Imaging Software NIS-Elements



Imaging Software

NIS Elements

Advanced Solutions for your Imaging World

Nikon offers a total software solution for image capturing, archiving, and analysis

Why NIS-Elements?

NIS-Elements is an integrated software imaging platform which delivers comprehensive control of microscopes and peripheral devices, image capturing, documentation, image analysis and data management of up to six dimensions. This system contributes to experiment efficiency with intuitive image analysis features and database building capabilities developed to handle archiving and management of large numbers of multidimensional image files. Unified control of the entire imaging system offers significant benefits to users for cutting-edge research, such as live cell imaging.

Total Imaging Solution

Designed to serve the needs of advanced bioresearch, clinical, industrial and documentation professionals, NIS-Elements provides a totally integrated solution for users of Nikon and other manufacturers' accessories by delivering automated intelligence to microscopes, cameras, and peripheral components. The software optimizes the imaging process and workflow and provides the critical element of information management for system-based microscopy.



Highest Quality Optical Performance

The world-renowned Nikon CFI60 infinity optical system has effectively set a new standard for optical quality by providing longer working distances, higher numerical apertures, and the widest magnification range and documentation field sizes. Nikon's new objectives and accessories are specifically engineered for digital imaging.

Diverse Line of Powerful Digital Cameras

Nikon offers a full line of digital cameras, addressing the varied needs of users in multiple disciplines. Each Nikon digital camera is designed to work seamlessly with Nikon microscopes, peripherals, and software. With Nikon Digital Sight (DS) series cameras, even novice users can take beautiful and accurate microscopic images. For the advanced researcher, high resolution image capturing and versatile camera control is fast and simple.



NIS-Elements AR is optimized for advanced research applications, featuring fully-automated acquisition and device control through full 6D (X, Y, Z, Lambda (Wavelength), Time, Multipoint) image acquisition and a wide range of image analyses.

NIS-Elements BR is suited for standard research applications, photodocumentation of fluorescent samples and image analysis, including intensity and counting measurements. It features acquisition and device control through 4D (up to four dimensions can be selected from X, Y, Z, Lambda (Wavelength), Time, Multipoint) acquisition.

NIS-Elements D supports color documentation requirements in bioresearch, clinical and industrial applications, with basic measuring and reporting capabilities.

Main Features

	AR	BR	D SDI
Window style	(Multiple Document Interface)	(Multiple Document Interface)	(Single Document Interface)
Dark color scheme	0	0	_
Industrial simple GUI	—	—	0
Camera control	0	0	0
Microscope control	0	0	0
Nikon made peripheral control	0	0	0
Non-Nikon peripheral control	0	0	0
Live image capture	0	0	0
Time-lapse image capturing (T)	0	0	\bigtriangleup
Z-series image capturing (Z)	0	0	0
Multichannel image capturing (λ)	0	0	—
Multipoint image capturing (MP)	0	0	0
Multidimensional image capturing	Up to 6D	Up to 4D	_
Stimulation experiment	•	—	—
RAM capture	0	—	_
HDR image capture	0	•	•
AVI live-stream capture	0	0	0
Objective calibration	0	0	0
Capturing data savings (Meta-data)	0	0	0
Image filtering	0	Δ	\bigtriangleup
Binary	0	Δ	
LUT (look up table)	0	0	0
Histogram	0	0	0
Manual measurement	0	0	0
Auto measurement	0	0	۲
Intensity line profile	0	0	0
Intensity surface plot	0	0	0
Time (intensity) measurement	0	•	—
3D measurement	•		
Volume measurement	0	—	_
Database	•	•	•
Macro	0	\bigtriangleup	\bigtriangleup
Advanced interpreter	0	•	•
Report generator	0	0	0
Live compare	0	•	•
Volume view	0	Δ	\bigtriangleup
EDF (Extended depth of focus)		•	•
3D surface view	•	•	•
Ratio view	0		_
2D real time deconvolution	•	—	_
2D deconvolution		-	—
3D deconvolution	•	_	
3D blind deconvolution	•	_	_
TTL/analog IO	•	•	•
Object classifier		-	—
2D/3D object tracking	•	—	_
Calcium & FRET		-	—
General analysis	•	_	
HC Template	•	-	_
JOBS Editor	•	-	
N-SIM analysis	•	_	_
N-SIM offline analysis	•	—	
N-STORM analysis		-	_
N-STORM offline analysis*		—	
Metalogical analysis	-	—	•
Illumination sequence	•	_	_
Stage incubator control		_	—
Dual camera support	•	_	_

 \bigcirc : Full function △ : Limited function —: Not available ● / ▲ : Option

* N-STORM analysis is required.

Image Acquisition

NIS-Elements offers the most suitable image acquisition for various applications with the integrated control of the camera, motorized microscope and peripheral devices.

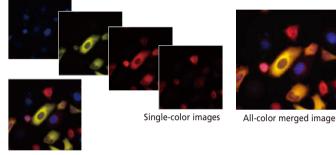
Br

Z-series

Multichannel (multi color)

NIS-Elements can acquire full bit depth multi-color images, combining multiple fluorescence wavelengths and different illumination methods (DIC, phase contrast etc.), while offering independently scalable channels.

Optical Conf.		Name	Comp. Color	T Pos.	
DAPI	-	DAPI		Al	1
FITC	-	FITC		Al	
TRITC		TRITC		Al	
OIC		DIC		AI	



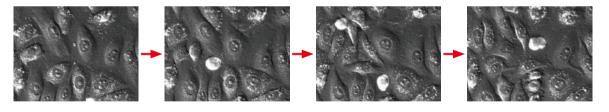
Specified-color merged image

Time Lapse

Time lapse imaging in NIS-Elements is easily configurable simply by setting the time interval and duration of capture.

The Perfect Focus System of the motorized inverted microscope Ti-E enables high-accuracy image capture without focus drifting even during extended time time-lapse experiments.

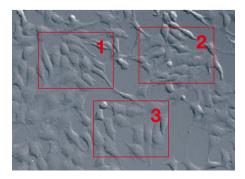
Phase	Interval	Duration	1	Loops
# #1	10 min	24 hour(s)		145
1				



Multipoint Experiments

NIS-Elements' motorized stage control offers automated travel to multiple stage points of the sample of a multi-well plate or dish. Stage points are memorized and can be saved and loaded for future imaging sessions.

