

NIS-Elements AR

User's Guide

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Table of Contents

1. Welcome to NIS-Elements AR documentation	1
2. Installation and Startup	3
2.1. NIS-Elements AR Installation	3
2.2. Command Line Options	9
2.2.1. Syntax and Example:	9
2.2.2. Switches:	9
3. Application Appearance	11
3.1. NIS-Elements AR Screen	11
3.1.1. Menu Bar	12
3.1.2. Application Tool Bars	14
3.1.3. Status Bar	14
3.1.4. Document Window	15
3.2. Modifying Layout	18
3.2.1. Layouts	18
3.2.2. Layout Manager	19
3.2.3. Docking Panes	24
3.2.4. Appearance Options	27
3.3. Organizer	28
3.3.1. Image Filter	29
3.3.2. Operations with Images	31
3.3.3. Sorting of Images	32
3.3.4. Grouping of Images	32
3.3.5. Adjusting the Organizer Layout	32
4. Setting the Hardware Up	33
4.1. Working with Cameras	33
4.1.1. Live Signal from Camera	35
4.1.2. Capturing Images	35
4.2. Optical Configurations	36
4.3. Objectives	39
4.3.1. Managing Objectives	39
4.3.2. Objective Calibration	41
4.3.3. Units	43
4.4. User Management	45
5. Capturing	49
5.1. Single Image Capture	49
5.2. ND2 Acquisition	50
5.2.1. Time-lapse Acquisition	51
5.2.2. Multi-point Acquisition	51
5.2.3. Z-series Acquisition	54
5.2.4. Multi-channel Acquisition	55
5.2.5. Combining N Dimensions	56
5.3. Capturing to Ring Buffer	61
5.4. Capturing AVI movies	63
6. Image Documents	65
6.1. NIS-Elements AR Documents	65
6.1.1. Image Layers	65
6.1.2. Document Types in NIS-Elements AR	66
6.1.3. ND2 Documents	67

6.2. Working with Documents	78
6.2.1. Opening Saved Files	78
6.2.2. Switching Between Loaded Documents	79
6.2.3. Closing Documents	79
6.2.4. Copying Channels by Drag and Drop	80
6.2.5. Image Formats	81
7. Image Analysis	83
7.1. Histogram and Look Up Tables	83
7.1.1. Histogram	83
7.1.2. LUTs	85
7.2. Thresholding	95
7.2.1. RGB Mode	95
7.2.2. HSI Mode	97
7.2.3. Intensity Mode	97
7.2.4. MCH Mode	97
7.3. Binary Layer	98
7.3.1. Controls (horizontal tool bar)	98
7.3.2. Drawing tools (vertical tool bar)	98
7.3.3. Binary Layer Color and Transparency	101
7.3.4. Erasing Single objects	101
7.3.5. Multiple Binary Layers	101
7.4. Measurement	102
7.4.1. Calibration	102
7.4.2. Rough Measurement	102
7.4.3. Manual Measurement	103
7.4.4. Automated Measurement	104
7.5. Regions of Interest - ROIs	106
7.5.1. ROI in Time - Moving ROIs	107
7.6. Measurement Features	110
7.7. Mathematical Morphology Basics	124
7.7.1. Connectivity	126
7.7.2. Structuring Element = Kernel = Matrix	126
7.8. Object Count	131
7.8.1. Thresholding the Image	131
7.8.2. Applying Restrictions	131
7.8.3. Results	133
7.9. Time Measurement	135
7.10. Tracking (-TR- option)	137
7.10.1. Automatic Tracking	137
7.10.2. Manual Tracking	139
7.10.3. Object Redefinition	140
7.10.4. Visualization	140
7.10.5. Measurement	141
7.10.6. Graph Properties	143
7.11. User Macros	144
7.11.1. Recording a Macro	144
7.11.2. Writing/Editing a Macro	144
7.11.3. Creating a Macro from History	145
7.11.4. Saving a Macro	146
7.11.5. Running a Macro	146
7.11.6. Breaking a Macro	147

7.12. Creating Reports	148
7.12.1. Working with Report Objects	150
7.12.2. Working with Report Templates	152
7.12.3. Creating Reports from Database	153
7.13. Creating Movies	155
7.13.1. Capturing AVI Movie	155
7.13.2. Converting ND2 to AVI	158
7.13.3. Creating AVI from a Sequence of Images	159
7.13.4. Movie Maker (-LO- option)	160
7.14. Exporting Results	164
8. Cameras	169
8.1. Common Settings	169
8.2. Advanced Settings	172
8.3. Camera ROI on Live Image	173
8.4. Controlling Cameras by Macro	174
8.4.1. How to Set Camera Properties	174
9. Devices	175
9.1. Logical Devices	175
9.2. Nikon Eclipse 90i/80i Microscope	180
9.3. Nikon AZ100 Microscope	181
9.4. Nikon Eclipse LV Series Microscope	182
9.5. Nikon Eclipse L200 Microscope	183
9.6. Nikon Eclipse MA200 Microscope	184
9.7. Nikon MM 400/800 Microscopes	185
9.8. Nikon TE2000 Microscope	186
9.9. Nikon TI Microscope	187
9.9.1. Using TI Recipe	188
10. Additional Modules	191
10.1. Database	191
10.1.1. New Database	191
10.1.2. New Connection	192
10.1.3. Database Tables	192
10.1.4. User Permissions	192
10.1.5. Database Backup	193
10.2. Extended Depth of Focus	194
10.2.1. Align Sequence	194
10.2.2. Create the focused image	194
10.2.3. View the result	194
10.2.4. Edit Area in Focused Image	197
10.2.5. Real Time EDF	197
10.3. Deconvolution (-LO- option)	199
10.3.1. Deconvolution (Standard)	199
10.3.2. Blind Deconvolution	200
10.3.3. Fast (Wiener) Deconvolution	201
10.4. High Dynamic Range (-LO- option)	202
10.5. Deconvolution Module (legacy)	204
10.6. AutoQuant Blind Deconvolution Module	206
10.7. AutoQuant Real-Time 2D Deconvolution Module	209
10.8. Metalog - Grain Size Analysis Module	212
10.8.1. Features of the Grain Size Analysis Module:	212
10.8.2. Quick Guide	212

10.9. Metalo - Cast Iron Analysis Module	213
10.9.1. Features of the Cast Iron Analysis Module:	213
10.9.2. Quick Guide	213
10.10. Calcium and FRET module	214
10.10.1. Capturing FRET image, FRET calibration	214
10.10.2. FRET Method	217
10.10.3. Creating FRET image	217
10.10.4. [Ca ²⁺] Ion Concentration Measurement	218
10.11. The Advanced Interpreter (API) Module	221

1. Welcome to NIS-Elements AR documentation.

This help file describes capabilities of the NIS-Elements AR laboratory image analysis system. Chapters and paragraphs marked by the following symbols describe features available only if certain module or device was installed.

(-A1- option)

Requires the **[Nikon A1 Confocal Microscope]** device.

(-DB- option)

Requires the **[Database]** module. See [Database].

(-DEC- option)

Requires the one of the **[Deconvolution]** modules.

(-EDF- option)

Requires the **[EDF]** module. See [Extended Depth of Focus].

(-FRET- option)

Requires the **[Calcium and FRET]** module.

(-LO- option)

Requires one of the **[Local Option]** features to be installed. See Step 2.

(-MET- option)

Requires the one of the **[Metallography]** modules.

(-NDA- option)

Requires the **[Advanced ND Acquisition]** module

(-TR- option)

Requires the **[2D Object Tracking]** module. See [Tracking (-TR- option)].

(-6D- option)

Requires the **[6D Acquisition]** module

2. Installation and Startup

2.1. NIS-Elements AR Installation

Quick Start

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

The Installation DVD-ROM Content

The installation DVD-ROM contains:

- NIS-Elements AR software setup file
- Drivers and utilities for the HASP Key
- Drivers for selected cameras
- User's Guide in the PDF file format
- Sample image database
- Sample ND2 image sequences

The Installation Process

Note

You have to possess the administrator rights to your computer to be able to install NIS-Elements AR successfully.

- 1) When the installation DVD is inserted, a selection window appears automatically.



Select the software package to be installed. Select the one you have got the license for and which is properly coded in your HASP key. The installation wizard welcome-window appears. Click **[Next]** to continue.

- 2)

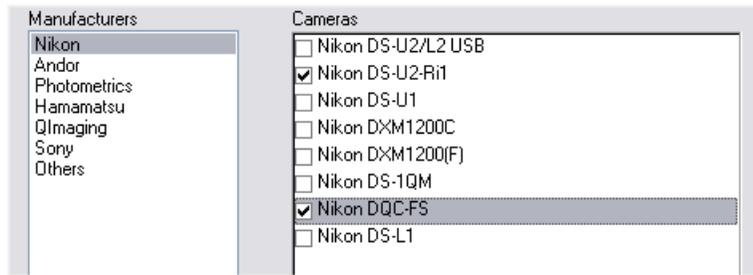


Select whether to install **[Local Option]** or not. Define the folder where NIS-Elements AR should be installed. We recommend to use the predefined directory. If you want to change the directory anyway, press the **[Browse]** button and select a new one. Otherwise click **[Next]**.

Note

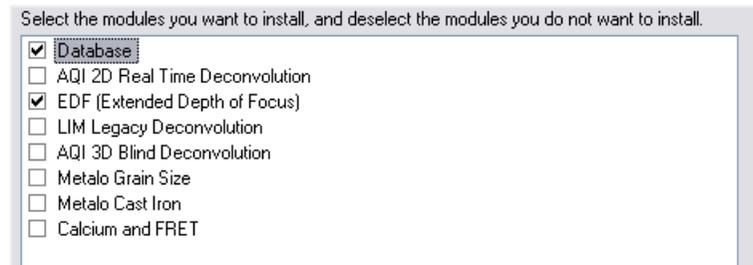
The [Local Option] installation provides some advanced features which did not pass the quality assurance procedure yet. We recommend to wait until they are released officially.

3)



Now, select the cameras which will be used with NIS-Elements AR.

4)

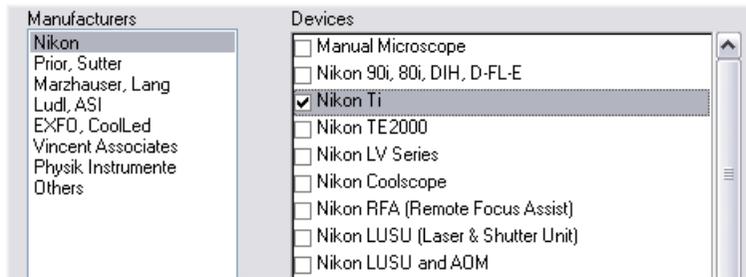


If your licence contains some additional modules besides the NIS-Elements AR core software, please select them in this window.

Note

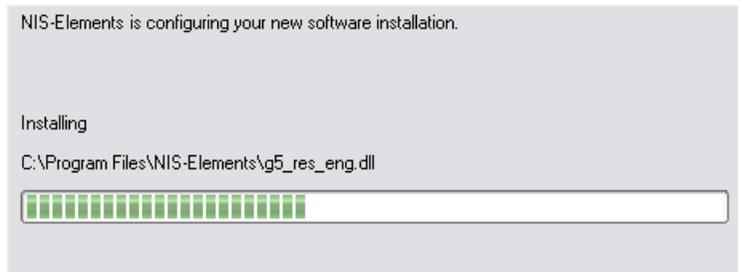
Any module selected will be installed along with NIS-Elements AR automatically. However, you might not be licensed to use it. The module will run after you get the corresponding code registered in your HASP key.

5)



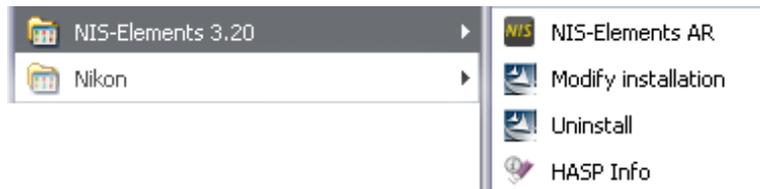
Select the devices which will be used with NIS-Elements AR. Finish the installation by clicking the **[Install]** button.

6)



The setup creates a new program group in the **[Start]** menu containing the following items: NIS-Elements AR 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 AR 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 AR 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 AR main system installation.

- Go to [Start menu > Programs > NIS-Elements AR] program group.
- Select the **[Modify Installation]** command.
- Follow the installation wizard instructions. Select the check-boxes 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 AR installation directory (e.g. C:\Program files\NIS-Elements AR \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 AR 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 the NIS-Elements AR installation is finished. The utility called HASPinfo is installed to the NIS-Elements AR directory. It enables the user to view information about the software licence and is accessible via the **[Help > HASP Info]** menu command.

2.2. Command Line Options

When starting NIS-Elements AR from the command line (or when editing the desktop shortcut properties), you may append some parameters to the main command and thereby modify the startup behavior:

2.2.1. Syntax and Example:

“NIS-Elements AR executable” **[switch#1] [parameter#1] [switch#2] [parameter#2]** etc. For example:

“C:\Program Files\NIS-Elements AR \NIS-Elements AR.exe” -f “C:\Images\starting_image.jp2”

This command runs NIS-Elements AR and opens the **[starting_image.jp2]** at the beginning.

Note

- *By default, only one instance of the application is allowed to run. When the application is running and a command similar to our example is called, it will be applied to this running instance. This does not apply when the [-q] switch is engaged.*
- *Some switches do not have parameters.*

2.2.2. Switches:

-?

Displays a help screen with the description of switches.

-c “Command”

The application runs the specified internal NIS-Elements AR command.

-f “Filename”

The application tries to open the image pointed to by the **[Filename]** parameter.

-g “Grabber name”

The image grabber (driver) of the specified Grabber name will be used and no camera-selection window will show at startup. 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”

Set the parameter value to “last” and the most recently used grabber/camera will be loaded.

-gn

No grabber driver will be loaded. The “passive” version of the software will start.

-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 grabber/driver. The available HW Units are listed in the **[Acquire > Select Camera]** window. Set the para-

meter value to “last” and the most recently used HW Unit will be loaded. When using this option you must also use the **[-g]** switch.

-i “Config Name”

The application creates a registry entry in "Laboratory Imaging\Platform\Config Name" to load from and save its configuration to.

-s “Settings File”

The application will use the specified Settings File to load and save its settings.

-l “Language”

The application will run in the specified language (if available). Use 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 the **[Macro File]** parameter.

-passive

The application will not connect to any device on startup (NIS-Elements AR will start with blank Device Manager). Camera selection is not affected by this option.

-p “Position”

The application window will be placed according to the parameter:

- **[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.

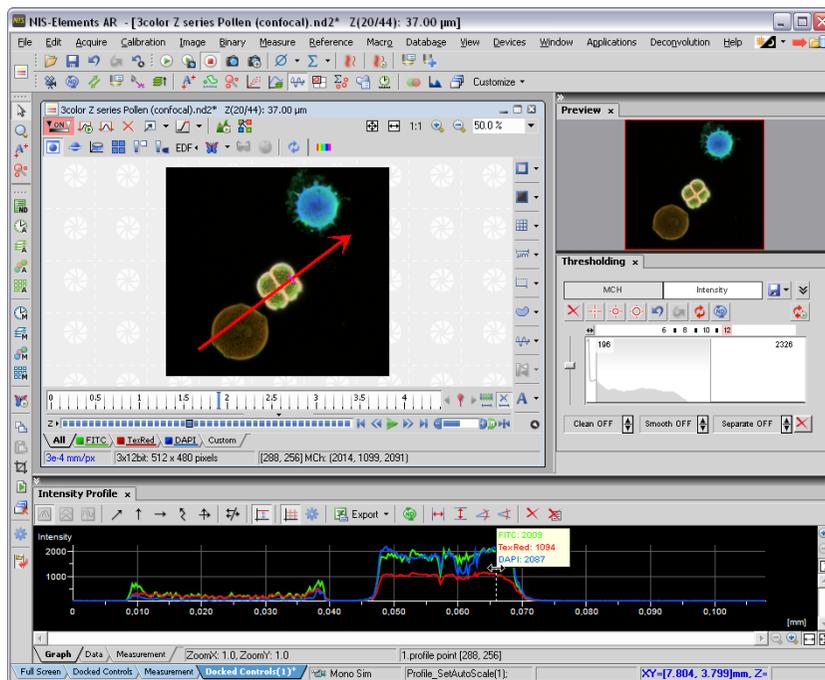
-q

A new instance of the application will run.

3. Application Appearance

3.1. NIS-Elements AR Screen

The following items can be found within the NIS-Elements AR screen:



- The top **[menu bar]** containing pull-down menus with sets of commands.
- **[Application tool bars]** (left and top) where buttons representing various menu commands are gathered for quick access.
- The **[status bar]** displaying useful information.
- **[Document tool bars]** (right and top) which enable to customize the view of each opened image individually.
- Docked or floating **[control windows]** - see more description of docking windows in the [Modifying Layout] chapter.

3.1.1. Menu Bar

All commands sorted according to their purpose are placed in the following menus:

Standard screen

File

Concerns operations with files such as Open, Save, Import, Send, etc.

Edit

Concerns operations with the current document. Copy, Paste, Undo, General Options and other commands are available.

Acquire

Concern image acquisition.

Calibration

Concerns document/objective calibration and optical configurations.

Image

Concerns operations with images, color transformations, resizing, etc.

Binary

Concerns operations with the binary layer - contains thresholding and morphology commands.

Measure

Concerns manual and automatic measurement.

Reference

Concerns operations with reference images. To display all reference images, use the **[View > Thumbnails]** command.

Macro

Concerns user macros, macro creation, etc.

Database (-DB- option)

Concerns database connections, database tables, database backup etc.

View

Gathers commands concerning the appearance of the screen, displayed control windows, zoom, etc.

Devices

Contains commands needed to handle all connected devices (except cameras).

Window

Concerns opened document windows. It enables to select a window to be displayed or select a predefined windows documents layout.

Applications

Gathers commands of additional modules if there are some installed.

Help

Enables access to additional information about the system such as help, HASP Info, application log, etc.

Organizer

In **[Organizer]**, most of the menus are hidden, but the following is added:

Tools

Contains commands for filtering and sorting images.

3.1.2. Application Tool Bars

There is a default set of tool bars each containing number of buttons. There is also one fully customizable tool bar - the main left tool bar - to which any button can be added (or removed from it). Every button or a tool bar can be hidden by user using the **[Layout Manager]**. Please see [Modifying Layout] for further details.

3.1.3. Status Bar

The status bar at the bottom of the screen displays the following information:

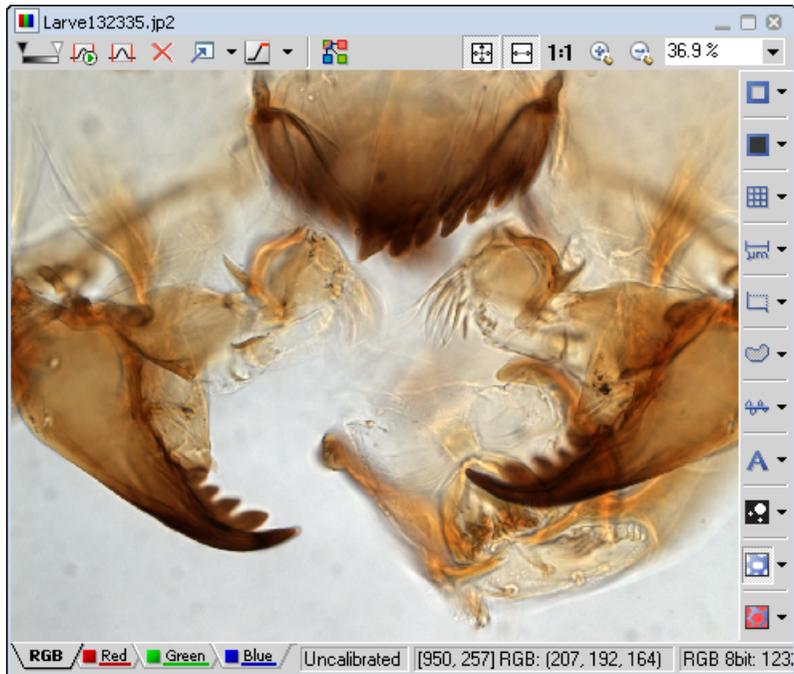


The Application Status Bar

1. Available layouts (you can hide the layout tabs by de-selecting the **[Edit > Options > Appearance > Show Layout Tabs]** option)
2. Type of the current camera
3. The most recently performed command, or the FPS/Exposure info in case of live image.
4. Name of the current objective.
5. Current coordinates of XY(Z) stage.

3.1.4. Document Window

Tools affecting the appearance of the current document (image) are gathered within the document tool bars. Although tool bars can be customized, the default ones contain the following buttons:



LUTs

-  **[Enable LUTs]** button applies LUTs to the image. See [LUTs].
-  **[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.
-  **[Show LUTs window]** button opens the window with LUTs.
-  **[Pixel Saturation Indication]** button turns on/off pixel saturation indication without setting on/off LUTs. Select the highlighting color from the nearby pull-down menu.

Split Components

-  **[Split Components]** button turns ON a special view, where color channels of the image are displayed separately (tiled).

Zoom Controls

-  **[Fit to screen]** button adjusts zoom to view the whole image within the NIS-Elements AR screen.
-  **[Best Fit]** button adjusts zoom to fit the NIS-Elements AR image window in one direction but to fill the screen.
- 1:1 **[1:1 Zoom]** button adjust zoom so that one pixel of the image matches one pixel of monitor.
-  **[Zoom In]** Increases magnification of the image.
-  **[Zoom Out]** Decreases magnification of the image.

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 use 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.
-  **[View LUT Intensity]** button displays the scale of intensities used inside the image. It works on monochromatic images or a single image channel.
- **A** **[Show Annotations]** button displays the vector layer which typically consists of annotation objects (text labels, arrows) and measurement objects.

-  **[View Binary]** button displays the binary layer of the document.
-  **[View Color]** button displays the color layer of the document.
-  **[View Overlay]** button displays the color layer and the binary layer in overlay.

Tip

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 document window displays the following information:



0.34 $\mu\text{m}/\text{px}$	[990, 997] RGB: (111, 111, 144)	RGB 8bit, 1280 x 1024 pixels
------------------------------	---------------------------------	------------------------------

The Document Status Bar

- Image calibration
- Pixel coordinates of the mouse cursor along with channel intensities
- Color mode (RGB, Monochromatic, etc.)
- Image bit depth (8bit, 12bit, 16bit, etc.)
- Image size in pixels

3.2. Modifying Layout

Having a well organized application layout can help you make the work with NIS-Elements AR very effective. The live image, image documents, various control windows (Camera Settings, Measurement, Histogram, LUTs, etc.) can be arranged within the application window. The control windows as well as tool bars can be floating or docked on sides of the screen. Multiple monitors setup is also supported. User commands may be added to the main menu, and tool bar buttons may be added/removed.

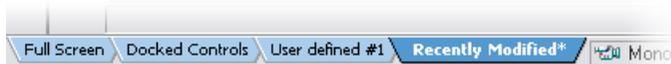
3.2.1. Layouts

A layout in the context of NIS-Elements AR is a set of options describing the arrangement of control windows, tool bars, and menu items. Blue tabs representing active layouts appear in the application status bar. The following layouts are placed there by default:

- **[Full Screen]**
- **[Docked Controls]**
- **[Measurement]**

Other layouts can be added and managed via the Layout Manager. To hide/show the layout tabs within the status bar, go for **[Edit > Options]**, select the **[Appearance]** tab and (de)select the **[Show Layout Tabs]** option.

To Create a New Layout



- Modify the current layout so that it suits your concept of work.
- An 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. If you do not need to create a new layout but would like to save the changes made, just right click the current (asterisk-marked) tab and select the **[Save]** command from the menu.
- Write the new layout name and confirm it by **[OK]**.
- A new tab appears and the layout is saved to the list of layouts.

To Reload Previous Layout Settings

You may want to undo the changes made to the layout. Mostly, it can be done by the **[Reload]** command.

- Right click the asterisk-marked (recently modified but not saved) tab and choose the **[Reload]** command.

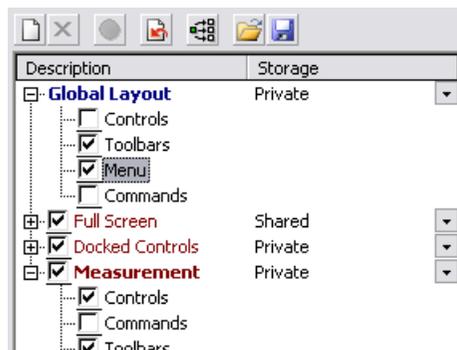
- The application restores the last saved state of the layout.

3.2.2. Layout Manager

Call the **[View > Layout > Layout Manager]** command. The Layout Manager appears.

3.2.2.1. Layouts

The list of currently available layouts is placed on the left side of the layout manager. Each layout may contain information about controls, tool bars, menu, and commands to be performed when switching between layouts.



The List of Layouts

Modifying the Layout Settings

- Select items within the **[Global Layout]** list which you want to be shared by all layouts.
- The check marks on the left of the layout names indicate the layout visibility. Select the ones you want to display in the application status bar.
- If an item within some particular layout is not selected, it means you do not want to customize it and the global settings - if selected within the **[Global Layout]** - will be used. If the item is not selected within the Global Layout either, the settings of the most recently active layout will be used.
- Set whether the layout is **[Private]** or **[Shared]**. If set **[Private]**, it will not be visible for other users. See [User Management].
- Customize each item according to your needs (see below).

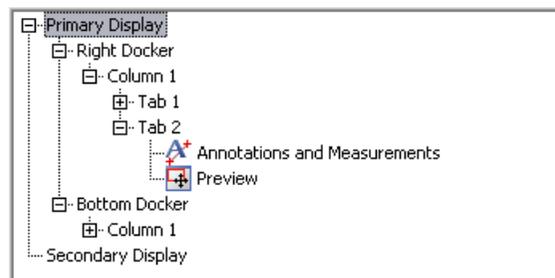
Layout Manager Tools

-  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
-  The **[Load Default]** button loads original settings of the selected pre-defined layout (**[Full Screen]**, **[Docked Controls]**, **[Measurement]**) so that it looks just like after the program installation.
-  The **[Apply to All]** button ensures that the executed changes will be applied to all layouts.
-  The **[Import]** button enables to load a previously saved set of layouts from an XML file.
-  The settings of all layouts can be saved to an external XML file. Use this **[Export]** button and define the destination file name.

3.2.2.2. Controls

The layout of docking panes and the control windows arrangement has to be modified directly in the application. The right part of the window provides only the overview of the structure.

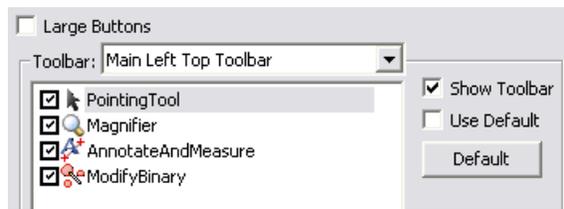


Tree View Levels

There are 5 levels in the tree view:

1. **[Display]** (primary, secondary, etc.)
2. **[Docker]** (top, bottom, left, right)
3. **[Column]** (controls can be placed in a docker side by side)
4. **[Tab]** (one column can take multiple controls “layered”)
5. **[Controls]** in each tab.

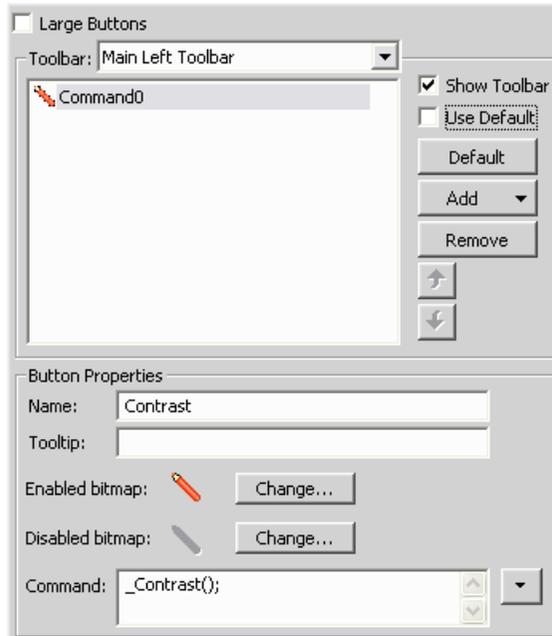
3.2.2.3. Modifying Tool Bars



- Select the **[Toolbars]** item within the list of layouts. The right-side part of the window changes.
- Choose one of the tool bars which you would like to modify from the **[Toolbar]** pull-down menu.
- If selected, de-select the **[Use Default]** option on the right.
- Any button of the tool bar may be hidden by de-selecting it. No buttons can be added to any of the tool bars except the **[Main Left Tool Bar]**.
- The whole tool bar can be hidden by de-selecting the **[Show Toolbar]** check box.
- There are two sizes of buttons available. Select the **[Large Buttons]** option to use the larger one. This setting is shared by all tool bars.

