GE Healthcare

IN Cell™ Analyzer 1000

Operating Instructions

Original Instructions





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1 Introduction

Purpose of the Operating Instructions

The Operating Instructions provide you with the instructions needed to handle the IN Cell Analyzer 1000 in a safe way.

Prerequisites

In order to operate the IN Cell Analyzer 1000 safely and according to the intended purpose the following prerequisites must be met:

- You should have a general understanding of the use of a personal computer. running Microsoft[™] Windows[™] in the version provided with your product.
- You should be acquainted with the use of general laboratory equipment and with handling of biological materials.
- You must read the Safety Instructions in Chapter 2 of these Operating Instructions.
- The system should be installed according to the instructions in *Chapter 3* of these Operating Instructions.
- You must read and understand these Operating Instructions.

In this chapter

This chapter contains important user information and a general description of the IN Cell Analyzer 1000 and its intended use.

1.1 Important user information

1.1 Important user information

Read this before using the IN Cell Analyzer 1000



All users must read the Safety Instructions in *Chapter 2* of these Operating Instructions before installing, using or maintaining the system.

Do not operate the IN Cell Analyzer 1000 in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

The IN Cell Analyzer 1000 is an Isothermal Titration Calorimeter system designed for bio-molecular interaction studies in research applications.

The IN Cell Analyzer 1000 system is intended for research use only and shall not be used in any clinical procedures or for diagnostic purposes.

Safety notices

These Operating Instructions contain WARNINGS, CAUTIONS and NOTICES concerning the use of the product, with meanings as defined below.



WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.



CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.



NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

- **Note:** A Note is used to indicate information that is important for trouble-free and optimal use of the product.
- *Tip:* A Tip provides information that can improve or optimize your procedures.

Typographical conventions

Software texts and commands are identified by **bold italic** text. A colon is used to separate menu levels (e.g. *File:Open* refers to the *Open* option in the *File* menu).

1.2 Regulatory information

This section lists the directives and standards that are fulfilled by the IN Cell Analyzer 1000.

Manufacturing information

Requirement	Content
Name and address of manufacturer	GE Healthcare UK Ltd Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
Name and ID of notified body	INTERTEK SEMKO AB, NB 0413
Place and date of declaration	Uppsala, Sweden, Oct. 2009
Identity of person authorized to sign Declaration of Conformity	See EC Declaration of Conformity.

CE Conformity

Directive	Title
2006/42/EC	Machinery Directive (MD)
2006/95/EC	Low Voltage Directive (LVD)
2004/108/EC	ElectroMagnetic Compatibility (EMC) Directive

1 Introduction

1.2 Regulatory information

International standards

Standard	Description	Notes
EN 61010-1, IEC 61010-1, CAN/CSA-C22.2 no. 61010-1	Safety requirements for electrical equipment for measurement, control and laboratory use	
EN 61326-1 (CISPR Group 1, Class A)	EMC emissions and immunity requirements for measurement, control and laboratory use	Harmonized with 2004/108/EC
EN-ISO 12100-1, 12100-2	Safety of machinery – Basic concepts, general principles and design	Harmonized with 2006/42/EC
EN-ISO 14121-1, 14121-2	Safety of machinery – Principles of risk assessment	Harmonized with 2006/42/EC

CE marking

CE

The CE marking and the corresponding Declaration of Conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked instruments, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation or explicitly authorized by GE Healthcare.

Regulatory compliance of connected equipment

Any equipment connected to the IN Cell Analyzer 1000 should meet the safety requirements of EN 61010-1/IEC61010-1 or relevant harmonized standards. Within the European Union, connected equipment must be CE-marked.

1.3 Instrument



Figure 1-1.

The Services Cabinet contains the following:

- Computer
- Lamp power supply unit
- 24-volt DC power supply unit
- Main power service rail
- Power cabling connections
- Liquid handling power supply (Kinetics option)
- OZ Amplifier (OZ option)
- Ludl encoder control box

The optical unit contains the following:

• Inverted microscope and base plate

Plate delivery mechanism(1)



Figure 1-2. Plate delivery system (1).

- Objective nosepiece
- Auto focus and stepper motor
- Liquid handling mechanism (Kinetics option)
- Heaters (Kinetics option)
- Optigrid paddle (OZ Option)

Xenon Lamp Assembly

This is a high intensity light source used for microscope observations only. It should not be used for any other purpose. Do not disassemble the lamp, as this will affect the performance of the instrument. To replace the bulb, contact a GE Healthcare service engineer. The lamp housing is shown below.



Figure 1-3. Lamp housing inside enclosure.

2 Safety instructions

This chapter describes safety compliance, safety labels, general safety precautions, emergency procedures, power failure and decommissioning of IN Cell Analyzer 1000.