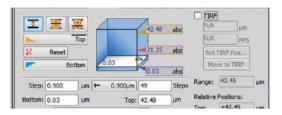
Point Name	X [mm]	Y [mm]	Z [um]		PFS
#1	2,425	0.356	1.48		4125
✓ #2	1.200	0.248	1.23		4152
🖌 #3	0.567	0.862	0.53		4222
#4	1.600	0.248	0.83		4123
¥ #5 ->	1.542	0.887	<-	Offset All X,Y,Z	4 <

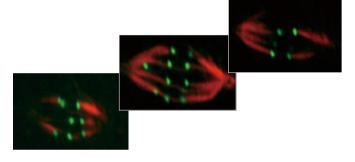




Ar Br D

Through motorized focus control, NIS-Elements reconstructs and renders 3D images from multiple Z-axis planes.





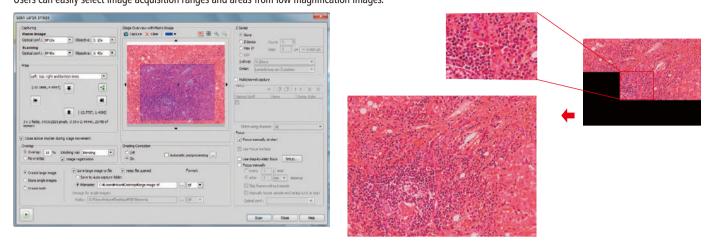




Rr D

Image Stitching (Large image)

Large Image Acquisition generates a single high-magnification wide field-of-view image by automatically stitching multiple adjacent frames from a multipoint acquisition using a motorized stage or from multiple single images captured from a previous session. Users can easily select image acquisition ranges and areas from low magnification images.



Option (Ar Br)

Multidimensional imaging

ND Acquisition

NIS-Elements captures images in a combination of multiple dimensions such as Time-Lapse, Multichannel, Z-series, and Multipoint. It is also possible to create and manage the acquisition of a multi-dimensional dataset with a thirty-minute time lapse of two wavelengths and a Z series across each well of a multiwell plate.

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ND Simultaneous Stimulation

NIS-Elements enables image acquisition during photo stimulation.

Apperment: IDStmulation Save to File Path: CVProgram Files/ht/5 Elenents/Kimages/ Browse Filename: rd010.nd2 Record Data Time schedule (Mono Sin / DirTs). Shutter Device) Acquisition: Interval Jouration Loops Vio delay 10 min Stimulation/Bleaching: Wait Position Duration Sec Static 2 sec [5 M	1				
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Filename: md010.nd2 Record Data Thre schedule (Hono Sm / D/TSL Shutter Device) Acoustion: Interval Duration Loops No delay 10 min V ??? Stimulation/Sleaching: Wait Position Duration 3 sec V Static V 2 sec [Save to	Fle						
Time schedule (Mono Sim / D/TSL Shutter Device) Acoustion: Interval Duration Loops No delay 10 min ¥ 777 Stimulation/Sleaching: Wait Position Duration 3 sec ¥ Statc ¥ 2 sec	Path:	C:¥Progra	m Files¥NIS-EI	ements¥Imag	es¥		Browse	
Acquisition: Interval Duration Loops No delay 10 min ¥ ??? Stimulation/Bleaching: Wat Position Duration 3 sec \$ State \$ 2sec [Filename:	nd010.nd	2			Reco	rd Data	
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Perform Time Measurement (0 ROIs)	3 sec		State		Zsec			
	Perform	Time Meas	rement (0 RO	ls)				



ND Sequential Acquisition

ND Stimulation

NIS-Elements allows various sequential imaging experiments to be combined with other functions, such as simultaneous photo stimulation and imaging, or

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	Action	Description	
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	ND Accusition	 Time(50 loops), 	
	Seq. Stimulation	Acquisition 10 x	1s, Stimulation 30c Defne
#3 #4		Time(20 loops),	Multipoint(3), Lambd Define
#3 #4	ND Acquisition		

* Available dimensions vary depending on the package.

Option (Ar)

NIS-Elements controls photo stimulation and image acquisition.

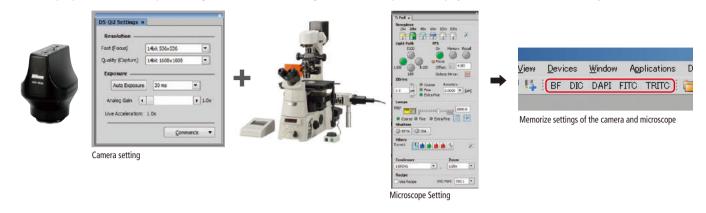
mmenti NDSti 25 30 35 Brows .ndz Record Data.. no Sm / INTSL Shutter Device) + (1 + + 1 × × Duration Loops Accuston Interval Durator Accuston Isec Isec Stimulation No delay Sec Accuston No delay 30 sec • 2 • 11/A • 777 #2 erform Time Measurement (0 ROIs se Active Shutter when Id-Advanced >> Load * Save * Kannoun* # Runnow

Option (Ar)

Optical Configuration



Presets or 'Optical Configurations' can be saved for each observation method such as FITC fluorescence and DIC imaging, memorizing the settings of the microscope, camera and peripheral devices. The optical configurations are created through a one click set up and are displayed as icons in the tool bar for easy access and use.



Movie Capture, Fast Image Capture

NIS-Elements has several options to observe and capture a sample's change and fast movement.

Fast Time Lapse

Fast Time Lapse is designed for ultra high-speed cameras. The hard disk drive can be used together with PC memory to enable a longer acquisition time.

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		Memory and Disk	
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RAM Capture

RAM Capture allows for acquisition at the fastest possible rate of the camera. A RAM buffer is utilized to enable capture and retrieve a high speed time lapse, which aids in the capture of fleeting events such as calcium sparks, motility and translocation.

AVI Live-Stream Capture

AVI Acquisition automatically captures live data into an easily exportable and viewable AVI format.

