used for every database entry added to the table.

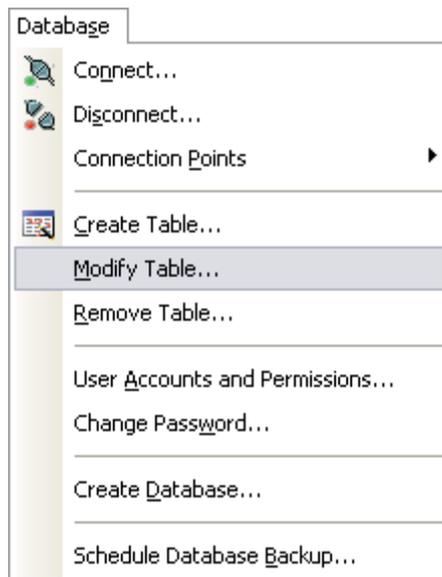
## **Managing Tables**

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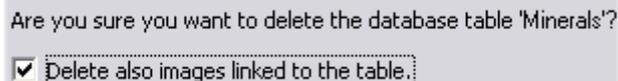
After you have created one or more tables, you can manage them easily using the [Remove Table] and [Modify Table] commands from the Database menu. Each command opens a dialog window.

The Modify Table command asks you to select a table to modify and opens the table properties window.

The Remove Table command removes a table from a database. However, the database images may optionally not be deleted. A directory to store images of each table is created during the table creation. The directory has a name that matches the name of the table and is placed in the directory where the database file is stored. Copies of the source files from your disk are created in there for database purposes.



If you check the [Delete also images linked to the table] check box during the table removal, all these copies are deleted from your disk. Otherwise, the copies remain untouched.



## Adding Images to Database

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You have successfully created a database with at least one table. Use it to store pictures with descriptive information. Every time you choose the [File > Save to Database] command, you will be prompt to pick a table to write in. The [Save] button invokes a window to fill the database entry information and saves the current image into that table. View the database records in the [Organizer] view (the [F10] hotkey).

## Connecting to a Database

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### Connection Points

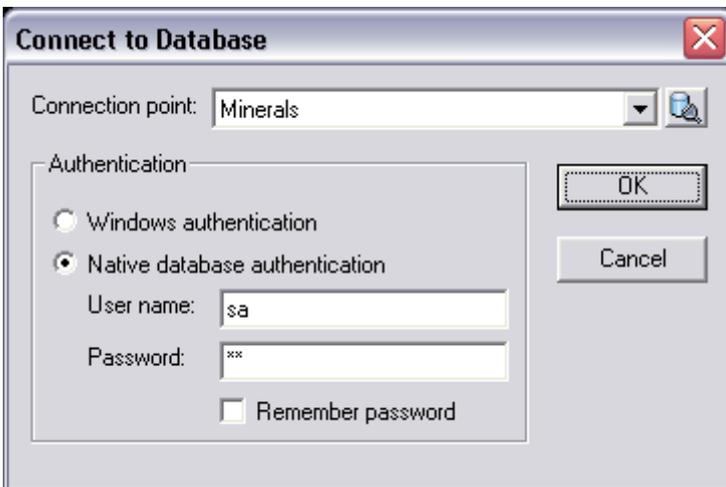
---

Connection points are intended to ease work with databases. A connection point has similar functionality to file shortcuts or links. It can be named arbitrarily and you can access the database through it. It is allowed to create and use one connection point per database here.

Let`s suppose you have a database created. Launch the [Create Connection Point] command. Write the connection point name and select the path to the database file in the wizard that opens (once you have created the connection point, you can manage user accounts and permissions of the related database by selecting the [User Accounts and Permissions] command from the Database menu). You can also create more or delete some connection points in the connection points manager (Database menu > Connection Points submenu > Manage Connection Points command).

## Connecting to Database

Use the [Connect] command to connect to a database. Select the connection point (representing the database) from the combo box. In case this combo box is empty, press the icon on the right side to create a new connection point.

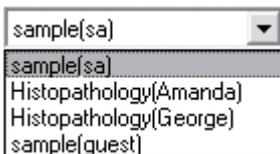


### Authentication

To access the database, a user authentication is needed. If the [Windows authentication] option is chosen, the username and password of your windows account are used when logging in. [The Native database authentication] requires entering the username and password which is valid for the database you want to connect to. Click [OK] to connect.

### Active Connections

You can view the list of active connections in the [Organizer] view (F10 key shortcut). The [Disconnect] menu command displays the dialog box where you can select the connection point to be disconnected. Disconnecting the point closes the way to save images to the corresponding database for the user. Still, you can access the other databases through other connection points.

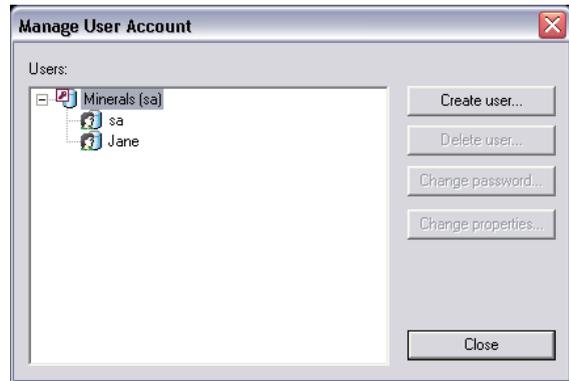


Every user who possess rights to the database administrator account (with the username „sa“ by default) can manage other user accounts using the [User accounts and permissions] menu command.

## **User Accounts and Permissions**

The [Create user] button displays a dialog box (see below). The [Delete user] button displays a confirmation dialog box - when [OK] is pressed, the user is deleted.

The Change password button enables to set a new password for the selected user. The dialog box with two editboxes (the new [Password] and its [Confirmation]) appears. The [Change properties] button opens an advanced dialog box where you can set various user rights.



## **Create New User Account**

The user creation allows you to choose between the [Regular user] (this option enables to create an arbitrarily named user) and the [User from Windows account] (it is possible to choose from users, who have a windows account on the current computer).

Write a new user name to the [User name] box or select existing user from the list of windows accounts. You can add any comments to the [Description] box. Enter a [Password] twice ([Confirm Password]) and [Create] the new user.

*Multiple connections to databases using different user accounts are permitted. If some intended operations are unavailable, check whether you are logged under the correct user account with adequate rights.*

## User Permissions

Having sufficient privileges, you may change the other user's accounts properties by pressing the [Change properties] button in the [User Accounts and Permissions] window. The dialog box, where it is possible to set user rights for various actions performed on a database, opens. To set or ban the rights, click into the check boxes. There are three main sections in the dialog window: the [User rights], [Tables] and [Fields].

The dialog box 'Table and Field Properties for 'Jane'' contains the following sections:

- Description:** A text input field.
- User rights:** A group box with four checkboxes: 'Can create user' (checked), 'Can delete user' (unchecked), 'Can modify user' (unchecked), and 'Can create table' (checked). 'Can delete table' is also present but unchecked.
- Tables:** A table with columns: Table, View, Create record, Delete record, Create field, Delete field, Delegate rights. Rows include 'Cytogenetics' and 'Polymers', both with all permissions checked.
- Fields:** A table with columns: Field, View, Modify value, Modify type. Rows include 'Sample', 'Type', 'Magnification', 'Camera', 'Method/Technique', 'Description', and 'Date', all with 'View' and 'Modify value' checked.
- Note:** A text area with the placeholder 'Some hint should be here.'

### The User rights section

This section is used to define, what actions are allowed for the selected user in relation to other users and all databases. Decide, whether the user can delete, modify, create user accounts or delete and create tables. The „[sa]“ (database administrator) user's properties cannot be modified nor that account can be deleted by other users.