Modifying the Main Left Tool Bar

Custom user buttons can be added to the main left tool bar. You can define your own buttons which run single macro functions or execute macros. Select the **[Main Left Toolbar]** from the pull-down menu.



Lets say that we very often use the **[Image > Contrast]** command. It is useful to add a shortcut button to the tool bar. Press the **[Add]** button, and choose **[Command]** from the pull-down menu:

- A new command - **[Command0]** - is added to the list.
- Now, attach the NIS-Elements AR command: Open the pull-down menu on the right side of the **[Command]** edit box and click **[Command List]**.
- A list of commands appears. Choose **[_ Contrast()]**.
- Confirm the selection by **[OK]**.

It is possible to create a sequence of commands, by repeating this procedure.

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

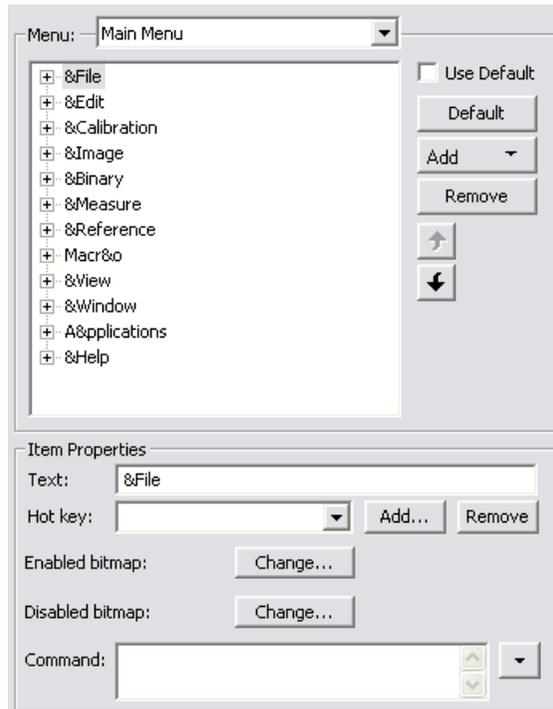
It is handy to define a tool-tip (a text that appears when the mouse cursor is placed over the icon) for your command. Simply write the text into the **[Tooltip]** box. You can change the position of the command in the

tool bar using the arrow buttons. The **[Default]** button discards your changes and inserts the default set of commands to the tool bar.

3.2.2.4. Modifying the Menu

The **[Main Menu]** and some of the **[Context Menus]** accessible by right-click within the application window may be modified. Items of the context menus can be hidden by de-selecting them similarly to the tool bars. The main menu can be modified as follows:

Modifying the Main Menu



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

Note

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. The [Use Default] check box, when selected, applies the default settings to the menu.

Item Properties

Text

This is the text which appears in the pull-down menu. You may add “&” before any letter - such letter will be considered a keyboard shortcut 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. Press [Remove] to remove the selected hot key.

Enabled/Disabled bitmap, Command

These fields serve for assigning a bitmap image and a macro function to the menu command. It works the same way as when modifying the Main Left Tool Bar (described above).

3.2.2.5. Commands

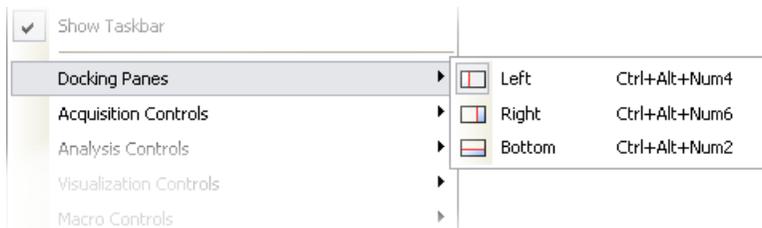
These settings enable to run a command or a macro right before and after you switch to the layout.

3.2.3. Docking Panes

Docking panes are square spaces inside the application window, where you can place (dock) any of the control windows. There is one docking pane available on each side (Right, Bottom, Left).

To Display a Docking Pane

- Go to the [View > Docking Panes] sub-menu and select the pane you would like to display.

**Note**

The [Docking Panes] sub-menu can be also displayed by right-clicking into the empty application screen.

- The docking pane appears, either empty or with some window(s) docked inside.

- Repeat this procedure to display more docking panes.

Handling Control Windows

Various control windows can be displayed docked within the docking panes or they can be floating. See the following picture:



The Docked Control Window Caption

To handle the control windows (CWs), you can:

Add CW to a docking pane

Right click inside a docking pane (3) and select the control window to be displayed from the context menu. If the window is already opened somewhere else (in another docking pane or floating), it closes and moves to the new destination.

Close CW

Click the **[cross]** button (2) in the right top corner of the tab.

Drag CW

Drag any CW by the tab and drop it somewhere. If you drop it by the edge of a docking pane, it will create another column of this pane. If you drop it over the caption of another CW, it will be docked in the same pane as a new tab. If dropped somewhere else, the CW will be floating.

Note

A color frame appears when you place the mouse cursor dragging a CW over the edge of a docking pane or a caption of another CW. It indicates that if you drop it, its placement will be handled automatically.

Minimize the docking pane

Click the arrows in the top left corner. The pane minimizes to a stripe by the edge of the screen. It can be restored to its original position by double clicking this stripe or by clicking the arrows again.

Close the docking pane

Click the **[cross]** button (4) in the docking pane caption. Or you can right click the pane and unselect the **[Docking View]** option.

Dock/undock CW

To dock (and undock) a CW, double click its tab.

Display CW

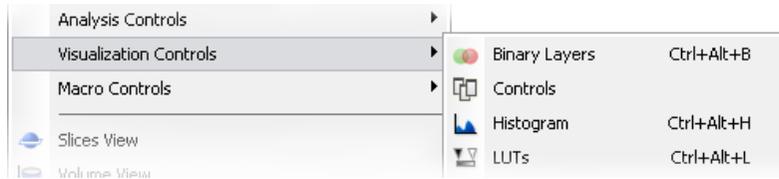
Another way to display a CW is to go to the **[View]** menu and select the desired control window. The controls are sorted to several groups:

Acquisition
Analysis

Visualization

Macro

After that, the CW appears floating on the screen.

**Shrink/Expand dockers**

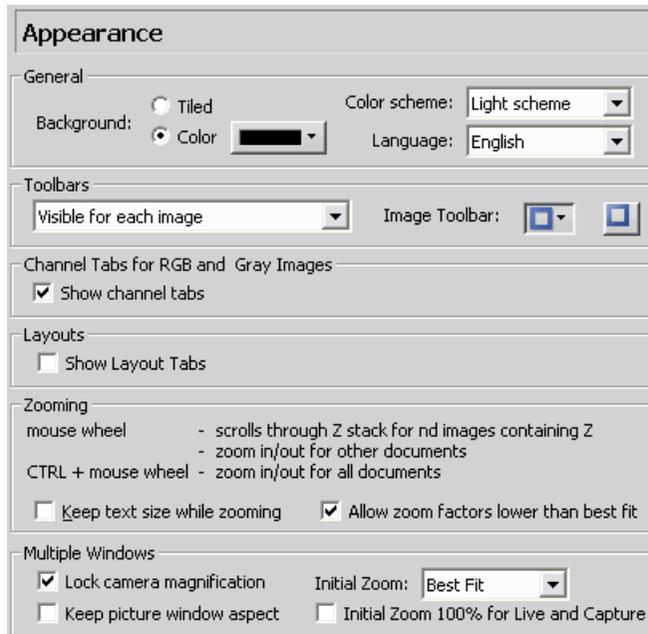
Having more docking panes opened, a situation where there is not enough room for the control windows can occur. In such case, the **[Shrink]** and **[Expand]** commands shall be used.



- Right click the pane you would like to shrink/expand. A context menu appears.
- Select the **[Expand]/[Shrink]** command. When one of the panes shrinks, the neighbouring pane expands to the emptied corner and vice-versa.

3.2.4. Appearance Options

General appearance adjustments can be made in the **[Options]** window. Run the **[Edit > Options]** command and switch to the **[Appearance]** tab.



The following properties can be adjusted here:

- Application Background
- Color Scheme Selection
- Application Language
- Document Tool Bars Visibility and Style
- Channel Tabs Visibility
- Layout Tabs Visibility
- Initial Zoom of Images
- etc.

3.3. Organizer

Apart from the main application layout used for capturing and image analysis, NIS-Elements AR provides an extra layout called **[Organizer]**. Organizer makes the work with image files and databases as easy as possible.

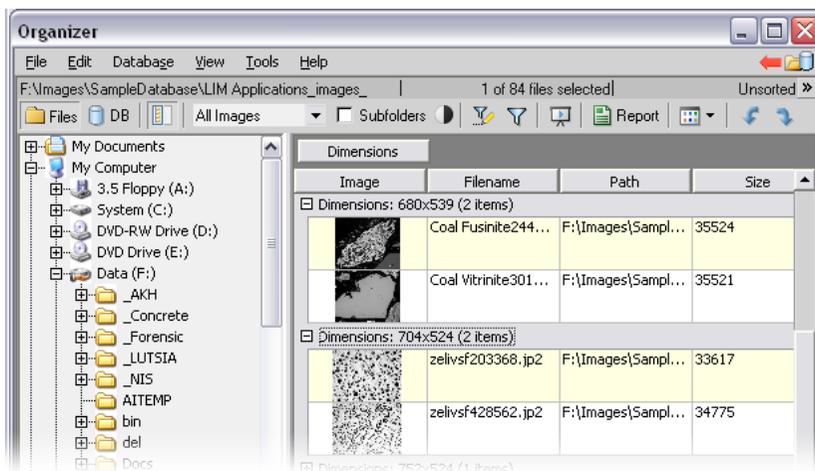
To activate it, click the  button located in the top right corner of the application window or press **[F10]**.

The screen is by default divided into two identical panes, each pane switchable between two modes: the **[Files View]** and the **[Database View]**.

-  **[Files]** - This button switches the pane to show a directory tree and images from the selected folder (and optionally its sub-folders).
-  **[Database]** (-DB- option) - This button switches the pane to show the database structure and lists images from the currently selected database table.

To switch from one pane to the other, use the **[View > Next Pane (F6)]** command. To copy files between the panes, simply drag the images from one side to the other side.

Features Available in the Files View

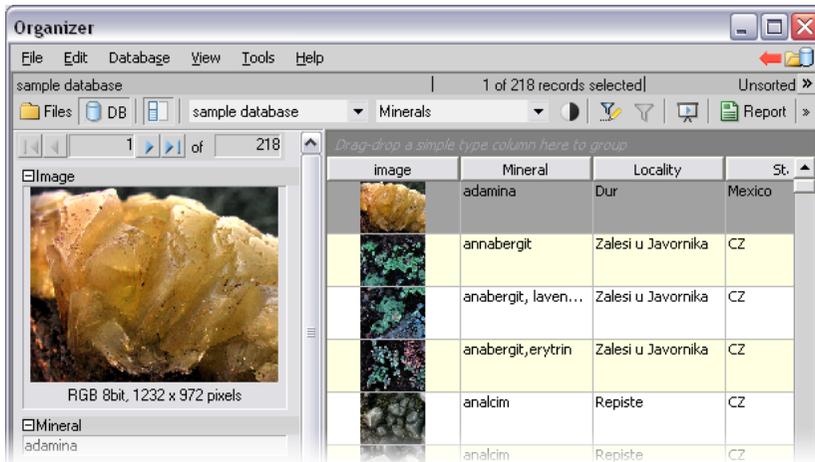


The Files View

-  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 and only the one will be displayed. Or, **[All Images]** can be displayed.

- Subfolders If this check-box is selected all images from included sub-folders are displayed.

Features Available in the Database View (-DB- option)



The Database View

- 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 database connection name and enables to switch between active connections.
- This pull-down menu selects a database table to be displayed.

3.3.1. 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 conditions may be applied.

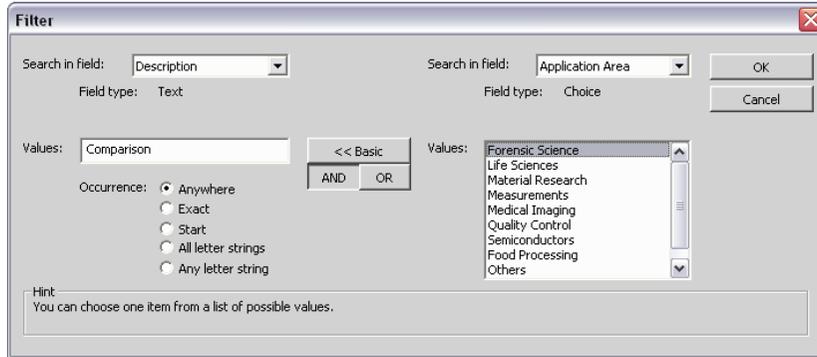
- Pressing this button invokes the filter setup window.
- This button activates the filter.

Basic Mode

This mode enables you to filter files according to one condition.

Advanced Mode

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



1. **[Search in field]** - select the field where NIS-Elements AR should search for a given expression.

Note

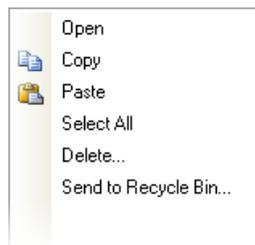
When using a filter in the [Files] view, the fields from file-properties are listed in the menu. In the [Database] view, the list shows names of fields from the current database table.

2. 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.
3. If the field type is **[Text]**, the **[Occurrence]** setting determines the way of evaluating the expression:
 - **[Anywhere]** - If the given sequence of characters is found anywhere in the field, the system will evaluate it as a match. For example: you have entered "set" to the **[Values]** field. The filter will select records with the following field values: "set", "reset", "settings", "preset", etc.
 - **[Exact]** - If the given sequence of characters exactly equals to the content of the field, it is evaluated as a match. Fields containing the "set" value only will match.
 - **[Start]** - If the entered string is found at the beginning of a field, it passes the condition. For example: fields containing "set", "settings", "setup" will match.
 - **[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 quoted. If this option is selected, only records where all of those expressions appear (anywhere) will match.
 - **[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.

3.3.2. Operations with Images

- To open an image from the **[Files]** view, double click its thumbnail. NIS-Elements AR will close **[Organizer]** and 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 images 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.



Thumbnail View

Press this button and select the size of displayed image thumbnails. Selecting the **[Details with preview]** option will display images below each other with all available information aside.



Rotating Images

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.

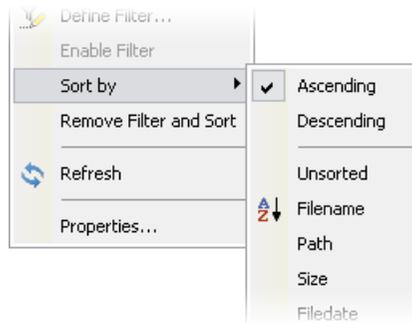


Autocontrast

Press this button to turn the **[Apply autocontrast to thumbnails]** option ON. It enhances the image thumbnails automatically. Dark images gain details.

3.3.3. Sorting of Images

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



3.3.4. 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 to the others. See [fig. The Files View] (the Dimensions column is grouped).

3.3.5. Adjusting the Organizer Layout

The pane size is adjustable. To resize it:

1. Place the mouse cursor over the dividing line in the middle.
2. The cursor becomes an arrow with two tips.
3. Drag it left or right to the new position.

Also, the following buttons appear in the top bar:

-  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).

4. Setting the Hardware Up

4.1. Working with Cameras

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

Setting Up the Camera

1)

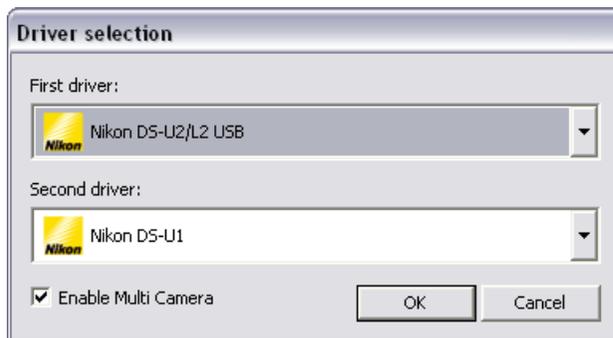
Selecting the Driver

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



Multiple Cameras

In some cases, two cameras can be connected to one system. If so, check the **[Enable Multi Camera]** option and select the **[Second driver]** from the list.



The following set of buttons appears in the top tool bar when multiple cameras are used:



2)

Selecting a 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.

3)

Adjust the Camera Settings

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.

4.1.1. Live Signal from 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 AR provides two different camera modes: **[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. This mode is usually set to low resolution, therefore the frame rate is high. It is used for searching the specimen or when focusing manually.



Capture - Quality

This tool bar button runs the **[Quality]** live mode. This mode should be set to high resolution which reduces the camera frame rate. It is applied every time the **[Capture]** button is pressed.

4.1.2. 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 procedure when the live image is active, NIS-Elements AR automatically freezes the image. Let's see the difference between the **[Capture]** and **[Freeze]** commands:



Capture

When pressed, the camera exposure runs till the end of the current frame, and the next frame is captured and displayed on the screen (it is the first frame with the complete exposure after you have pressed the **[Capture]** button).



Freeze

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

Note

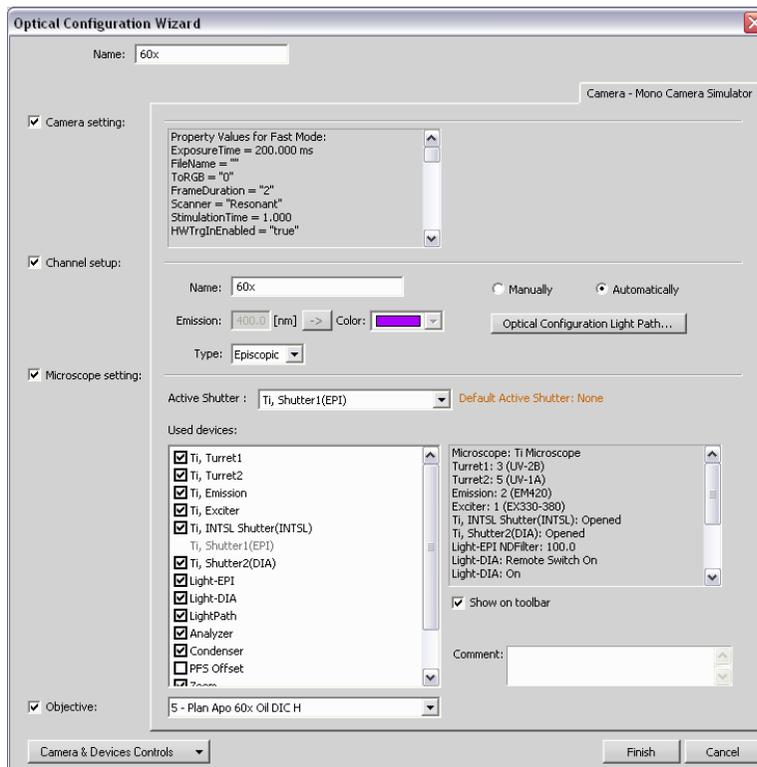
If you are using the [Fast] mode and press [Capture], NIS-Elements AR will automatically switch to the quality mode to capture the image. When the image is captured, it is opened on the screen as a new document.

4.2. Optical Configurations

Typically, laboratory computer image analysis systems consist of a computer, a camera, and a microscope equipped with certain 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 AR. In addition, it is possible to integrate single settings of all these devices into one compact set called **[Optical Configuration]**. It is recommended to create several optical configurations containing particular devices settings. Then a single click can completely change the current hardware configuration.

Creating New Optical Configuration

- 1) 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.
- 2) Choose the **[Calibration > New Optical Configuration]** command. The following window appears: Adjust the settings of the devices to match the intended state which will be saved to the optical configuration.



- 3) Type the name of the new optical configuration to the **[Name]** field. We recommend to use a short descriptive name, the name will be displayed in the main tool bar.

- 4) In the left column, select which device settings to associate with the Optical configuration.

Camera setting

A list of the current camera properties appears on the right. It is being updated dynamically.

Channel setup

These settings determine how channels of newly captured images will be named and what color will be assigned to them. The available properties depends on the current camera setup (color/mono, triggered acquisition engaged or not, dual view engaged or not, etc.). In the mono camera mode, you can either assign the name, the emission length and color to the channel **[Manually]**, or leave this task to NIS-Elements AR (the **[Automatically]** option). In such case, information of the light path (emission wavelength) will be used to determine the channel name and color.

Microscope setting

Select which parts of the microscope shall be included in the configuration by checking them in the **[Used devices]** dialog box. If there is more than one shutter available and you would like to associate a shutter with the optical configuration, select which one is the **[Active Shutter]** from the pull-down menu.

Objective

An objective mounted to a motorized nosepiece can be included in the configuration. Select the objective from the pull-down menu. Objectives which are currently assigned to any position of the nosepiece are listed. See [Objectives].

Note

The objective must be assigned to a nosepiece position via the microscope control pad or the nosepiece control window first.

- 5) If some of the device settings still need to be adjusted, click the **[Camera & Devices Controls]** button and select the appropriate control window from the pull-down menu. Adjust the settings within the control window, the optical configuration will be updated automatically.
- 6) Click **[Finish]** to save the new optical configuration and to close the window.
- 7) You can create more optical configurations by repeating the procedure. The optical configurations are saved to registry immediately and can be exported to an XML file (**[Calibration > Optical Configurations > Backup]**).

Managing Optical Configurations

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

- Create, duplicate, rename, delete, copy settings and switch between optical configurations.
- Modify optical configuration properties.
- Import and export optical configurations to/from an XML file.

Operations with Optical Configurations

Once created, the configuration appears in the list and can be shared with other users by changing the **[Private]** option to **[Shared]** (see [User Management]). The following operations can be performed on the selected configuration:

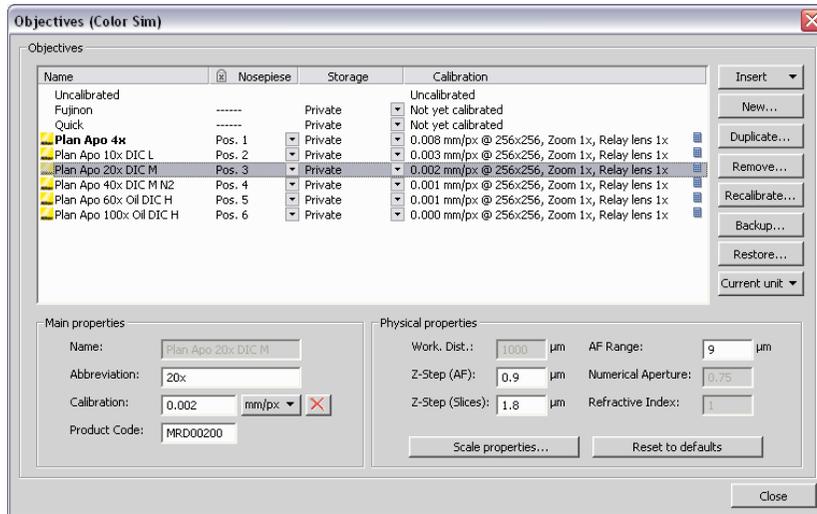
- It can be deleted by pressing the **[Remove]** button. A confirmation dialog box appears.
- Its name can be changed by the **[Rename]** button. A button with the configuration name appears in the tool bar (if the **[Show on toolbar]** option is selected).
- The configuration settings may be transferred to another optical configuration by the **[Copy to]** button. Press the button and select the optical configuration to be overwritten with the current one.
- A copy of the configuration can be made via the **[Duplicate]** button.
- The configuration can be applied by the **[Set As Active]** button.
- The settings of all optical configurations can be exported to an external XML file using the **[Backup]** button.
- The previously exported optical configurations settings can be loaded from the XML file via the **[Restore]** button.
- The list of optical configurations can be ordered manually via the arrow buttons.
- Each configuration can be arbitrary modified within the right-side portion of the window.

4.3. Objectives

If you want to perform measurements on captured images it is wise to calibrate the objectives used to capture the images. Whenever an image is captured through a calibrated objective, the image inherits its calibration.

4.3.1. Managing Objectives

Run the **[Calibration > Objectives]** command. The **[Objectives]** window appears:



A list of used objectives can be created within the Objectives window. For each objective, the objective name, position in the changer, storage status and the calibration are displayed. A **[Calculator]** icon next to the calibration value indicates that the calibration has been calculated from objective properties. If the calculator

icon is missing, it that means the calibration has been performed manually. Use the buttons on the right side to manage the objectives:

Insert

Click the button and select one of the predefined objectives from the list ordered by magnification.

New

Press this button to create a custom objective. The new objective will be added to the list. Then, define the **[Main properties]** and the **[Physical properties]** of the objective.

Duplicate

If zoom is used, the objective calibration must be re-calculated accordingly. Use this button to make a copy of the objective and define the zoom factor in the window which opens.

Remove

This button deletes the selected objective.

Recalibrate

Starts the calibration of the selected objective. See [Objective Calibration].

Backup

Enables you to export the complete list of objectives to an XML file. The standard **[Save As]** window appears.

Restore

This button enables you to import a complete list of objectives from an external XML file previously created by the **[Backup]** button.

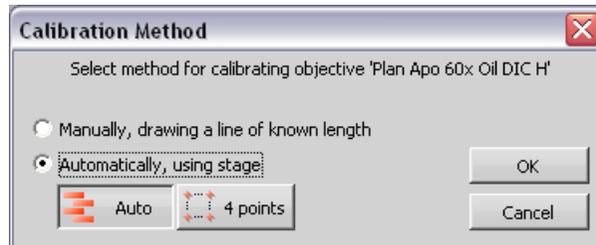
Current Unit

This button invokes a pull-down menu where units for the whole application can be selected.

Edit the properties of the selected objective in the bottom part of the window. Press the **[Close]** button to finish this window.

4.3.2. Objective Calibration

After you press the **[Recalibrate]** button in the **[Objectives]** window or when creating a new custom objective, the following window appears:



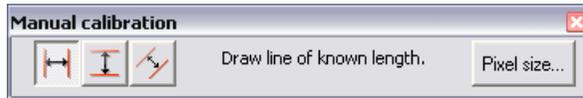
Select one of the calibration methods:

- The **[Manual]** calibration lets you draw a distance into a picture and assign real length to it (see below).
- If a motorized XY stage is available, the **[Auto]** and **[4 points]** automatic methods appear.

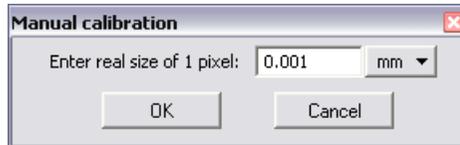
Press **[OK]** to continue...

Manual Calibration

- 1) When performing a manual calibration, the live image starts automatically and the following window appears:



- 2) Select one of the icons to draw a distance to the image. If you know the precise calibration value (px/unit), press the **[Pixel Size]** button to enter the real size of one pixel:



Enter the calibration value, select units, and confirm the calibration by **[OK]**. If you do not know the pixel size, you will have to continue with the calibration on live image:

- 3) Insert a calibration slide to the microscope stage.
- 4) The distance is defined by placing lines (Horizontal, Vertical, Parallel) to the image. Choose the orientation of the lines by pressing the appropriate button.

Note

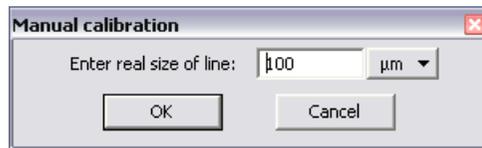
If you are sure the camera angle is 0, 90, 180, or 270°, it is recommended to select either vertical or horizontal lines. Otherwise, select the parallel lines.

- 5) Click into the image to place the first line. Place the second line in the intended position by another click.

Note

You can modify the line position while holding the mouse button. After you release the button no further changes can be made. When "Parallel lines" were selected, draw the first line by clicking twice inside the image. The line can be moved and adjusted arbitrary by mouse. When satisfied, finish the first line 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.

- 6) The following dialog box appears:



- 7) Enter the distance between the two lines and select correct units.
8) Press **OK**. The objective is calibrated now.

Automatic Calibration

The **[Auto method]** is fully automatic. NIS-Elements AR moves the motorized stage, acquires two images, and calculates the calibration from the shift of the images. If you select the **[4 points method]**, the system draws four points on the screen (subsequently) and asks user to move one significant part of the specimen to each position. After all four steps are completed, the calibration is calculated from the moves of the stage.