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		kdvanced>	>
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Rate: Max Fps -	Duration:	Infinite	

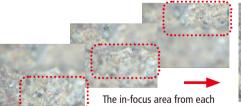
Option (Ar Br D)

Extended Depth of Focus (EDF)



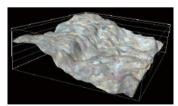
With motorized focusing

The EDF function selects the in-focus area from multiple Z-stack images, and produces one all-in-focus image. The composite image can be viewed and rotated as a virtual 3D image, as it contains Z-axis information.



frame is used to produce one all-in-focus image.

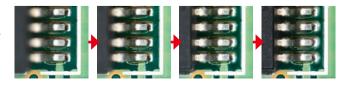






With manual focusing

An all-in-focus image is created in real-time in synchronization with the rotation of the focus knob. While the focus is manually adjusted, the in-focus areas of the image at different depths are successively captured and combined for the EDF image.



Br



High Speed Device Operation by Triggering

*Some cameras and devices are not compatible with these functions. For more information, please contact Nikon or an authorized representative.

Triggered Acquisition

Triggering external devices directly from the camera enables synchronized control of various devices such as the laser unit without passing through the PC. This allows for the fastest performance of the system components for multi-wavelength excitation in TIRF observation.



Triggered Acquisition x DIL-807 • Camara Multi-Excitation Device . ILI4A Lambd Setur 4 Line Exposure Focus Offset Image: State of the state o 0.0.0 ***** 33 ms 10 % 0 µm 15 % 33 ms 0.8 un 🗹 # 3 Line: 640 m Use different exposures Advanced Settings Enable Triggerin

Ti-recipe

This function enables the HUB-A controller of the motorized inverted microscope Ti-E to control both image acquisition and the fluorescent shutter by directly connecting the camera and a HUB-A controller without passing through a PC. As a result of optimizing the communication times of all connected devices, acquisition times for multi-dimensional datasets are reduced.





Camera exposure signal is connected to DSC port for faster experiments:	DSC1	-
Ti-recipe setting window		

NI-DAQ Control

Option (🗛 📴)

TTL and analog signal input/output with NI-DAQ Control enables experiments using various triggerable devices. Device triggering minimizes imaging time lag when used to control a Piezo Z device, shutter and wavelength switching, as it performs at the faster rate of the device without software communication delay. The calibration of analog signal output from devices to such as temperature enables data to be measured and observed during experiments.



Shutter, Piezo Drive, Light Source, Filter Wheel, etc.

Illumination Sequence Option



This module provides an easy-to-use graphical user interface for setting and running advanced real-time (triggered) acquisition experiments.

It is a universal interface for camera-to-device triggering. An item drag-drop function makes it easy to create any complex illumination or stimulation procedure in a matter of minutes.





Shutter, Piezo Drive, Light Source, Filter Wheel, etc.

Available devices		Installed devices
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Pree resourcest Analog inputs Analog Outputs TTL I/O: 3	17	Configure
Triggering		
PCI-6221 (Dev1):	/Dev1/PFI0 -	
PCI-6713 (Dev2):	/Dev2/PFID	
PCI-6723 (Dev3):	Dev3/PFI0	Close

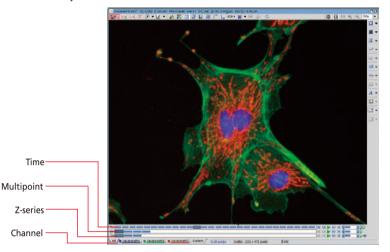
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Display and Data Processing

Various methods are available for displaying and processing captured images and datasets.

Multi-dimensional Image Display

NIS-Elements displays time lapse, multi-channel, multiple X, Y, Z positions in an intuitive layout, which allows for automatic playback and the ability to select subsections of the data to be saved as a new file.





Merge Channels

Multiple single channel images (ex., two from three-channel acquisition images) can be merged together to create an overlay of full depth separately scalable images. With AR and BR, images can be merged by simply dragging the tab of one image onto another image. With D, images are merged by selecting each image for red, blue, green and brightfield channels.



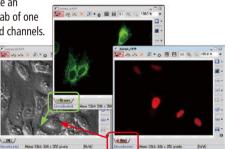


Image Processing

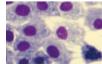
Image Filtering, Color Adjustment

*Usable functions vary depending on the package.

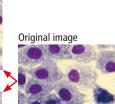
With NIS-Elements image processing tools, it is possible to modify image display and feature extraction using various filters for, for example, sharpness, smoothing and detection. White balance and RGB/HIS balance adjustment are additional available options.



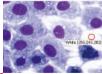




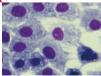
Smoothing







Color adjustment



RGB balance adjustment

Ar Br Option (D) Arithmetic operation (Image arithmetic)

NIS-Elements enables arithmetic operations such as addition, subtraction, multiplication and division on an image or between multiple images. Arithmetic operation between multiple images is also possible.



Arithmetic operation (Image averaging)

NIS-Elements reduces the noise of an image by averaging multiple sequential images such as time-lapse images. Rolling averaging that does not reduce frame rate is available as well.

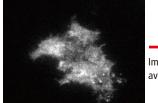
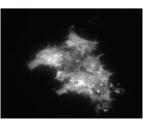


Image averaging



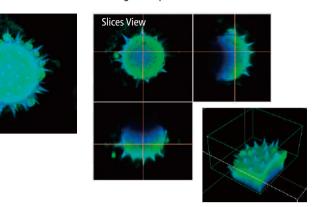


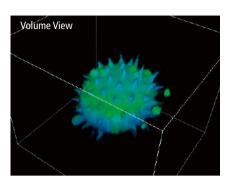
Z-Series Image Display (Volume View)

Projection

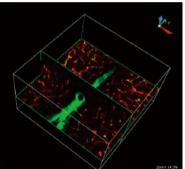
* Volume view and slice view are only possible with AR and BR.

Z-series images can be displayed in various formats such as max. and min. projections, X-Z axis and Y-Z axis cross-sectional slice view and 3D volume view. Rotatable 3D volume rendered views from 3D datasets are easily converted to an AVI or MOV format for file sharing and export.



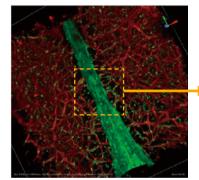


A combination of an AR package and a graphic board that supports DirectX 10 or later enables higher and more complex image display than ever.