The 'User rights' section contains the following checkboxes:

- Can create user
- Can delete user
- Can modify user
- Can create table
- Can delete table

### The Tables section

Displays a list of existing tables. It is possible to set rights to view, create and delete whole records, as well as rights to create and delete single fields of a table. When the [Delegate rights] check box is checked, the user is allowed to modify permissions of other users for that table.

Table	View	Create record	Delete record	Create field	Delete field	Delegate rights
Cytogenetics	<input checked="" type="checkbox"/>					
Polymers	<input checked="" type="checkbox"/>					

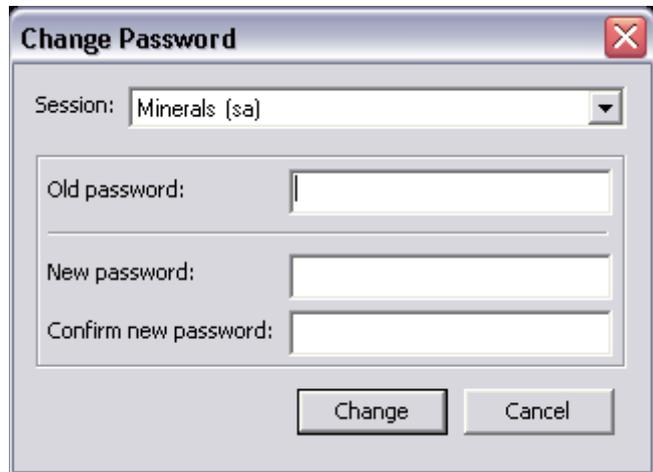
## **The Fields section**

More detailed settings for each table are listed here, if the table was selected in the [Tables] section. You can only see the fields that your privileges allows you to view. You can set, if the user will be able to view or modify every single field.

Field	View	Modify value	Modify type
Sample	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Type	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Magnification	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

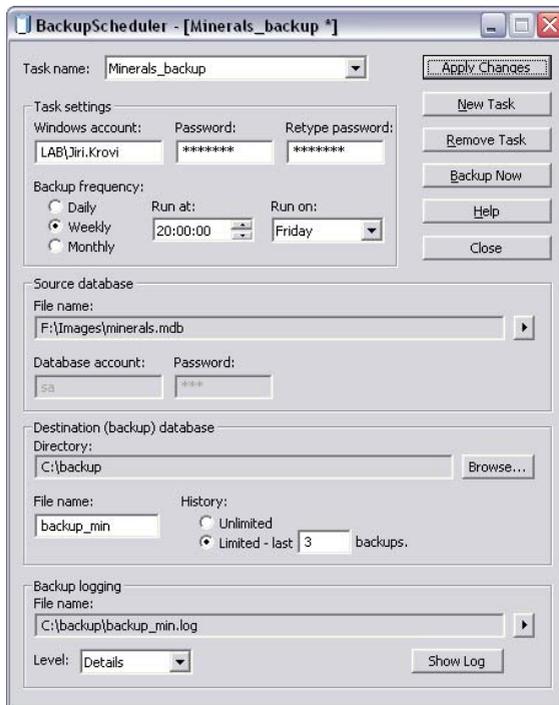
## **Changing Password**

You can change a password to a database via the [Change password] command from the Database menu. Enter your old password, and a new one twice to prevent a typewriting mistake. The [Change] button finishes the operation. A user with a privilege to „[Modify user]“ can change the other user`s passwords via the User Rights and Permissions menu command.



The image shows a 'Change Password' dialog box with a title bar containing a close button (X). The dialog contains a 'Session:' dropdown menu with 'Minerals (sa)' selected. Below this are three text input fields: 'Old password:', 'New password:', and 'Confirm new password:'. At the bottom right, there are two buttons: 'Change' and 'Cancel'.

## Database Backup



A possibility to backup the database is essential and strongly recommended for any serious work. The [Database Backup Scheduler] enables the system to backup the database automatically once in a precisely specified time interval, so you do not have to be afraid to loose any data. The Database Scheduler uses the standard Windows „[Scheduled Tasks]“ tool (Start > Control Panel > Scheduled Tasks). Once you schedule a backup, there is only one condition to perform it succesfully: The computer must be [ON] at the scheduled time.

## Backup Settings

To set a new periodic backup, click the [New Task] button. In the dialog window that appears, specify the task name and click [OK]. If you already have some task that suits your needs, it is possible to copy its properties by checking the [Based on] check box and choosing it s name from the list.



**Task settings**

Windows account: Password: Retype password:

[LAB\Jiri.Krovi] [\*\*\*\*\*] [\*\*\*\*\*]

Backup frequency:

Daily      Run at:      Run on:

Weekly      [20:00:00]      [Friday]

Monthly

Since the task is based on the Windows component, it is necessary to run it under a Windows user account. Either a local or a domain account is allowable (the domain name „[LAB]“ is used in our example). After filling in the password, decide, how often the backup shall be performed. Daily, weekly, and monthly intervals with a precision of seconds are available.

Furthermore, the location of three following files on a disk is needed. Use the [Browse] buttons on the right side of the textboxes to specify the paths to the [Source database] file (the file to be backed up), the [Destination (backup) database] (a directory to which the backup file will be written) and the [Backup log] file where the information about the backup process is stored. The specification of all three destinations is mandatory. Without it, the backup cannot start.

**Source database**

File name: [F:\Images\minerals.mdb] ▶

**Destination (backup) database**

Directory: [C:\backup] [Browse...]

**Backup logging**

File name: [C:\backup\backup\_min.log] ▶

### **Detailed Backup Settings**

In the Source database settings, there are the Database account and Password text boxes. They are not editable and are for information only.

Database account: Password:

[sa] [\*\*\*]

### **Backup History**

File name: [backup\_min]      History:

Unlimited

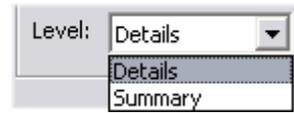
Limited - last [3] backups.

When performing a backup periodically, the already existing (the latest) backup file`s content is moved to a new history file and a suffix with the iteration number is attached to its name. The new backup file is created.

This way, an unlimited number of the history backup files is being created on a disk. When you limit the number of history files (e.g. to prevent the disk capacity overflow), each time the scheduler replaces the latest file with the new one, it also renames all the other backup files accordingly. As a result, the file names and their number on a disk remain the same (although their content changes).

## **Backup Logging**

The amount of information to be written to the backup log file is determined by the [Level] choice. The [Details] option records changes of all files of a database compared to the previous backup. If you choose to record the [Summary] only, possible critical errors and backup process results are included in the log file.



A screenshot of a software interface showing a dropdown menu for the 'Level' property. The menu is currently open, displaying three options: 'Details', 'Details', and 'Summary'. The top 'Details' option is highlighted with a blue background. The label 'Level:' is visible to the left of the dropdown box.

# The Extended Depth of Focus Module

The EDF module allows you to combine existing Z-stack images into one focused image picking the focused regions from each frame and merging them together. The "Align" algorithm ensures correct fitting of pixels even for images acquired via macro optics, where objects are slightly shifted or scaled during the z-stack acquisition.

When you start NIS-Elements with the EDF module installed, the following items appear in the [Applications] menu:

- Open File Sequence
- Align Sequence
- Create Focused Image
- Edit Area in Focused Image
- Real Time EDF
- Real Time EDF Manually
- Options

Open (or acquire) an ND2 document that includes the Z dimension. If you use the [Open File Sequence] command to get the Z stack of images, a new ND document is created automatically.

## Align Sequence

---

Small shifts of the images can occur as a result of hardware inaccuracies during the Z series acquisition. To get reliable results out of the EDF module, the sequence that is to be processed should be aligned first. Use the **Align Sequence** command to automatically correct possible shifts.