4.3.3. Units

NIS-Elements AR supports the following units:

- pixels
- nanometers
- micrometers
- millimeters
- centimeters
- decimeters
- meters
- kilometers
- inches
- 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 which are then used to display all values (e.g. measured length/area). There are two ways of how to select the desired units:

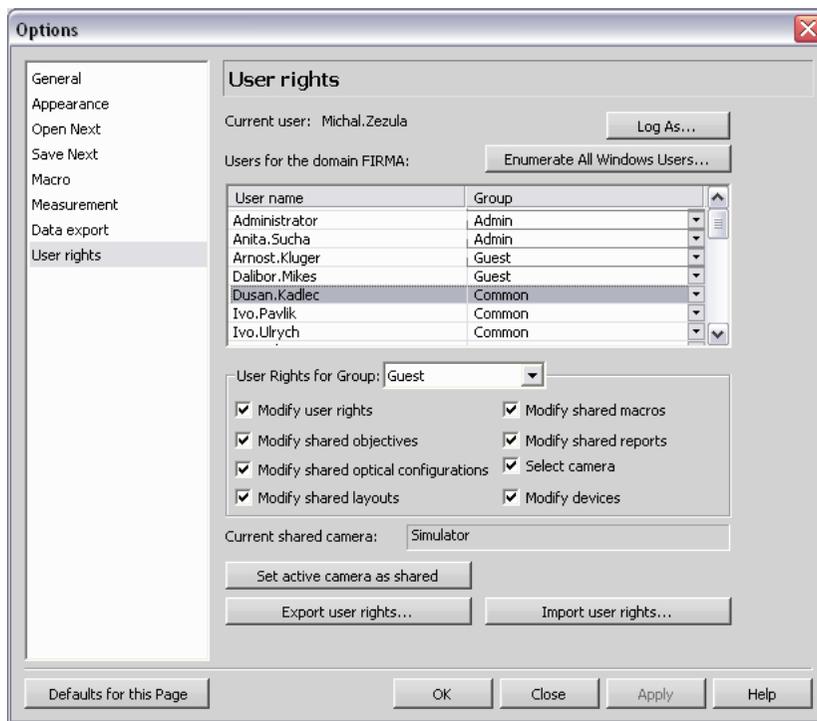
- Right click the document status bar where the calibration is displayed. Select the units and precision from the context menu.
- Or, click the **[Current unit]** button located in the **[Calibration > Optical Configurations]** command window and select the units.

4.4. User Management

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

- Admin
- Common
- Guest

Run the **[Edit > Options]** command and select **[User Rights]** in the left column, the following 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 the permission to modify them can do so.

User Rights Options

Current user

Displays the current MS Windows user.

Log As

Opens the **[Login As]** dialog which enables you to login as a different user with stronger user rights for the purpose of changing User rights settings.

Enumerate All Windows Users

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

User Rights for Group

From this pull-down menu, select the group you would like to modify permissions of. You have to have the **[Modify users]** permission to do that.

Modify user rights

Users can modify permissions of all groups in this window, and apply the **[Set active camera as shared]** option.

Modify shared objectives

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

Modify shared optical configurations

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

Modify shared layouts

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

Current shared camera

This field informs you which of the available cameras is set as **[Shared]**. Users without the **[Select**

camera] permission will only be able to use this camera.

Set active camera as shared

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

Export/Import user rights

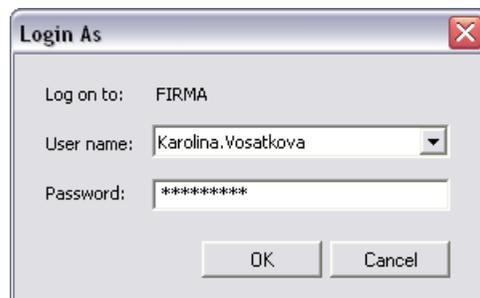
These buttons enable anyone to save/load complete settings of users and user groups to/from an external XML file. Standard **[Open]** and **[Save As]** windows appear. Having the data exported may be useful when copying the settings to other computers.

Defaults for this Page

Pressing this button restores the default settings of this window. All your changes will be lost.

Log As

NIS-Elements AR user rights management primarily utilizes MS Windows user accounts to recognize the users. However, for the purposes of NIS-Elements AR user rights administration, you can log in temporarily under a different (MS Windows) account. Press the **[Log As]** button in the **[Users]** tab in the **[Options]** window. A dialog box appears:



Select the user name you want to log-in as. Enter a valid password and press **[OK]**. The new user identity is available only while the User manager is opened, it does not have any effect to other NIS-Elements AR functionality or other applications.

5. Capturing

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

5.1. Single Image Capture

- Turn the connected camera and other devices ON and start NIS-Elements AR.
- When the application is started display the **[Camera Settings]** control window (**[Acquire > Camera Settings]**).
- Switch camera to the **[Live-Fast]** mode and adjust the camera 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. This 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 toolbar, by invoking the **[Acquire > Capture]** command.
- A new image is opened and named “Captured” automatically.

5.2. ND2 Acquisition

NIS-Elements AR being a multi-purpose imaging system can be used as a handy tool to study objects, live organisms, processes, etc. The universal **[ND2]**(N-dimensional) file format is what makes this possible. One **[ND2]** file can contain multiple images organized according to what type of acquisition they came from. There are the following acquisition types:

Note

Each acquisition type gives a name to the corresponding dimension of the resulting ND2 file. For example, we can speak about two-dimensional TZ ND2 file, which means the file contains the Time-lapse dimension and the Z-series dimension.

Time-lapse - T

A sequence of images can be captured over a period of time and create a time-lapse document.

Multi-point - XY

Images from different areas of the slide can be acquired.

Z-series - Z

Several techniques utilize Z-stacks of images. Such Z-stack can be for example converted to a 3D model of the specimen.

Multi-channel - λ

Fluorescence signals can be captured selectively using excitation and emission filters, each of them creating a different color channel.

Large image - M (-6D- option)

When the area of interest exceeds the camera field of view, you can capture a large image composed of multiple image frames and let it be stitched by an automatic algorithm.

An ND2 document can be created (captured) either containing one dimension or combining several of them.

- Single-dimensional documents can be created automatically (using motorized accessories) or manually using commands from the **[Acquire]** menu.
- To create a multi-dimensional document, use the **[Applications > ND > Define/Run Experiment (ND) (-6D- option)]** command (ND module required).

Special Options

Special options can be applied to ND experiments:

Auto Focus

Automatic focusing can be used during the experiment. You can select the auto-focus method that best meets your needs. It can be determined whether the focusing shall be performed at the beginning of

the whole experiment or at the beginning of each phase. The **[Define]** button shows a window where you can define parameters of the selected focusing method.

Closing Shutter

The active shutter can be closed between single image acquisitions. Just select the **[Close active Shutter...]** option in the experiment window.

Running Macro Commands

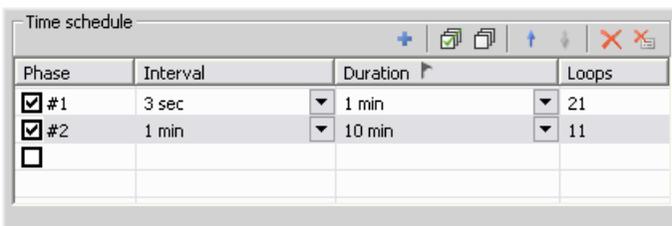
You can define a command (or a macro) to be run in various stages of the experiment. Select the timing in the **[Advanced]** section of the experiment window and enter the command to be run.

In/Out signals (-LO- option) (-6D- option)

An ND2 experiment can control some accessory devices but can be controlled by another accessory devices at the same time by sending/receiving Analog/TTL signals. This feature requires additional hardware (NI DAQ controller set). If the NI DAQ PCI card is installed, an extra tab called **[Inputs/Outputs]** appears within the ND experiment window. See **[Applications > ND > Define/Run Experiment (ND) (-6D- option)]**.

5.2.1. Time-lapse Acquisition

Detailed studying of long-lasting processes is enabled by the time lapse acquisition mode of NIS-Elements AR. The achievable experiment duration is limited by the hardware abilities of your PC only. Invoke the **[Acquire > Capture Timelapse > Capture Automatically]** command to set the experiment up:



Phase	Interval	Duration	Loops
<input checked="" type="checkbox"/> #1	3 sec	1 min	21
<input checked="" type="checkbox"/> #2	1 min	10 min	11
<input type="checkbox"/>			

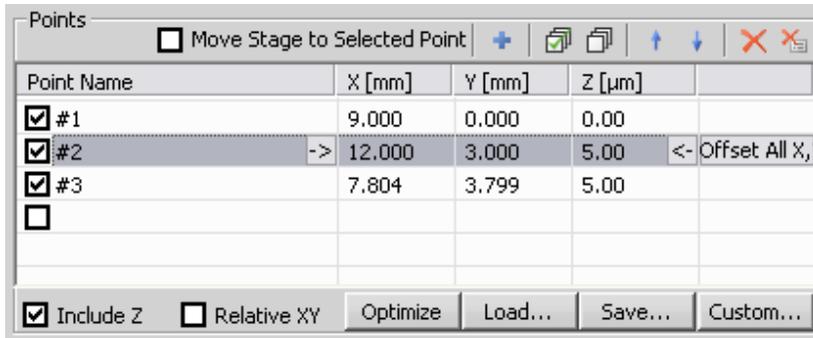
The **[Time schedule]** table enables you to define consecutive time phases where duration, interval between single images, and number of images of the phase can be adjusted. The **[Interval]**, **[Duration]**, and **[Loops]** settings are bound together, so you just need to set two of these parameters.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

The flag mark indicates the Duration/Loops priority. For example: if the camera exposure time exceeds the defined **[Interval]** between frames, than the experiment settings are not achievable - only the marked column will be pushed to be correct. You can set the priority column by clicking on its caption.

5.2.2. Multi-point Acquisition

Automatic multi-point acquisition is available when a motorized XY(Z) stage is connected to the system. 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. Run the **[Acquire > Capture Multipoint > Capture Automatically]** command.



To Define a Multipoint Array:

- Move the stage to the first position.
- Press the **+** **[Add New]** button. A new line containing current coordinates of the stage 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:

- Make sure the **[Move stage to selected point]**  **<<<** (-LO- option) is pressed. This button ensures that the motorized stage moves to the coordinates of the currently selected point.
- Click inside the line you would like to change.
- Move the Z drive to the new position.
- Click the **[<-]** button.

Note

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

Offset All

The offset all button can shift the XY coordinates of all points in the same way:

- Make sure the **[Move stage to selected point]**  **<<<** (-LO- option) is pressed. This button ensures that the motorized stage moves to the coordinates of the currently selected point.

- Select one point of the list.
- Move the XY(Z) stage to a new position (define the offset).
- Press the **[Offset All]** button.
- The coordinates of all points change - the same shift which you made with the stage is applied to them.

Special Options

Relative XY

Check the **[Relative XY]** item to consider all coordinates as relative with respect to the current stage position. Any of the points may be used as the reference point, just right-click it and select **[Set this point as a reference position]**.

Optimize

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

Custom

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

Redefine reference Z after Auto Focus/PFS

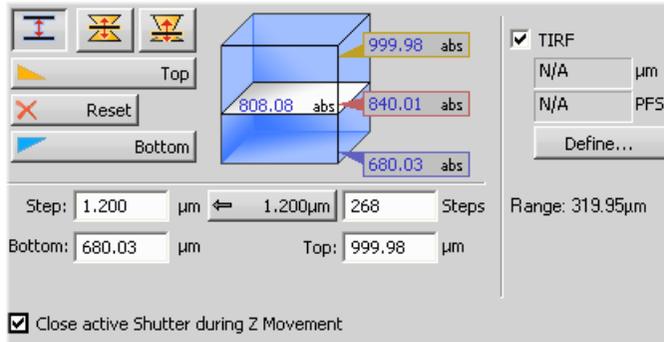
Check this option to redefine reference Z position after performing autofocus or PFS (if AF or PFS is turned on).

Split Multipoints (-LO- option)

Select this box to perform the **[File > ND > Split Multipoints]** command directly after the ND2 experiment.

5.2.3. Z-series Acquisition

Automatic capturing of images from different focal planes of the specimen can be performed. Run the **[Acquire > Capture Z-Series > Capture Automatically]** command. There are three different approaches of how to set the experiment:



There are the following ways of defining the Z drive range:

Range by Top/Bottom Position and Step

- 1) Press the  button, the cube turns blue.
- 2) Run the Live camera signal. In case you have two Z drives, select the **[Z device]** from the pull-down menu.
- 3) 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.
- 4) Define the **[Step]** size in μm or the number of steps to be captured.

Note

In this mode the [Home position] is assigned to the middle one of all the defined steps automatically (e.g. for 5 steps, it is the third step, etc.). The [Reset] button discards the Top, Home, and Bottom positions settings.

Range by Home Position and Range

- 1) 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 μm .
 -  **[Asymmetric mode]** - The range is specified by two values, **[Below]** - the distance below the home position, and **[Above]** - the distance above the home position.
- 2) Run the Live camera signal. In case you have two Z drives, select the **[Z device]** from the pull-down menu.

- 3) 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.
- 4) Specify the scanning **[Range]**.
- 5) Define the **[Step]** size in μm or the number of **[steps]** to be captured.

Defining TIRF position

When the TIRF system is enabled, the TIRF option appears in the right portion of the window. To set the TIRF position:

- Click the **[Define]** button. A window appears waiting for you to move the Z drive.
- Move the Z drive to the correct TIRF Z position.
- Click **[OK]**. The TIRF position value appears in the field above the **[Define]** button.

Using TIRF

To enable TIRF:

- Select the **[TIRF]** check box within the Z-Series experiment settings
- At least one value of the **[Z phase]** column of the Multi-channel acquisition setup has to be set to **[TIRF]**. (See [Multi-channel Acquisition].)

5.2.4. Multi-channel Acquisition

Fluorescence (multi-channel) pictures can be acquired. Run the **[Applications > ND > Define/Run Experiment (ND) (-6D- option)]** command and select the **[Lambda]** tab. There you can set the multi-channel experiment.

Optical Conf.	Name	Comp. Color	T phase	Z phase
Apo 4x	Brightfield	Brightfield	All	All
	Channel1		All	All
			All	All

Channels Setup

Specify how many channels are to be captured. 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 pull-down menu in the Optical Configuration column. The **[Comp. Color]** column specifies the color tone, in which the channel will be displayed.

Special Options

Camera

When you are in a multi camera mode, you can select the camera to capture each channel.

T phase

If there are T dimensions engaged in the experiment, another column called T Phase appears in the table. You can reduce the number of captured channels by selecting **[First]** or **[n-th]** (the channel is included in the first or every n-th time phase only).

Z Phase

If there is a Z dimension engaged in the experiment, another column called **[Z Phase]** appears in the table. You can reduce the number of captured channels by selecting the **[Home]** option - the channel will be captured within the home position of each Z series only. Also, if a **[TIRF]** system is available, the **[TIRF]** option appears as an option. If selected, the channel will be captured within the TIRF position of each Z series only (the TIRF position can be set within the Z-Series acquisition setup window).

Note

The phase settings (T and Z) can be combined in any way, but at least one channel must be included in every T or Z loop which means that the [All] option must be selected in both columns for at least one channel.

Merge Cameras

Available only in multi camera mode. When using two cameras, two ND documents will be created by default. If it the **[Merge Cameras]** option is selected, only one ND document is created. This ND document contains the maximum parameters from the two cameras (width, height and bit depth per component).

Stretch Camera images to Same Size

Available only for multi camera mode. When the two cameras differ in resolution, the camera images will be stretched to the same size.

Manual Filter Change

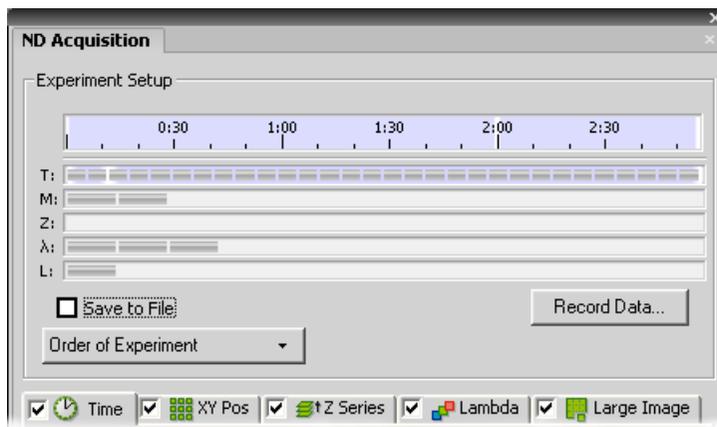
The **[Wait for user before changing to next channel]** advanced option enables you to switch the wavelength filters manually during the multichannel experiment. Use this option if there is no automatic filter changer available.

5.2.5. Combining N Dimensions

The dimensions can be combined together creating an N-dimensional document as the result:

- Run the **[Applications > ND > Define/Run Experiment (ND) (-6D- option)]** 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.
- Press the **[Order of Experiment]** button and select the item which defines the desired order of loops in the ND document.
- 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 afterwards. To do so, select the **[Save to File]** check-box. The path and the file name shall be defined if saving to a file is preferred.

Note

When saving to a file, the size of the resulting ND file is not limited by the amount of RAM installed to your PC, but by the amount of free space on your hard-drive.

- Click the **[Run now]** button to start the acquisition.

Save to File

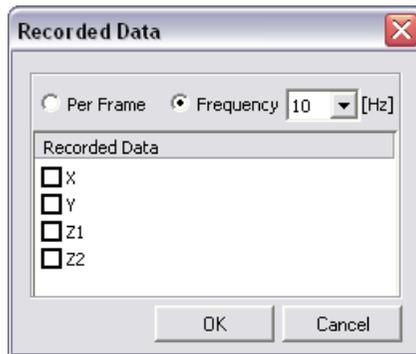
An image can be captured and saved manually after the experiment is finished, or it can be saved to harddisk “on the fly”:

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

Recorded Data

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

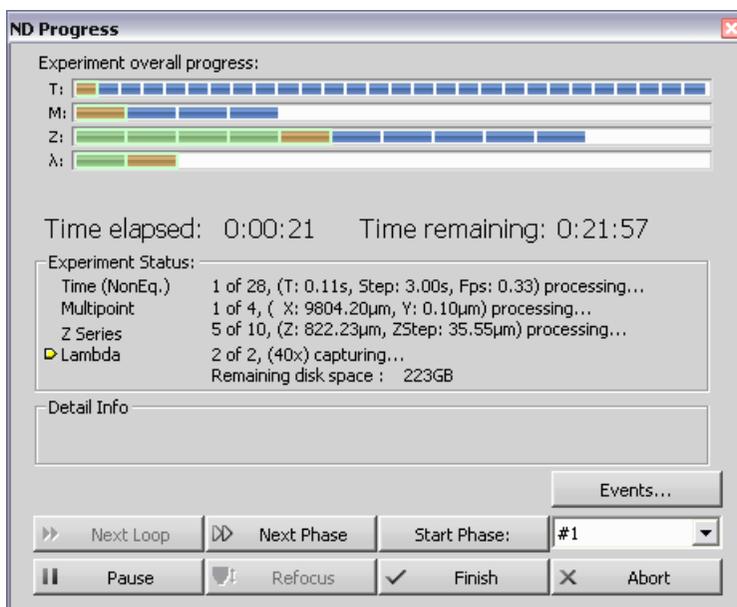
- Press the **[Record Data]** button. The following window appears:



- Select whether to record the data for each frame (**[Per Frame]**) or with a certain **[Frequency]**.
- The data to be recorded shall be check-marked.
- To view the recorded data of an opened ND2 file, invoke the **[File > Image Properties]** command and switch to the **[Recorded Data]** tab.

ND Experiment Progress

The following window appears while the experiment is running:



The window displays the experiment overall progress, time elapsed from start of the experiment, estimated remaining time, remaining free space on the disk where the captured ND document is being saved, and detailed messages about the experiment work-flow such as: “waiting for next loop”, “stage moving” etc.

You can control the running experiment by the following buttons:

Next Loop

When the time interval of one time loop is long enough, the system captures all the included dimensions and waits for the scheduled beginning of the next loop. In such case the **[Next Loop]** button becomes enabled and you can click it to start the next time loop ahead of the schedule.

Next Phase

After you press this button, the experiment jumps to the beginning of the next time **[Phase]** after the current loop is finished.

Start Phase

Use this button to start the time phase selected in the combo box. Select the phase first and press the **[Start Phase]** button.

Pause/Continue

The experiment can be paused by the **[Pause]** button. Let it go on by pressing the same button.

Refocus

If there is a need to focus manually, press the **[Refocus]** button. It pauses the ND experiment and runs **[Live]** camera signal.

Finish

This button stops the experiment and saves captured data.

Abort

Use this button to stop the experiment without saving the captured data.

Events

If a remarkable event occurs during the experiment, the user can press a shortcut key and the information will be saved to the resulting ND2 document. You can define the key-shortcuts after you press the **[Events]** button. See [ND2 Documents].

5.3. Capturing to Ring Buffer

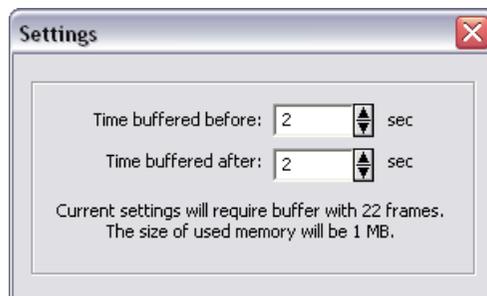
The Capturing-to-RAM technique enables user to record sequences displaying very quick actions - lasting tens of milliseconds. Make sure that the RAM Capturing buttons are displayed on the **[Grabber & Devices ToolBar]** (see [Modifying Layout]), or use the corresponding commands in the **[Acquire > RAM Capture]** menu.

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 seconds).



- Click on the **[Settings]** button.
- 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 **[Circular buffer ON]**  button to enable recording of Live image to RAM.
- The ring buffer functionality gets activated.

Capture the Image Sequence

- Press the **[RAM Capture]** button.

- A new document window containing the captured image sequence opens.

Note

When the Camera is switched to Live mode, the circular buffer (ring) button indicates the buffer activity. If the [RAM Capture] button is pressed afterwards, the captured image sequence will contain frames from the whole time interval before and after the button was pressed. If you press the button while in the Frozen mode or without the time buffer turned ON, only the [Time buffered after] portion of the sequence will be captured.

5.4. Capturing AVI movies

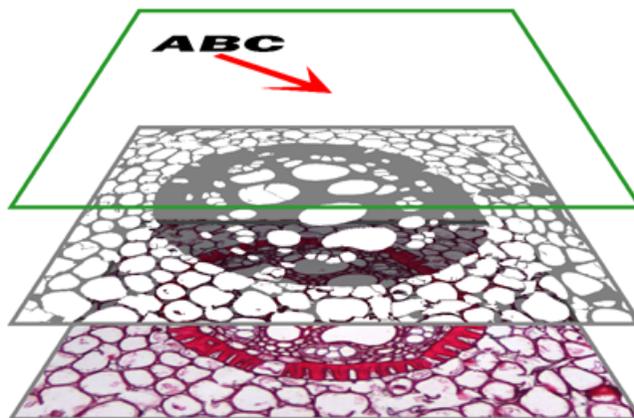
AVI acquisition is an easy way of recording actions in real time. It produces *.avi video files, which can be played by any standard video player, so you do not need NIS-Elements AR to view the resulting video. Please see [Creating Movies].

6. Image Documents

6.1. NIS-Elements AR Documents

6.1.1. Image Layers

Each NIS-Elements AR document can consist of the following image layers:



Annotation layer

In this layer, vector objects are stored. The results of interactive and automatic measurements, text labels and other annotations can be included.

Binary layer(s)

The binary layer is usually the result of thresholding. By thresholding, you distinguish objects of interest from background. A picture of night sky can make a nice example - shining stars may be “thresholded” and compose the binary layer. Then, by means of automatic measurement, you can e.g. count them. More than one binary layer can be placed over one image.

Color layer

The color layer contains the image captured by a camera. It can handle images with the depth of up to 16 bits per color component. The size of this layer determines the maximal size of the other layers.

Note

When saving an image, only some file formats are capable of saving all the layers. The other image formats will save the content of the color layer only. See [Image Formats].

Working with the Annotation Layer

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

A

This button of the right document tool bar turns the visibility of annotation layer ON/OFF. Right-clicking the button displays a context menu with the following options:

- **[Clear All Objects]** will delete annotation and measurement objects.
- **[Clear Annotation Objects]** will delete all annotation objects.
- **[Clear Measurement Objects]** will delete all 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.

Working with the Binary Layer

There are the following buttons at the bottom of the right document tool bar.



This button displays the binary layer (only). It can be edited by hand using the binary editor (press **[Tab]**) . See [Binary Layer] .



The binary layer can be displayed together with the color layer using the overlay mode. Several 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 the color image (only).

6.1.2. Document Types in NIS-Elements AR

RGB Documents

Images acquired by a color camera typically consist of three components that represent red, green and blue channel intensities. You can display a single color channel using the tabs located in the bottom-left corner

of the 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 (RGB), multichannel images can be composed of arbitrary number of user-definable color channels.

Note

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

ND2 (multi-dimensional) Documents

An ND2 document is usually a sequence of images. One **[ND2]** file can contain multiple images organized according to what type of acquisition they came from. There are the following acquisition types, also called dimensions available: Time-lapse, Multi-point, Z-series, Multi-channel, Large-image. The dimensions can be combined together.

6.1.3. ND2 Documents

6.1.3.1. Control Bar

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, loops of each dimension are indicated by rectangles. In case the multi-channel dimension is included and the number of channels does not exceed 10, the channels are indicated by the color tabs at the very bottom of the document window.

Browse the ND document by clicking inside the time line. You can also display a single loop by selecting the corresponding blue rectangle. There are some examples, how the ND2 document control bar can look like in different cases:



A T/Z/multi-channel document with some loops selected.



A T/Z/multi-channel document containing 11 channels and the time line hidden



An HDR multi-exposure document

Playing Controls



Play Sequence

Plays all images of the dimension at selected speed. If a selection is applied, only the selected images will be included in the playback.



Stop Playing

Stops playing the sequence at the last displayed frame.



Previous Position

Displays the previous frame of the dimension.



Next Position

Displays the next image of the dimension.



Decrease/Increase Playing Speed

Changes the playback speed by one step down/up



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.

Event and Selection Controls

 Insert User Event

This button inserts an event marker to the current frame.

 Move to Previous/Next User event

Click these arrows to browse through the frames where user events occur.

 User Event Selection

This button selects frames of the ND2 file according to the **[Event Playing Range]** settings - right click this button and pick the **[Event Playing Range]** command from the context menu to view/modify them. A simple window appears. Define the number of frames/seconds which will be selected around each event.

 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 while holding the **[Ctrl]** key down. Right-click the navigation bar to invoke the context menu. A selection can be adjusted, deleted, or the nd2 file can be cropped.

Note

ND2 files may contain extremely large number of frames so 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 have selected the first and the last time phase with the [Shift] key down, but preserves the [Select Every...] setting.

 Disable Selection

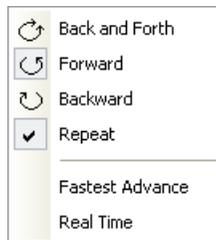
Press this button to de-select all frames of the ND document.

Tips

- Right-click the selection to invoke a context menu. The 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 control bar (T>, Z>, ...).
- Place the cursor over one of the dimensions. A tool-tip which displays statistics of the dimension appears.

Playing options

Right click the speed bar to display the following context menu and select the playing mode.

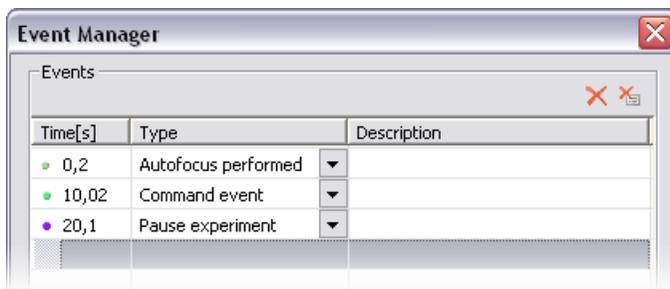
**6.1.3.2. Events**

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 while the ND experiment is running, others must be defined by user (by the  button).

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.



6.1.3.3. Views

There are several views which can display the ND2 documents in various ways. 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.

Note

When you switch the view, a new document window opens by default. This behavior can be changed within the [Edit > Options] command window so that only one view of a document will be opened at a time.