Cut Plane XY+YZ



High Resolution Display Mode

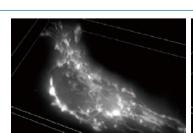
Deconvolution

3D Deconvolution

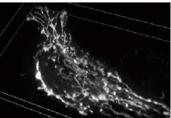
Haze and blur of the acquired fluorescence image can be eliminated. By reassigning out-of-focus intensities back to the spatial locations to where they originated, the intensity of the image is kept and allows for quantitative analysis. Algorithms for wide-field fluorescence, point-scanning confocal and spinning-disk confocal images are available.

2D Deconvolution

The 2D deconvolution module can be applied to a live image or an already acquired dataset. The module also allows the elimination of out-of-focus blur from live images and multidimensional images.

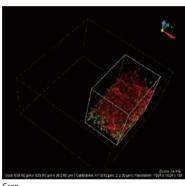


Before deconvolution



Option (Ar)

After deconvolution



Crop

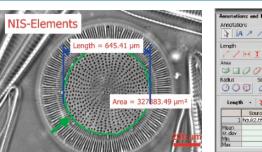
Mouse Brain Sample mounted in RapiClear 1.47 (SunJin Lab Co., Taiwan) to render the tissue optically clear.

Cut Plane Any

Measurement and Analysis

Manual Measurement (Interactive Measurement) and Image Annotation

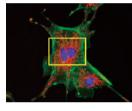
Interactive Measurement allows easy measurement of length and area by drawing lines or an object directly on the image. The results can be attached to the image, and also exported as text or to an Excel spreadsheet. Annotations such as arrows, circles, squares, text are also available display options.

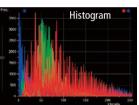


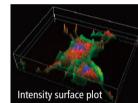


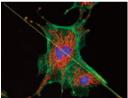
Histogram/Intensity Line Profile/Intensity Surface Plot 🛛 🖬 🖻

Histogram measurement measures the intensity distribution of pixels across the whole image or a defined region. An intensity line profile measurement shows the intensity distribution on a defined line. The Intensity Surface plot shows the intensity distribution of an image with the height of the *z*-axis line.





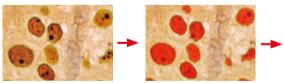


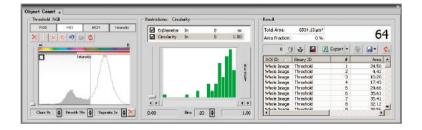


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Auto Measurement (Object Counting) **M D** Option ()

Auto measurement measures the number or area of objects which are extracted from images by the creation of a binary layer through thresholding using RGB/HIS or intensity values. The results can be listed or exported as text or an excel file. It is possible to save and reuse thresholding parameters.





ROI Statistics

Ar Br

*Usable functions vary depending on the package. Common pixel measurements such as area, maximum or minimum intensity are possible with the user defined ROI

(Region Of Interest). ROI or multiple ROIs statistic results for a single image or a multi-dimensional dataset are displayed and easily exported as text or an excel file.



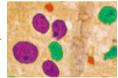
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Feature	ROI	Binary
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Mean Intensity	120.54	0
Min Intensity	63.00	Ċ
Max Intensity	206.00	0
Sum Intensity	5287736.00	0
StDev Intensity	28.36	0
Signal/Background	3.14:1	0

Classifier

Object Classifier

Option (🔤)

Object classifier uses objects identified by thresholding along with additional features such as shape factors, and other statistical methods including nearest neighbor and neural networks for

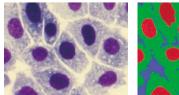


classifying objects into multiple categories. It is also possible to teach the module based on interactive 'picking' of image pixels.

Pixel Classifier

Ar Br Option (D)

This function classifies each pixel in the image with RGB/HIS and intensity across the whole image. Results are reported in percentage and it possible to save and reuse parameters across a large sample of images. Multiple binary layers are also displayed with multiple colors on the image and are available with other analysis tools within the software package.





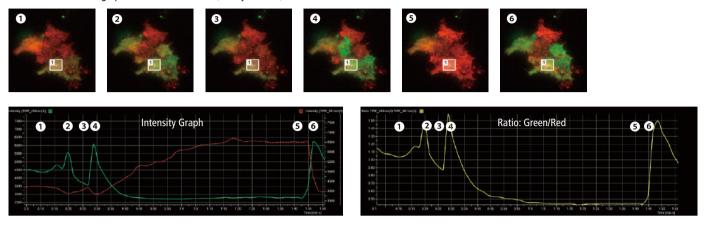




Ar Option (Br)

Time (Intensity) Measurement

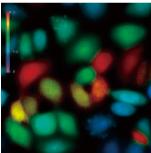
Time measurement creates a graph of sequential intensity changes while time-lapse imaging or from captured time-lapse images. Ratio view function* allows the measurement of the ratio of two wavelengths across multiple ROIs and shows the ratio value by pixel. Numeric data and graph images are exportable and the measurements on the graph are available as well. (* Only with AR)



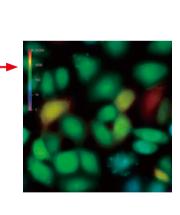
Calcium & FRET

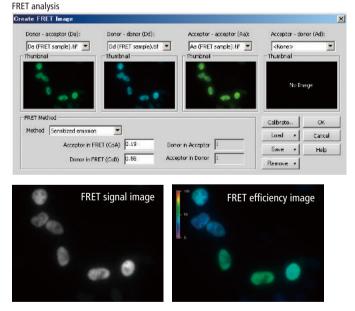
Option (Ar)

Ca²⁺ ion concentration calibration of the ratiometric fluorochrome Fura2, for example, is available using an easily configurable wizard. Corrected FRET image and FRET efficiency, reported in percentage is also available using three filter sets (three types of excitation–fluorescent combination: "Donor – Donor," "Acceptor – Acceptor" and "Donor – Acceptor") and two bleed-through factors.



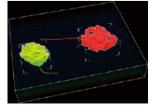






2D/3D Object Tracking

Tracking of an object utilizes the threshold of objects over time and produces measurements such as velocity, acceleration, and distance from a specified origin. The tracking module offers both automated tracking and manual tracking methods.