## Select the method

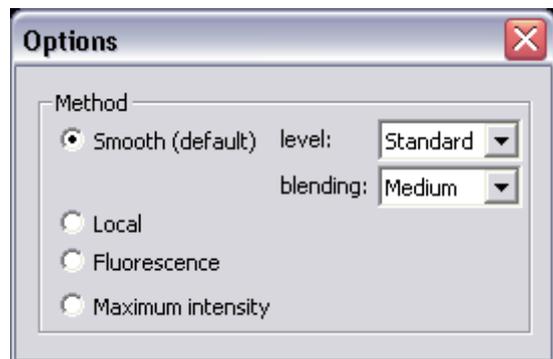
---

Once the sequence is aligned, invoke the [Options] command to select the preferred method of creating the focused image. The following dialog window appears:

### **Smooth (default)**

The focused image is created step by step. In each step, this method computes the focus criterion for each pixel and compares them. It has two parameters which you can change according to the needs of a sequence.

- **Level** - Affects the smoothness of the focus criteria in the image. Higher



settings mean that the focused areas in the image will affect larger neighborhood. The default setting is Standard.

- **Blending** - When the focus criteria in the source frames are similar, the pixels from those frames can be combined and the transitions between the focused regions in the result image will be smooth. The higher blending you select, the more differing pixels will be combined.

### **Local**

This method computes the focus criteria in another way than the Smooth method. It finds the focused regions of the source frames and copies them to the result image. An additional processing of the regions removes the badly detected ones. However, borders (transitions) between some regions might be clearly visible at the end.

### **Fluorescence**

Similar to the Local method, but it uses different focus criteria optimized for fluorescence images.

### **Maximum intensity**

The focus criterion is the intensity of each pixel. The pixels with the highest intensities of all frames are copied to the result image.

## **Creation of the focused image**

---

When the method is selected and the sequence is aligned, the only thing to do is to run the [Create Focused Image] command. The focused image will be created and appended to the ND2 document (*when the ND2 file is saved to disk the focused image is included.*)

### **Viewing of the result**

---



After the focused image is created, all buttons of the EDF tool bar (located inside the document window) become enabled. There are three ways to observe the resulting focused image, as:

- A common Color/Gray image
- An anaglyph - to be viewed using special 3D glasses.
- A 3D model, using so called Surface View.



This button displays the focused result image.

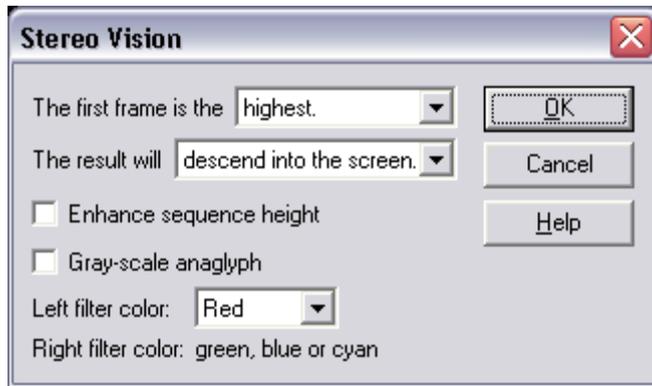
### **Creating anaglyph**

---



Using this button, you can create three-dimensional stereo images (anaglyphs). These images shall be observed with special glasses having red and green or blue filters. This provides a 3D impression. Click the nearby arrow button and select the [Anaglyph options] command.

The following dialog box appears.



**The first frame is the**

Defines the direction of the z-axis sequence. Can be *highest* or *lowest*

**The result will**

Defines the stereovision impression, which you will experience using special red-green glasses. Can *descend into the screen* or *rise from the screen*.

**Enhance sequence height**

Enhances the depth feeling.

**Gray-scale anaglyph**

Creates a gray-scale anaglyph. If not checked, the anaglyph is color.

**Left filter color**

You can select the color of your glasses. This option is crucial to get the right 3D impression.

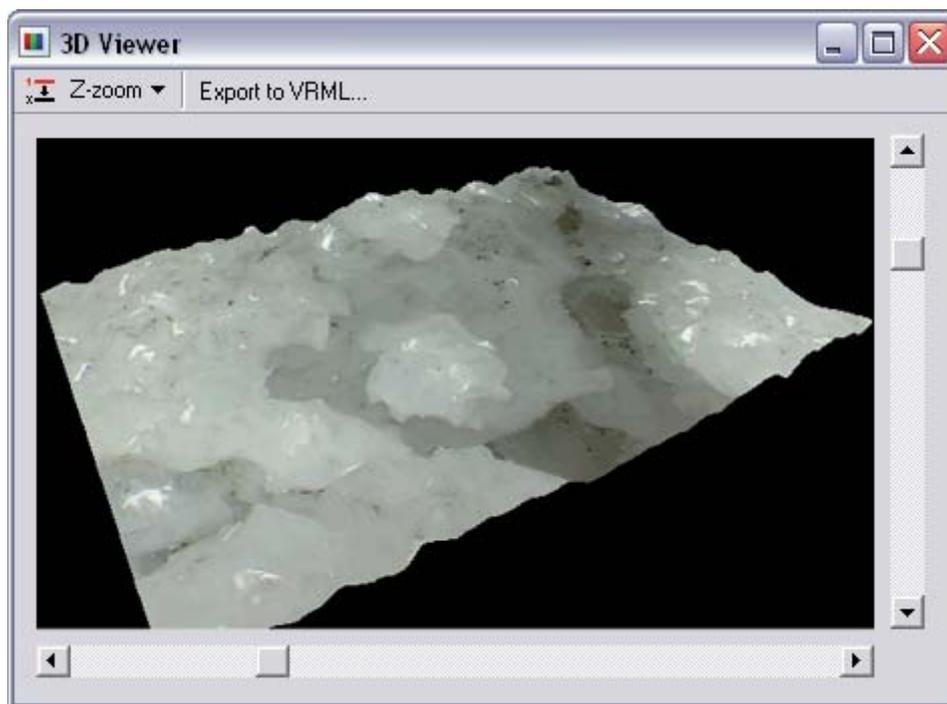
## Exploring the 3D model

---



This button runs the built in 3D viewer.

The 3D viewer displays the surface of a captured object three-dimensionally.



- For proper view, it is necessary to set whether the first frame in the sequence is the lowest or the highest one (set this by the leftmost icon).
- You can also adjust the sequence height for more credible look via the [Z-zoom] combo box.
- Navigate the view by sliders on sides or by mouse. A mouse wheel serves for zooming.

The 3D model can be exported to VRML (Virtual reality modeling language) format for distributing and viewing outside of NIS-Elements software. This format is platform independent and is used mainly on the internet.

To export the image to VRML, press the [Export to VRML] button. Two files will be saved. One that contains the 3D information (WRL) and the other that is a 2D texture (JPG). Mind, that both files must be distributed (e.g. placed on the web) together!

To view files in VRML format some VRML viewer is needed. The viewer is a plugin to your web browser. There are several viewers available for free:

- **Cosmo Player** - <http://ovrt.nist.gov/cosmo>
- **Cortona** - <http://www.parallelgraphics.com>

## **Edit Area in Focused Image**

---

This advanced option enables you to draw an area inside the image and select the Z slice to be used in the resulting focused image.

- Display the focused image.
- Select the [Edit Area in Focused Image] command.\* The cursor changes.
- Draw an area inside the focused image which should be affected. Finish drawing by right-click.
- Use the mousewheel or arrow keys to browse Z slices within the area.
- Finish the procedure by right-click or [Enter].

\* - *when you have the area defined, the selection can be inverted by pressing [G].*

## **Real Time EDF**

---

The [Real Time EDF] command is integrating the whole functionality of EDF. It captures a Z-sequence, aligns the images (optionally), and creates the focused image. When the [Real Time EDF] command is invoked, the [Capture Z Series] dialog window appears with the [Align images] check box added. There you can set whether to align the images or not.