Main View

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

Maximum/Minimum Intensity Projection View

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. (Requires Z or T dimension)

Slices View

This view displays orthogonal XY, XZ, and YZ projections of the image sequence. (Requires Z or T dimension)

Volume View

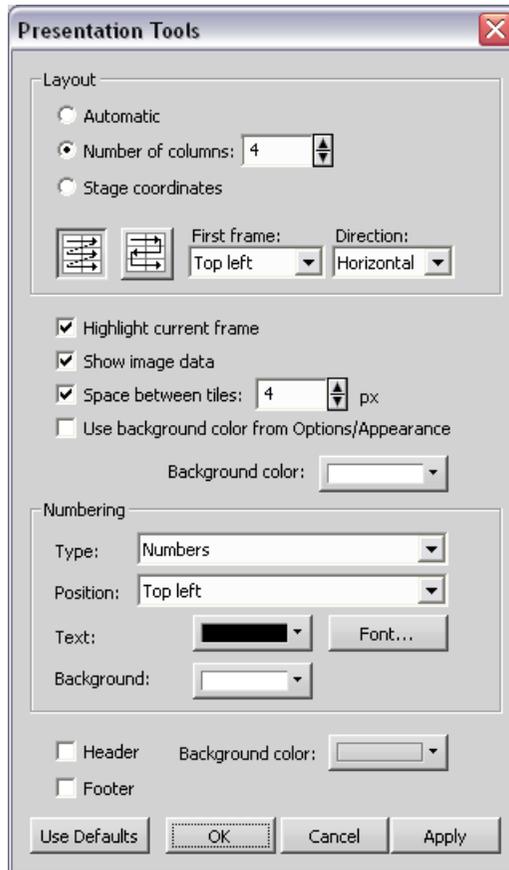
This view creates a 3D model of the acquired object. (Requires Z dimension)

Tiled View

This view displays frames of the selected dimension arranged one next to other. (Requires Z, T or XY dimension)

6.1.3.4. Tiled View Options

You can change the way the tiles are displayed. Press the **[Layout Settings]**  button in the top document tool bar. The following window appears:



Layout Options

Automatic

Calculates the number of columns automatically.

Number Of Columns

Define the number of columns by entering a value.

Stage Coordinates

Frames of a multi-point ND document can be displayed in the layout matching the XY stage positions which were used during the acquisition.

Direction of drawing

Define whether the thumbnails are displayed in rows or a meander. Also define in which corner shall the first frame be placed.

Numbering

Numbers may appear on the tiles if you wish. Set the options here:

Type

Select whether and how the numbers/letters are displayed in tiles:

- None
- Numbers
- Uppercase letters
- Lowercase letters

Position

Specify the text area position within the tile.

Text

Define text color and select a font.

Background

Define background color of the text area inside.

Other Options

Highlight current frame

Check this item to highlight the currently selected frame by a red frame.

Show image data

Check this item to display the information under each frame.

Space between tiles

Check this item to keep the tiles apart by a distance. Define the space size between the tiles in pixels.

Use background color from Options/Appearance

Check this option to use the default background color for the tiled view. Otherwise, you can pick any color from the **[Background color]** palette.

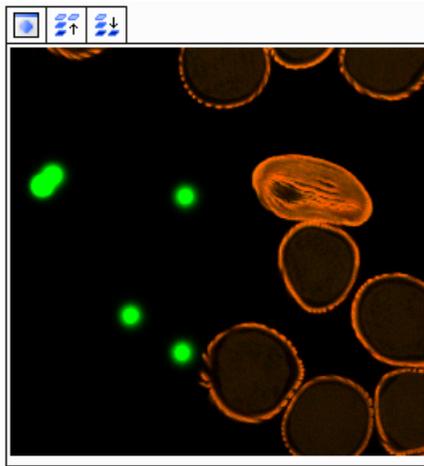
Header/Footer

A header/footer can be displayed within the tiled view. Select the check-box to enable this feature. When you place the cursor over the header/footer area, a button appears, press it to switch to edit-mode.

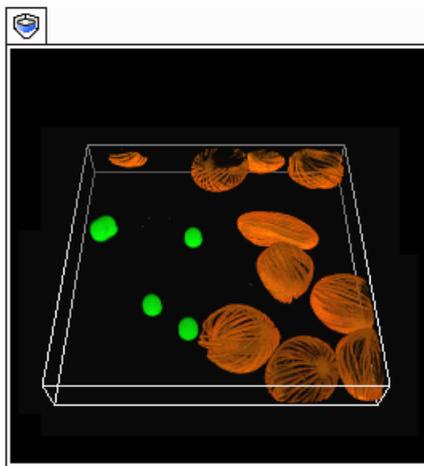
Background Color

Select the header and footer background color.

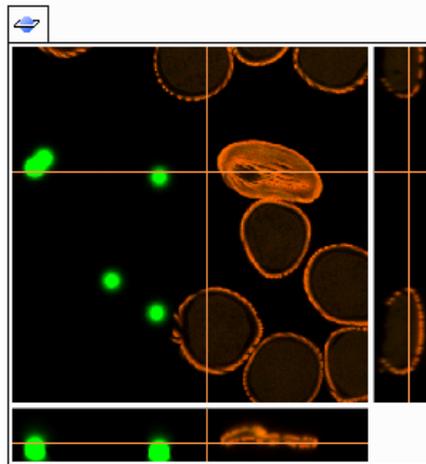
6.1.3.5. View - Examples



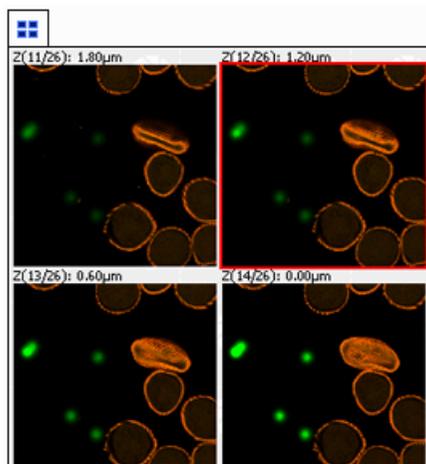
Main View, Max/Min Intensity Projection View



Volume View



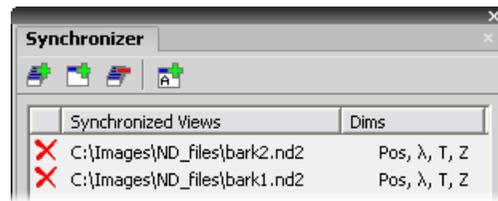
Slices View



Tiled View

6.1.3.6. Synchronizer

Synchronizer enables to compare (run and view) two or more ND2 documents at the same time. It automatically synchronizes view of all documents added to the list. Run **[View > Visualization Controls > Synchronizer]**:



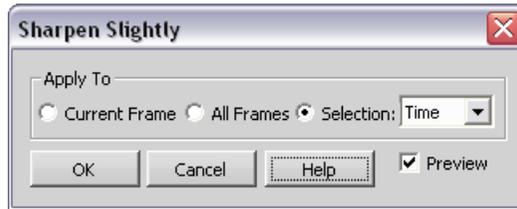
- Open at least two ND documents you would like to observe.
- Use the **[Add All]**  button to add them to the synchronizer table. In case there are more documents opened, use the **[Add Current View]**  button instead. The ND2 file being added must be active.
- The names of selected documents appear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

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 synchronizer, click the reset button  next to its name.

6.1.3.7. ND2 Documents Processing

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



Single Image

The operation will be performed on the current frame of the ND2 document.

All Images

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

Selected Dimension

The operation will be performed on all frames of the selected dimension.

Note

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 loop.

If you selected some of the frames/loops of the ND document by mouse, the **[Selection]** option appears in the pull-down menu. Opt for it to process only these selected (green highlighted) frames/loops.

6.2. Working with Documents

It is possible to work with multiple image documents opened at the same time.

6.2.1. Opening Saved Files

NIS-Elements AR offers several ways to open an image file, using either:

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 in the main tool bar

Organizer

An image can be opened by double clicking its filename within **[Organizer]**. Press **[F10]** to display the Organizer mode. Please see the [Organizer] chapter for more details.

Recent Files List

You can quickly access the last opened images using the **[File > Recent files]** menu.

Open next/previous/first/last Commands

These commands enable you to continuously open the next or previous images from a particular directory or a database table. The commands are located in the **[File > Open/Save Next]** menu.

Auto Capture Folder

The **[Auto Capture Folder]** is a control window that can be displayed by calling the **[View > Acquisition Controls > Auto Capture Folder]**. It displays images of a selected folder. To change the folder click the  button in the top left corner of the control window and browse for another folder. Any image of this folder can be opened by double-clicking its thumbnail.

Any File Manager

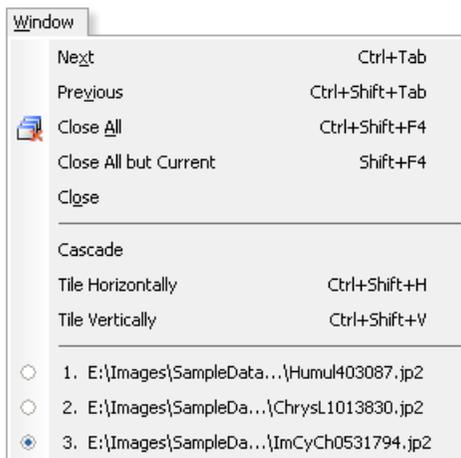
During installation, NIS-Elements AR creates file associations to files that are considered its native format for storing images (JPEG2000 , ND2). The JPEG2000 (JP2) and ND2 image files can be then opened in NIS-Elements AR just by double clicking their names within any file manager or the desktop.

Opening Files in Progressive Mode

If the image to be opened (a single frame in case of ND2 documents) is too big - so that it can not be loaded to RAM in one piece - you will be asked whether to open it in a **[Progressive Mode]**. This means that only a thumbnail of the image will be loaded to RAM. When zooming-in to view image details, the actual image data of the portion needed will be loaded to RAM progressively (piece by piece). However, many image processing functions and commands are disabled in this mode.

6.2.2. Switching Between Loaded Documents

Commands for managing the opened images are grouped in the **[Window]** menu. The recently opened files are listed in its bottom part. The currently displayed image is indicated by the selected radio button. 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).



Document windows can be also arranged automatically using the **[Tile horizontally]** or **[Tile vertically]** commands. This will change the size and position of the opened documents and they will be arranged next to each other in the selected direction.

6.2.3. Closing Documents

- The currently displayed image can be quickly closed by pressing the cross button 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 AR will display a confirmation dialog box, offering to save the changes.
- Use the **[Window > Close All but Current]** command to close all opened documents but keep the current one opened.

6.2.4. Copying Channels by Drag and Drop

1)



Drag one of the channel tabs and drop it:

- to another image, so it will become a multichannel image;
- inside the NIS-Elements AR application window in order to create a new document

2) Even the **[All]** and **[RGB]** tab can be copied.

Note

When extracting a channel from Live image, it does not freeze the camera signal.

ND2 Documents

- 1) 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.
- 2) 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.
- 3) **[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.
- 4) Drag one of the channel tabs of a **[single image]** and drop it **[to an ND2 document]**. The channel will be copied to every loop of the ND2 file.

Note

This behavior 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)

6.2.5. Image Formats

NIS-Elements AR supports a number of standard file formats. In addition, NIS-Elements AR 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. It also maintains all image layers of course.

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 AR 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 AR 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 software. Nowadays, all its features (and more) are provided by the JPEG 2000 format.

ICS/IDS image sequence

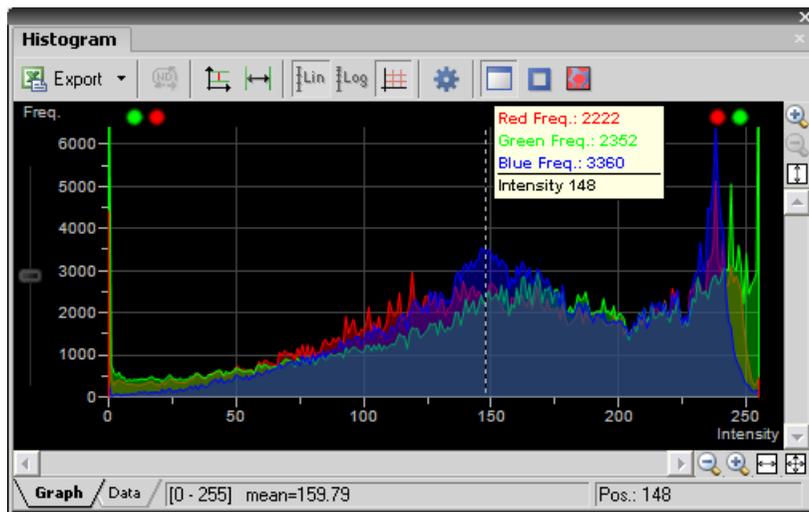
ICS/IDS image sequences are generated by some microscopes and consist of two files: the ICS file with information about the sequence; the IDS file containing the image data. The ICS file must be stored in the same directory together with the IDS file.

7. Image Analysis

7.1. Histogram and Look Up Tables

7.1.1. Histogram

A histogram displays frequencies of pixels of a certain intensity value. The intensity values range from 0 to 255 (for 8bit images). A separate curve is created for each color channel. Run the **[View > Visualization Controls > Histogram]** command to display the histogram control window:



Source Data

Source data of the histogram can be viewed if you swap to the **[Data]** tab in the bottom-left corner of the window. The area from where the histogram is calculated can be restricted. Select one of the following histogram modes:



The data are obtained from the whole image.



The data are obtained from within the probe.



The data are obtained from within the current ROI.



The data are obtained from the image parts under the binary layer.

Export



The source data or the histogram image can be exported to an external file. Display the **[Export]** pull-down menu and select a destination. Click the button to perform the export. 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 applied.

Note

Please, see the *[Exporting Results]* chapter for further details.

Appearance

The histogram can be zoomed in and out using the zoom buttons on sides of the window. There are also other options to adjust the graph appearance:

- **[Left slider]** - there is a slider along the Frequency axis. Drag it in order to stretch the view of either lower or upper values of the axis.
- **[Drawing style]** - There are two ways the histogram can be drawn:
 - **[Raw Data]** - tries to draw the source data precisely to the graph
 - **[Trend Style]** - interpolates the data so that the histogram lines appear smoother.
-  **[Auto Scale Vertical]** zooms the graph of each channel separately to fit the available area. 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.
-  **[Graph Linear]** displays the linear scale on the Y axis.
-  **[Graph Logarithmic]** displays logarithmic scale on the Y axis.
-  **[Show Grid]** turns ON/OFF the grid in the background.

The graph appearance can be modified. Press the **[Options]**  button - a window appears where the following settings can be adjusted:

Colors

Graph background and axes colors can be selected.

Pen Width

Set the width of the histogram(s) line 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 color in %.

Vertical/Horizontal AutoScale, Show Grid

These options equals the corresponding buttons of the histogram tool bar.

Interpolation method

Select the way of drawing the graph line. The **[Linear]** (smooth) and **[Quick]** (precise) options are available.

AntiAlias

Smooths 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.

Overrun Indication

If certain amount of pixels with maximum/minimum intensity values (black/white pixels) are spotted in the image, color dots appears above the graph. The color indicates which channel is affected. When you place the mouse cursor over the dots, a tool tip message appears with details about the percentage of under/over-exposed pixels. The percentage limit setting is loaded from the LUTs settings (the color dots do not appear unless the set percentage of black/white pixels is exceeded).

Note

If the overrun concerns more than three channels (considering multi-channel images), only one white dot is displayed instead of many color dots.

7.1.2. LUTs

A look-up table is a useful tool for image color and brightness modifications. You can use LUTs to enhance images for observation purposes so the color modifications will be non-destructive to the image data. LUTs settings are saved along with the image document. If required, the LUTs settings can be applied to the image data by pressing the  button. The following actions are available within the LUTs window:

- Adjusting the **[Gamma parameter]**. The gamma curve is drawn in gray over each graph. You can drag the middle point and move it up or down, or enter an exact value to the **[G:]** field above.

- Adjusting the input intensity range. The input intensity range can be restricted by moving the black and white triangular sliders to the center. All pixels with values outside of the modified input range will be set to maximum/minimum values and the remaining pixels will be approximated in order to fill the output (full) range.

Note

This is the way how images can be equalized. For example, if the image is very dark and most of the histogram is located to the left while the right part of it is flat, you may move the white slider(s) to the position where the histogram line starts to rise. This will incredibly brighten the image and reveal details which were previously hidden in a dark.

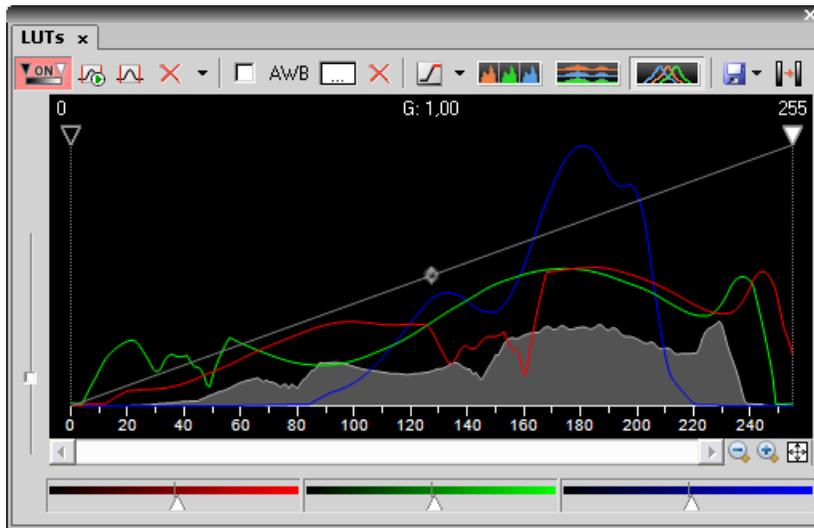
- Optimal (automatic) zoom of each graph can be set by right-clicking on one of the sliders.
- You can adjust the histograms height non-proportionally by moving the slider on the left of the window up and down.

Tips

- Right-click the graph area and (de)select **[Draw trend style]**. When ON, the LUTs curves will be smoothed to display the data trends rather than represent the actual image data values.
- The position of the black, white and Gamma sliders can be reset by a double click.

Different controls will be available when applying LUTs to monochromatic, RGB, multi-channel or spectral image. When LUTs are active, the LUTs button in the top-left corner of the document window is highlighted red.

7.1.2.1. LUTs on RGB Images



There are 3 separate windows for each of the RGB channels. All channels are controlled at the same time by default. To control each channel separately, press and hold the **[Shift]** key while you drag the sliders or adjust Gamma.

LUTs Tools



Enable/Disable LUTs

This button applies LUTs to the current image.



Keep Auto Scale

Press this button to run the auto scale procedure permanently (on the live image). When you turn this button OFF, the settings remain as if the **[Auto Scale]** button was pressed only once.



Auto Scale

This button adjusts the white slider position of all channels automatically with the purpose to enhance the image reasonably. If you have selected the **[Use Black Level]** option from the **[Settings]** pull-down menu, the black slider will be affected too.



Reset

Discard all LUTs settings and turn LUTs OFF by pressing this button.

Settings

Display the pull down menu and select one of the following commands:

- **[Use Black Level]** - Check this item to ensure that the black slider will be affected by the auto scale functions .
- **[Settings]** - opens the **[Auto Scale Settings]** window. See Auto Scale Settings.

Keep Auto White Balance Check Box

Check this option to switch the Auto White Balance functionality permanently (on the live image).

AWB

Press this button to perform the Auto White Balance operation once.

Auto White Balance Color (...)

This button Opens the **[AWB Color]** window and enables you to select a color shade which the system shall eliminate (make white). See AWB.



Reset Components

Discard all AWB settings with this button.



Color Oversaturation

Switch this button ON and the system highlights all pixels with values reaching maximal values. Select colors to highlight the pixels with in the pull-down menu.



These buttons arrange histograms of single channels next to each other horizontally or vertically.



(-LO- option)

This button arranges histograms of single channels overlapped. Use sliders at the bottom of the graph window to amplify or reduce the green/red/blue component display in the view.



Save/Load LUTs

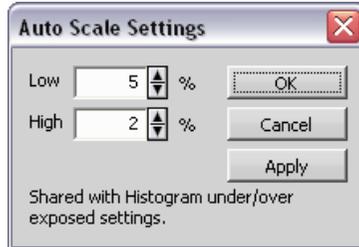
This pull-down menu enables user to handle LUTs settings in various ways. They can be saved to a **[*.lut]** file and loaded later. Or, LUTs settings saved along with an image document can be loaded directly from this document by the **[Reuse LUTs from File]** command. Or you can copy/paste the settings within the documents opened in NIS-Elements AR (the copy/paste .

Modify Image Through LUTs

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.

Auto Scale Settings

Press the arrow next to the **[Reset LUTs]** button and a pull-down menu appears. Invoke the **[Settings]** command.



The **[Low]** and **[High]** fields determine how many of all pixels of the picture are left outside the sliders when **[Auto Scale]** is applied (0-10%).

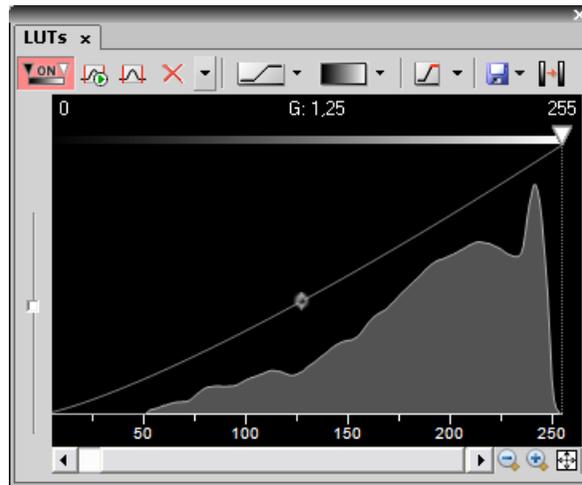
AWB

White color in some images may suffer from having a color tint. The AWB (Auto White Balance) mode adjusts the image to get pure white instead of this tint. Similarly to LUTs auto scale, the AWB function can be used once, or permanently on the live image. If you know the tint your "white" color has, you can select this color by the color picker that appears after pressing the **[...]** button:



Choose the color by mouse. You can also enter the RGB values to the fields below. A preview of the chosen color appears in the rightmost rectangle.

7.1.2.2. LUTs on Monochromatic Images



All features mentioned above are also valid for monochromatic images, except that the **[AWB]** function is not available. There are some other features added when in “mono” mode.

Mapping Mode

The Mapping mode pull-down menu enables you to select how the intensities overreaching the white slider setting will be displayed. Choose the mapping mode:

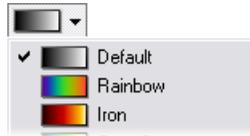


In the **[Contrast mode]**, the pixels with values higher than the white slider level will be displayed white, unlike in the **[Window mode]** where they will be displayed black.

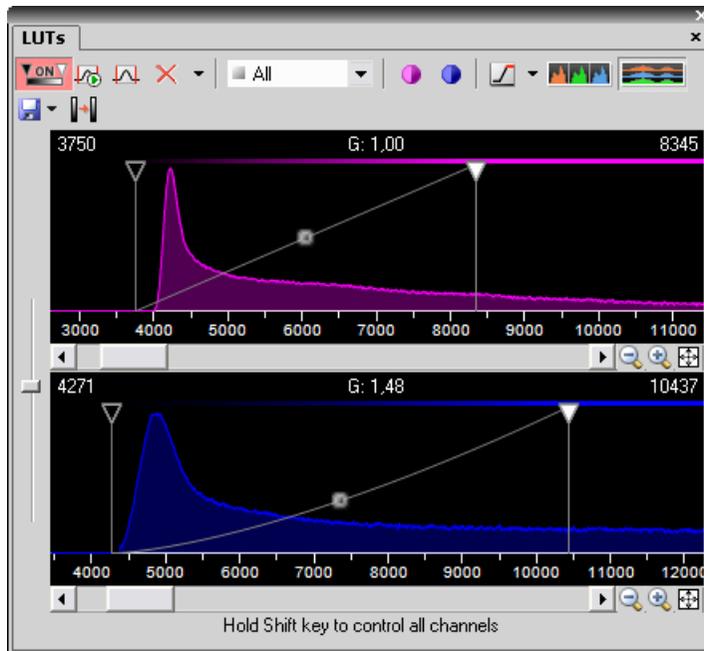
Gradient

Mapping monochromatic images to pseudo-color gradients is often used to enhance details in the image which would otherwise not be obvious. This button indicates the currently selected gradient. When

pressed, a pull-down menu appears where you can select the color scheme you would like to use. Try a few of the schemes to see which one suits best.



7.1.2.3. LUTs on Multichannel Images



LUTs window on two-channel image

Most of features mentioned above are also valid for multi-channel images. There are some other features added when in “multi-channel” mode. Each channel is controlled separately by default. To control all channels at the same time, press and hold the **[Shift]** key while you drag the sliders or adjust Gamma.

When the image contains up to three channels, all channels can be visible at one time and you can handle them similarly to the RGB mode. When there are more than three channels, only one channel is displayed at one time. A pull-down menu in the tool bar enables you to select the channel to be displayed.

 Next to the channel selection pull-down menu, there is this **[Auto Scale]** button. By pressing it, the system will automatically adjust only the current channel settings.

7.1.2.4. LUTs on Spectral Images

When observing a spectral image, the graph inside the LUTs window does not show the image histogram(s), but reveals another information about the image. Intensity values are on the Y axis and spectra is on the X. There are two curves in the graph. The top (white) curve displays the maximal intensity and the color curve displays the mean intensity of each channel.

Note

Spectral image consists of many channels, each representing a single wavelength.

The LUTs behavior depends on the selected displaying mode. There are four displaying modes. You can select the mode either by a pull-down menu in the LUTs tool bar, or within the document top tool bar.

- **[True Color]**
- **[Custom Color]**
- **[Grouped Color]**
- **[Gray Scale]**

In the bottom part of the LUTs window, **[Gain]** and **[Black Level]** settings can be adjusted either for all channels together or for each group separately - depending on the displaying mode. Gain brightens the image and the Black Level works just like the left (black) on RGB/mono images.

Note

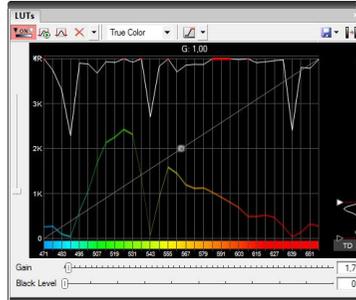
Each slider (gain or black level) offers a range of values to be set by moving it, but any higher value can be entered in the field next of the sliders.

TD channel

A spectral image can contain additional TD (transmitted detector) channel. Its basically a standard monochromatic image. LUTs settings of this channel appear on the right edge of the LUTs window.

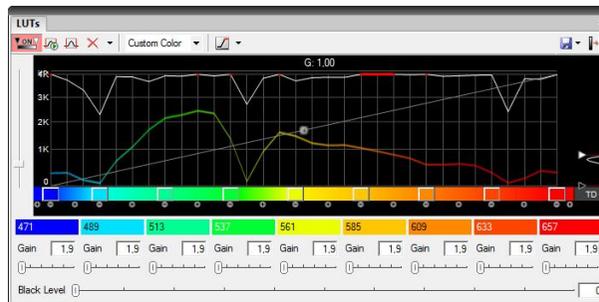
True Color Displaying Mode

You can switch to this displaying mode by clicking the  button located in the document tool bar.



Custom Color Displaying Mode

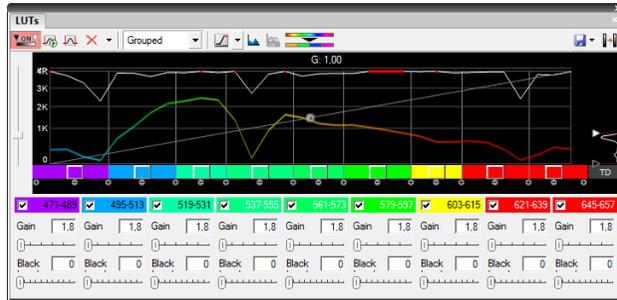
You can switch to this displaying mode by clicking the  button located in the document tool bar. You can assign custom colors to sections of the spectrum. Sections (colors) can be added/removed by clicking the **[+]** and **[-]** buttons placed below the spectral graph. To change the section color, click the button located in the color stripe. A window appears where you can select the color from a palette or define it by **[Hue]** or **[Wavelength]** settings.



Grouped Displaying Mode

You can switch to this displaying mode by clicking the  button located in the document tool bar. This view enables you to reduce the number of channels multiple-times. You can adjust the number of groups by

the **[+]** and **[-]** buttons and also change each channel width by moving the white horizontal dividers. Gain and Black Level for each group can be adjusted.



Gray Scale Displaying Mode

You can switch to this displaying mode by clicking the  button located in the document tool bar. The image is displayed in gray scale, but various color gradients can be applied to it.

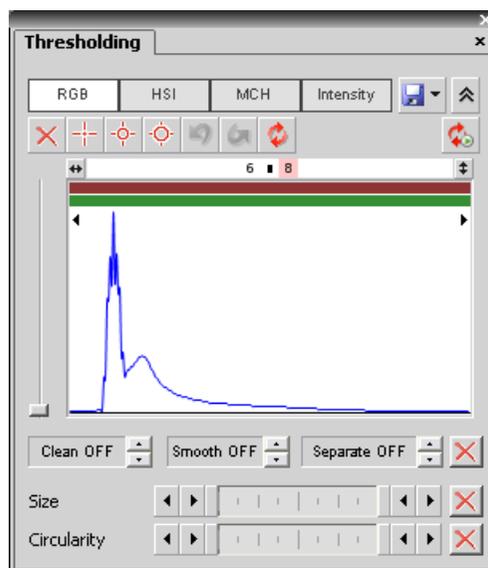


7.2. Thresholding

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 objects to be analyzed from background. To display the thresholding control window, run the **[View > Analysis Controls > Thresholding]** command. Thresholding can be performed in the following modes:

7.2.1. 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.
-  This button erases the threshold settings (no binary objects are created).
-  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.
-  Undo - reverses the previous threshold operation.
-  Redo - returns the threshold as it had been before the **[Undo]** button was used.
-  Rethreshold image - use this button to update the binary layer according to the current settings. The purpose of this button is to obtain the original binary layer in case it has been modified e.g. by a command from the binary layer.
-  Keep Updating Binary - press this button to keep the binary layer up-to-date all the time. You may find this function useful especially when thresholding is applied to the live image.
-  Threshold ND image - when an ND2 document is opened, this button appears. Define the threshold limits on the current frame and use the button to apply it on the whole ND2 document.