2.1 Safety precautions

Introduction

Before installing, operating of maintaining the system, you must be aware of the hazards described in the user documentation. Follow the instructions provided to avoid personal injury or damage to the equipment.

The safety precautions in this section are grouped into the following categories:

- General precautions
- Flammable liquids
- Personal protection
- Installing and moving the instrument
- System operation
- Maintenance

2.1 Safety precautions

General precautions



WARNING!

Provide proper electrical power to the instrument. This should be 100 – 240 Volt, 50/60 Hertz alternating current, with a Ground Fault Circuit Interrupter (GFCI). Some power strips, such as the one provided by GE Healthcare with your instrument, contain a GFCI. All power plugs and cords should be 3-prong, grounded cables or outlets.



WARNING!

In case of fire, unplug the instrument.



WARNING!

Do not operate the IN Cell Analyzer 1000 in any other way than described in the IN Cell Analyzer 1000 and/or Auto-IN Cell Analyzer 1000 manuals.



WARNING!

The IN Cell Analyzer 1000 uses extremely high voltage. Ensure that all power has been shut off prior to opening the lamp housing.



WARNING!

Make sure the rear power connector is always accessible.

WARNING! Use caution spilled on or

Use caution when using solutions near the instrument. If any liquid is spilled on or around the instrument, unplug the instrument immediately and wipe it up. If there is any possibility that liquid may have leaked into the instrument case, contact GE Healthcare immediately. Do not plug the instrument into any electrical outlet until the problem is resolved.

WARNING!

Using controls, making adjustments, or performing procedures other than those specified in the IN Cell Analyzer 1000 documentation can result in hazardous exposure to high voltage or moving parts. Exposure to these hazards can cause severe personal injury.

WARNING!

This instrument is not designed to the Medical Devices Directive 93/42/ EEC and should not be used for medical purposes and/or in the diagnosis of patients.

NOTICE!

The Pifoc element of the IN Cell Analyzer 1000 is extremely sensitive. Any undo force or twisting action can damage the Pifoc crystal. Adhere to the warnings marked on both the turret and Pifoc element.

Using flammable liquids

WARNING!

A fume hood or similar ventilation system shall be used when flammable or noxious substances are used.

WARNING!

Fire Hazard. Before starting the system make sure that there is no leakage.

Personal protection

WARNING!

Always use protective glasses and other personal protective equipment (PPE) appropriate with the current application, to ensure personal safety during operation.

WARNING!

The operator should always follow proper laboratory procedures in handling and disposing of volatile or hazardous solutions.

2.1 Safety precautions

Installing and moving the instrument

WARNING!

Power cord. Only use power cords delivered or approved by GE Healthcare.

WARNING!

Do not block the ventilation inlets or outlets on the system.

WARNING!

Access to power switch and power cord. Do not block the rear and side panel of the instrument. The Power switch must always be easy to access. The power cord must always be easy to disconnect.

System operation

WARNING

Laser Safety: The Auto focus sensor should not be opened by the user or service technician. Tamper-proof screws have been employed on the case of the Auto focus sensor to prevent access to harmful invisible laser radiation. Do not modify the Auto focus case or attempt to remove the tamper-proof screws. The laser employed in the Auto focus sensor is a 780 nm 5 mW laser.

WARNING!

Hot surfaces: The microscope lamp house(s) and its surroundings become extremely hot during lamp illumination. Do not touch any of these surfaces while the lamp is lit or immediately after the lamp has been extinguished.

CAUTION

Pinch hazard: Your fingers could be pinched if they are near the stage when it is in motion.

CAUTION

Waste tubes and containers shall be secured and sealed to prevent accidental spillage.

Maintenance

WARNING!

Replace fuses ONLY with same type fuses. Several spare fuses are provided with the original shipment and the power receptacle is labeled with the correct type.

WARNING!

Repairs, alterations or modifications must only be carried out by a GE Healthcare specialist, or with explicit directions from a GE Healthcare technician. Removal or modification of any cover or component could result in an unsafe or easily damaged instrument. The GE Healthcare service department will be happy to answer any questions and provide parts and service when necessary.

WARNING!

Only spare parts that are approved or supplied by GE Healthcare may be used for maintaining or servicing the system.

WARNING!

Disconnect power. Always disconnect power from the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.

WARNING!

Hazardous chemicals during maintenance. When using hazardous chemicals for cleaning, wash the system with a neutral solution in the last phase or step.

WARNING!

Decontaminate the equipment before decommissioning to ensure the removal of all hazardous residues.

2 Safety instructions

2.2 Labels

2.2 Labels

Labels on the instrument

Below are examples of the identification labels attached to the IN Cell instrument.

xx-xxxx-x	x	INCell Analy	zer xxxx
Code No: Serial No Mfg Year:	XXXXXXXX XXXXXXX 2009	Voltage: Frequency: Power max: Fuse:	
\mathbb{N}	X @		
Made in Sw	eden GE He 751 84	althcare Bio-Scien I Uppsala Sweden	ces AB

Figure 2-1. Marking label.