If the [Real Time EDF Manually] command is used, not requiring to possess a motorized Z-drive, the [Acquire > Capture Z Series > Capture Manually] dialog window appears with the [Align images] check box added. It behaves the same as the [Real Time EDF] procedure except for the manual way of capturing the Z sequence.

*Please, see more details about the dialog window in the [ Z Series Capturing ] chapter.*

# Deconvolution Module (AR)

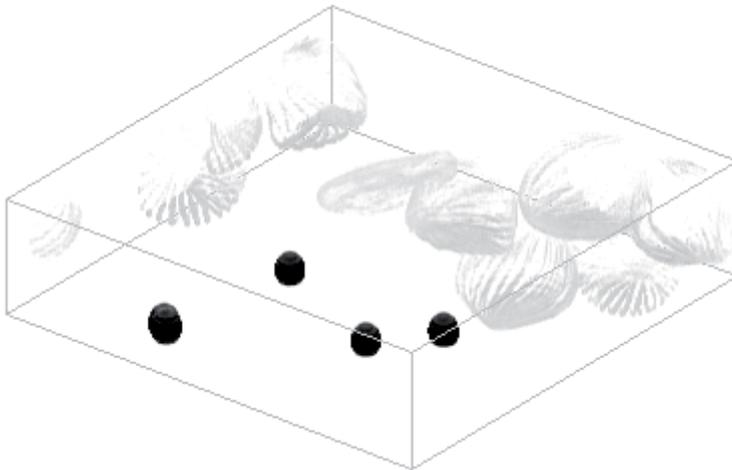
## Introduction

---

### What is deconvolution for?

---

Deconvolution is a mathematical algorithm for removing unsharpen parts of image that lays out of the focused plane.



### How does it work?

---

The algorithm uses image information from different Z-slices. The input is a set of Z-slices captured by a fluorescence microscope. The output is another set of slices, but, the image information of each slice which does not belong to its focus plane is suppressed.

The algorithm used is: constrained iterative - maximum likelihood estimation algorithm (the actual name of the algorithm is "Richardson-Lucy")

## Results

---

The resulting image should look similarly as if captured by a confocal microscope.

## Get a Z sequence of images

---

### Open a Z series ND2 document.

---

Invoke the [ File > Open ] command and select an ND2 document to be opened. It must contain the Z dimension.

### Acquire Z-series

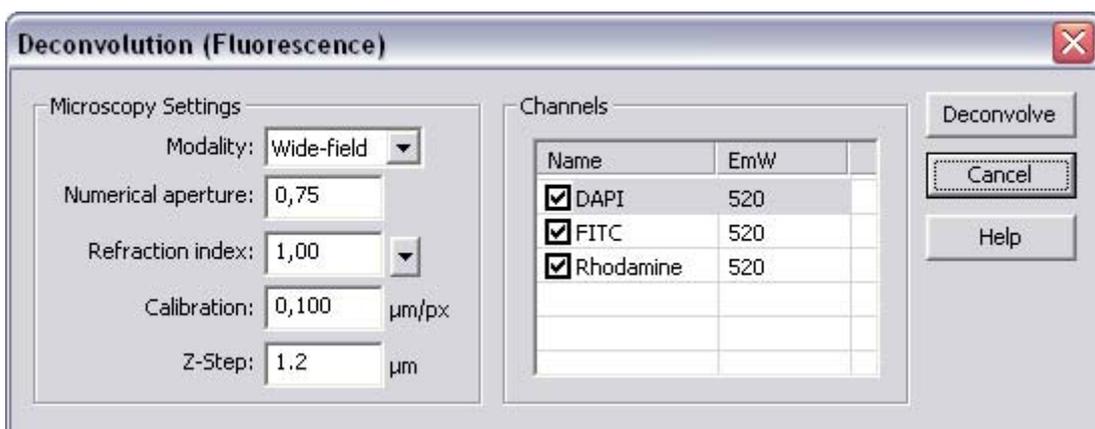
---

Another option is to acquire a sequence of Z slices using the [ Acquire > Capture Z-Series Automatically ] or the [ Acquire > Capture Z-Series Manually ] commands.

### Deconvolve the Z Stack image

---

Run the [Applications > Deconvolution > Deconvolve Z Series] command and set the deconvolution parameters:



- Select your microscopy **Modality** , wide-field or confocal.
- Fill-in the **numerical aperture** (NA) value of the objective used.
- Fill-in the **refraction index** of the immersion medium used. There are some values predefined in the nearby menu (air, water, immersion oil, glycerin).
- Fill-in the calibration field. Mind that the **deconvolution doesn't work on uncalibrated sequences** .
- Pick the channels to deconvolve in the **Channels** field.
- Fill-in the emission wavelengths ( **EmW** ) of the channels. These values don't have to be accurate because they affect the result only slightly.
- Fill-in the **Z-Step** distance between the slices of your sequence.\*
- Press the **Deconvolve** button.

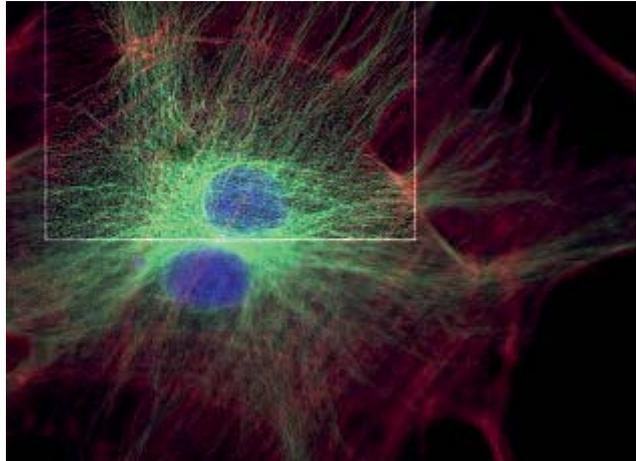
\* - It is important to fill the same value that was used during the Z Series acquisition. The

*deconvolution will not work properly without a reasonable Z-Step value. When you are about to capture a Z sequence, you should [ set the Z-step value ] to [Suggested step size] or smaller in order to get enough detail for the deconvolution algorithm to work fine.*

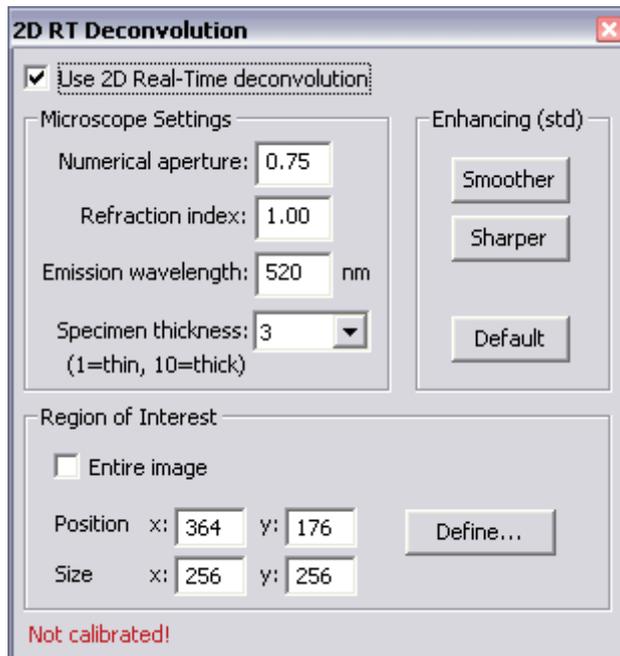
# The 2D Real-Time Deconvolution Module (AR)

Deconvolution is an image processing technique that removes inaccurate features in an image which are caused by the optical path. It is usually used for enhancing blurred images. RT Deconvolution algorithm enables high speed processing, therefore it is very suitable for use on Live signal from cameras. Real-time deconvolution can be applied to Live image continuously or when capturing only. It can be also used for processing saved images.