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 thresholding range and drag it left or right.

The two last behaviors 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 is updated only once after the mouse button is released. Such operation saves some computing and therefore 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

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]** - smooths the binary image contours.
- **[Separate]** - separates objects.

See [Mathematical Morphology Basics].

7.2.2. HSI Mode

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 HSI (Hue, Saturation, Intensity) color space and that the **[Saturation]** and **[Intensity]** channels can be switched OFF. Therefore you can threshold over the **[H]**, **[HS]**, or **[HI]** channels only. Switch the channel off by de-selecting the check box in the top-left corner of the channel histogram.

7.2.3. Intensity Mode

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

7.2.4. 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.

- If there are many channels, only one channel histogram is displayed. Switch between the channels by selecting their names from the bottom pull-down menu.
- The binary layer(s) can be displayed in two modes:
 1.  Each channel can create its own binary layer
 2.  One layer can be created as an intersection of all channels binaries.

7.3. Binary Layer

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 **[Tab]**. New buttons appear on the tool bars:

7.3.1. 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 horizontal tool bar are simplified versions of mathematical morphology functions:

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

Please see the [Mathematical Morphology Basics] chapter.

7.3.2. Drawing tools (vertical tool bar)

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 )
- Drawing of any object which 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 automatic drawing tools (threshold, auto detect) have a changeable parameter. It can be modified by **[+]** and **[-]** keys or by mouse wheel.
- The scene can be magnified by the UP/DOWN arrows when mouse wheel serves another purposes.
- You can drag a magnified image by right mouse button.
- A line width can be set in the top tool bar.
- Hints are displayed in the second top tool bar.

Drawing Tools

-  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 movable circle/ellipse. If you grab the ellipse by the center, you can move it. If you grab it by 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. **[F4]**
-  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.
- **A** Text tool. Displays dialog box for defining text parameters.
- **[Commands]** tool. The last button in the left tool bar displays the currently selected tool. You can invoke a pop up menu and select one of the additional commands:

Commands

-  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.
-  **[Smooth]** - see the [Mathematical Morphology Basics] chapter.
-  **[Contour]** - see the [Mathematical Morphology Basics] chapter.
-  Inverts the binary image. Foreground becomes background and vice versa.

7.3.3. Binary Layer Color and Transparency

When in overlay mode:

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

7.3.4. Erasing Single objects

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.

7.3.5. Multiple Binary Layers

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.

7.4. Measurement

7.4.1. Calibration

Calibration of the image is of crucial importance to measurement. A distance will be assigned to one pixel so that correct and realistic measurements can be performed and objects of different images could be compared to each other. A correct calibration has to be made before you begin to measure.

7.4.2. Rough Measurement

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.

Select Graticule Type

The type of the graticule ruler is indicated by a picture on the **[Graticules]** button placed on the right document tool bar. 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

Define Graticule Properties

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

Graticules Density

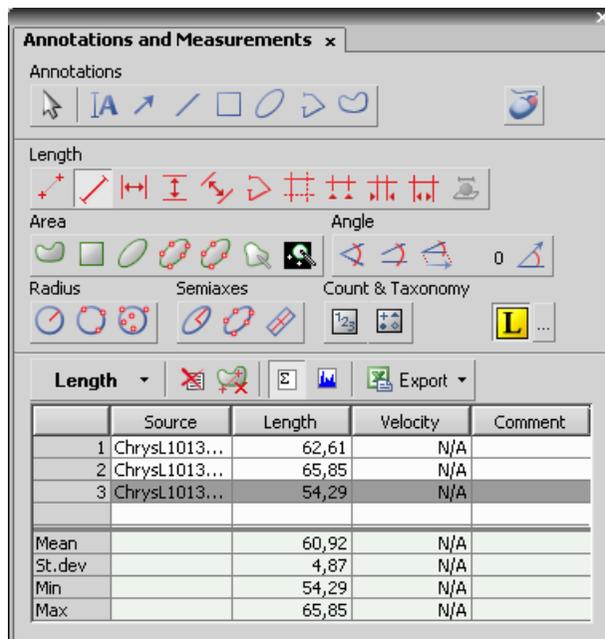
The density value - the closest distance between two line intersections of the graticule - can be set, or you can let NIS-Elements AR adjust it automatically according to the current zoom factor. The units selection depends on the image calibration (calibrated/uncalibrated).

Measure the Image

The graticule measurement provides 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 **[Copy Graticules to Binary]** command.
- A **[Graticule Mask]** can be created from the current binary layer using the **[Copy/Add Binary to Graticule Mask]** 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.

7.4.3. Manual Measurement

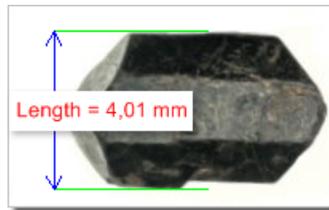


Length, area, angles, taxonomy, counts, circle radius, and ellipse semiaxes can be measured manually over an image. The results are being recorded to a simple statistics table, which can be exported to a file or clipboard. Also, the data can be presented as a graph.

- 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.

Example Procedure

Measurement of the image of a grain:



- 1) Select the **[Horizontal parallel lines]**  tool.
- 2) 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.
- 3) Repeat this to place the second line on the bottom edge of the crystal.
- 4) When finished, an arrow is drawn between the lines, and the result of the measurement is attached. A record with the measurement type and the measured value is added to the results table.

Please find details about the measurement tools in the electronic documentation of NIS-Elements AR.

7.4.4. Automated Measurement

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

- Optical system calibration (see the [Optical Configurations] chapter)
- Image acquisition. (see the [5 Capturing] chapter)
- Defining threshold, creating the binary layer (see the [Thresholding] chapter)
- Performing the automated measurement

- Results presentation

Image Layers Involved in Measurement

The the binary layer and also the color image can get involved in the automated measurement:

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

Object/Field Measurement

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

Object Measurement

Groups of neighbouring pixels of the binary layer are called objects. Results of the object measurement are usually the 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 area (measurement frame, ROI). Such information can be 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

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

Region of Interest - ROI

ROI is a user defined area of the image. Whenever the ROI is displayed (by the **[Measure > Use ROI]** command), it is also applied to restrict the area of measurement.

See **[Measure > Simple ROI Editor] [Measure > Raster ROI Editor]**

Measurement Frame

The measurement frame is a re-sizable rectangular frame which serves for bounding the area accepted for measurement. Turn the measurement frame ON via the **[Measure > Use Measurement Frame]** command.

Binary objects touching edges of ROI or the measurement frame can be treated differently (excluded from the measurement or included). This behavior can be specified within the **[Edit > Options]** window.

Statistics and Data Presentation

NIS-Elements AR 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 and ROI Features]** windows. Results of all automated measurements are presented and can be exported from the **[View > Analysis Controls > Automated Measurement Results]** control window.

7.5. Regions of Interest - ROIs

In laboratory imaging experiments, users are often interested in just a part of the image. This is when ROIs come to help in NIS-Elements AR. If a ROI is turned ON, only the ROI area of the image is evaluated by the automatic measurement commands while the rest of the image is left ignored. We distinguish the following types of ROI:

Types of ROI

Camera ROI

A rectangular ROI which restricts the live camera image. See [Camera ROI on Live Image].

Vector ROI

A ROI created by the simple ROI editor. It can be freely resized and rotated. See [Measure > Simple ROI Editor].

Raster ROI

A ROI created by the raster ROI editor (similar to the binary layer editor - see [Binary Layer]). The raster ROIs behave the same as the vector ROIs, but can not be resized or rotated. See [Measure > Raster ROI Editor].

Usage of ROIs

The ROIs can be also distinguished according to their purpose. Right-click the ROI and select its purpose from the context menu:

Standard ROI

A standard ROI restricting the area of measurement used by the automatic measurement functions.

Background ROI

The ROI specifying the background characteristics. This ROI is used as the data source by the [Image > Background > Subtract Background Using Background ROI] command.

Reference ROI

A special ROI used by the [View > Analysis Controls > Time measurement] control window to reduce the influence of photo-bleaching on the resulting data.

Stimulation ROI

Confocal microscopes use this type of ROI to aim stimulation lasers.

ROIs in time

When performing time-lapse experiments, the observed objects may be moving. If a ROI has been created over such object, you can make it move along with the object. Right-click the ROI and select one of these options:

ROI is global

Position of a global ROI throughout different frames of the ND2 file is static

ROI changes over time

Position of a moving ROI can differ frame by frame. It is definable on an ND2 document. See [ROI in Time - Moving ROIs].

7.5.1. ROI in Time - Moving ROIs

To define a moving ROI:

- Open or capture a time-lapse ND document.
- Display the first frame of the document and open the vector ROI editor by the **[Measure > Simple ROI Editor]** command.
- With one of the editor tools, draw the ROI around an object. Close the ROI editor.
- Right-click the ROI and select **[ROI Changes Over Time]** from the context menu.
- Play the image sequence and if the object of interest starts to move out of the ROI, pause the replay.
- Now it is possible to drag the ROI to the new position. If the ROI is created by the vector editor, its size and rotation can be also modified.
- Play the sequence further and drag the ROI to a new position each time the object in the image moves.

Note

ROI positions between two user-defined frames are interpolated. If the object of interest was moving at constant speed with a linear trajectory, then it would be sufficient to define the ROI positions only in the first and the last frame of the image sequence.

Tips on Moving ROI

ROI Tracking

Motion characteristics of a manually defined moving ROI can be measured using the **[View > Analysis Controls > Tracking (-TRC- option)]** control window. Just click the **[Add Moving ROIs]**  button to add the current ROIs to the table of objects.

Automatic ROI Definition

Automatic **[Tracking]** features may be utilized to define moving ROIs. Already tracked objects may be converted to moving ROIs by clicking the **[Binary to ROI]**  button. See **[View > Analysis Controls > Tracking (-TRC- option)]**.

ROI Multiple Selection

Use the Ctrl key to select/unselect more than one ROI. When you have selected more ROIs, press and hold the **[Alt]** key while dragging a single ROI. Other selected ROIs will not be moved but remain selected.

Editing ROI in time

You can edit ROIs in time using the **[Edit ROIs in time]** command, which is accessible from the contextual menu of the  **[Turn ROI on/off]** button (in the right document toolbar).



Use provided tools to edit or create a new ROI:

Move

This tool enables to move the ROI to a different place in the image. As you place the cursor over the ROI, color of the selected ROI is indicated near the mouse cursor (this proves very useful when you have defined many ROIs, or they are overlapping). Simply drag and drop the ROI to another position.

Pick

Use this tool to select directly in the image which ROI you want to edit.

Draw

Use this tool to draw a freehand curve which defines new region of interest. Press the right mouse button to close the curve and finish the definition.

Detect

Enables to redetect ROI or to detect a new ROI (when the **[New]** button is pressed, or New item is selected from the pull down menu).

New

Press this button to create a new ROI. According to which detection tool is selected, you can either start drawing a new ROI, or click inside the image to let the application detect the ROI automatically.

ROI picker

Select which ROI you want to edit from the pull down menu.

After Edit

Specify which action is taken after a ROI has been edited. The **[Jump Forward]** option displays another time frame according to set number of frames in the Skip pull down menu.

Skip

Choose how many frames are skipped after an object is edited (and the **[Jump Forward]** option is selected). Skip 1 frame just moves to the nearest next frame.

Delete

Press this button to delete selected ROI.

Undo/Redo

Moves back and forth through the history of changes.



Help

Displays help file.



Finish

Press this button to finish definition and close the dialog.

7.6. Measurement Features

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

AcqTime

Calling the Grab Sequence command, the AcqTime is set to zero at the beginning. Then, NIS-Elements AR 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 AR .

Measurement Type: object, field

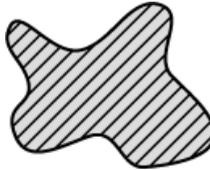
Image Type: binary

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.

Measurement Type: object, field, interactive

Image Type: binary



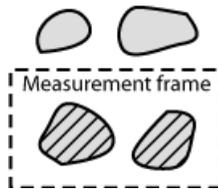
AreaFraction

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.

$\text{AreaFraction} = \text{Area} / \text{MeasuredArea}$

Measurement Type: field

Image Type: binary



Blue

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

Measurement Type: interactive

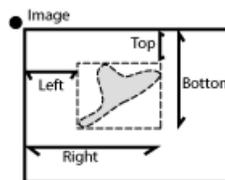
Image Type: color

BoundsLeft, BoundsRight, BoundsTop, BoundsBottom

These 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.

Measurement Type: object

Image Type: binary

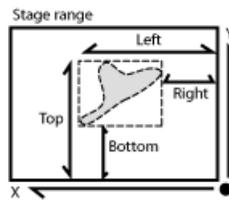


BoundsAbsLeft, BoundsAbsRight, BoundsAbsTop, BoundsAbsBottom

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.

Measurement Type: object

Image Type: 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.

Measurement Type: object

Image Type: binary

BrightVariation

It is the usual standard deviation of brightness values.

Measurement Type: object, field

Image Type: 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.

Measurement Type: object

Image Type: 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.

Measurement Type: object

Image Type: 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.

Measurement Type: object

Image Type: 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.

Measurement Type: object

Image Type: binary

CentreXpx

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

Measurement Type: object

Image Type: binary

CentreYpx

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

Measurement Type: object

Image Type: 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.

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

Measurement Type: object

Image Type: binary

Class

NIS-Elements AR 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 AR 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.

Measurement Type: object, field, interactive

Image Type: binary

DensityVariation

DensityVariation is derived from density values. It is a usual standard deviation of density values.

Measurement Type: object, field

Image Type: color/binary

Elongation

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

$$\text{Elongation} = \text{MaxFerret} / \text{MinFerret}$$

Measurement Type: object

Image Type: binary

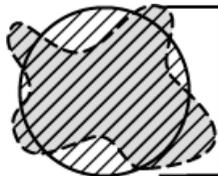
EqDiameter

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:

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

Measurement Type: object, interactive

Image Type: 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.

Measurement Type: object, field, interactive

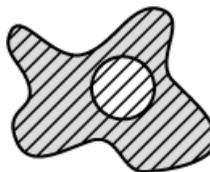
Image Type: color

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.

Measurement Type: object

Image Type: binary



FillRatio

FillRatio is the ratio of Area and FillArea:

$$FillRatio = Area / FillArea$$

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.

Measurement Type: object

Image Type: 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.

Measurement Type: object, field

Image Type: color

Green

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

Measurement Type: interactive

Image Type: 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.

Measurement Type: object, field

Image Type: 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.

Measurement Type: object, field

Image Type: color/binary

SumBrightness

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

Measurement Type: object, interactive

Image Type: 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:

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

Measurement Type: object, field, interactive

Image Type: color/binary

SumIntensity

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

Measurement Type: object

Image Type: color/gray

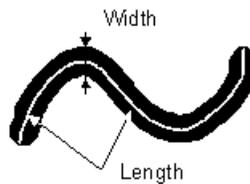
Length

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.

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

Measurement Type: object, interactive

Image Type: binary

**LineLength**

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

Measurement Type: object

Image Type: color/gray

Luminance

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

Measurement Type: object, field, interactive

Image Type: color

MaxFeret

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

Measurement Type: object, interactive

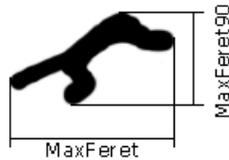
Image Type: binary

MaxFeret90

The MaxFeret90 is a length projected across the MaxFeret diameter.

Measurement Type: object, interactive

Image Type: binary

**MaxIntensity**

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

Measurement Type: interactive

Image Type: 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.

Measurement Type: object, field, interactive

Image Type: color/binary

MeanBrightness

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

Measurement Type: object, field, interactive

Image Type: color/binary

MeanDensity

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

Measurement Type: object, field, interactive

Image Type: color/binary

MeanIntensity

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

Measurement Type: object, field, interactive

Image Type: 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.

Measurement Type: object, field, interactive

Image Type: 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.

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

Measurement Type: object, field

Image Type: binary

MeanRed

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

Measurement Type: object, field, interactive

Image Type: color/binary

MeanSaturation

MeanSaturation is a statistical mean of saturation values of pixels.

Measurement Type: object, field

Image Type: 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.

Measurement Type: object, field

Image Type: 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 $\alpha \pm$ equals the projected length of object at angle $\alpha \pm$, $\alpha \pm (0,180)$; NIS-Elements AR calculates Feret's diameter for $\alpha \pm = 0,10,20, 30, \dots, 180$.

Measurement Type: object, interactive

Image Type: binary

MinIntensity

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

Measurement Type: interactive

Image Type: color/gray

NumberObjects

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

Measurement Type: field

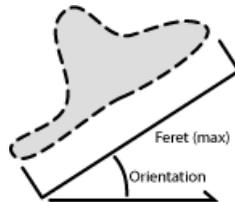
Image Type: binary

Orientation

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

Measurement Type: object

Image Type: 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

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

Measurement Type: object, field, interactive

Image Type: binary



Red

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

Measurement Type: interactive

Image Type: color

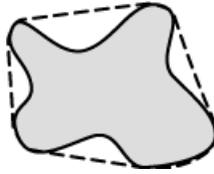
ShapeFactor

This parameter is to define whether the object is rough or not.

$$\text{Shape factor} = 4 \pi (\text{area}) / (\text{Convex hull perimeter})^2$$

Measurement Type: object

Image Type: binary

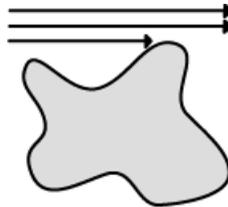


StartX

StartX is the x co-ordinate of a pixel on the object boundary. When you scan the image from the origin as indicated (left->right), the first pixel of the object you hit is the one with StartX and StartY coordinates.

Measurement Type: object

Image Type: binary



StartY

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

Measurement Type: object

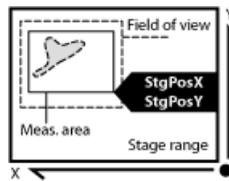
Image Type: binary

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.

Measurement Type: object, field

Image Type: 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.

Measurement Type: object, field

Image Type: 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.

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

Measurement Type: field

Image Type: binary

Time

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

Measurement Type: object, field

Image Type: color/binary

VolumeEqCylinder

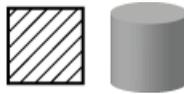
This parameter is based on the rod model. Length is interpreted as height and Width as the base diameter of a cylinder. Bases are considered to be spherical.

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

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

Measurement Type: object

Image Type: binary



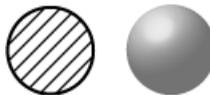
VolumeEqSphere

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.

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

Measurement Type: object

Image Type: 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.

Measurement Type: object, field, interactive

Image Type: color

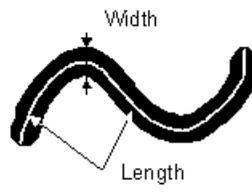
Width

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:

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

Measurement Type: object

Image Type: binary



7.7. Mathematical Morphology Basics

The binary image as a result of thresholding often needs to be modified before a measurement is performed. Edges of the objects can be smoothed, holes in the objects filled etc. by using the mathematical morphology commands.

Note

“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 twice the thickness of the border to be added, these objects become merge together. If a hole is smaller than twice the thickness of the border, 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. Very close objects may be connected together.

**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. After applying the **[Fill Holes]** transformation, objects become homogeneous.

**Contour**

This transformation converts objects into their contours.

**Smooth**

Smooth affects rough edges of the objects makes them smooth.

**Morpho Separate Objects**

This transformation detects standalone objects that are connected together and isolates them.

Homotopic Transformations

Homotopic transformations 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, even if they are very near each other, will become again two objects without holes, but likely with a different shape and size. Opening, Closing, Erosion and Dilation are not homotopic trans-

formations. Typical homotopic transformations in NIS-Elements AR are: **[Skeletonize]**, **[Homotopic Marking]** and **[Thickening]**.

7.7.1. Connectivity

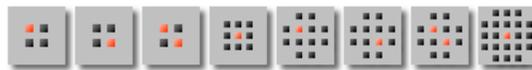
Applying the above mentioned transformations has some limitations due to digital images rasterization.

When speaking about binary image processing, a binary image is a set of pixels where values 1 represent objects and values 0 represent background. In the square grid of the image, two possible connectivities can be used for processing - the 8-connectivity or the 4-connectivity. Look at the picture below. If the 8-connectivity is used, the two pixels represent one object. If the 4-connectivity is applied, there are two objects in the picture. NIS-Elements AR works with the 8-connectivity model, so all pixels neighboring by the corner belong to one object.



7.7.2. Structuring Element = Kernel = Matrix

When applying Erosion, Dilation, Opening or Closing, one of the parameters which determines the transformation result is the selection of kernel (structuring element, matrix) type. There are the following kernels used in NIS-Elements AR:



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

7.7.2.1. Erosion

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

Move the midpoint of the kernel to every point of the image. Each time, look at the neighboring pixels of the kernel 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 kernel, set the midpoint to background(0).

7.7.2.2. Dilation

You can imagine the dilation as the following algorithm:

Move the midpoint of the kernel to every point of the image. Each time, look at the neighboring pixels of the kernel 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).

7.7.2.3. Open and Close

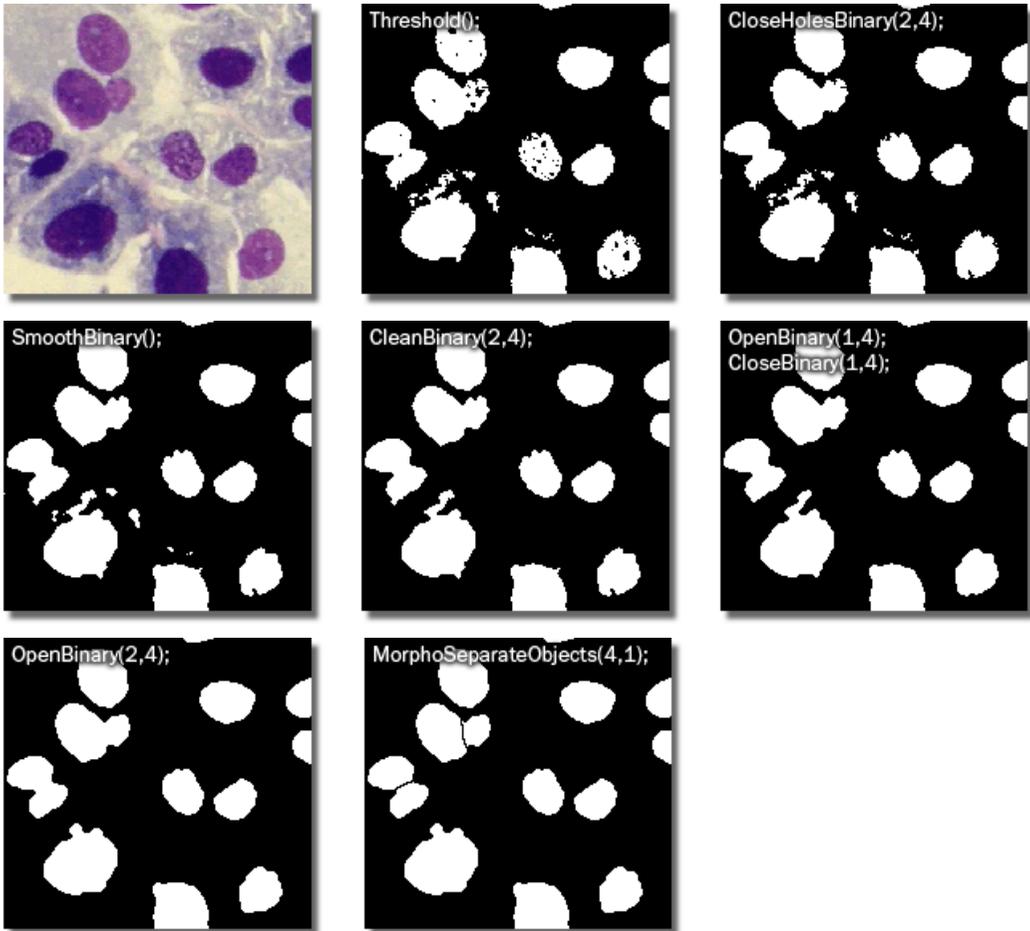
Open is performed by eroding the image and then applying a dilation to the eroded image. On the contrary, Closing is performed as a dilation followed by erosion.

7.7.2.4. Repetition issues

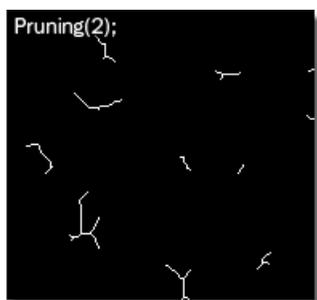
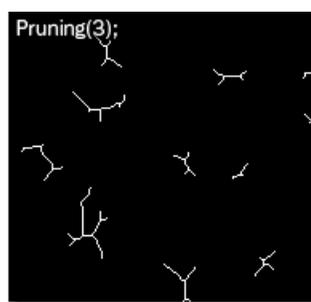
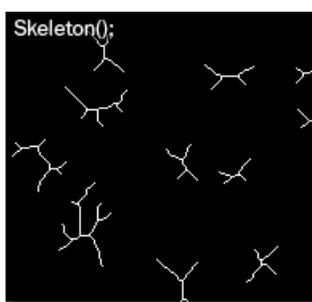
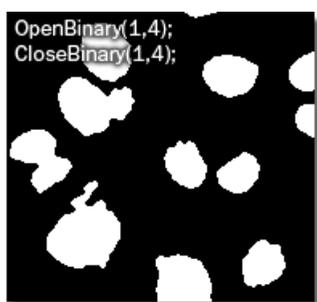
If the midpoint is not in the center, applying erosions or dilations by odd number of iterations causes image to shift by 1 pixel. Normally, the total image shift would be determined by the number of iterations (in pixels). NIS-Elements AR eliminates this shift: it changes the position of the midpoint 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.

7.7.2.5. Examples

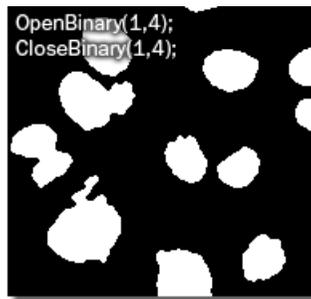
Please see the following examples of some of the binary functions applied to an image. In the following sequence of images, the functions were applied subsequently:



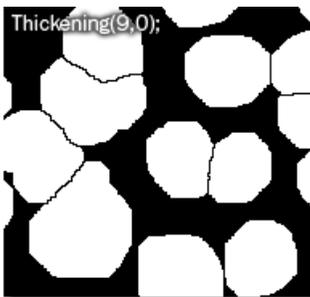
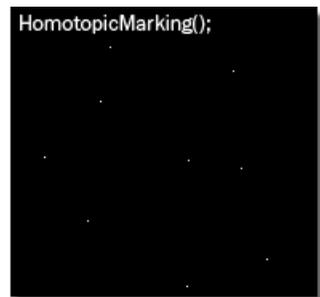
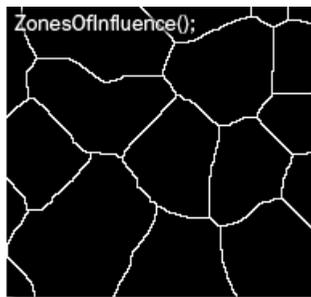
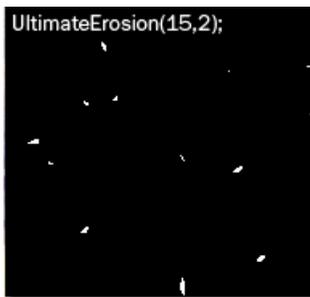
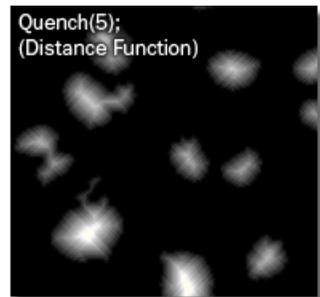
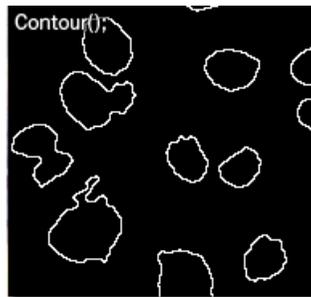
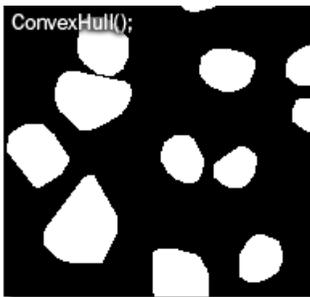
See another sequence of functions:



The following examples were created by calling each of the functions separately:

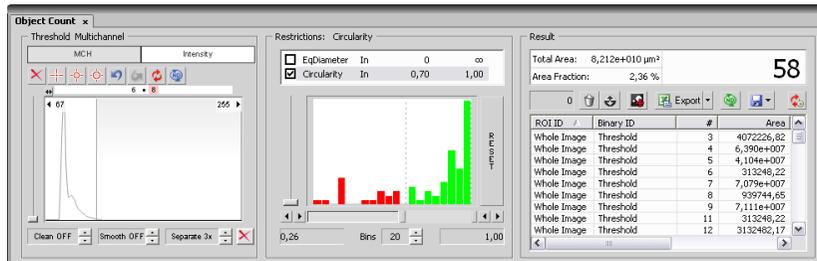


Original Image



7.8. Object Count

The **[Object Count]** tool enables users to threshold the image, automatically measure the binary objects, and export the measured data to a file in a straightforward way. Object count can be performed even on Live image. Run the **[View > Analysis Controls > Object Count]** command to display the following control window:



7.8.1. Thresholding the Image

The threshold limits shall be defined by picking object-typical colors from 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. The threshold limits are indicated in the histogram and can be further modified by dragging the limit lines by mouse.