Figure 2-2. Laser warning label.

Laser Auto focus

The IN Cell Analyzer 1000 uses a Class I laser-guided Auto focus device. The laser is contained within the Auto focus device, which is integrated with the microscope nosepiece.

WARNING!

The Auto focus sensor should not be opened by the user or service technician. Tamper-proof screws have been employed on the case of the Auto focus sensor to prevent access to harmful invisible laser radiation.

Symbols used in safety labels

C	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	Warning! Read the user manual before using the system. Do not open any covers or replace parts unless specifically stated in the user manual.
CE	The system complies with applicable European directives.

Labels concerning hazardous substances

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact and authorized of the manufacturer for information concerning the decommissioning of equipment.
This symbol indicates that the product contains hazardous materials in excess of the limits established by the Chinese standard SJ/T11363-2006. Requirements for Concentration Limits for certain Hazardous Substances in Electronics.

Emergency procedures

In an emergency situation, do as follows to stop the run:

Step	Action
1	Disconnect the equipment from the power outlet.
2	Check that the mains power source is providing power (by connecting another electrical item to the power outlet). If the mains power source does not work, contact your local maintenance department before proceeding.
3	Check that the mains power cable is connected correctly and that the plugs are firmly located in the sockets.

2.3 Recycling procedures

Step	Action
4	Test the unit again by switching it on.
5	If the unit still does not work, contact your local GE Healthcare service engineer, because the IN Cell Analyzer 1000 instrument contains a non-operator replaceable fuse.

Power failure

IN Cell Analyzer 1000

- 1 Close the IN Cell Analyzer 1000 software and shut down the computer.
- 2 Switch off the lamp power supply and then the instrument. Remove all mains cables from the power outlets.

2.3 Recycling procedures

The equipment shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.

Disposal, general instructions

When taking the IN Cell Analyzer 1000 system out of service, the different materials must be separated and recycled according to national and local environmental regulations.

Recycling of hazardous substances

The IN Cell Analyzer 1000 instrument contains hazardous substances. Detailed information is available from your GE Healthcare representative.

Disposal of electrical components

Waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of GE Healthcare for information concerning the decommissioning of equipment.

3 Installation

NOTICE

The IN Cell Analyzer 1000 instrument must be installed by trained GE Healthcare personnel.

3.1 Site requirements

The IN Cell Analyzer 1000 with Computer Controller requires about 1.2 meters of normal bench space (ca. 70 cm wide). This location should be away from strong drafts, room temperature fluctuations, intense sunlight, vibrations and strong electrical or magnetic fields (as may be produced by an NMR, microwave oven, large motors or refrigeration units). In addition the mains power source (100 to 240 VAC) should be properly grounded and free from voltage fluctuations, harmonic distortions, power dips and spikes. The AC power line should be dedicated to the IN Cell Analyzer 1000 system and should not share that power with additional equipment.

Table 3-1. Power supply requirements.

AC Mains Rea	quirements
Specification	Requirement
Voltage	100 to 240 VAC
Frequency	50/60 Hz

It is emphasized that room temperature fluctuations (i.e. maximum 2.5°C) due to the cycling on/off of heating and cooling systems, strong air currents, sunlight directly on the instrument and through space electromagnetic waves may cause subtle performance problems.

Table 3-2. Environmental operating requirements.

Environmental (Operating) requirements			
Temperature	10 to 40°C		
Humidity	\leq 70% Relative Humidity		
Atmospheric Pressure	700 hPa to 1060 hPa		

3.2 Transport

The system will be delivered in three separate crates, the size and weight of each is given below.

Table 3-3. Crate sizes.

Crates	Size (mm)	Weight (kg)
Box 1 – IN CELL Analyzer 1000 Gen 1.1 Optical Unit	870 × 700 × 685	145
Box 2 – Service Cabinet	553 x 600 x 670	98.5
Box 3 – Auxiliary Components	101 × 730 × 84	67.5

3.3 Unpacking

Contact your GE Healthcare representative upon delivery of the instrument.

Check the equipment for any apparent damage before allowing installation to start.

Document any damage and contact your local GE Healthcare representative.

NOTICE

The IN Cell Analyzer 1000 instrument must be unpacked and installed by trained GE Healthcare personnel.

3.4 Assembly

Contact your GE Healthcare representative upon confirming receipt of the instrument components.

3.5 Connections

A GE Healthcare representative will connect the instrument during the assembly process.

Figure 3-1. Service cabinet.