*There is an example of Real time deconvolution applied to rectangular region of interest.*



When you start NIS-Elements with the 2D Real-Time Deconvolution module installed, the [RTDeconv] section appears in the [Applications] menu with the [ Show RT Deconvolution Setup ] and the [ Enhance Current Image... ] commands. Click the first command to display the following control window.



## **Use 2D Real-Time deconvolution**

---

This option switches on/off the deconvolution on Live image. When a static document is opened, the **Apply** button appears instead of this option.

## **Microscope Settings**

---

Microscope Settings are the input parameters for the deconvolution.

### **Numerical aperture**

NA of the objective lens. This value is written on the objective itself.

### **Refraction index**

Refraction index of the lens immersion medium (typically 1.00 for air, 1.33 for water, 1.51 for immersion oil, 1.61 for glycerin).

### **Emission wavelength**

Emission wavelength of the fluorescent specimen in nm. Use 520nm if unsure.

### **Specimen thickness**

Describes how thick the is the specimen being imaged. The value is typically between 1 and 5. Use 1 for optically flat mono-layers, and 5+ for thicker specimens.

## **Enhancing**

---

Enhancing group controls the noise amplification vs. image smoothness. The default setting is suitable for low-noise images.

### **Smoother**

Press this button to decrease the noise.

### **Sharper**

Press this button to increase the sharpness of the image.

### **Default**

Return to default (std) setting.

## **Region of Interest (ROI)**

---

Region of interest specifies the part of the image where the deconvolution will be done. Reducing the area for the deconvolution will increase its speed.

### **Entire image**

Check this option if you want to deconvolve the entire image.

**Position (x, y)**

Top-left corner of the rectangle representing the ROI.

**Size (x, y)**

Width and height of the rectangle representing the ROI.

**Define...**

If you press this button, you can draw and/or adjust the rectangle with mouse directly in the image.

**"Not calibrated!"**

---

It is necessary to have the image calibrated in order for the deconvolution to work. If you see this message, you should calibrate the image or the optical configuration you use.

# AutoQant Deconvolution Module (AR)

## Introduction

---

### What is deconvolution?

---

Deconvolution is a mathematical algorithm for removing unsharpen parts of image that lays out of the focus plane.

### How does it work?

---

The algorithm uses image information from different Z-slices. The input is a set of Z-slices captured by a fluorescence microscope. The output is another set of slices, but, the image information of each slice that does not belong to its focus plane is suppressed.

### Getting a Z sequence of images

---

- Invoke the [ File > Open ] command and select an ND2 document to be opened. It must contain the Z dimension.
- Acquire a sequence of Z slices using the [ Acquire > Capture Z-Series Automatically ] or the [ Acquire > Capture Z-Series Manually ] command.

### Results

---

The resulting image should theoretically look similarly as if captured by a confocal microscope.

## To deconvolve a Z Sequence

Run the [Applications > AutoQuant Modules > Deconvolution] command and set the deconvolution parameters:

### Microscopy Settings

These values which give information about the microscope settings are usually retrieved from the captured document (except [Modality]). However, they can be modified by user.

#### Select microscope Modality:

- widefield fluorescence
- transmitted-light brightfield
- laser scanning confocal
- spinning disk confocal
- multi-photon fluorescence

#### Numerical Aperture

Fill-in the [NA] value of the objective used.

#### Refraction index

Fill-in the [refraction index] of the immersion medium used. There are some values predefined in the nearby pull-down menu (air, water, immersion oil, glycerin).

#### Calibration

Fill-in the [Calibration] field. Mind that **deconvolution doesn't work on uncalibrated sequences**.

#### Z-Step

Fill-in the [Z-Step] distance between the slices of your sequence.

**AutoQuant Blind Deconvolution**

Microscopy Settings

Modality: Widefield Fluorescence

Numerical aperture: 0,75

Refraction index: 1,00

Calibration: 0,100 µm/px

Z-step: 1,2 µm

Channels

Name	EmW	SA
<input checked="" type="checkbox"/> DAPI	520	0
<input checked="" type="checkbox"/> FITC	520	0
<input checked="" type="checkbox"/> Rhodamine	520	0

Emission wavelength: 520 nm

Spherical aberration: [Slider]

Apply to all

Deconvolution Settings

Number of iterations: 10

Noise level: Low

Faster processing/Reduced resolution

Deconvolve Cancel

## **Channels**

---

Pick the channels to be processed by selecting check boxes within the table of image channels. The channel name, emission wavelength, and spherical aberration are also retrieved from the document or can be modified by user.

### **EmW**

These values don't have to be accurate because they affect the result only slightly. To modify EmW, select the channel to which you are setting the emission wavelength, and fill the new EmW value inside the [Emission wavelength] field.

### **Spherical Aberration**

To modify the spherical aberration setting, select the channel to modify, and move the slider left/right. The button above the slider resets the [SA] value.

## **Deconvolution Settings**

---

### **Number of iterations**

Specify how many times you would like to perform the deconvolution algorithm.

### **Noise level**

Select the level of noise present in the images (low, medium, high)

### **Faster processing/ Reduced resolution**

Resolution of the result can be reduced on behalf of the processing speed (the resolution will be reduced temporarily during the processing. Afterwards, the original resolution will be renewed).

# Calcium and FRET module (AR)

Installing the Calcium and FRET module adds several tools regarding the FRET analysis method to the system:

- A control window suited for capturing FRET images.
- Automated FRET calibration algorithm.
- The [Create FRET Image] dialog window for "passive" creating FRET images from files.

## Capturing FRET image, FRET calibration

---

Run the [Applications > FRET > Capture FRET Image] command to display the main FRET control window. Select the appropriate settings and capture the FRET image by the [Capture FRET] button.

## Hardware Setup

---

The FRET control window consists of several sections. In this section, optical configurations which will be used during the acquisition should be assigned to FRET channels.

### Camera Setting

In this pull-down menu, select an optical configuration that contains [Camera settings] which shall be used during the FRET image acquisition. If [None] is selected, the current camera settings will be used.

### Channels

The FRET image can contain 2-4 channels. Here you can assign an optical configuration to each of them. It is recommended that the optical configuration contains only the filter changer settings.

### Dual View

If the Dual View device is available, it can be used for channel splitting. Then, only one optical configuration for each excitation wavelength will be used.

Hardware Setup

Camera setting

Optical configuration: None (use the current)

Channels

Use Dual View for emission splitting

Donor - acceptor (Da): Da

Donor - donor (Dd): Dd

Accepter - acceptor (Aa): Aa

Accepter - donor (Ad): Ad

Load Save

*The Dual View device splits the emitted light to two beams filtering different wavelengths, and each of them is captured by only a half of the camera chip.*

## **Load/Save**

The channels settings may be saved to the system by the [Save] button. Once saved under some user defined name, this name appears in the [Load] pull-down menu ready to be loaded. The settings can also be loaded from an external FRET image.

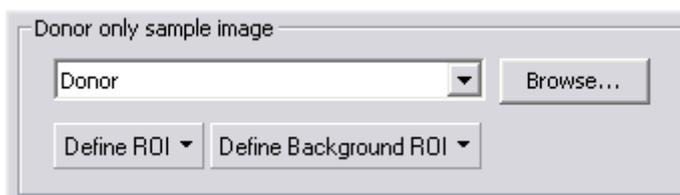
## **FRET Calibration**

---

Separate images of the D-labelled and the A-labelled samples must be be acquired in order to calibrate the FRET method correctly.