Index	Time [s]	Position X [µm]	Position Y [µm]	Position Z [µm]	Distance [µm]	Velocity [µm/s]	Acceleration [µm/s ²]	Path Length [µm]	
1	0.000000	32,980999	25.647466	0.000000	0.000000.0	N/A	N/A	0.000000.0	
2	60.000000	33,054185	25 596928	0.000000	0.088940	0.001482	N/A	0.088940	
3	120.000000	33.228145	25.628420	0.000000	0.176788	0.002946	0.000024	0.265728	
4	180.000000	33.343192	25,686851	0.000000	0.129035	0.002151	-0.000013	0.394762	
5	240,000000	33,578441	25,746873	0.000000	0.242785	0.004046	0.000032	0.637548	
6	300.000000	33,593850	25.772921	0.000000	0.030265	0.000504	-0.000059	0.667813	
7	360,000000	33,688604	25,827052	0.000000	0.109126	0.001819	0.000022	0.776939	
3	420.000000	33,790325	25.769797	0.000000	0.116727	0.001945	0.000002	0.893666	
9	480,000000	33,917277	25,782998	0.000000	0.127637	0.002127	0.000003	1.021303	
10	540,000000	33,971434	25,746410	0.000000	0.065357	0.001089	-0.000017	1,086660	
11	600,000000	33,983979	25,602417	0.000000	0.144538	0.002409	0.000022	1.231198	
12	660,000000	34,190742	25,544193	0.000000	0.214804	0.003580	0.000020	1,446003	
13	720.000000	34,409811	25.577152	0.000000	0.221535	0.003692	0.000002	1.667538	
14	780,000000	34,521361	25,500699	0.000000	0.135235	0.002254	-0.000024	1,802773	
15	840,000000	34.692465	25,624591	0.000000	0.211248	0.003521	0.000021	2,014021	
16	900,000000	34,756459	25.641291	0.000000	0.066137	0.001102	-0.000040	2.080158	-
•									۱

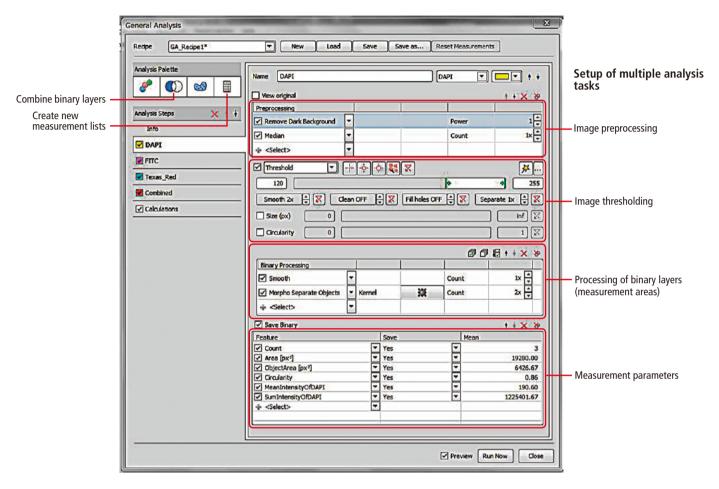


GA (General Analysis)

Option (Ar)

General Analysis streamlines the setup of image analysis combining functions, such as image preprocessing and thresholding, processing of binary images and features, and measurement settings.

- GA creates a new measurement area by combining multiple binary layers, and creates a new measurement parameter by applying these custom measurement settings.
- Each setting can be stored as a recipe that can be rerun for routine analysis under the same conditions across multiple datasets.

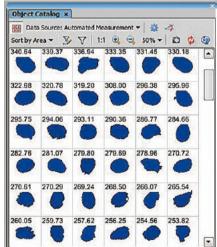


Output of analyzed results

Automated Measurement results

Current fi 199 obje		S	tore Data		Curr Store	UDject Dat	a 💽 🕺	× 1		E Exp	ort • 🛪	¢ @	9
Drag-sta	1		heenim	n/here	to orace						199 objects	, current field	i (µm
4>	\$0	F	ObjID	ND.M	A T	MeanIntensity	Circularity	Length	Width	MaxFeret	MeanPh	MinFeret	E
1	New	1	12	1	186.01	1285.19	0.874	20.28	9.17	20.28	N/A	11.59	
116	New	1	132	1	185.79	1259.96	0.853	19.97	9.30	19.97	N/A	12.68	
176	New	1	200	1	185.06	1090.23	0.396	32.67	5.66	19.57	N/A	14.22	
10	New	1	21	1	184.32	1266.93	0.818	20.37	9.05	20.37	N/A	11.41	
37	New	1	48	1	182.73	1269.58	0.617	22.30	8.20	20.63	N/A	14.28	4
20	New	1	31	1	182.41	1255.16	0.829	17.90	10.19	17.90	N/A	13.88	
26	New	1	37	1	182.20	1282.40	0.906	18.22	10.00	18.22	N/A	12.89	1
	blew.		22		101.04	1797.55	0.920	71.07	0 70	71.97	AL/A	10.65	_
Mean	a she have				214.14	1230.37	0.817	19.85	10.24	20.05	N/A	13.77	
St.dev					100.77	60.90	0.125	5.33	3.15	5.24	N/A	3.84	
Min					4.86	1024.16	0.336	3.10	1.51	3.10	N/A	2.15	
Max					719.41	1314.45	0.989	37.24	20.49	36.41	N/A	26.63	
	1							123	-				

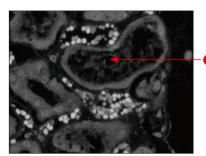
Object catalog



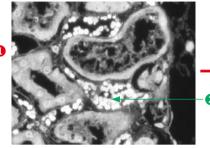
Others

HDR (High Dynamic Range) Image Acquisition

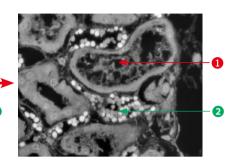
HDR creates an image with appropriate brightness in both the dark and bright regions in a sample by combining multiple images acquired with different exposure settings. It is also possible to create HDR image using multiple captured images.



300-msec exposure: **1** area is underexposed



600-msec exposure: 2 area is overexposed

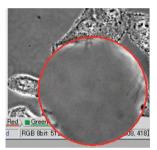


HDR image: captures both () and () areas with optimal exposure

Background Compensation



Background correction uses previously captured images to correct uneven background brightness while imaging or of captured images.