External Connections

The unit has 2 main electrical inlets at the rear and 2 main power switches on the front. The 3 connecting cables between the cabinet and the instrument may be connected in only one way, as the cable ends are keyed to be accepted in only one of each of the three receptacles. Also connected at the rear of the cabinet are the keyboard, mouse, and monitor (second is optional), and printer. An earthed (ground) wire is installed.

Figure 3-2. Connections on the back of the instrument.

3.6 Spare parts and accessories

For correct up to date information on spare parts and accessories, visit: www.gelifesciences.com

4 Operation

4.1 Operation overview

Step	Action
1	Switch the instrument on.
2	Ensure any manually adjustable hardware is in place.
3	Select or create an Acquisition Protocol.
4	Acquire an image stack.
5	Select or create an analysis protocol.
6	Load and analyze the image stack.

CAUTION

Before using the IN Cell Analyzer 1000, ensure that the system is clean and dry. Any detected problems should be dealt with before the system is started up. If there is a spill or some of the modules have condensed water, these should be dried clean and the system left for a few hours before using.

4.2 Starting the instrument

1 Switch on the instrument using the On-Off switches in the Service Cabinet.

Figure 4-1. Service cabinet closed.

- 2 Ensure that the dichroic is correct (type and position).
- 3 Ensure that the required objective lens is correctly positioned by turning the turret until you feel it click into place.
- 4 Switch on the lamp power supply by turning the key clockwise. Warm-up time is 15 min.

Figure 4-2. Key used to switch on lamp power.

Note: The lamp must be switched on before operating the computer.

- 5 Switch on any printer.
- 6 Double-click on the icon for the IN Cell Analyzer 1000 Instrument software. The IN Cell Analyzer 1000 software will then start.

4.3 Defining an acquisition protocol

- 1 To run the Acquisition Protocol Wizard, open Application > Acquisition Protocol Manager...
- 2 When the Acquisition Protocol Manager dialog box appears, press New....

rotocol name: ho	
New Import	Export Delete Rename Edit
Protocol name:	hg
Available in current setup:	Yes
Microscopy type:	Fluorescence
Plate	Matrical_384_0.17mm plate
Camera:	CoolSnap HQ
Objective:	Nikon_4x_PlanApo
Fields per well:	1 1000000
Auto focus search:	Search ±1.0µm, accuracy ±0.4µm
Combining:	2 at 50.00μm
Direction:	Horizontal Snake
Number of active wells:	384
Time series:	Off
Expected running time:	Off

Figure 4-3. Acquire protocol manager dialog box.

You will then be asked whether you want to **Use currently selected protocol as a template** (if one has previously been defined). If you select **No**, the Acquisition Protocol Wizard starts with default settings. If you select **Yes**, the Acquisition Protocol Wizard starts with the parameters of the currently selected protocol.

Enter a name for the protocol. Type a name into the entry field. All existing protocol

Protocol Name				
Specify a name fo	r the new protocol	and enter a descri	ption.	
Protocol name:				
Description	1			#
Description				

names are listed in the dropdown list.

Figure 4-4. Acquisition Protocol Wizard - Protocol Name.

3 Specify a password to associate with the protocol. Although you will be able to run the Acquisition Protocol without a password, the assigned password must be entered in order to modify it.

Protocol Passwor	1	
Specify and verify	a password, which will be required to m	odify the protocol.
Password:	[
Verify password:		
Coursel 1	Alberty Martha	1 1 100

Figure 4-5. Acquisition Protocol Wizard - Protocol Password.

4 Specify which microscope objective to use during image acquisition. All installed objectives are listed, along with a summary of their optical parameters.

bjective Specify which objective y objective parameters is dis	ou are using from the drop-down list. A summary of splayed below.
bjective name: NIKON	4X_PLANAPO
Objective name:	NIKON 4X PLANAPO
Magnification:	4x
Numerical aperture:	0.2
Refractive index:	1
Focal length (microns):	50000
Objective height (mm):	0
Camera type:	74
Camera width:	640
Camera height:	512
Camera duplication:	1
Camera binning:	1

Figure 4-6. Acquisition Protocol Wizard - Objective.

5 Specify which microplate is to be used during image acquisition. The *Well Plate* page of the wizard will list at least one plate type.

arehealten marertt			
Plate name:	Greiner uClear		
Plate name	Greiner uClear		
Valid	Yes		
Number of wells	96 (8 x 12)		
Well shape	Round		
Plate height	14.00 [mm]		
Top-left well center	(14.38, 11.24) [mm]		
Spacing	(9.00, 9.00) [mm]		
Well size	6.50 (mm)		
Bottom thickness	250.0		
Bottom height	3.4		
Bottom interface	Plastic		
Well volume	350.0		

Figure 4-7. Acquisition Protocol Wizard - Well Plate.

6 If your system is equipped with an optional plate heater, the *Plate Heater* dialog box allows you to use plate heating during acquisition. If plate heating is required, check the *Use plate heating* box and specify the target temperature (between 25–42°C).