- Insert the D-labelled sample. Press the [Capture Donor Only Sample] button.
- A new document will be captured and named "Donor".
- Insert the A-labelled sample. Press the [Capture Acceptor Only Sample] button.
- A new document will be captured and named "Acceptor".
- Press the [Calibrate] button to display the calibration dialog.



- Select the "Donor" image and the "Acceptor" image in the appropriate pull-down menus.
- The [Define ROI] and the [Define Background ROI] buttons enable to distinguish the foreground/background parts of the images.
- Draw the background/foreground ROIs.
- Confirm the calibration by [OK].

*Any image file can be used for the calibration. The pull down menu displays the currently opened images. Other images can be reached via the [Browse] button. If a non-FRET image is used, a dialog window appears where you should map the image channels to FRET channels and select the FRET method.*

## **FRET Method**

---

In this section, select one of the two available FRET methods.

### **FRET coefficients**

There can be 2 or 4 coefficients used according to the method. Their values are results of the

calibration. If the calibration was not performed, you can put the coefficients in by hand. The coefficients settings can be saved/loaded similarly to the channels settings.

## **Creating FRET image**

---

The FRET image can be created from images saved on harddisk too. Run the [Applications > FRET > Create FRET Image] command. A dialog window appears. The FRET image creation principles are the same as if capturing the FRET image except that you provide the module with mono images from harddisk (instead of capturing them via a camera).

- Select a mono image from the pull-down menus for each FRET channel. Their previews appear.
- Select a method, calibrate the module, modify the coefficients (similarly as if capturing the FRET image). Confirm the settings with [OK].
- A new FRET document is created and opened within the application screen.

## **Calcium Calibration**

---

The module provides the methods for the efficient calcium ion concentration measurement. Calcium calibration, graph display, and image display in terms of  $\text{Ca}^{2+}$  concentration is available. All constants necessary for calcium ion concentration calculation can be defined manually within the [Ratio Properties] dialog window. Or, the  $R_{\min/\max}$  and  $F_{\min/\max}$  constants can be picked from the graph.

# Devices

Before you get to work with NIS-Elements, all hardware accessories should be connected properly to the system. In most cases, the following basic procedure is sufficient to connect a device successfully:

- Install NIS-Elements, and select the appropriate devices during the installation.
- Connect the device to a PC and switch the device ON.
- Run NIS-Elements, and run the [Devices > Manage Devices] command to open the Device Manager.
- Use the [Add] button to add the device to NIS-Elements.
- Select the device in the devices list and press the [Connect] button.
- Select logical devices to be activated.
- Configure device-specific settings using the [Configure (*physical*) Device] and the [(*logical*) Device Parameters] buttons.
- [Close] the Device Manager.

## Logical Devices

---

NIS-Elements handles hardware accessories using the concept of logical devices. There are some features of different hardware devices which equal, and therefore can be controlled equally. Such features are called "logical devices" for purposes of NIS-Elements. A typical logical device is [Stage XY]. Different microscopes can be equipped with different XY motorized stages, although - regarding the user interface - they behave equally. One physical device (a piece of hardware) can contain one or more logical devices, the list of which appear in the Device Manager after connecting it to NIS-Elements.

## Available Logical Devices

---

### Analyzer

The analyzer is a polarizing filter placed in the optical path before the specimen. The logical device offers two states: ON (inserted) and OFF (extracted).

### Aperture

This logical device is used for controlling apertures in the light path. It is used in complex microscopes rather than as a standalone device. Typically it contains two controls. One for switching it ON and OFF (typically a button), and the other for specifying its size (typically an edit-box or a slider).

### Condenser

A condenser is a two-lens combination located next to the light source in the optical path. Its purpose is to collect light and direct it onto the object being examined. The corresponding logical device relates to a changer of different condensers.

## **Filter**

This logical device controls filter changer movements. There can be several filter changers connected to NIS-Elements at a time. Each filter changer needs to be set up - filter types shall be assigned to positions of the changer:

- Display the filter changer control window ([Devices > Filters & Shutters] or [Devices > Microscope Control Pad]).
- Click the the setup ([...]) button, a dialog window appears.
- Select one of the available positions which the filter will be assigned to.
- Select the filter name from the [Predefined]/[Custom] list of filters.
- Click the [<-Set] button
- The filters can be moved within the already defined positions using the [Up/Down] arrow buttons.

*When browsing the list of filters, details about the currently selected filter are displayed in the right-most portion of the window.*

## **Light**

This logical device is used for controlling the specimen illumination remotely. There is not any standard dialog box for the light control. Each device handles this logical device via a specially designed user interface - typically containing one button for ON/OFF switching and a slider for the intensity regulation.

## **Light Path**

Some microscopes have more than one port where it is possible to attach a light source or a camera. This logical device can switch the light between these ports.

## **Microscope**

This logical device is used to group standalone logical devices used in microscopes. To control the logical devices of a microscope from one control window, select the [Devices > Microscope Control Pad] command.

## **Nosepiece**

This logical device serves for controlling microscope objective changers. There can be three nosepiece types attached to a microscope:

- **Manual** - it can not be controlled via the [Microscope Control Pad] or the [Nosepiece] control window.
- **Intelligent** - the current nosepiece position is indicated, but can not be controlled.
- **Motorized** - such nosepieces can be fully controlled via the [Microscope Control Pad] or the [Nosepiece] control window.

## **Assigning objectives**

- To assign an objective (or to change an attached objective assignment) to a position, click the setup ([...]) button.
- A dialog window appears which enables you to select on of the available objectives to the corresponding position.
- Each objective has its specifications displayed in the table. These specifications are not editable.

### **ND Filter**

A neutral-density filter is a light absorbing filter whose absorption spectrum is moderately flat. It is used to reduce the light intensity within the optical path. The logical device offers two states: ON (inserted) and OFF (extracted).

### **PFS**

Perfect Focus System - this logical device corresponds to the PFS physical device available with Nikon TE2000/TI microscopes.

### **Shutter**

This logical device can control shutters installed in your system. This device is handled via the [Devices > Filters & Shutters] control window or straight from the microscope control pad.

### **Zoom**

This logical device is used for controlling the zoom factor. Run the [Devices > Zoom Configuration] command to adjust the zoom settings.

# Nikon Eclipse 90i/80i Microscope

The 80i/90i (fully armed) Microscope Control Pad consists of the following portions:

## Nosepiece

This portion depends on the type of the connected nosepiece:

- **Manual** - it remains hidden
- **Intelligent** - it indicates the current nosepiece position, but can not be controlled.
- **Motorized** - it can be fully controlled.