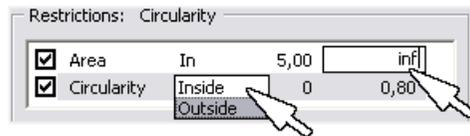
Please, refer to the [Thresholding] chapter for further details on thresholding.

7.8.2. Applying Restrictions

The number of objects that are included in the results table can be restricted by defining limits. Objects which do not fit these limits will be left out of the result table.

- Right click to the restrictions field to select one or more of the available measurement features.
- 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 by double clicking the indicated value.

- Decide whether the defined interval will be excluded or included - this is done by setting the **[Inside/Outside]** option next to the feature name.
- The nearby check box indicates whether the restriction is currently being applied or not. If applied, the histogram below is color, otherwise it is gray.



Note

Infinitude can be defined by entering "oo" or "inf".

Reset

The **[Reset]** button on the right side discards restriction settings of all features.

Bins

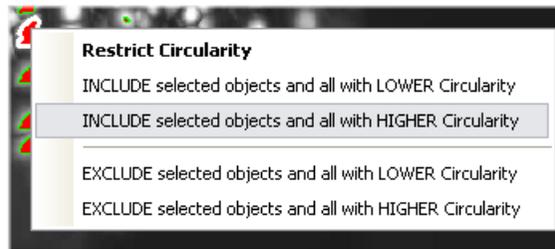
This setting determines the number of columns in the histogram.

Restrictions from the Image

A reference object can be picked from the image and used to set the limit values.

- Select the restriction feature you would like to set by mouse. Lets assume you have selected **[Circularity]**.
- Right click the thresholded object which is going to be used as source. A context menu appears.
- Select whether to **[INCLUDE]** or **[EXCLUDE]** the selected object and all others with higher/lower circularity.

- The restriction will be applied and indicated by colors within the image.



7.8.3. Results

The following results appear in the results table:

- **[Total Area]** is the area of the whole image in square pixels or square *calibration units*.
- **[Area Fraction]** is the ratio between **[Total Area]** and the area of the thresholded objects.
- **[Number of objects]** - number of objects after applying restrictions.
- **[Table of results]** - measurement results for each object are displayed in this table. Select the columns to display/hide from the context menu which appears when you right-click one of the column captions.
- **[#]** - number of selected objects is displayed in the left part of the tool bar. One or more objects can be selected by mouse while holding the **[Ctrl]** or the **[Shift]** key.

Tool Bar

There is a tool bar above the results table which contains the following buttons:

-  **[Delete selected objects]** removes the selected objects. The objects can be selected by mouse while holding **[Ctrl]**.
-  **[Invert selection]** deselects objects selected by mouse and vice versa.
-  **[Generate binary]** erases binary objects which do not meet the restrictions settings.
-  **[Export ND data]** - when an ND2 file is opened, the thresholding, measurement, and restrictions are applied to a single frame. Pressing this button will apply it to all frames of the ND2 document and export the results to a file or clipboard according to the current export settings.

-  **[Save/Load Object Count configuration]** invokes a pull-down menu which enables you to load/save the current object count settings from/to an external file (*.counting).
-  **[Keep updating count]** refreshes the measured results each time the binary layer changes (e.g. when the threshold is modified).
- **[Export]** - 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.

7.9. Time Measurement

The time measurement tool enables to record average pixel intensities within ROI(s) during a time interval. The measurement can be performed on Live camera signal or on a saved ND2 document. Before the measurement is started, regions of interest (ROIs) shall be defined and turned ON. Run the **[View > Analysis Controls > Time measurement]** command to open the Time Measurement control window.



Quick Guide

- Enable the Measurement ROI by the **[Measure > Use ROI]** command.
- Run the **[Acquire > Live - Fast]** command to display the live camera signal.
- Define ROI(s) within the image. Time measurement will be performed under the ROI areas. Use the **[Measure > Simple ROI Editor]** command or the **[Define]** tool bar button to open the vector ROI editor and use the **[Measure > Raster ROI Editor]** command to display the raster ROI editor. See [Regions of Interest - ROIs].
- Once the ROIs are defined, press the **[Measure]** button - the measurement will start.
- Press the **[Stop]** button to finish the measurement.
- Export the results via the **[Export]** button. See [Exporting Results] for further details.
- A snapshot of the graph will be created if you press the  **[Create Snapshot]** button.

Note

If there are too many ROIs defined and the graph gets confusing, a single ROI (and the corresponding graph) can be highlighted. Right click the ROI name and select [Bookmark this ROI] from the context menu.

Multi ROIs, Multi Dyes

The **[Multi ROIs]** and **[Multi Dyes]** buttons in the top right corner swap between two measurement modes:

-  **[Multi ROIs]** - Multiple ROIs can be defined. In this mode, data for each ROI are available.
-  **[Multi Dyes]** - In this mode, data for each color channel (averaged from all ROIs) are available.

Creating Meta-Data Graphs

The **[Time Measurement]** control window can be used to create a graph of any meta-data included in the ND2 file - the temperature for example.

- Open or capture an ND2 document containing meta-data.
- Define ROIs.
- The **[Left/Right]** combo boxes become enabled. Choose the data you want to display in the graph. And start the measurement with the **[Measure]** button.
- After the measurement finishes, you can overview the graph in the **[Graph]** tab.

Graph Display

Use the following buttons to adjust displayed part of a graph.



No Split

The graph is not split.



Split Horizontally

Components defined in the **[Left]** and **[Right]** pull down menus are displayed separately in two graphs.



Range 0-bitdepth

Range of Y axis is set from 0 to maximal value of image bit depth (e.g. 255 for 8 bit image).



Range 0-max

Range of Y axis is set from 0 to maximal value measured in the component.

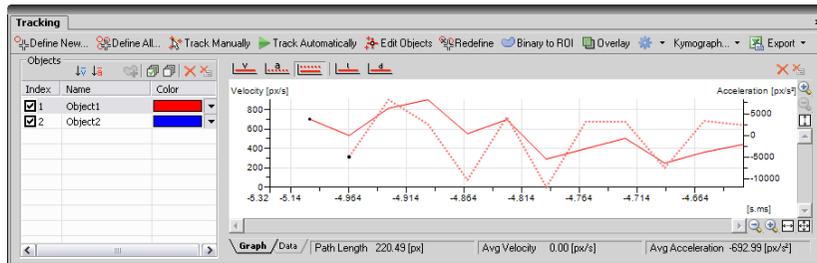


Range min-max

Range of Y axis is set from minimal to maximal value measured in the component.

7.10. Tracking (-TR- option)

Motion characteristics of objects such as living cells can be measured automatically or manually using the **[Tracking]** control window. Run the **[View > Analysis Controls > Tracking]** command to display the control window:



7.10.1. Automatic Tracking

Define the objects to be tracked. There are two ways to do so:

Note

The higher contrast between the objects and the background, the better results the tracking will bring. If the contrast in your image sequence is not sufficient, you may need to perform some image processing before the tracking.

Object Definition - One by One

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.

Object Definition - All Together

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. Afterwards, some deleting of mis-detected objects may be needed.

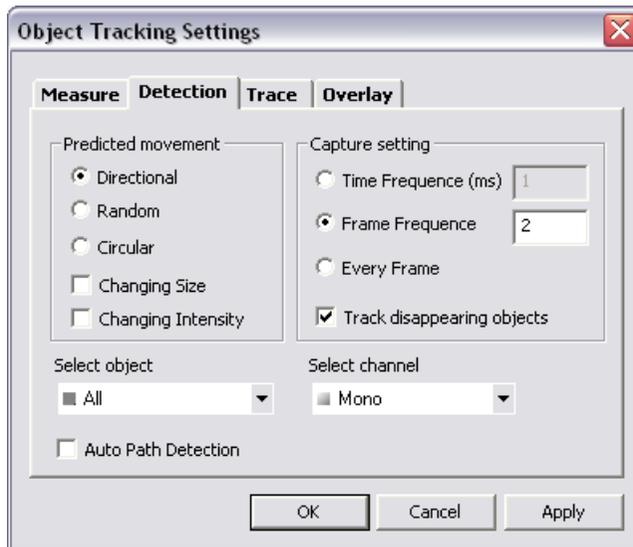
Tracking

When 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 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 work-flow 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 window and select which channels shall be used for the tracking 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 regain the trail of such lost objects. If this option is disabled, the object track will be finished where the object has been detected last.

Tracking ROIs

There are two issues regarding regions of interest and tracking:

- Binary objects which you have defined by the **[Define New]** or **[Define All]** buttons can be converted to ROI objects. Press the  **[Binary to ROI]** button to start the conversion. A set of ROIs will be created for each frame of the ND2 file.
- A ROI area defined on an ND2 sequence may be either global (the same on all frames) or moving - defined per frame in time. If you have a moving ROI defined, you can perform the tracking using the **[Add moving ROIs]**  button. See [ROI in Time - Moving ROIs]

7.10.2. 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 the object 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.
- In this way, you define the trajectory point #2 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.

7.10.3. Object Redefinition

Some objects, especially if they are living cells, can change their image characteristics - fade, change size, etc - during the motion. In such cases, the automatic tracking algorithm may get confused and lose 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 - the tracking algorithm uses the new values from the frame where redefinition occurred on.

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]** option from the **[Settings]** pull-down menu.
- Click the point you would like to modify and drag it by mouse to the right position.

7.10.4. 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 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. The movement direction and the object velocity become obvious.

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

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 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.

To create a 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.

To create a kymograph on selected objects

- 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.

7.10.5. 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.

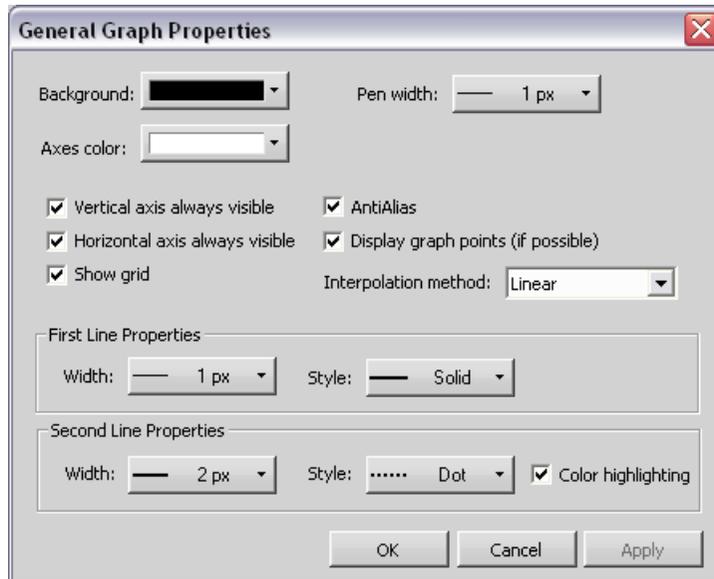
- Click the **[Define]** button. The window disappears.
- Place the Reference point to the image by mouse. The window reappears.

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.

7.10.6. Graph Properties

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



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).

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.

7.11. User Macros

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

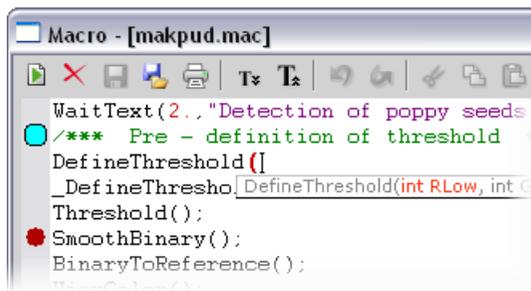
7.11.1. Recording a Macro

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

- Start the NIS-Elements AR macro recorder by selecting the **[Macro > Record]** command.
- Perform the series of actions you would like to record.
- Finish the recording by the same command again (its name changes to “Stop Recording”)
- 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.

7.11.2. Writing/Editing a Macro

If you possess programmers skills, a macro can be written by hand in the built-in macro editor. Run the **[Macro > Edit]** command to display it.



```

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

```

The macro editor provides:

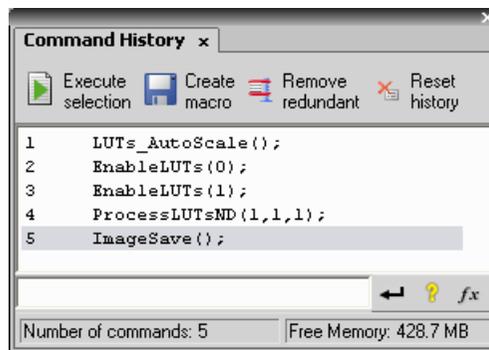
- Direct output to a printer.
- Undo/Redo functionality.
- Command insertion from the list of available commands.

- Interactive command names list. Press **[Ctrl+Space]** to display a simplified list of all commands while typing the macro.
- Syntax hints (parameter types and names) appear as you type a command name.
- 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 the state of variables and therefore inspect the macro functionality thoroughly.
- Syntax highlighting.
- Help on commands with detailed description.

7.11.3. Creating a Macro from History

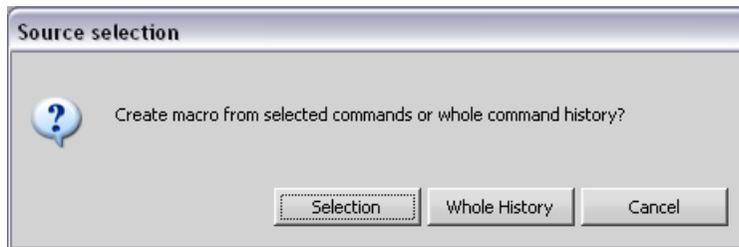
You can create a macro using the list of recently performed commands.

- 1) Run the **[Macro > History]** command to display the **[Command History]** control window:



- 2) Push the **[Create Macro]** button.

- 3) Select what portion of the command history will be used in the macro:



Selection

This option will use only commands which you have previously selected by mouse within the **[Command History]** window. Group selection (holding **[Shift]** or **[Ctrl]**) is available.

Whole History

All commands listed in the **[Command History]** window will be used for the new macro.

- 4) After you selected one of the options, the macro editor appears containing the new macro source code. Use the editor to fine-tune it.

Note

The [Remove Redundant] command is automatically performed before the macro is opened in the editor.

7.11.4. Saving a Macro

Use the **[Save Macro]**  and the **[Save Macro As]**  commands in the macro editor to save the created macro.

7.11.5. Running a Macro

NIS-Elements AR provides several ways to run a macro.

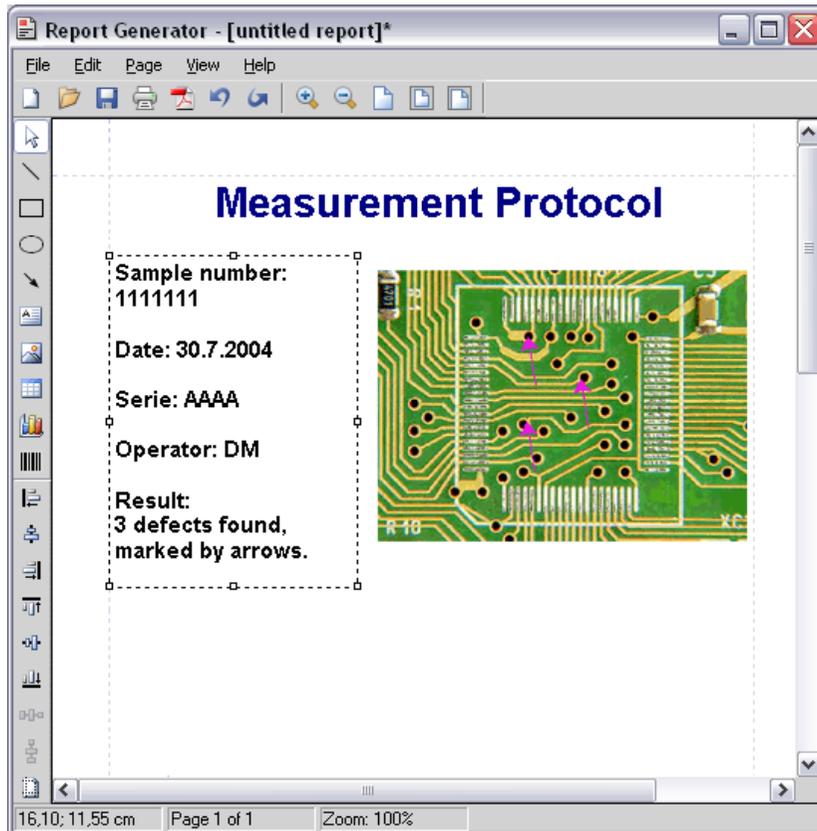
- You can run the current macro loaded to NIS-Elements AR by choosing the **[Macro > Run]** command or by pressing **[F4]**.
- A **[hotkey]** combination (Ctrl+Alt+1,2,3 ... 9) can be assigned to it in the **[Macro > Options]** window.
- You can run a macro at the beginning of the NIS-Elements AR session, by assigning the **[StartUp]** flag to the macro in the **[Macro > Options]** window.
- You can run a macro saved to a disk by using the **[Macro > Run From]** command.
- A tool bar button can be assigned to run a macro. See [Modifying Layout].

7.11.6. Breaking a Macro

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

7.12. Creating Reports

Report Generator enables the user to create customized reports containing images, measured data, user texts, graphics, or database entries. PDF files can be created directly from NIS-Elements AR. When you invoke the **[File > Report > New Blank Report]** command, a **[Report Generator]** window 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
-  Insert bar code
-  Align - aligns selected objects to the position indicated on the button by the line
-  Center - aligns the selected objects to center
-  Distribute - distributes the objects uniformly along the specified direction
-  Determines whether to apply the align commands relatively to the whole page or the selected objects only.

Context Menu Commands

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

- If there are more than one objects selected, the **[Group]** command makes them to behave as a single object.
- The **[Align or Distribute]** commands enable moving objects within the page or align them with another object(s).
- The **[Resize object]** commands 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 window where object properties such as color, background color, border size and color, object size, font, aspect ratio, alignment, and shape can be adjusted.

Insert images, text fields and other objects to the report.

7.12.1. Working with Report Objects

When you double-click any of the report objects, a window appears where the properties can be adjusted. Some properties are common to 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.
- Line widths can be adjusted within the range of 0,25 to 6 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 pull-down 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 window. It shows the **[current scale/original image dimensions]** ratio (the image must be calibrated to use this feature).
- 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 or displayed.
- 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 **[CTRL]** 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 icon.
- If the **[Relative to Page]** option is turned **[ON]**, the objects will be aligned to the edges or the 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 outer 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]** sub-menu 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 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 a macro.
- **[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.

7.12.2. Working with Report Templates

A report template is a layout defining the appearance of future reports, 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 Blank Report]** command. An empty report appears.
- In the Report Generator, select **[File > Change to Template]**.
- 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 or tables, the current image to the image field, etc.).
- Save the report template (*.rtt) via the **[File > Save]** command.

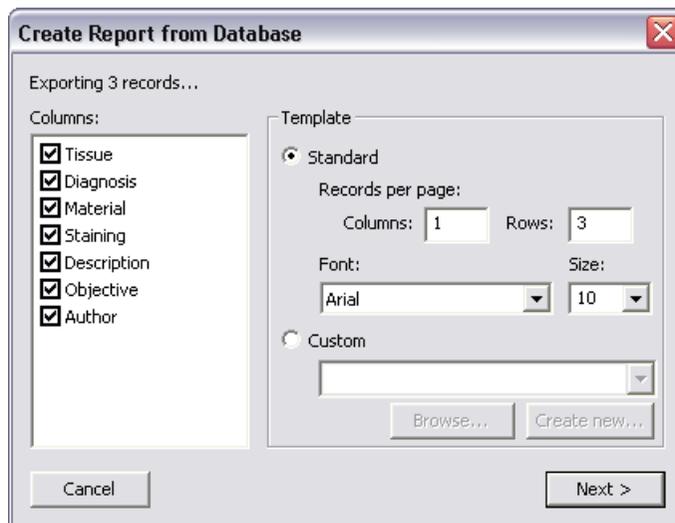
To Create a Report from Template

- If the template is opened inside the Report Generator, run the **[File > Create Report]** command. Otherwise, use the **[File > Report > New Report from Template]** command inside the main application window 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.

7.12.3. Creating Reports from Database

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 AR to **[Organizer]** by the **[View > Organizer Layout]** command.
- Select one or more images. These images will be inserted to the report.
- Click the **[Report]** button on the main tool bar. The following window appears:



- In the **[Columns]** section, you can select fields, which will be included in the report. If you are exporting images from a database, the database table fields will be listed. If you are exporting images from a directory, image description items will appear.
- The **[Template]** portion of the dialog specifies the layout details. 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, a user report template (*.rtt) can be opened and used for the report creation. If such report does not exist yet, you can create it using the **[Create New]** button.

If 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 of images spaced according to the **[columns/rows]** settings.

- Edit the top-left cell of the image grid. You can change the text/image boxes position, size, and mapping. During report creation, all 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.

7.13. Creating Movies

Movies is one of the possible ways to present data captured using NIS-Elements AR. The following functionality is available:

- A real-time movie of a time-lapse process can be captured.
- A sequence of standalone images can be converted to a movie.
- An ND2 file can be converted to a movie.
- A 3D model or a surface view of an object can be set in motion and make a nice presentation movie.

Movies are saved as an **[AVI]** which can be played back by any standard video player so you do not need NIS-Elements AR to view them.

7.13.1. Capturing AVI Movie

- Display the AVI Acquisition control window by the **[Acquire > AVI Acquisition]** command.
- Adjust the advanced settings, especially define the file name and the destination folder.
- Click the **[Record]** button. The live image appears and recording starts 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 window appears. Select one of the codecs available in the system or use the **[Default]** option. Remember that the codec you choose will be required for the AVI playback as well (when playing the video on other computers).

Note

Video codec is a software tool which can code and decode video files. We recommend to use one of the following codecs:

Cinepack

Provides maximum compatibility and small files.

Intel Indeo

Provides the best quality/compression rate.

Microsoft YUV

A non-compression (maximum-quality) codec.

Size

The size is determined by the resolution of the connected camera, but can 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.

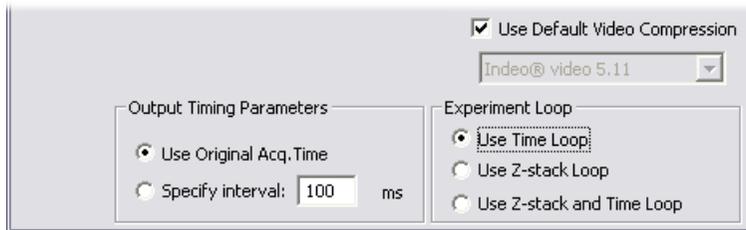
No matter what size is selected, the video always displays the whole scene. If the defined size has different aspect ratio than the live image, the empty space is filled with black.

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.

7.13.2. Converting ND2 to AVI

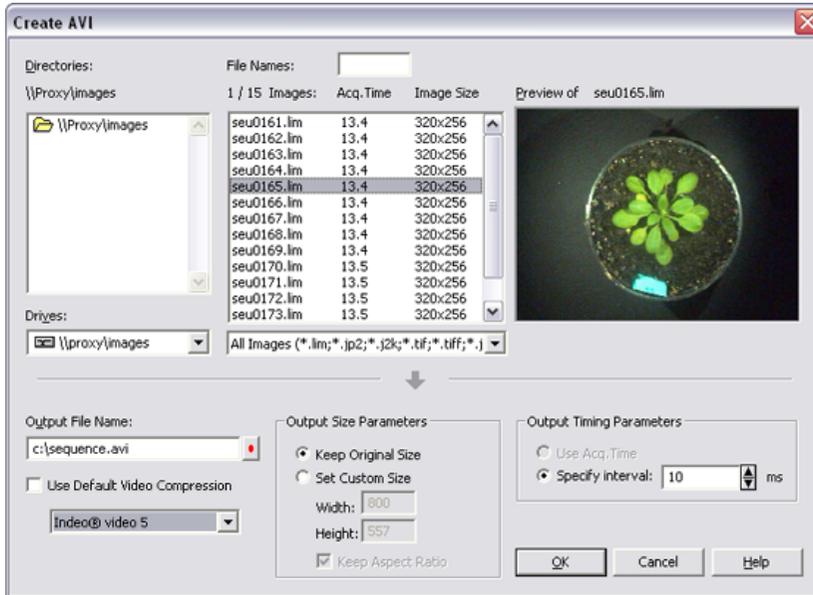
You can easily create an AVI movie from an ND2 document. Run the **[File > Save As]** command and instead of saving the file as an ND2, simply select the AVI format in the **[Save As Type]** pull-down menu. The **[Save As]** window expands as follows:



Adjust the AVI file settings and press **[Save]** to finish. Please see the **[File > Save As]** command description for more details.

7.13.3. Creating AVI from a Sequence of Images

You can convert a sequence of images to AVI by the **[File > Sequences > Create AVI]** command. After you invoke the command, the **[Create AVI]** dialog box appears:



Create an AVI file:

- Select a folder to look in for images and choose the images. 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.
- Define the output file name and adjust all the parameters of the output file dimensions and timing. To select a video codec to be used, de-select the **[Use Default Video Compression]** option. The default codec (Intel Indeo) will be used otherwise.
- Click **[OK]** to confirm the settings. An **[AVI Creation Preview]** window appears.
- After the sequence is processed, press the **[Close]** button to finish. The file is saved automatically to the previously specified location.

See **[File > Sequences > Create AVI]** description for more details.

7.13.4. Movie Maker (-LO- option)

[3D Volume View] and **[EDF 3D Surface View]** (-EDF- option) objects are about be brought to life once the NIS-Elements AR movie-maker is started. The Movie Maker control window appears after you press the

 button located on the document tool bar (Volume View or Surface View must be must be active). The user is supposed to create a set of key-frames. The rest of the movie will be created by interpolation between these key frames. This gives the user absolute freedom in how the movie will look like.

Movie Maker Controls

When you start the movie maker, the following bar appears at the bottom of the document view.



1. The **[Clapper]** button. By this button, the director's mode can be turned ON/OFF. See Directors Mode.
2. Time line. By clicking to the time line you can browse the movie frames. When a key frame is added, a bold line appears in the time line to indicate its position.
3. **[First Frame]**, **[Play]**, **[Last Frame]** buttons.
4. **[Settings]** - by this button, you can define the movie length and the frame rate.
5. **[Create Movie]**
6. The key frame buttons. The existing key frames may be browsed by pressing the **[Previous/Next Key Frame]** buttons. The **[Add/Update Key Frame]** button inserts a key frame to the current position on the time line.
7. **[Presets]** - a predefined rotation can be loaded. When you press the button and select the rotation type, the existing key frames settings will be overwritten.
8. **[Import/Export Key Frames]** - this enables you to save and load the key frame settings. A file with the **[*.KEY]** extension will be created during the export.

How to Create a Movie

- Run the Movie Maker by the  button.
- Adjust the movie settings by the **[Settings]** button a window appears where the movie duration and frame rate can be defined.

- You can use one of the predefined presets or import some previously defined key frames by the **[Export/Import]** button.
- Either enable or disable the Director's mode by the clapper button. See Directors Mode.
- Go to the beginning of the time line and define the initial zoom, position, rotation or component appearance of the object. This step is not necessary, but very much recommended. Press the **[Add/Update key frame]** button to fix the initial appearance.
- Browse the time line, modify the object position each time, and set key frames by clicking the **[Add/Update key frame]** button. Press the **[Play Movie]** button for a preview.
- When all the key frames are properly set, press the **[Create Movie]** button. The movie is created and the AVI file will open in a new document window.
- Save the new file by the standard **[File > Save As]** command.