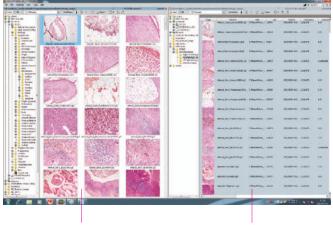
Before compensation

After compensation

Database



Using the organizer function, captured images are displayed in thumbnails for easy retrieval of the desired image. By simply clicking on the thumbnail image in this view, the image is easily opened. Sorting and filtering this database of images and datasets using acquisition details such as objective settings, date and author is an easy method for data management as well.



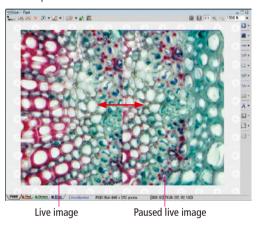
Organizer

Database

Live Image Comparison

Ar Option (Br D)

Live Compare enables easy image comparison between a sample image and a live image. Live observation side by side with a paused live image is also available in split screen mode.



Report Generation

Images captured with NIS-Elements have information such as acquisition details and analysis results, allowing export and PDF conversion of the image and the associated image header and data information.

E 0 4 8 8 5 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Measurement P	rotocol
4	for Papel of 2 James 1321	

User Rights/Control

For safe system management, it is possible to individually limit each user authorization using the user account of Windows[®] (such as the Administrator or Guest) or the user account of NIS-Elements. It limits the authorization and modification of the device settings (microscopes, cameras or others), optical configuration and layout editing.



MS Elements



High-Content Acquisition and Analysis

HC Template for the High Content Analysis System





The HC Template is a dedicated software module within NIS-Elements.

- It allows fully automated acquisition and analysis of a large number of high-content, multi-dimensional images with integrated control of the high-speed motorized focus and stage of Ti-E motorized inverted microscope, camera and peripheral devices.
- HC Template within NIS-Elements allows for quick experimental setups with several autofocusing options and an immediate view of measurement data well by well during acquisition and via a heat map for trend observation and further analysis.



- The microscope-based High Content Analysis System combined with the Ti-E offers a wide range of interchangeable options, including a full range of camera models, such as high-speed and high-definition cameras, as well as a choice of magnifications and fluorescence filters.
- High-speed peripheral devices are optionally available to boost throughput of integrated devices. These include the high-speed piezo Z-objective positioner for fast autofocusing and the automatic plate loader for auto analysis of multiple well plates.

High Content Analysis System

Microscope	Motorized inverted microscope ECLIPSE Ti-E
Compatible well plate types	6-, 12-, 24-, 48-, 96-well plates, 384-well plate (for fluorescence observation)
Multiple FOV experiments	Center, Covering, Random, Random+Center and Regular pattern in each well
Illumination methods	Fluorescence, Phase contrast, DIC
Image acquisition	Multichannel, Time lapse, Multidimensional imaging
Acquisition speed	2 min/96 well plate (1 point/well, 30 msec/shot, without Z stacking, PFS (Perfect Focus System))
Data visualization	Plate view, Sample labeling, Heat map, Graphing
Recommended objectives	S Fluor 4x, Plan Fluor 10x, S Plan Fluor ELWD 20xC/40xC (phase contrast objectives can also be used for image acquisition)
Recommended imaging devices	Camera : DS-Qi2 (Nikon), Zyla sCMOS (Andor technology), iXon3 DU-897 (Andor technology) Confocal microscope : A1+, A1R+, C2+ (Nikon)



High-speed Piezo Z-objective Positioner (Option)

For fast autofocusing

	-
Z travel range	Up to 200 µm
Z travel speed	30 msec
Repeatability	±5 nm
(Physik Instrumente (P	I) GmbH & Co. KG)

Ĵ.

Automatic Plate Loader (Option) For automatic imaging with multiple well plates

manuple wen plates	
Number of well plates	Up to 20 plates
Loading speed	30 sec/plate
Barcode reader option	1D and 2D barcode
(Prior Scientific Instruments Ltd	d.)

Job Wizard - Fixed Sample

Fixed Sample



- 23

Sequential HC Template workflow from acquisition to analysis

Experimental setups

The wizard format simplifies settings for each experimental parameter. It also enables sequential operational settings, such as well plate definition, acquisition patterns in a well, wavelengths, autofocus and image analysis.

> Define general job parameters Z-stack

- Sample labelling
- Autofocus
- •Sending task completion by e-mail or SNS

Well plate setting

- Define well plate to use
- Select well plate for image capture • Define XY image capture pattern inside a well

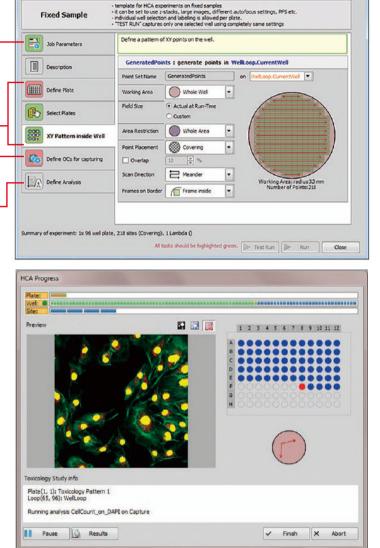
Define optical configurations for image capture

Define analysis

(Set the custom image analysis using General Analysis on page 12.)

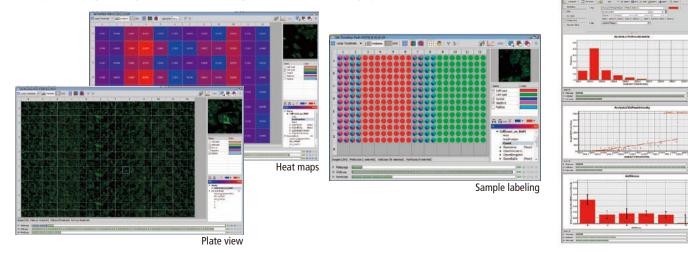
Progress display

Current well-plate acquisition point is displayed in real time along with a live image. Image analysis is conducted during image acquisition, allowing for immediate observation of data collection and experimental status.



B Results display

Various formats are available for displaying results. Plate view is a centralized view that shows acquired images and all associated data. Sample labeling manages image data by linking cells by name, type and quantity of reagent. Heat maps visualize trends in measurement results and analytical values. Graphical displays of analytical values such as histograms, scatter plots and bar graphs are also available.