## Lamp

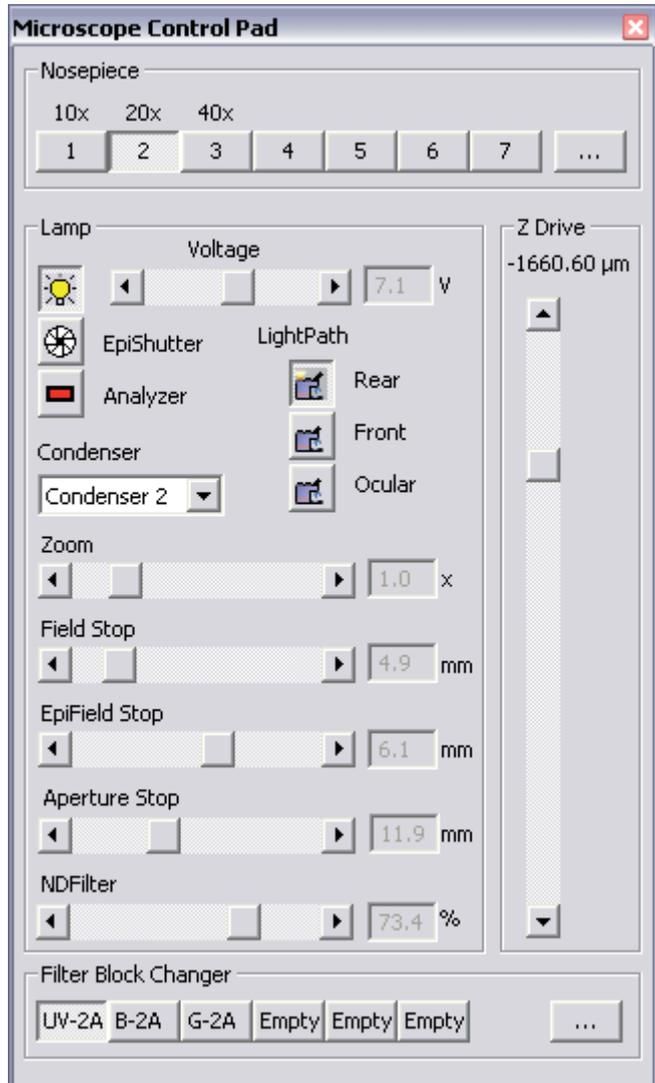
The light path accessories that affects light intensity can be controlled here. The condenser turret is controlled by selecting the desired condenser from the pull-down menu.

## Z Drive

The Z drive movements are indicated/performed via the scrollbar. Tolerance (precision) can be set in the pull-down menu. The higher the tolerance is, the faster the Z drive moves, though less precisely.

## Filter Block Changer

The standard motorized filter changers control.



# Nikon AZ100 Microscope

When AZ100 is connected to NIS-Elements, the manual remote control gets disabled automatically. The AZ100 Microscope Control Pad consists of the following portions:

## Objectives

The objectives portion of the pad indicates the nosepiece status: the currently selected objective and objectives magnification. The objective changer cannot be controlled by NIS-Elements.

## Zoom

Zoom magnification can be changed by moving the scroll bar. The current zoom factor is indicated above. Since the zoom device is a part of the microscope equipment, concerning optical configurations, its settings are automatically treated as an inseparable part of [microscope settings].

## Condenser

The green button indicates the current condenser lense position (Low/High). The condenser cannot be controlled.

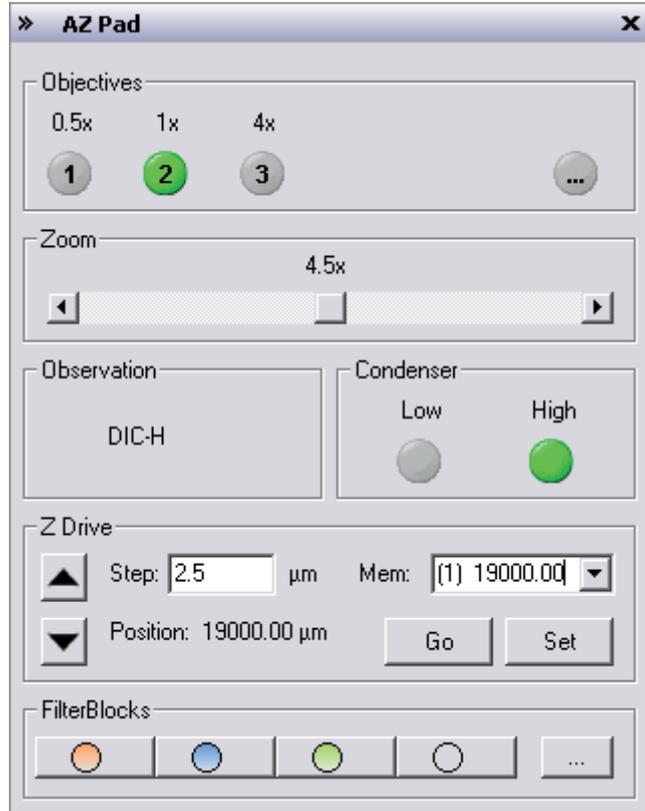
## Z Drive

The Z drive can be controlled via the microscope control pad in the following way:

- The [up/down arrows] move the Z drive by a defined [Step].
- The current position can be memorized by pressing the [Set] button. The position value will be written to the pull-down menu.
- To move the Z drive to a memorized position, select the desired value from the pull-down menu and press the [Go] button.
- The current position is indicated below the [Step] edit box.

## FilterBlocks

The currently selected filter of the four available filter positions is indicated in the Filter Blocks portion of the pad. The filter changer cannot be controlled by NIS-Elements. Filters assignment may be changed after pressing the [...] button.



# Nikon Eclipse LV Series Microscope

The LV (fully armed) Microscope Control Pad consists of the following portions:

## **Nosepiece**

The motorized objective changer can be controlled via this portion of the dialog window.

## **Accessories**

Intensity of episcopic and diasopic light sources can be controlled.

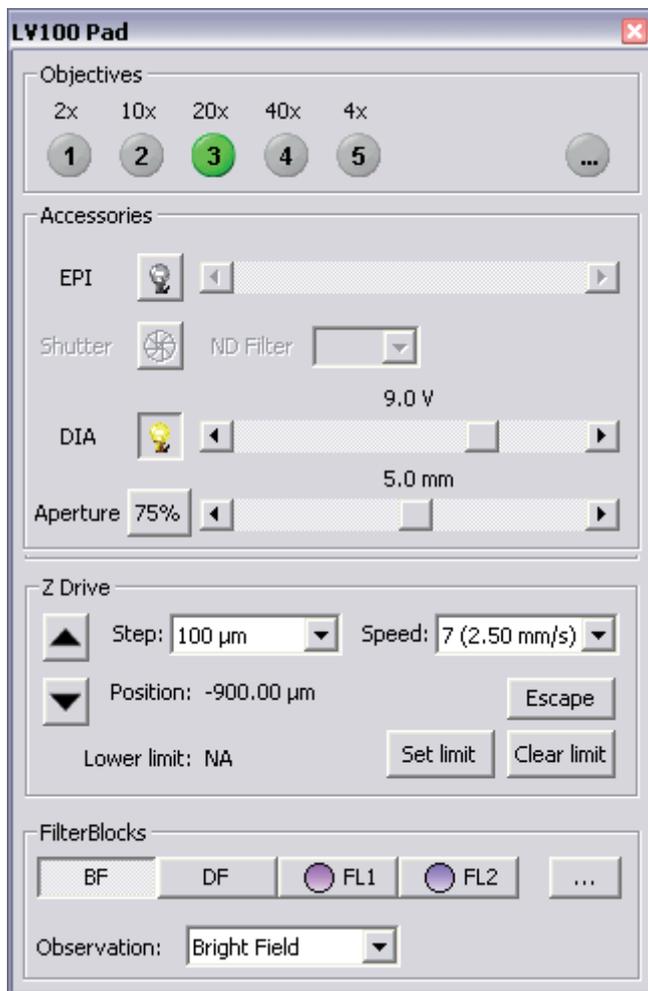
## **Z Drive**

The motorized Z drive is controllable too. Clicking the arrow buttons moves Z drive by the step defined in the pull-down menu. The [Escape] button clears the objectives of the stage reach. The bottommost position (lower limit) of the Z drive can be set using the [Set Limit] button. The [Clear Limit] button discards the setting.

*Please make sure to set [Lower limit] before using the microscope. Otherwise, the objective can accidentally hit the stage when the motorized Z drive moves.*

## **Filter Blocks**

There are 4 available positions in the changer. The first two have fixed filters for bright field (BF) and dark field (DF) microscopy. The other two are available for any filters to be inserted.