Plate Heater			
Specify whether the plate he target temperature if applica	eater should be ble.	turned on before a	cquisition and specif
Use plate heating			
Specify target temperature	28.0	*C	

Figure 4-8. Acquisition Protocol Wizard - Plate Heater.

Well Labels page is used to define a labelling scheme for each row and column of wells.

Figure 4-9. Acquisition Protocol Wizard - Well Labels.

7 **Well definition** - Use the schematic diagram to designate wells as active or blank, and to define the origin well (i.e., the starting point for image acquisition). By default, all wells are initially designated as active.

Figure 4-10. Acquisition Protocol Wizard- Well Definition.

8 Microscopy type - If the transmitted light module has been fitted, the fluorescence, brightfield, phase contrast, or DIC imaging modes can be selected, along with the required number of fluorescence imaging modes.

Figure 4-11. Acquisition Protocol Wizard - Microscopy.

If the camera has been set up with multiple configurations (such as with different Gain or Binning factors), select the configuration you wish to use.

Camera	· · · · · · · · · · · · · · · · · · ·
parameters is displayed belo	are using from the drop-down list. A summary of camera w.
Camera name:	oolSnap HQ 💌
Description:	Gain 2 no binning
Gain:	2
Width:	1300
Height	1028
Binning:	1x1
Support OZ Module:	Yes
OZ Module Image Quality:	Normal
Flat Field Correction	Not established
Use flat field correction if a	available.

Figure 4-12. Acquisition Protocol Wizard - Camera.

9 Filter Selection and Display page is used to select excitation and emission filters for fluorescence studies and to specify a number of camera settings and parameters.

	and visuals	filters for e	to be used ach filter.	rrom	i the list belo	w. spe	ciriy (ne exp	osure time	e, orrset
	Excitation	filter:	Emission f	ilter:	Exposure: (msec)	HWAF offset:	Visual (μm) iked	s	OZ Module
λ1	360_40	-	460_40	•	1	0			<u>a</u> [
32	535_50	-	620_40		1	0			
23	620_60	•	700_75	•	1	0		•	200
λ4	620_60	-	460_40	•	1	0			<u>8</u> T
	Fused im	nage:		1	Enable f	used im	age	Def	ine
								Auto offs	et

Figure 4-13. Acquisition Protocol Wizard - Filter Selection and Display.

10 **Time Series** page is used to specify the time intervals between acquisitions for a kinetic experiment. If a liquid handling procedure is to be defined, it will first be necessary to add a time series. In doing so, sufficient time intervals must be left between image acquisitions to enable the liquid handling procedure to be completed. There are two types of time series available, termed **Spit and Stare** and **Look Walk Look** modes.

ime Series Specify parameters for tim	e series acquisition		
Acquire time series	Add	Modify	Delete
Time			
0.000			
Hada Tone And Chan		Cananda	
Mode: Tabicana anale		Jeconus	

Figure 4-14. Acquisition Protocol Wizard - Time Series

If the Liquid Handling Module is fitted, your IN Cell Analyzer 1000 Instrument contains a liquid handling system for dispensing or aspirating a reagent from 96-

L iquid Handli Specify the li	ng quid handling events. Press ''H	lelp'' for more information.	
	+		
		Measure	

and 384-well plates and for cleaning the system using a wash station.

Figure 4-15. Acquisition Protocol Wizard - Liquid Handling.

11 **Z dimension** feature (*Figure 4-16*) allows you to collect a set of images at different focal planes. Check the **Enable acquisition** of images in Z-dimension box and enter the required Number of Slices (images), the Starting position, and the Step size in the corresponding fields. Up to 50 slices are available and the starting position can be between -100 and +100 microns from the HWAF position.

Z dimension		
Specify whether you want to acquire images in z o	limension	
Enable acquisition of images in Z-dimension		
Number of Slices:	0	-
Starting position (relative to HWAF position):	0	microns
Step:	0	microns

Figure 4-16. Acquisition Protocol Wizard - Z dimension.

12 **Acquisition Options** page is used to specify the manner in which the images are to be acquired. The schematic Well Layout diagram provides a representation of the entire well and the arrangements of the field of view(s) selected.

Specify how images are to be	acquired. Press	"Help" for more i	nformation.
Flexible number of fields	Acquire until	0	cells are found
Fields to be acquired: 1	Attr	ange fields.	
Direction of Acquisition			
C Horizontal	/ertical	-Well Layout	
Horizontal Snake C N	/ertical Snake		
Acquisition Pattern			
Acquire at well center			
C Acquire at a specified locatio	n		
C Acquire randomly in specified	active region		
Active Region		- 1	
Exclusion margin: 0	-000		
Julius Brown	0.000		and a second
Active region: 🗰 Lenter	C Edge		Edit

Figure 4-17. Acquisition Protocol Wizard - Acquisition Options.