Directors Mode

In the directors mode it is not needed to press the **[Add key frame]** button every time the position of the object is changed. The director's mode detects all changes made to the object position and adds a key frame to the time line whenever such change is detected. A typical work-flow would look like this:

- Turn ON the directors mode by the **[Clapper]** button. The time line gets highlighted with red color.
- Click to the time line where a certain position of the object shall occur.
- Move the object by mouse or adjust it in some way (zoom, crop, etc.) - at this moment, the key frame will be added to the time line automatically.
- Click to the time line where another position shall occur, and so on...

The director's mode shall be used when you know precisely how the movie shall look.

Special Options

A contextual menu appears whenever you right-click on any key frame or between two key frames (a segment) within the time line.

Set key frame time

Sets the selected key frame position - moves the key frame to this position.

Set as new start of the movie

Sets the selected key frame as the first frame of the movie and moves all other key frames proportionally. Key frames prior to the selected one will be deleted.

Set as new end of the movie

Sets the selected key frame as a new end of the movie and moves all other key frames proportionally. Key frames past the selected one will be deleted.

Copy/Paste key frame

Enables to copy and paste key frames.

Delete key frame

Deletes the selected key frame.

Delete all key frames

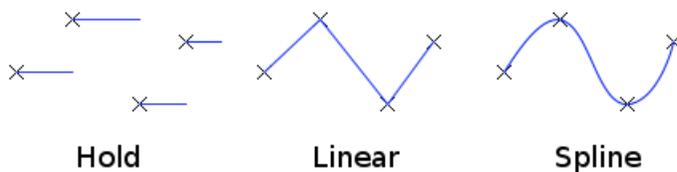
Deletes all defined key frames.

Set time segment duration

Sets the segment duration. Positions of all other key frames will be moved proportionally.

Hold/Linear/Spline

Select the type of interpolation to be used between two key frames. Set **[Spline]** for smooth changes (motion, lightness etc.)

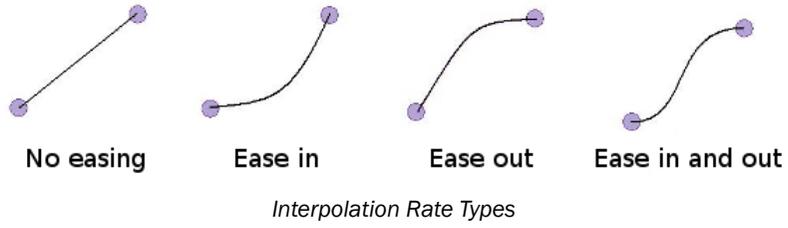


Interpolation Types

Ease in/out

Easing defines the interpolation rate between two key frames. No easing means constant rate of interpolation. Use the **[Ease in]** option for the first time segment to increase slowly the rate of change. On

the contrary, the **[Ease out]** option serves the best at the end of the movie to make the motion slowly stop. Of course, you can get the benefit of combining them.



7.14. Exporting Results

Some image analysis data and measurement results can be transferred out of NIS-Elements AR so they could be used further. There is a standard **[Export]** pull-down menu in some of the control windows (intensity profile, histogram, measurement, etc...) which enables the export.



The set of commands of the menu differs according to the type of data (tables or graphs) which can be exported. Click the arrow button to display the pull-down menu. When you select the destination, the pull down menu hides and the icon of the selected export type 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.

To File

Tabular data can be exported to an MS Excel sheet (xls) or a text file (tab delimited txt).

To Clipboard

Data tables and graph images can be exported (copied) to MS 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

Tabular data and graph images can be exported to NIS-Elements AR 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 **[Web Browser]** option), or the HTML code can be copied to Windows clipboard - ready to be inserted to any HTML/text editor.

To Printer

Tabular data can be printed directly. This option opens a standard **[Print]** dialog window where it is possible to select a printer and print the data.

Data Export Options

Run the **[Edit > Options]** command and switch to the **[Data export]** tab or press the **[Settings]**  button within the export pull-down menu. There are two tabs available:

Global Settings Options

Data export

Global Settings | Data export

Export into Microsoft Excel application

Use currently opened file or create a new one

Open or create specific file

Start at column: and row: A1

Export text files into folder

Append exported data after previous exports

Export also column headers if not appending

Activate target application after export

Export into Microsoft Excel application

Select this option if you want to export the data to MS Excel application. You can choose if the data will be exported into currently opened file or a new file will be created. Or you can set to export the data to a specific file. If this file does not exist, it will be created. Choose the path and name of the file to be created/opened.

Set the **[Start at column]** values (column and row indexes). These values mark the first cell in the MS Excel application which will hold the exported data.

Export text files into folder

Select this item if you prefer to export the data to a text file. Press the **[...]** button and specify the target folder and the file name.

Append exported data after previous exports

If you check this item the existing data will not be overwritten by the new data.

Export also column headers if not appending

Include the titles of columns. If the **[Append exported data after previous exports]** is selected, no headers will be included in the export.

Active target application after export

If you check this item the exported file is opened in the default application (MS Excel or the default text editor) automatically.

Data export Options

You can modify the amount of data included in each export type.

The screenshot shows the 'Data export' dialog box with the following content:

Global Settings | Data export

ManualMeasurement ▾

Id	Export Name	Sheet Name
1	Length	Length
2	Area	Area
3	Radius	Radius
4	SemiAxes	SemiAxes
5	Angle	Angle
6	Length3D	Length3D
7	Counts	Counts
8	Taxonomy	Taxonomy

Options for: Angle

- Source
- Angle
- ND.T
- ND.Z
- ND.M
- Comment

Select All | Clear All

The control windows which contain the Export to file functionality are listed in the top pull-down menu. Select the control window of which the export shall be modified.

Export Name

A control window such as **[View > Analysis Controls > Annotations and Measurements]** stores information about the measurement in multiple tables according to the measurement type (area, length, etc.). In this column, you can select the table you would like to modify.

Sheet name

In this column you can define an arbitrary name which will be used to name a sheet or a txt file to which the data will be exported.

Options for:

This section of the window enables you to select the items to be included/excluded from the selected table during export.

8. Cameras

A digital camera mounted to a microscope port or a macro-scope stand records images of the observed scene on a light-sensitive sensor, and transfers them to a computer. NIS-Elements AR supports various cameras differing from each other in resolution, frame-rate, sensor type, etc. Despite these differences, controlling different cameras is similar. The following features are included in the **[View > Acquisition Controls > Camera Settings]** window depending on a particular camera type.

8.1. Common Settings

Fast (Focus)/Quality (Capture) Formats

Usually, cameras can provide several image resolutions. NIS-Elements AR enables to create two presets of the resolution settings - **[Fast]** and **[Quality]**. The formats then differ in image size, and consequently in frame rate (number of frames per second - fps). The higher the resolution is, the lower frame rate can be achieved. Available resolutions depend on the camera type.

Binning

The binning mode provides considerably enhanced camera chip sensitivity by integrating more elements (pixels) together. E.g.: **[binning x4]** integrates the signal from the area of 4x4 chip elements to one pixel of the resulting image. Smaller resolutions and faster frame rates are achieved using the binning modes.

Exposure Mode

Exposure mode sets how the Exposure time is calculated. Generally, there are automatic and manual modes:

- **[Auto Exposure]** - Calculates an optimal combination of **[Exposure]** time and **[Gain]** in order to achieve optimal exposure. Image quality is the priority, therefore longer exposure times are chosen. Usually, the auto exposure can be run once by pressing the **[Auto Exposure]** button, or continuously by selecting the nearby check box.
- **[Manual Exposure]** - User selects both **[Exposure]** time and **[Gain]** manually.

Exposure

When in **[manual exposure]** mode, this option controls the exposure time, which is expressed inversely ($1/x$ second). It is the time of charge accumulation in a camera chip between two adjacent frames.

Prolonging the exposure time increases the brightness of the image as well as the image quality (the less gain, the less noise), and decreases frame rate.

Gain

Controls the sensitivity of a camera. Increasing the gain increases brightness of the image, but decreases quality (the more random noise, the more streaky noise and color unevenness), and increases the frame rate indirectly by enabling shorter exposure times.

Averaging

Averaging is a commonly used technique of decreasing noise in the image. In this method 2, 4, 8 or 16 consecutive frames are averaged together.

Offset

Sets the brightness of the image. It is a constant additive (positive or negative) changing all pixel values of the image. With negative offset value the dark image areas become pure black. Considering fluores-

cence microscopy, an appropriate offset setting can create continuous black background and thus help to enhance (together with gain or illumination enhancement) the contrast.

Contrast

Affects dynamics of how the luminosity is rendered. There are several modes for different illumination (contrast) scene situation.

Gamma

The gamma correction maps the intensity of live signal exponentially to the gamma parameter. For $\gamma < 1$, dark portions of the image are enhanced whereas for $\gamma > 1$, image parts of higher intensities are enhanced.

AE Compensation

In automatic exposure modes, the compensation affects how optimum exposure settings (Exposure time and Gain) are calculated. The Compensation value is expressed in Exposure Values (EV). Setting the compensation to + 1.0 EV makes the image twice brighter (e.g. doubles the Exposure time or Gain).

AE Lock

This option makes the automatic exposure mode to lock the current exposure settings (Exposure time and Gain).

Sharpness

Some camera settings provide the sharpness control which affects how sharp edges in the image appear. Too much sharpness leads to oversaturated edges.

Hue

Hue shifts image colors across the rainbow.

Saturation

The amount of saturation determines how colors are rendered. More saturation produces richer colors. Less saturation makes the colors gray.

White Balance

There are usually Red, Green and Blue Gain properties that control how colors are rendered. It is used to eliminate a color cast from white areas.

Auto White Balance (AWB)

The AWB button performs an automatic white balancing. It adjusts the red, green, and blue image components in order to get a neutral white color.

8.2. Advanced Settings

Separate Channel Settings

Having a color camera, influence of the settings described above on each color channel can be adjusted. In such case, three edit boxes with the default values set to 1.0 are displayed below the feature setting. These values determine the influence of the feature on each color channel.

Using Probe

The probe enables you to determine a small image area that serves as the data source area for LUTs, histogram etc. It also affects the AWB (auto white balance) and the AE (Auto Exposure) features of the cameras that support it. When the probe functionality is not supported, the AWB and AE algorithms are computed from the whole image.

Live Acceleration

Would you like to increase the frame rate of the live signal? Move the slider to a desired multiplier. The system then automatically shortens the exposure time (so the frame rate rises), and the loss of intensity is compensated by gain (software multiplication).

Target Maximal Intensity

Restricts the maximal image intensity after the auto exposure was applied. The value represents a percentage of the whole camera dynamic range.

Maximal Exposure

This is a safeguard of the time of the Auto Exposure. For quick exposure, it is convenient not to set this value too high.

Overillumination Tolerance

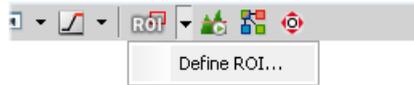
Sets how many pixels should be white after the Auto exposure is performed. Use lower values (0.01%) for very bright (shining) objects (fluorescence). For common bright field, even 1% may be a good value. Optionally, you can define the absolute number of white pixels.

Use Current ROI

The currently defined region of interest, set by the **[Define ROI]** command, can be switched ON/OFF by checking this item.

8.3. Camera ROI on Live Image

You can specify a camera ROI on a live image by selecting the **[Define ROI]** command placed in the document tool bar next to the ROI button. In a window that appears, set the dimensions and the position of the ROI rectangle. The live image is then restricted to the defined area when the **[ROI]** button is pressed.



8.4. Controlling Cameras by Macro

Every camera can be controlled from within a macro by changing its properties. There is a `CameraGet_*` and `CameraSet_*` macro function available for each camera property (e.g. `CameraGet_ExposureTime()`). The number of functions (beginning with `CameraSet_/Get_`) and their actual names depend on the type of camera(s) you have currently connected to NIS-Elements AR.

CameraGet_

The [**CameraGet_...**] functions retrieve current values of the properties determined by the function name postfix. So the `CameraGet_Exposure(int Mode, double *Exposure);` function retrieves the current value of Exposure of the specified Mode.

CameraSet

The [**CameraSet_...**] functions allows you to adjust camera properties. So the `CameraSet_Exposure(int Mode, double *Exposure);` function sets a new Exposure value for the specified Mode.

8.4.1. How to Set Camera Properties

Instead of describing each property function, we will show you a universal way how to handle a camera by macro:

- 1) Display the [**View > Acquisition Controls > Camera Settings**] control window and find the property you would like to control.
- 2) Make a change of the property from within the control window. For example, change the exposure time.
- 3) Display the [**View > Macro Controls > Command History**] window and see the last function which has been called and its parameters. This is the function you will control the exposure time with.
- 4) Experiment with the camera control window in order to determine the right parameter values of the functions to be called within your macro.

Note

See the [*Function Reference > Acquire > Camera > Properties*] section of the online help. Some of the most common functions are described there.

9. Devices

Before you get to work with NIS-Elements AR, 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 AR, and select the appropriate devices during the installation.
- Connect the device to a PC and switch the device ON.
- Run NIS-Elements AR, and run the **[Devices > Manage devices]** command to open the Device Manager.
- Use the **[Add]** button to add the device to NIS-Elements AR.
- 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.

9.1. Logical Devices

NIS-Elements AR 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 AR. 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 the connection is established.

Available Logical Devices

Analyzer

The analyzer is a polarizing filter placed in the optical path between the specimen and the lamp. 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. Two parameters can be typically set for aperture devices, the state (ON/OFF) and aperture size.

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 to the specimen 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 AR 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 settings button, a 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.

Note

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 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 read by the application, but can not be controlled.
- **[Motorized]** - such nosepieces can be fully controlled via the **[Microscope Control Pad]** or the **[Nosepiece]** control window.

See Assigning objectives .

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. You can also rename

the shutter by running a command which appears in the contextual menu in **[Filters & Shutters]** control window, **[Device Manager]** window, or in the device toolbar contextual menu.

Zoom

This logical device is used for controlling the zoom factor. Run the **[Devices > Zoom Configuration]** command to adjust the zoom settings.

Stage XY

Stage XY enables the movement of specimen within X and Y axes. The system offers user to control stage movements and brings some extra functionality.

Stage Z

The Z Drive device enables movement throughout the Z axis direction.

TTL/Analog Input, Output

There are six types of logical devices available when the NI DAQ controller set is installed: TTL Input, TTL Output, Analog Input, Analog Output, Calibrated Analog Input, Calibrated Analog Output. Each of these logical devices represents one connection to an external device. The number of connections depends on the actual hardware setup.

Assigning objectives

- To assign an objective (or to change an attached objective assignment) to a position, click the setup  button in the microscope control pad.
- A window appears which enables you to select one of the available objectives to the corresponding position.
- Each objective has its specifications displayed in the table. These specifications are not editable.

Using two independent Z Drive devices

Workstations can be equipped with two independent Z drive systems, one slow (or coarse) and the other fast (or fine). Typically the first one is used for specimen manipulation in Z axis, the second one for auto-focusing.

Absolute Z

The current positions of both Z drives (Z1, Z2) and the absolute Z position (sum of Z1 and Z2) are displayed in the main status bar. If there is not enough space for all these values, only the **[absolute Z]** value is displayed (Z1 and Z2 still appear in a tool-tip). The absolute Z value appears in the Z Series Setup.

Active Z

The concept of **[Active Z]** enables user to select the preferred Z drive:

- The active Z drive is used when performing auto-focus.
- The **[Devices > Enable Mouse Joystick Z in Live]** command applies to the active Z.

You can select the Active Z device in the **[Devices > Mouse Joystick and Auto Focus Z]** menu.

Regular Z drive + Piezo Z drive

If you have a regular Z drive and a Piezo Z drive installed, some functionality is added:

- The **[Home Piezo]** button is added to the **[Z Series Setup]** window when Piezo Z is selected.
- The **[Devices > Keeps Z position and centers Piezo Z]** and the **[Devices > Move Piezo Z to Home Position]** commands appear in the **[Devices]** menu

The button and the **[Devices > Keeps Z position and centers Piezo Z]** command provide corresponding functionality. They move the Piezo Z device to its home position, and compensate this shift by moving the second Z drive in order to maintain the absolute Z position.

9.2. 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

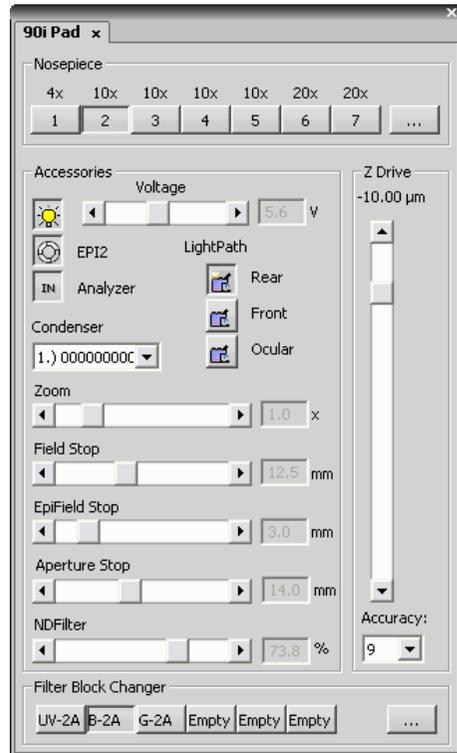
The light path accessories that affects light intensity can be controlled here. The condenser turret is controlled by selecting the desired condenser from the pull-down menu.

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.



9.3. Nikon AZ100 Microscope

When **[AZ100]** is connected to NIS-Elements AR, 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 can not be controlled by NIS-Elements AR .

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 lenses position (Low/High). The condenser cannot be controlled.

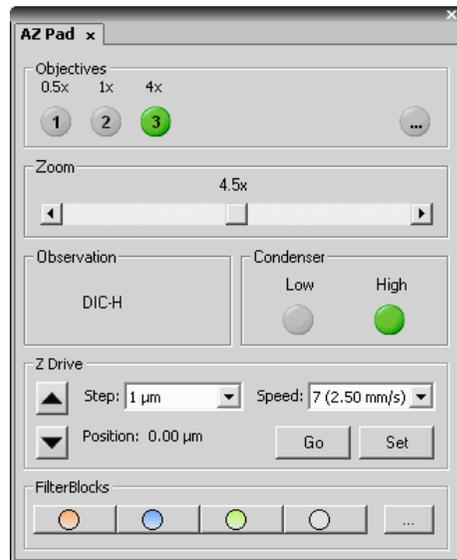
Z Drive

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 AR . Filters assignment may be changed after pressing the **[...]** button.



9.4. 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 window.

Accessories

Intensity of episcopic and diasopic light sources can be controlled.

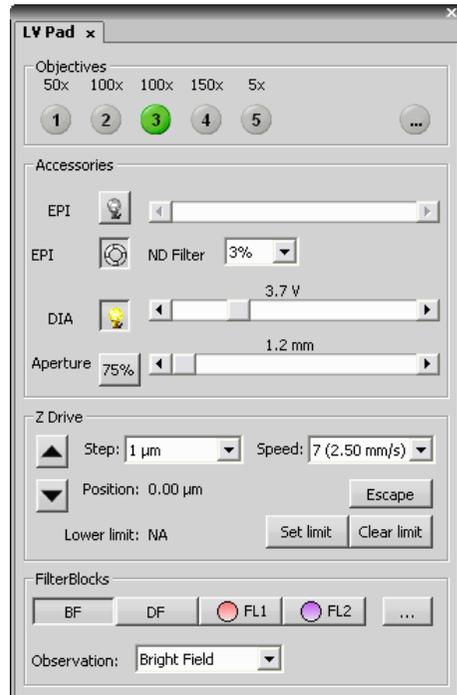
Z Drive

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 moves the objectives out 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 while 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.



9.5. Nikon Eclipse L200 Microscope

The L200/L300 Microscope Control Pad consists of the following portions:

Nosepiece

The motorized objective changer can be controlled via this portion of the window. If a move from position 1 to position 6 is ordered, a warning appears. This behavior shall prevent the high magnification objectives to strike the XY stage.

Light

A halogen lamp or the **[Nikon Intensilight]** illumination device can be attached to the EPI port of the microscope. Both lights can be controlled from here (if selected in the Light Control pull-down menu). If two halogen lamps are attached, the light control works as the EPI/DIA switcher.

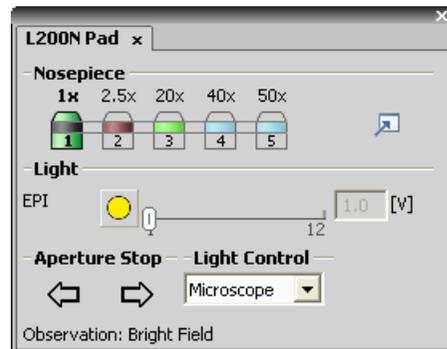
Aperture Stop

These two arrows control the EPI aperture stop equally to the buttons on the microscope body.

Light Control

You can select from where the lamps will be controlled:

- NIS-Elements AR - from the Control Pad
- **[Microscope]** - from the microscope body



9.6. Nikon Eclipse MA200 Microscope

The MA200 Inverted Metallurgical Microscope Control Pad consists of the following portions:

Nosepiece

The motorized objective changer can be controlled via this portion of the window. If a move from position 1 to position 5 is ordered a warning appears. This behavior shall prevent the high magnification objectives to strike the XY stage.

Light

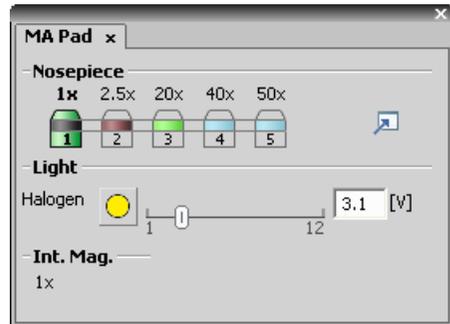
A halogen lamp or the **[Nikon Intensilight]** illumination device can be attached to the microscope and controlled from here. If the halogen lamp is attached to the microscope, internal (manual) or external (via NIS-Elements AR) control can be selected on the back of the microscope body.

Shutter

The shutter is a part of the Intensilight device and can be controlled from here.

Intermediate Magnification

This portion of the pad indicates the current state of the intermediate magnification device. There are three predefined magnifications (1x, 1.5x, 2x).



9.7. Nikon MM 400/800 Microscopes

A measuring microscope connected to NIS-Elements AR 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 AR

Z Drive

The Measuring Microscopes Z drive may be moved using the **[View > Acquisition Controls > XYZ Navigation]** control window.

9.8. 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 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

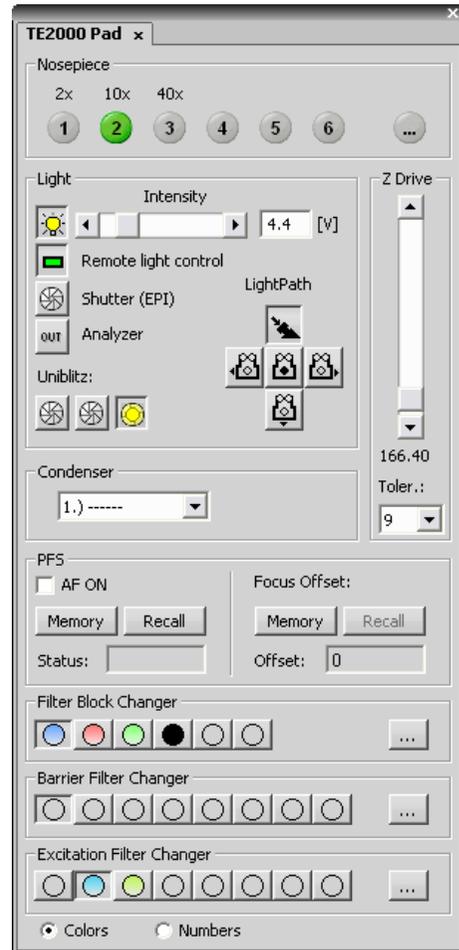
The Z drive position is indicated, or can be controlled via a scrollbar.

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.



9.9. 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 AR and the microscope RCP. Press  to display the nosepiece settings window.

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.

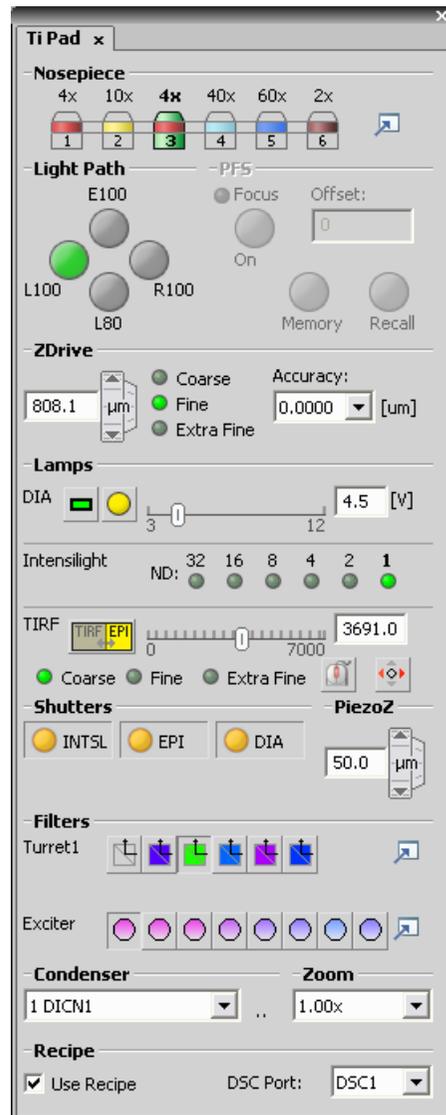
Z Drive

Movements of the integrated Z drive are performed by the up/down arrows. Accuracy can be set in the pull-down menu on the right. The Accuracy 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 via the control 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. You can set the set TIRF control sens-



itivity (coarse, fine, or extra fine) in the Microscope Control Pad. TIRF can be controlled by mouse wheel or joystick X axis (or both).

Shutters

Register the correct shutter types in the RCP. Then, the shutters can be controlled by NIS-Elements AR

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.

Recipe (-AR- option), (-BR- option)

TI recipe is a special feature provided by the HUB-A. It provides hardware acceleration of an ND capturing experiment. Mark the check box to enable the acceleration and select the port to which the camera is connected. See below.

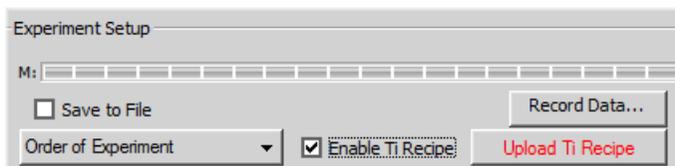
9.9.1. Using TI Recipe

To enable the TI Recipe functionality, the following hardware configuration is necessary:

Hardware requirements

- **[HUB-A]**
- **[Andor DU-897]** camera connected to the DSC1 or DSC2 port of the HUB-A.
- A shutter device (or two) connected to HUB-A. **[Nikon Intensilight]** and **[Sutter Lambda SC]** devices are supported.

When connected correctly, the **[TI Recipe]** check box becomes available within the ND experiment window.



- Define the ND experiment.
- Select the **[Enable Ti Recipe]** check box in the **[Applications > ND > Define/Run Experiment (ND) (-6D- option)]** command window.
- Click the **[Upload Ti Recipe]** button to upload the experiment settings to HUB-A.
- Run the acquisition.

When the acquisition is started, the experiment settings are sent to HUB-A where they are converted to a set of hardware instructions. The acquisition is then controlled by these instructions instead of being controlled by the software.

There are the following limitations of the experiment complexity:

- Maximum of 100 XY positions (multi-point dimension) can be handled.
- Maximum of 9 different channels can be handled.
- The total number of HUB-A instructions (after conversion from the experiment definition) must not exceed 1000.

10. Additional Modules

10.1. Database

This chapter will give you an overview of what the capabilities of the database module are.

NIS-Elements AR supports MS Access, MS SQL, and Oracle databases. The capabilities of the database module are following:

- The system can create and control MS Access databases (*.MDB), the images are stored on hard disk, only links to them and other data are stored in the database file.
- The system can connect to and control a running MS SQL or Oracle SQL server database, the images are stored on the server machine within the database. This concept requires a standalone SQL system and is recommended when working with large amounts of data. Administering and back-ups are handled by the SQL server.

Note

*An *.INI file must be created by the [sql_util.exe] tool placed in the installation directory (this is done by the administrator during installation). Such INI file provides the connection between your PC and the remote SQL database. Whenever we talk about the MDB file in this manual, the same applies to the INI file accordingly.*

After you install the Database module, the **[Database]** menu appears in the main tool bar.