# Nikon MM 400/800 Microscopes

The measuring microscope connected to NIS-Elements provides two logical devices:

## **Nosepiece**

Two types of nosepiece can be attached to the microscope:

- **Manual** - cannot communicate with the computer. Each time you switch the objectives, you should also select a position in the Nosepiece control window accordingly.
- **Intelligent** - cannot be controlled by the system, but when you change the objective manually, it indicates the change in the Nosepiece control window automatically.

After adding the MM microscope to the system via Device Manager, the [Nosepiece] logical device becomes available. Display its control window by the [View > Acquisition Controls > Nosepiece] command. The type of the nosepiece must be defined via the hardware control pad in order to be recognized by NIS-Elements

## **Z Drive**

The Measuring Microscopes Z drive may be moved using the [View > Acquisition Controls > XYZ Navigation] control window.

# Nikon TE2000 Microscope

The TE2000 (fully armed) Microscope Control Pad consists of the following portions:

## **Nosepiece**

The motorized objective changer can be controlled via this portion of the dialog window.

## **Light**

Complete light path accessories can be controlled here. Switch the light between ports as needed.

## **Condenser**

The condenser turret is controlled by selecting the desired condenser from a pull-down menu.

## **Z Drive**

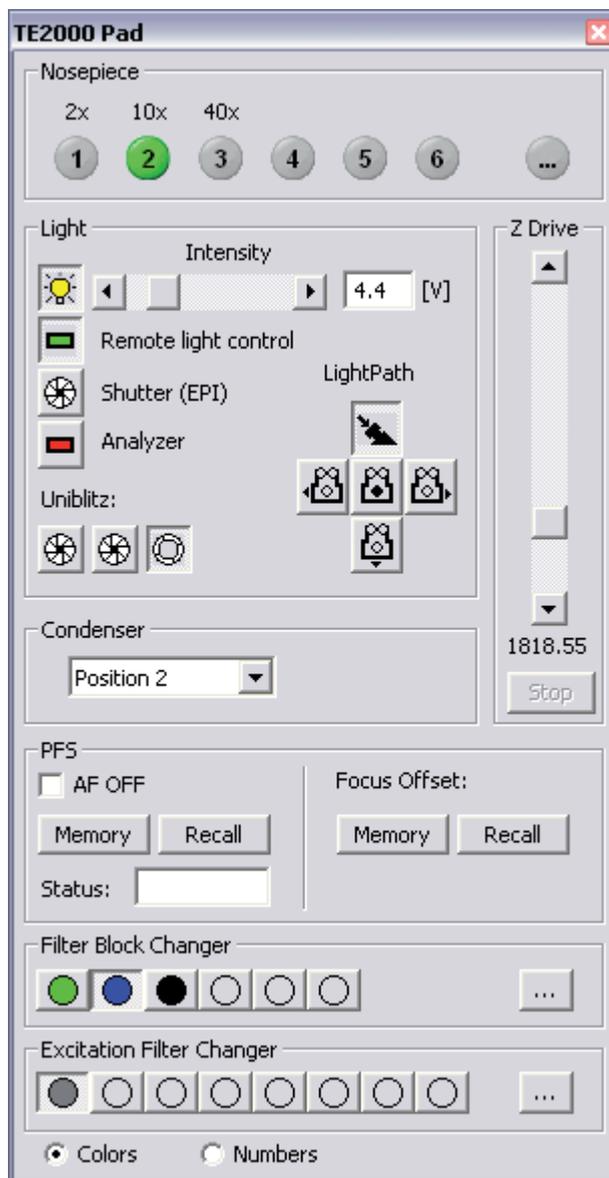
The Z drive position is indicated, or can be controlled, via a scrollbar.

## **PFS**

The functionality of the PFS-section of the control pad corresponds to the hardware controller.

## **Filter Changers**

Standard motorized filter changer controls. You can select whether filter colors or position numbers are displayed on the buttons.



# Nikon TI Microscope

The TI (fully armed) Microscope Control Pad consists of the following portions:

## Nosepiece

The nosepiece can be controlled here. The objective settings are being shared between NIS-Elements and the microscope RCP.

## Light Path

The light path control buttons correspond to the buttons placed on the microscope body.

## PFS

This optional accessory can be controlled via the pad. The offset value can be put straight in the edit box. When you are about to turn the PFS ON, make sure the [Focus] indicator shines. It indicates that the PFS is ready to get to the defined offset position.\* The [Memory] and [Recall] buttons equal the buttons on the microscope body.

\* - Vast shift of the Z drive can cause that PFS will not be able to refocus. That is when the [Focus] indicator stops shining.

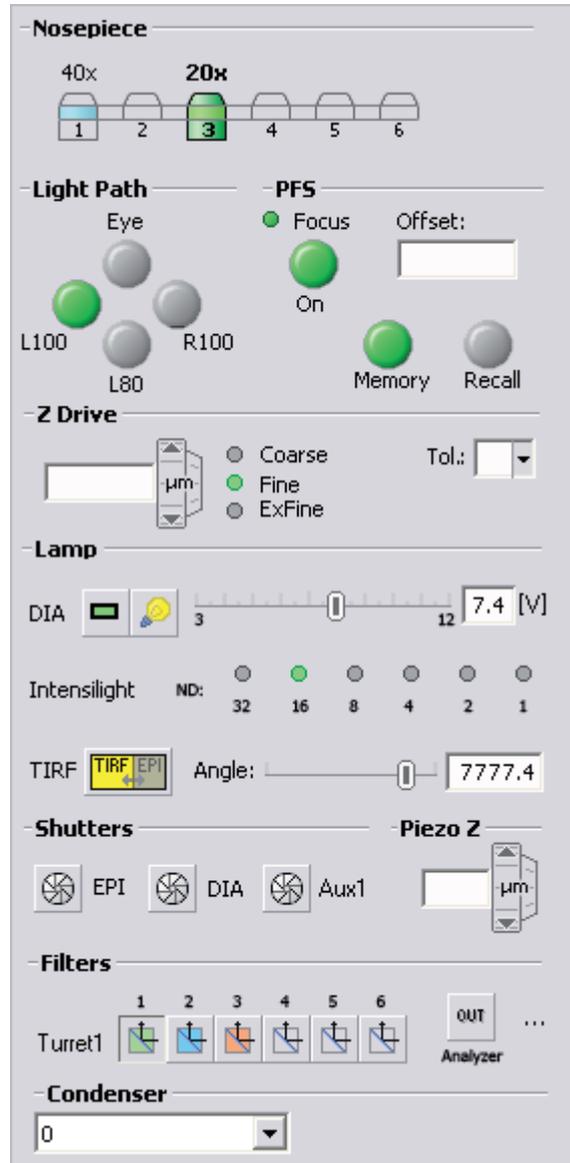
## Z Drive

The integrated Z drive movements are performed by the up/down arrows. Tolerance (precision) can be set in the pull-down menu on the right. The tolerance setting affects the Z drive speed slightly.

## Lamp

Three types of illumination devices can be attached: Diascopic lamp, Intensilight fiber illuminator, TIRF illuminator. All these devices are controllable by the pad.

**TIRF** - A shutter can be assigned to close every time the TIRF position is being changed. To do so, right-click the TIRF logical device within the [Device Manager] window and select the shutter type from the context menu.



### **Shutters**

Register the correct shutter types in the RCP. Then, the shutters can be controlled by NIS-Elements.

### **Piezo Z**

An optional piezo stage is controlled equally to the Z Drive device. The piezo Z component should be registered via RCP, and direct USB cable must be used between PC and the microscope body (not the HUB connection).

### **Filters**

The standard motorized filter changer control. The [Analyzer] button appears, when the [ANALYZER] filter type is assigned to one position of the turret. Press the [IN/OUT] button to insert/extract the analyzer.

### **Condenser**

The condenser turret is controlled by selecting the desired condenser from the pull-down menu.