13 Segmentation parameters are enabled when the *Flexible Number of Fields* checkbox is marked on the *Acquisition Options* dialog. Segmentation parameters are used to set the on-line cell counting parameters and should be optimized before acquiring images.

Segmentation Parameters	
Specify segmentation parameters. When a new Nu digitization to view the updated image. Press "Help	icleus wave is selected, perform "for more information.
Force cell count	<u></u>
Nucleus wave: D360_40x - HQ460_40M	
Minimum nucleus area: 100 µm2	
Segmentation method: Enhanced	
Cells found:	

Figure 4-18. Acquisition Protocol Wizard - Segmentation Parameters.

Phase contrast microscopy is a contrast-enhancing optical technique that can be used to produce high-contrast images of living cells in their natural state without being killed, fixed, and stained. The High pass filter controls the amount of detail that is filtered out of the image. The **DIC Settings** controls the settings for DIC

synthetic images.

ase Contrast and DIC Specify phase contrast and	d DIC parameters.	
Phase Contrast Settings		
High pass filter [0, 100]:	1.0	
🗂 Invert phase	polarity	
Illumination method	C Transmitted light C Reflected light	
DIC Settings Contrast angle (degrees):	135 式 Combiner prism(degrees):	0 🕂
Intensity modulation:	standard	
Absorption rate [0.0, 1.0]	[0.50	
Enable automatic image	e update	

Figure 4-19. Acquisition Protocol Wizard - Phase Contrast and DIC.

14 *Image Combination* creates a single focused image from a set of images acquired at different focal planes. Enter the size of the Z-axis step in the entry field.

Image Combination Specify image combination	parameters. Press "Help" for more informat	ion.
Use image combination		
Number of planes:	2 📫	
Define combining par	ameters	
Auto Z stepping:	50 μm	
		ä

Figure 4-20. Acquisition Protocol Wizard - Image Combination.

15 **Software Auto focus Search** feature uses sophisticated software algorithms to automatically find the Z-axis position with the sharpest focus. This software-based

Auto focus feature can be used instead of, or in conjunction with, the laser-guided Hardware Auto focus (HWAF) system.

Software Auto Specify softw	are aut	s Search o focus parameters. Press "Help" for more information.
☑ Use softwar	e auto	focus search
Search: ±	100	μm from current position
Initial step:	10	μm
Accuracy:	0.5	μm min. stepping interval
	6% of	the objective depth of field
🔲 Show liv	e image	a de la companya de l
		ă

Figure 4-21. Acquisition Protocol Wizard - Software Auto Focus Search.

16 **Output Formats** page of the Acquisition Protocol Wizard is used to specify the format of the output files.

Figure 4-22. Acquisition Protocol Wizard - Output Formats.

17 **Stack Destination** page is used to specify where to store the images as they are acquired.

stack Desti	nation				
Specify ba creation of	e path where th subfolder for ea	ne image files ch plate	are stored	and the namin	g method for
System	default				
D:\App	sVAL33_Releas	eVIMGSTAC	(\Copy of	hg .	
C Predefin	ed				
1					Browse
C Determi	ned at run time				
nage Stack F	older Naming M	ode			
For every plaimage stack	ate the system w file will be store	vill create a su d. Specify the	ubfolder of e method o	the base folder f naming these	, in which the folders:
C Use cur	ent date and tim	ne. Example: 1	2000.10.12	2.11.12	
C Use the	unique plate ide	ntifier.			
G Use a st	ran folder. Prev	ious stack in	this folder	will be overwrit	ten.

Figure 4-23. Acquisition Protocol Wizard - Stack Destination.

18 **Batch Analysis** allows you to analyze multiple image stacks (i.e., images from different plates) automatically, with little or no intervention. The Batch Analysis page of the wizard lets you put the image stack into the Batch Analysis Queue as soon as it has been acquired.

Batch Analys	is	
Specify whe queue.	ther you want to automaticaly add acquired experiment to batch analy	sis
🔽 Automatica	ally add to Batch Analysis Queue	
Batch Analysis	s Queue file:	
d:\apps\al33_	release\queue.txt	1
Analysis proto	cot	
	· · · · · · · · · · · · · · · · · · ·	1
		10

Figure 4-24. Acquisition Protocol Wizard - Batch Analysis.

19 To complete the Acquisition Protocol Wizard, press *Finish*. As you exit the wizard, the IN Cell Analyzer 1000 will re-display the Acquisition Protocol Manager dialog box A summary of the protocol is displayed. Press [*OK*] to exit and save the protocol or press *Run...* to run the protocol without going through the Start Acquisition Session Wizard.