Visual programming tool that enables creation of highly flexible experimental templates

JOBS Editor

Option (Ar)

JOBS Editor is a visual programming tool that can create experimental templates (JOBS) simply by dragging and dropping the settings for "tasks" in the sequence of experimental procedures, such as sample definition, image acquisition settings and analysis settings, into the JOBS Editor window.

The HC Template offers streamlined operation of high-throughput imaging/analysis. However, JOBS Editor enables easy creation of more complex and custom experimental templates, from image acquisition to analysis, by providing a number of available tasks, without the need of advanced data programming knowledge and the need for creating a macro.



For offline analysis of high-content data

Remote Database/JOBS Viewer* (for HC Template and JOBS Editor)

Option (Ar)

13

nts_V430'Drivers\te

(0 runs - 0 images) (1 runs - 0 images) (1 runs - 8 images) (29 runs - 301 ima

Action to perfor

Download to Loca Keep Local

Download Cancel

Enables image analysis and management of a large volume of high-content imaging data on an offline PC. Operating image acquisition and data analysis on separate computers boost total throughput. General Analysis module is required for offline image analysis.

*JOBS Viewer is not available in some regions.

- Remote Database allows offline exchange of data between computers and network servers.
- Using JOBS Viewer and/or General Analysis, images acquired through HC Template or JOBS can be analyzed on a dedicated offline computer.

Local database C:\P	ogramData (Labo	ratory Imaging Vob	s/jobsdb.dat		Remote database	C: Program Files WI	5-Element
Remote database Ci'P	ogram Files(VIIS-	Elements_V430\Driv	ers/test.dot		Local database	C: ProgramData/La	toratory 1
Project		Content	1		Project		c
• Project					• V Project		
Uve Cels		(D runs - 0 ima	oes)		1 Live	Cells	(0
HCA Fixed with WPL		(1 runs - 0 ima	ges)		HCA	Fixed with WPL	(L
		(1 runs - 8 ima	ges)		HCA	Fixed Samples	(1
		(29 runs - 301	images)		HCA	Live	(25
HCA Fixed		(0 runs - 0 ima	ges)		MCA		(0
		(1 runs - 0 ima	ges)		New New		(1
		(2 runs - 0 ima	ges)		☑ Live		(2
		(D runs - O ima	(D runs - O images)			d Samples 1	(0
			(1 runs - 0 images)		PFS_		(1
		face (1 runs - 0 ima	ges)			Plate: Create Focus Su	rface (1
Create PFS			(3 runs - 0 images)			te PFS Surface	(3
WellSelector			(4 runs - 9 mages)		Welt Welt		(4
HCA Fixed w	an wet i	(0 runs - 0 ima	p=4)		E I ICA	Fixed with WPL1	(0
	Uplo	ading Rules				Dow	nloadi
Present in Local	Present	in Remote	mote Action to perform		Present in Local		nt in Remo
X Absent V Present		ent	Keep Remote	-	X Absent	V Pre	asent
X Absent					V Present	X Ab	trest
X Absent	X Abso	int	Ubload to Remote				

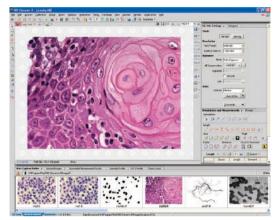
Data upload/download usir Remote Database

Advanced Solutions for your Imaging World

GUI Option

Industrial Simple GUI

With D package, the simple GUI mode provides controls for the most common operations such as image capture and simple measurement.

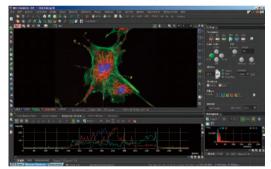


Standard GUI mode: Displays all functions of D package

Dark Color Scheme

Ar Br

This popular display option mode has a brightness level interface color palette suitable for use in a dark microscopy room.



Off-line Analysis and Viewer Software

NIS-Elements is compatible not only with Nikon products but also with third-party products such as high-sensitivity CCD cameras and peripheral devices. Third party devices and cameras are easy to integrate through the NIS-Elements intuitive install and device manager.

Off-line Package for Analysis

The NIS-Elements off-line software package offers analysis tools such as intensity measurements and object counting of tiff and multi-dimensional format images captured with Nikon's microscopes and third-party software.

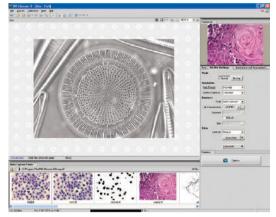
Viewer Software

This is free software for image display of single images and datasets captured using NIS-Elements. Possible views include Tile View, Max/Min Projections and 3D Volume View. Saving multi-dimensional files into TIFF format is available as well. The viewer is downloadable from the Nikon website.

Software Upgrade

SUA License

NIS-Elements can be upgraded for one year from the date of purchase. The Software Upgrade Agreement (SUA) License, which is purchasable in one-year license segments, extends the access to the latest version of NIS-Elements.



Simple GUI mode: Display only image capturing and measurement

Layout Manager



Layout manager enables customizing layouts of controls, toolbars and menus and application (image acquisition or measurement). Saving custom layouts is possible and accessible through one-click tab access.





License



MS Elements

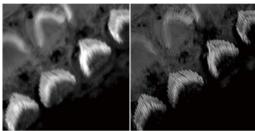
Supporting Broad Microscope Imaging

NIS-Elements is a common software platform for Nikon microscope systems, which allows the comprehensive control of wide range of functions for cameras, confocal imaging systems and super resolution microscopes.

C NIS-Elements C ER NIS-Elements C-ER

NIS-Elements C/NIS-Elements C-ER imaging software enables integrated control of the confocal imaging system, microscope and peripheral devices with a simple and intuitive interface. Various reliable analysis functions are also available.

NIS-Elements C-ER can provide resolution enhancement capability for confocal images. Without changing the conventional confocal image acquisition procedure, users can easily achieve enhanced resolution by utilizing unique image processing technology.