10.1.1. New Database

Before you can connect to a new database the structure of tables and fields, or at least the protection level shall be set:

1. Run **[Database > New Database (-DB- option)]** .
2. Select the database engine and locate the connection file (*.MDB to be created, or *.INI file created by the sql_util.exe). Click **[Next]** .
3. Select one of the database templates. Each template consists of several predefined tables. If you choose **[Blank]** , no tables will be created (later you will have to use the **[Database > New Table (-DB- option)]** command). Click **[Next]** .
4. Select the default protection of the database. The user accounts can be based on MS Windows accounts, or arbitrary number of database user accounts protected by password can be created. Or, the database can be fully accessible for all users - if the **[Not Protected]** option is selected. Click **[Finish]** .
5. The database connection will be automatically created and connected. You can browse the database with the **[Organizer]** .

10.1.2. New Connection

If there is an existing database created by NIS-Elements AR, you can connect to it via the **[Database > New Connection (-DB- option)]** command:

1. Run **[Database > New Connection (-DB- option)]** . A window appears.
2. Select the correct database engine and locate the corresponding connection file (MDB or INI).
3. Confirm the action with **[OK]** .

Connections to databases can be managed via the **[Database > Manage Connections (-DB- option)]** command.

10.1.3. Database Tables

A new table can be added to an existing database. Every database must contain at least one table. If you have created a blank database or you would like to add a table to any of the connected databases, do as follows.

1. Run **[Database > New Table (-DB- option)]** . A window appears.
2. Select one of the connected databases to which the table will be added.
3. Select one of the table templates. Table templates can be imported from the other connected databases. If you choose **[Blank]** , you will have to create the table structure from scratch (in the next step). Click **[Next]** .
4. Define the field properties in the window that appears.
5. **[Finish]** the table creation.

10.1.4. User Permissions

Database access rights can be set for individual users. As we mentioned above, there are two types of protection:

Password protection

Any number of database user accounts can be created. Then you can connect to a database by the user name and password.

Windows account protection

User permissions to databases can be set for different MS Windows users. The access to a database is then granted if only the user is currently logged in Windows.

Both protection types can be combined in one database. To manage the user accounts and permissions of a connected database:

Note

You have to be connected to the database under an account with sufficient user rights.

1. Run **[Database > User Accounts and Permissions (-DB- option)]** . A window appears.
2. Select the database of your interest. All user accounts of the database will appear in the list below.
3. Create new or select an existing user account.
4. Use the buttons on the right to manage the account properties, duplicate it (along with the permissions settings), change the password, modify the permissions (**[Change properties]**), or even delete it.

10.1.5. Database Backup

If you run one of the supported SQL server databases, it is a task for the database administrator to make backups (or it is usually configured to make the backups automatically). However, if you utilize the MS Access database engine, the NIS-Elements AR backup scheduler shall be used.

The capability to backup the database is essential for serious work. The Database Backup Scheduler enables to backup the MS Access 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 successfully: The computer must be turned ON at the scheduled time. Configure the backup via the **[Database > Schedule Database Backup (-DB- option)]** command.

10.2. Extended Depth of Focus

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.

When you start NIS-Elements AR with the EDF module installed, the following items appear in the **[Applications]** menu:

- **[Applications > EDF > Open File Sequence (-EDF- option)]**
- **[Applications > EDF > Align Sequence (-EDF- option)]**
- **[Applications > EDF > Create Focused Image (-EDF- option)]**
- **[Applications > EDF > Edit Area in Focused Image (-EDF- option)]**
- **[Applications > EDF > Edit Background (-EDF- option)]**
- **[Applications > EDF > Real Time EDF (-EDF- option)]**
- **[Applications > EDF > Real Time EDF Manually (-EDF- option)]**

Open (or acquire) an ND2 document that includes the Z dimension. If you use the **[Applications > EDF > Open File Sequence (-EDF- option)]** command to get the Z stack of images, a new ND document is created automatically.

10.2.1. 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 **[Applications > EDF > Align Sequence (-EDF- option)]** command to automatically correct possible shifts.

10.2.2. Create the focused image

When the method is selected and the sequence is aligned, the only thing to do is to run the **[Applications > EDF > Create Focused Image (-EDF- option)]** command. The focused image will be created and appended to the ND2 document.

10.2.3. View the result



After the focused image is created, all buttons of the EDF tool bar (located within the document tool bar) become enabled. There are three ways to observe the resulting focused image, as:

- A common Color/Gray image
- An anaglyph - to be viewed using 3D glasses.
- A 3D model, utilizing so called Surface View.

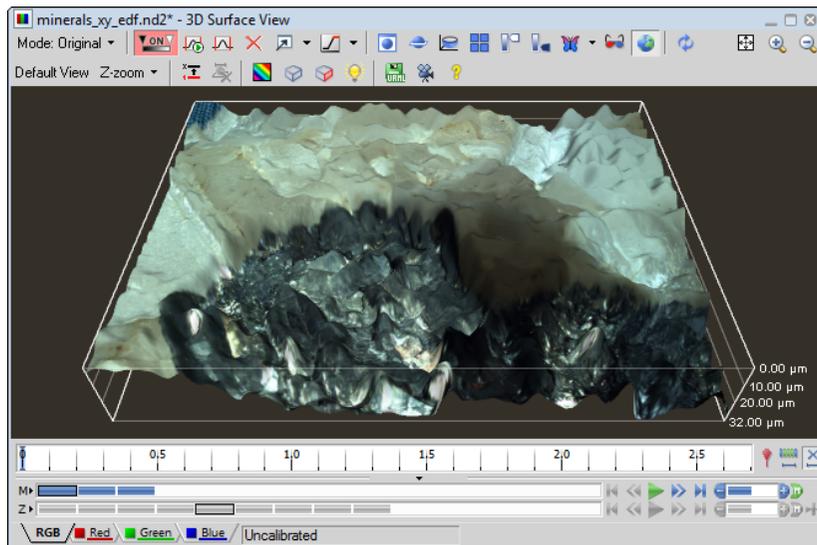
 This button displays the focused image.

Creating anaglyph

 Using this button, you can create a three-dimensional stereo image (anaglyph). This image shall be observed with special glasses having red and green or blue filters which provides the 3D impression.

Exploring the surface view

 This button runs the built in surface viewer. The surface viewer displays the surface of a captured object three-dimensionally. You can rotate the view by mouse or shift it by right-mouse. The mouse wheel serves for zooming.



Special Options

Default View

Displays the object in default rotation and zoom.

Z-zoom

Adjusts the sequence height for more credible look.



Top/Bottom First

Enables to set whether the first frame in the sequence is the lowest or the highest one.



Select background Color

Enables to choose color of the background.



Show Crop Plane

Enables you to crop virtually cut the surface by a plane. Press **[Ctrl]** to display the cropping plane and the axis of rotation. Hold the Ctrl key down and drag the cropping plane or the axis of rotation with left

mouse button to a new position. Press **[Ctrl]** and drag the plane by right mouse button to rotate the cropping plane.



Toggle Scene Lighting

Switches on/off the lighting of surface view.



Export to VRML

Exports the surface to VRML (Virtual reality modeling language). Two files will be saved. One that contains the 3D information (WRL) and the other 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> [<http://ovrt.nist.gov/cosmo>]
- **[Cortona]** - <http://www.parallelgraphics.com> [<http://www.parallelgraphics.com>]



Movie Maker

Runs the Movie Maker. See [Movie Maker (-LO- option)].

10.2.4. 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 **[Applications > EDF > Edit Area in Focused Image (-EDF- option)]** command. The cursor changes.
- Draw an area inside the focused image which should be affected. Finish drawing by right-click.

Note

The selection can be inverted by pressing [G].

- Use the mouse wheel or arrow keys to browse Z slices within the selected area.
- Finish the procedure by right-click or **[Enter]** .

10.2.5. Real Time EDF

The **[Applications > EDF > Real Time EDF (-EDF- option)]** command is integrating the whole functionality of EDF. It captures a Z-sequence, aligns the images (optionally), and creates the focused image. When the **[Applications > EDF > Real Time EDF (-EDF- option)]** command is invoked, the **[Capture Z Series]** window appears with the **[Align images]** check box added. There you can set whether to align the images or not.

If the **[Applications > EDF > Real Time EDF Manually (-EDF- option)]** command is used, not requiring to possess a motorized Z-drive, the **[Acquire > Capture Z Series > Capture Manually]** 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.

See [Z-series Acquisition].

10.3. Deconvolution (-LO- option)

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

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

The better we know the PSF parameters, the better the de-convolution results will be. The best way to know the PSF is to the image of a nano-bead and use it to represent the PSF shape.

Note

The background value of the PSF (nano-bead) image must be 0 in order for the deconvolution algorithms to work correctly.

There are the following deconvolution methods available in the deconvolution module all of which can be applied to either 2D or 3D (including Z-dimension) image documents:

10.3.1. Deconvolution (Standard)

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

$$C = A * B + N$$

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

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

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

Resources

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

10.3.2. Blind Deconvolution

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

$$C = A * B + N$$

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

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

$$C = A1 * B1 + N1$$

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

$$C = A1 * B2 + N1$$

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

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

Resources

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

10.3.3. Fast (Wiener) Deconvolution

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

$$C = A * B + N$$

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

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

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

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

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

$$A' = C * BInv$$

Resources

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

10.4. High Dynamic Range (-LO- option)

The High Dynamic Range module brings the functionality of creating HDR images within NIS-Elements AR. The following ways to create a HDR image are available:

Automatic capture

Call the **[Applications > HDR > Capture Reflection Free Image (HDR) (-LO- option)]** command and the HDR image will be captured using computer-estimated settings

Capture

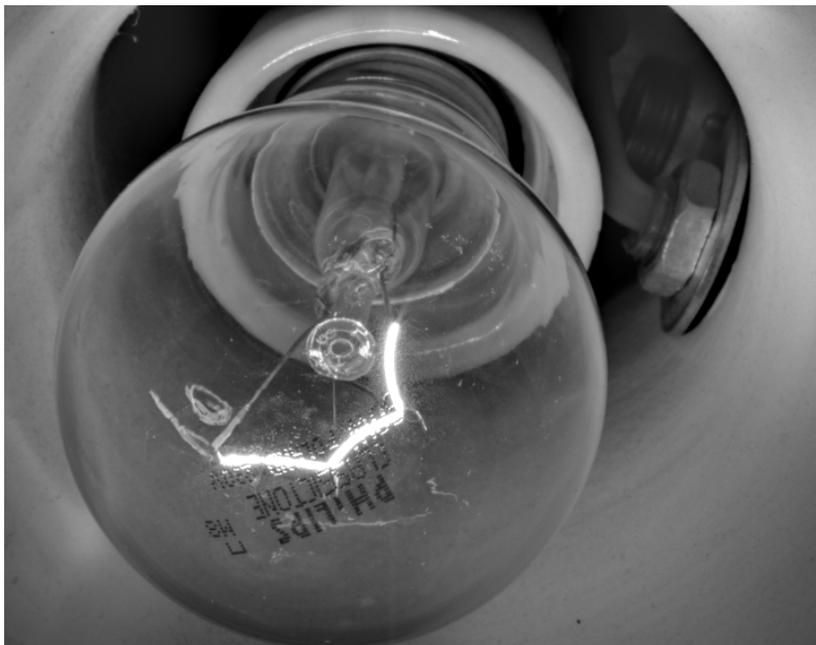
When the **[Applications > HDR > Capture HDR Image (HDR) (-LO- option)]** command is called, you will be asked to adjust the HDR acquisition settings such as number of exposures, exposure time range, camera settings, etc.

Create from files

If a sequence of images which were captured using different exposure times is available, the **[Applications > HDR > Create HDR Image (HDR) (-LO- option)]** command can load the images and merge them into a HDR image.

Create from an ND file

Similarly to the situation when the HDR file is created from a sequence of images, it can be as well created from the frames of an ND2 document. Just run the **[Applications > HDR > Create HDR from ND (HDR) (-LO- option)]** command.



A shining bulb captured with HDR

HDR module background

The HDR module is based on the image fusion technique which considers the edge response, the “well exposed” measure and the “well saturated” measure. The fusion works locally. That means the measures of quality are computed for every single pixel from its neighborhood over the whole picture stack. If we simplify the fusion process, we can say that pixels with the same coordinates are compared throughout the stack of images and the one with the highest quality measure is chosen to be used in the resulting image.

10.5. Deconvolution Module (legacy)

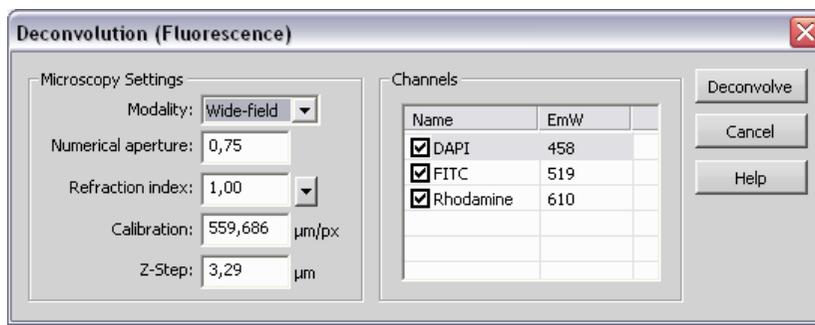
The imperfection of a microscope optical path causes convolution of the original image signal. The convolution makes the resulting image blurry. Deconvolution is a mathematical algorithm for repairing the blurred parts of the image. Some characteristics of the optical path (objective) shall be known in order for the deconvolution module to work properly. The algorithm used is “constrained iterative - maximum likelihood estimation algorithm” (the actual name of the algorithm is “Lucy-Richardson”).

Get a Z sequence of images

Open or acquire an ND2 file containing the Z dimension.

Deconvolve the Z Stack image

Run the **[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 drop-down 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.

Note

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.

- Press the **[Deconvolve]** button.

10.6. AutoQuant Blind Deconvolution Module

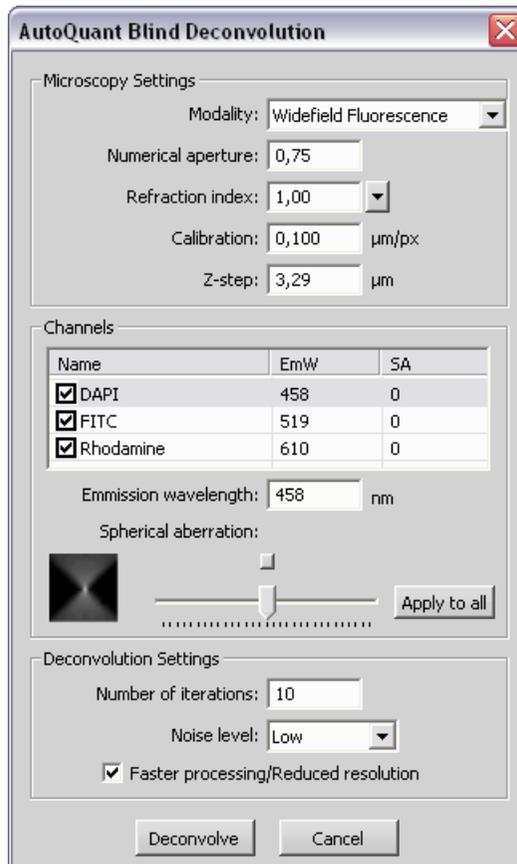
The imperfection of a microscope optical path causes convolution of the original image signal. The convolution makes the resulting image blurry. Deconvolution is a mathematical algorithm for repairing the blurred parts of the image. Some characteristics of the optical path (objective) shall be known in order for the deconvolution module to work properly.

Get a Z sequence of images

Open or acquire an ND2 file containing the Z dimension.

Deconvolve the Z Stack image

Run the **[Deconvolution > AutoQuant Modules > Deconvolution (-DEC- option)]** command and set the deconvolution parameters. 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.



Microscopy Settings

Modality

- widefield fluorescence
- transmitted-light bright field
- 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.

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

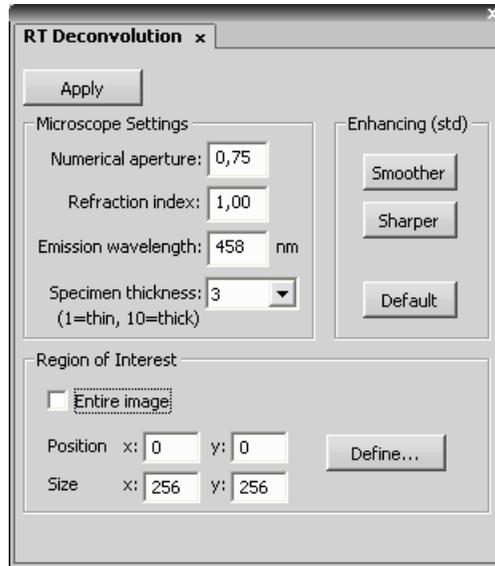
Resolution of the result can be reduced on behalf of the processing speed (the resolution will be reduced temporarily during the processing. Afterwards, the original resolution will be renewed).

10.7. AutoQuant Real-Time 2D Deconvolution Module

The imperfection of a microscope optical path causes convolution of the original image signal. The convolution makes the resulting image blurry. The Real-Time Deconvolution module algorithm enables high speed processing and therefore is very suitable for use on Live signal from cameras. Real-time deconvolution can be applied to the Live image continuously or just at capture-time. It can be also used for processing saved images.

When you start NIS-Elements AR with the 2D Real-Time Deconvolution module installed, the **[RTDeconv]** section appears in the **[Applications]** menu with the **[Deconvolution > RT Deconv > Show RT Deconv-**

lution Setup (Dec) (-DEC- option)] and the **[Deconvolution > RT Deconv > Enhanced Current Image (Dec) (-DEC- option)]** 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.

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.

The **[Enhancing]** portion settings affect the noise amplification vs. image smoothness. The **[Default]** setting is suitable for low-noise images. Press the **[Smoother]** button to decrease the image noise and the **[Sharper]** button to sharpen the image (but allow some noise).

Region of Interest (ROI)

Region of interest specifies the part of the image where the deconvolution will be performed. Reducing the area for the deconvolution will increase its speed. Check the **[Entire Image]** option to deconvolve the entire image. If this option is not selected, you can define the **[Position]** of the top-left corner of the ROI and its size in pixels. Or, the ROI can be drawn to the image by mouse. To do so, press the **[Define...]** button, draw the rectangle to the image and finish the definition by right-click.

“Not calibrated!”

It is necessary to have the image calibrated in order for the deconvolution module to work. If you see this message, you should calibrate the image or the objective you use.

10.8. Metalo - Grain Size Analysis Module

After the module installation, a new item called **[Metallography]** appears in the **[Applications]** menu. Select the **[Grain Size]** command to display the grain size measurement layout.

10.8.1. Features of the Grain Size Analysis Module:

- Measurement of single-phase structures (e.g. Austenite, Aluminium).
- Measurement of two-phase structures (e.g. Ferrite-Austenite, Ferrite-Pearlite).
- Planimetric, Linear, Circular, and Abrams measurement methods.
- Customization of the predefined measurement procedures.
- Support of ASTM and JIS standards (ASTM E112-96, ASTM E1382-97, JISG0551).
- Easy data export.

10.8.2. Quick Guide

- Open/acquire the image to analyze.
- Set the structure type and the detection procedure parameters within the **[Detection]** tab.
- Set the measurement procedure parameters within the **[Measurement]** tab.
- Run the **[Measure > Measure]** command.
- View the measured data within the **[Data]** tab and export it to one of the available outputs via the **[Export]** button. See [Exporting Results].

The analysis can be performed even on two or more images sequentially. After defining all the parameters, run the **[Measure > Measure Sequence]** command.

10.9. Metalo - Cast Iron Analysis Module

After the module installation, a new item called **[Metallography]** appears in the **[Applications]** menu. Select the **[Cast Iron]** command to display the cast iron measurement layout.

10.9.1. Features of the Cast Iron Analysis Module:

- Graphite classification according to ISO/ASTM/JIS standards (ISO 945, ASTM A247-06, JIS G5502).
- Ferrite/pearlite ratio measurement.
- Customization of the predefined measurement procedures.
- Easy data export.

10.9.2. Quick Guide

- Open/acquire the image to analyze.
- Set the detection procedure parameters within the **[Detection]** tab.
- Set the measurement procedure parameters within the **[Measurement]** tab.
- Run the **[Measure > Measure]** command.
- View the measured data within the **[Data]** tab and export it to one of the available outputs via the Export button. See [Exporting Results]].

Note

The analysis can be performed even on two or more images sequentially. After defining all the parameters, run the [Measure > Measure Sequence] command.

10.10. Calcium and FRET module

Installing the **[Calcium and FRET]** module adds several tools to the system:

- A control window suited for capturing FRET images.
- Automated FRET calibration algorithm.
- The **[Create FRET Image]** window for “passive” creation of FRET images from files.
- Calcium ion concentration measurement ability.

What is FRET?

- A non-radiative transfer of energy from a donor molecule to an acceptor molecule....
- Förster/Fluorescence Resonance Energy Transfer
- Fluorescence emission from an acceptor molecule due to non radiative energy transfer when a donor molecule was excited.

Conditions for FRET

- Donor’s emission must overlap acceptor’s excitation
- Donor and acceptor molecules must be <100 Å apart.
- Donor and acceptor molecules’ dipole orientation must be parallel.

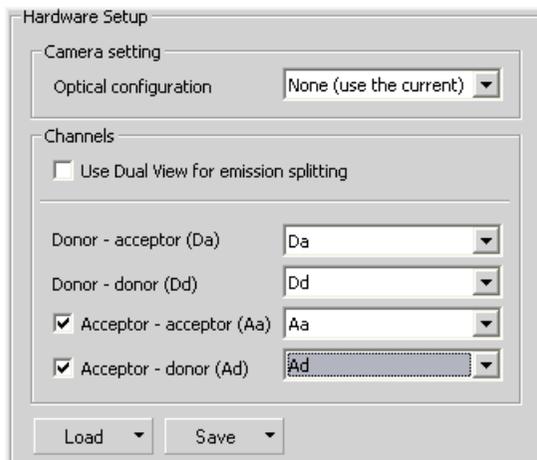
See also ???.

10.10.1. 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.



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

Acceptor - acceptor (Aa): Aa

Acceptor - donor (Ad): Ad

Load Save

Optical Configuration

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.

Note

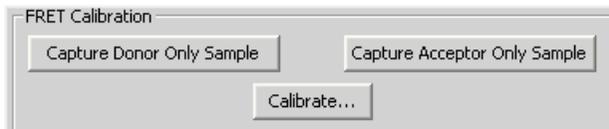
If the Dual View device is available, it can be used for channel splitting. Then, only one optical configuration for each excitation wavelength will be used. The Dual View device splits the emitted light to two beams filtering different wavelengths, and each of them is captured by only a half of the camera chip.

Load/Save

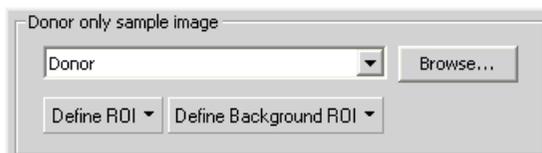
The channels settings may be saved to the system by the **[Save]** button. Once saved under some user defined name, this name appears in the **[Load]** pull-down menu ready to be loaded. The settings can also be loaded from an external FRET image.

FRET Calibration

Separate images of the D-labelled and the A-labelled samples must be 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**].

Note

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 window appears where you should map the image channels to FRET channels and select the FRET method.

10.10.2. FRET Method

In this section, select one of the two available FRET methods.

$$FRET_{CORRECTED} = Da_{FRET} - Dd_{FRET} \cdot \frac{Da_{DONOR}}{Dd_{DONOR}} - Aa_{FRET} \cdot \frac{Da_{ACCEPTOR}}{Aa_{ACCEPTOR}}$$

Sensitized Emission

$$FRET_{CORRECTED} = Da_{FRET} - \left(Dd_{FRET} - Aa_{FRET} \cdot \frac{Aa_{DONOR}}{Dd_{DONOR}} \right) \cdot \frac{Da_{DONOR}}{Dd_{DONOR}} - \left(Aa_{FRET} - Dd_{FRET} \cdot \frac{Dd_{ACCEPTOR}}{Aa_{ACCEPTOR}} \right) \cdot \frac{Da_{ACCEPTOR}}{Aa_{ACCEPTOR}}$$

Specified bleed through

The Xx_{FRET} members are average intensities of assigned FRET image components.

The $Xx_{DONOR/ACCEPTOR}$ coefficients are average intensities of the donor/acceptor-only images.

The $FRET_{EFFICIENCY}$ is calculated by the following formula:

$$EFFICIENCY [\%] = (FRET_{CORRECTED} / Dd_{FRET}) * 100$$

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.

- Donor in FRET (CoB) = Da_{DONOR} / Dd_{DONOR}
- Acceptor in Donor = Aa_{DONOR} / Dd_{DONOR}
- Acceptor in FRET (CoA) = $Da_{ACCEPTOR} / Aa_{ACCEPTOR}$
- Donor in Acceptor = $Dd_{ACCEPTOR} / Aa_{ACCEPTOR}$

10.10.3. Creating FRET image

The FRET image can be created from images saved on harddisk too. Run the **[Applications > FRET > Create FRET Image]** command. A 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.

10.10.4. [Ca²⁺] Ion Concentration Measurement

The **[Calcium and FRET]** module provides methods for an efficient calcium ion concentration measurement. See also ???.

Ratio Experiment Commons

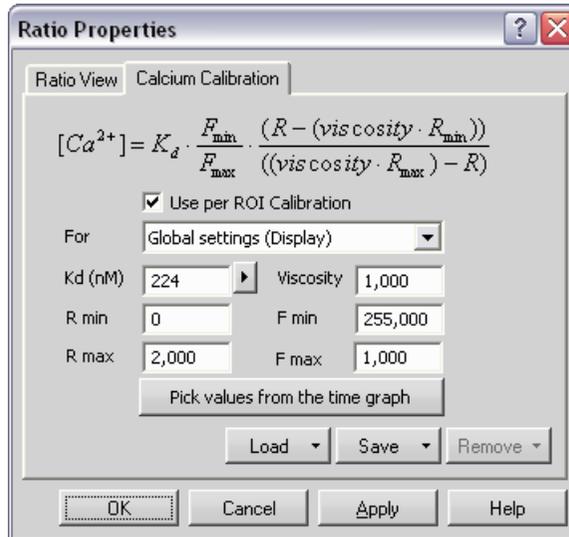
- Usually, **[Fura-2]** ratiometric fluorescent dye which binds to free intracellular calcium is used.
- Fura-2 is excited at 340 nm and 380nm
- Ratio of Emission of 340 and 380 correlated to the amount of intracellular calcium
- The ratio of the emissions at 340 and 380 wavelengths is directly correlated to the amount of intracellular calcium
- Why Use a Ratio? Because it is not as sensitive to changes in:
 - Dye concentration
 - Auto fluorescent background
 - Focus Drift
 - Ambient Lamp

Procedure

- 1) Define ROIs for the Ca²⁺ measurement.
- 2) Perform Time Measurement either during an ND2 experiment or on an existing ND2 file.
- 3) Turn Ratio View ON (**[View > Ratio View]**).
- 4) Go to the Time Measurement window and display the ratio graph in the **[Multi Dyes]**  mode.
- 5) Press the **[Define Calcium Calibration]**  button within the time measurement control window to pick the calibration values from the graph.
- 6) A wizard starts. Follow its instructions - pick the **[Rmin]** a **[Rmax]** values from the ratio graph.
- 7) The **[Ca2+]** graph display becomes available to be displayed in the time measurement window.
- 8) The **[Ca2+]** image channel view is created within the ND2 document. It visualizes the Ca2+ ion concentration via a color scale.

Ratio View Properties - Calcium Calibration settings

If you prefer to modify the Calcium Calibration by hand, run the **[View > Ratio Properties]** command. In the window that appears select the **[Use Ca²⁺ calibration]** check box (or it is already selected if the calibration procedure was performed beforehand). The **[Calcium Calibration]** tab appears.



All constants necessary for calcium ion concentration calculation can be defined manually within the **[Ratio Properties]** window, either for each ROI separately or globally (the **[Use per ROI Calibration]** option). Use the **[Pick values from the time graph]** to pick **[R min/max]** constants from the **[Time Measurement]**

graph - the time measurement must have been performed before. The **[F min/max]** values will be computed automatically.

[Ca²⁺]

Concentration of Calcium in nanoMoles.

K_d

Dissociation constant: 135nM at 20°C, 224nM at 37°C

F_{min}

The 380 intensity in the moment of **[R min]**.

F_{max}

The 380 intensity in the moment of **[R max]**.

R

Ratio of 340/380 intensity at the current time point

viscosity

“Constant” between 0.7 and 1.

R_{min}

The minimum ratio value of the experiment

R_{max}

The maximum ratio value of the experiment

The calibration settings can be saved for further use by the **[Save]** button (and loaded later by the **[Load]** button).

10.11. The Advanced Interpreter (API) Module

This module dramatically extends the macro programming capabilities. It adds the following functionality to the main application:

User interaction functions

Contains functions, which enable an interaction with user like: type value, text, combo boxes, list boxes and many other Windows standard controls with easy and intuitive code handling.

Support of importing functions from external DLLs

Functions from external DLLs can be imported at the start of the macro and used during the macro execution.