4.4 Preparations before start

- 1 Set the IN Cell Analyzer 1000 operation mode to **Assay Development** (**Mode** > **Assay development**).
- 2 Create a Plate or Slide Definition from the Applications menu if one does not exist.
- 3 Select the **Open Plate Manager** option from the option screen that appears when you enter A**ssay Development** mode.

'late/slide holder name:	reiner uClear	F
New	Export Delete Rename.	Edit
Plate name	Greiner uClear	
Valid	Yes	
Number of wells	96 (8 x 12)	
Well shape	Round	
Plate height	14.00 [mm]	
Top-left well center	(14.38, 11.24) [mm]	
Spacing	(9.00, 9.00) [mm]	
Well size	6.50 [mm]	
Bottom thickness	250.0	
Rattom beight	31	
•		

Figure 4-25. Plate Manager window

		Enter pla	ate parameters				
General			Well Layout				
Plate name:		_			No.	Interval	
			000	Rows:	0÷	_	mm
Plate height:		mm	888	Columns:	0.4		mr
Bottom thickness:		μm				Carlos and	
Bottom beight:			-Well Parame	eters	-Well Off	set	
Dottom Holgin.		10011		·····································		mm	
Bottom interface: Plas	tic	*			* *		
Well Volume:		μΙ		mm	. -	····•	
Plate name cannot be emi	ahu						

4 Press [*New...*] to bring up the *Create New Plate* or *Slide* dialog box.

Figure 4-26. Plate Editor window.

- 5 Select Plate and choose whether you want to 'Use the following as a template:' (if one has previously been defined). Click **OK** to go to the Plate Editor dialog box.
- 6 Run the Acquisition Protocol Wizard from the Applications menu.

4.5 Acquiring images

The IN Cell Analyzer 1000 uses the information specified by the Acquisition Protocol to capture images automatically, with little or no user intervention. The IN Cell Analyzer 1000 uses both laser-guided hardware and sophisticated software Auto focus algorithms to automatically find the best plane of focus at each well position. When acquisition is complete, the entire image set is stored to disk as a single image stack. Once an Acquisition Protocol has been defined, an image stack can be acquired. There are four ways to initiate the image acquisition process:

IN Cell Ana	lyzer 1000	×
IN	Cell Analyzer 1000	
# 0000 0000	C Start plate manager	
	C Start acquisition protocol manager	
100	Start acquisition session	
	Open existing image stack	
	OK Cancel	

Figure 4-27. Start acquisition session.

- Select the *Start acquisition session* option from the screen that appears when you enter Assay Development mode.
- Select the *Acquisition* option from the Mode menu.
- Select the Start Acquisition Session... option from the Application menu.
- Click the Acquisition wizard icon from the Application Toolbar.

Performing any of these operations launches the first page of the **Start Acquisition Session** Wizard 1 On the first page of the **Start Acquisition Session** Wizard, select one of the following options:

Acquisition		
Do you want to continue	acquiring an existing image stack	cor create a new one?
Create a new image	stack file	
C Continue with curren	it image stack file	
D:\Apps\AL33_Ship	ping\IMGSTACK\Hello World\A\	A.xdce
C Open an existing ima	age stack file	
		Browse

Figure 4-28. Acquisition Session Wizard - Acquisition.

- Create a new image stack file Select to create a completely new image stack.
- Continue with current image stack file Select to continue with the stack that is already open (filename is shown).
- Open an existing image stack file Select to open an existing stack. Press *Browse...* to locate the stack file.

2 Select the appropriate Acquisition Protocol from the **Protocol name** drop-down menu.

elect Protocol Select a protocol	from the dro	op-down list. A summary of the protocol's parame	ters i
displayed below.			
Protocol name:	default		
Protocol name:		default	_
Microscopy type:		Fluorescence	
File name:		None	
Plate		Greiner uClear	
Camera:		CoolSnap HQ	
Objective:		Nikon_10x_PlanApo	
Fields per well:		4 (2 × 2)	
Auto focus search	6	Off	
Combining:		Off	
Direction:		Horizontal Snake	
Number of active	wells:	96	
Number of acquire	ed images:	0 of 384	
Time series:		Off	
Expected running	time:	Off	

Figure 4-29. Acquisition Session Wizard - Select Protocol.

3 **Stack Destination** page is used to specify where to store the images as they are acquired and to specify a method for naming the folders.

tuck Destine	tion
Specify base creation of su	path where the image files are stored and the naming method for bfolder for each plate
 System de 	fault
D:\Apps\	AL33_Release\IMGSTACK\Copy of hg
C Predefined	
	Browse
C Determine	d at run time
age Stack Fold	ler Naming Mode
For every plate image stack file	the system will create a subfolder of the base folder, in which the will be stored. Specify the method of naming these folders:
C Use curren	t date and time. Example: 2000.10.12.11.12
C. Use the un	ique plate identifier.
s oscarcan	

Figure 4-30. Acquisition Protocol Wizard - Stack Destination.