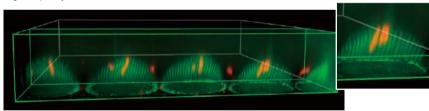
Conventional confocal image

image NIS-Elements C-ER image

Apical surfaces of auditory epithelia of mouse cochleae were stained by Atto-565-phalloidin at postnatal day 2. Photos courtesy of: Dr. Hideru Togashi, Division of Molecular and Cellular Biology, Kobe University Graduate School of Medicine.

N-SIM Analysis option

N-SIM Analysis option allows control of Nikon Super Resolution Microscope N-SIM, which can achieve an image resolution of 115nm (in 3D-SIM mode) and temporal resolution of up to 0.6 sec/frame using high frequency Structured Illumination.





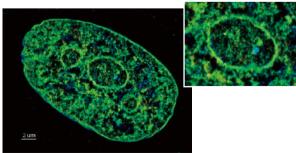
N-STORM Analysis option

Luminal surface of the organ of Corti at postnatal day 1. (Mouse)

Green: F-actin, red: acetylated-tubulin

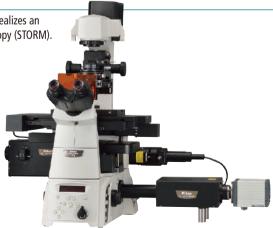
Kobe University Graduate School of Medicine

N-STORM Analysis option enables control of Nikon Super Resolution Microscope N-STORM, which realizes an incredible image resolution of approx. 20nm by utilizing STochastic Optical Reconstruction Microscopy (STORM).



Photos courtesy of: Drs. Kanoko Kominami. Hideru Togashi, and Yoshimi Takai, Division of Molecular and Cellular Biology.

A human fibroblast labeled with EdU-Alexa Fluor 647 to visualize DNA with 3D-STORM. Photo courtesy of: Jason Otterstrom, Ph.D., Melike Lakadamyaii, Ph.D., The Institute of Photonic Sciences (ICFO), Castelldefels



Ms Elements

NIS-Elements Supported Devices (ver. 4.50 or later)

Nikon Cameras

DS-Ri2 DS-Qi2 CCU: DS-U2/L2 (for camera head DS-2Mv/Vi1/ 2MBW/2MBWc/5M/5Mc/Fi1/Fi1c/Qi1/Ri1) CCU: DS-U3/L3 (for camera head DS-Vi1/Fi1/Fi1c/ Qi1/Ri1/Fi2) DQC-F5'2

Third-party Cameras

Photometrics Evolve QuantEM CoolSNAP HO2 CoolSNAP ES^{*2} Cascade 128+*2 Cascade II 512*2 Cascade 1K*2 Andor Technology Luca S, Luca R iXon+ 897, 888, 885 iXon X3 iXon Ultra iKon-M Clara Neo sCMOS Neo 5.5 sCMOS Zyla 5.5 sCMOS (3-Tap, 10-Tap) Zyla 4.2 sCMOS QImaging Retiga EXi Aqua/Blue Retiga 2000R - Mono/Color Retiga SRV + RGB-HM-S Slide Rolera EMC2 OICLick OICAM Hamamatsu ImagEM ImagEM 1K ImagEM X2 ORCA-R2 ORCA-Flash2.8 ORCA-Flash4.0 ORCA-Flash4.0 LT ORCA-D2 ORCA C9100-02, C9100-12 PCO pco.edge 3.1 (Only compatible with mono sensor) pco.edge 4.2 LT pco.edge 4.2 pco.edge 5.5 (Only compatible with mono sensor) Imaging Source DFK/DMK 31,41,51,72 series DFK/DMK 23U274 DFK/DMK 23UP031 DMK 23UM021 DFK 23U445 Others

TWAIN Device*2

Nikon Microscope Devices

Biological Microscope Ti (HUBC/A, HUBC/A-U, Ti-LAPP System*4) Biological Microscope TE2000 (Perfect Focus System) Biological Microscope 90i (ND filter, Stage, DIH-E/M) Biological Microscope 80i (DIH-E/M, D-FL-E, C-Box/C-Box2) Biological Microscopes Ni-E, Ni-U, Ci-E Fixed-stage Microscope FN1+ D-DH-E-A1* Stereo Microscope SMZ25/18/1270i/1270/800N Multizoom Microscope AZ100M Industrial Microscope LV-N Series*3 Measuring Microscope MM-400/800⁺² Metallographic Microscope MA200 Inspection Microscopes L200N/L300N C-HGFIE HG Fiber Illuminator Intensilight C-LEDFI Epi-FI LED Illuminator Laser Module LU-N Series LV-NCNT-N Nosepiece controller NI-SH-CON

Third-party Devices

Prior Scientific ProScan III ProScan II Prior PCI II OptiScan II ES10 NZ100,200,400 NanoStageZ FS107F Ludl Electronic Products MAC5000, MAC6000 Märzhäuser Wetzlar TANGO Desktop, Tango PCI **I STEP** Vincent Associates (Uniblitz) VCM-D1 Sutter Instrument Lambda 10-2, 10-3, SC, 10-B, XL Physical Instrument PI E-662, 665 (RS232) PI E-545+P-545.2R7 PI E-545+P-545.3R7 Photometrics Dual View ASI (Applied Scientific Instrumentation) MS-2000 FW-1000 SC-2000 National Instruments TTL Input/Output (NI Card) Tokai Hit WSKM OKO-Lab H301-T-UNIT-BL-PLUS + CO2-O2-UNIT-BL UNO-COMBINED-CONTROLLER Mad City Labs Nano-Drive Yokogawa CSU-W1*4 CSU-X1^{•4}

*1 Only compatible with 64-bit version OS

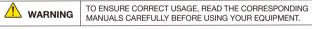
- *2 Not compatible with 64 bit version OS
- *3 Not compatible with LV-DAF Auto Focus Unit
- *4 Only compatible with NIS-Elements AR

Supported Operation System

Windows 7 Professional SP1 (32/64-bit version) * NIS-Elements Ar and C are only compatible with the 64-bit version.

NIS-Elements is compatible with all common file formats, such as JP2, JPG, TIFF, BMP, GIF, PNG, ND2, JFF, JTF, AVI, ICS/IDS. ND2 is a special format for NIS-Elements. ND2 allows storing sequences of images acquired during nD experiments. It contains information about the hardware settings and the experiment conditions and settings.

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. December 2015 ©2006-15 NIKON CORPORATION



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Printed in Japan (1512-06)T