4 **Plate Layout Information** dialog box allows you to preselect wells to be acquired. Select a layout file which contains the configuration for the acquisition.

The system car file. Well conter defined in the a	ormation include metadata describing plate layout (ex t description selected here will supersede we cquisition protocol. Please select the source	periment) in the stack Ill status information of this information. The
Layout name:	None	

Figure 4-31. Acquisition Session Wizard - Plate Layout Information.

5 Instrument Preparation page displays:

nstrument Prepar	ation				
Make sure you are correction and pla	e using the ol te heater info	bjective as spe ormation. Press	cified below Finish to pro	. Review the flat field oceed.	
Objective: N	NIKON_10K	_PLANAPO.			
at Field Correction -					
Established:		for diffe	erent set of f	iltersl	
Objective:					
🗖 Use flat field o	orrection for	this acquisition	session		
Retresh					
late Heating					
Current temperati	ure:	22.0			
Target temperatu	ae:	22.0			
Show plate he	eater status c	dialog before st	arting acquis	sition session	

Figure 4-32. Acquisition Session Wizard - Instrument Preparation.

• Specified objective

- Flat field Correction
- Plate Heating
- 6 The *Acquire Stack* dialog box displays a color-coded schematic diagram of the microplate and all the well locations are labelled. Click *Start* to begin the acquisition process.

Figure 4-33. Acquire stack window.

Optical Z-Sectioning Module (optional)

The optical z-sectioning module allows you to convert the illumination system of a conventional wide field microscope to a structured light imaging system.

Before taking optical z-sectioning images, the optical z-sectioning module must be calibrated. The steps are as follows:

- 1 Dock the plate.
- 2 Insert the optical z-sectioning calibration slide into the IN Cell Analyzer 1000.

Select *Application > Dock Plate*. The dock plate slides out. A window appears with the following message: Put the plate on the plate holder. Press *OK* when ready.

Place the calibration slide (part of the optical *z*-sectioning package) in a slide holder and insert the slide holder onto the plate holder. Click **OK**. The dock plate slides in.

3 Bring the optical grid pattern into focus.

Select Settings > Calibrate OZ Module. The following message appears: 'Special skills and knowledge are needed to properly calibrate OZ module. Do you want to continue?'

Click Yes. The Calibrate Optical Z-sectioning Module dialog box appears.

Stage Position (µm) X -30699.2 Z Y -3700.0 Move Move to XY Z Step XY Step Z Step	2336.5 e to Z Tools: Dock Plate <u>Dock Plate</u> <u>Dock Plate</u> <u>Dock Plate</u> <u>Dock Plate</u> <u>Dock Plate</u>
Benchmark Current S/N: 339 M Amplitude (level): 840	✓
DZ Module last calibration: 2006-0	2-27, 12:12:12

Figure 4-34. Calibrate optional Z-sectioning module dialog box.

Adjust the position of the slide so that the light beam from the objective is on the fluorescent part of the slide. Under XY Step, click the up and down arrows to move the slide in the Y direction. Click the left and right arrows to move the slide in the X direction.

Click **Rough Focus**. Rough Focus finds the approximate bottom of the calibration slide using the Hardware Auto focus (HWAF) feature. Rough Focus brings the bottom of the slide close to the focus point, to within \pm 200 µm.

Under **Z Step**, click the up and down arrows to fine-tune the focus of the grid lines in the Image View Area. Note that the image gets brighter as it gets closer to being in focus.

- 4 Check optical z-sectioning movement control.
- 5 Achieve maximum signal / noise (S/N) ratio.
- 6 Run the calibration software.

To acquire optical z-sectioning images, you use the same basic procedure as you would for acquiring wide field images. Do one of the following:

- 1 Create a new Acquisition Protocol.
- 2 Modify an existing acquisition protocol for wide field images and edit it.

4.6 Displaying an image stack

1 To view the image stack (i.e., all acquired images), press the Image stack and analysis icon. The Image Stack and Analysis option screen appears.

Current i	mage stack: D:\A\IMGSTACK\Hello World\A\A.xdce
•	 View current image stack
	C Open image stack file

Figure 4-35. Image stack.

- View current image stack Work with the image stack that is already open, if any.
- Open image stack file Load an image stack from disk

	1	2	3	4	5	6	7	8	9	10	11	12
A	0											
в	ŏ	ŏ	ŏ	Ď	ŏ	Ď	Ď	Ď	Ď	ŏ	ŏ	ŏ
С	Ŏ	Ŏ	Ŏ	Ď	Ŏ	Ŏ	Ď	Ŏ	Ď	Ŏ	Ŏ	Ŏ
D	Õ	Ó)	Ó	Ō	Ō)	Ď	Ó	Ó	0
E	0	Ó									Ó	
F	0	0										
G	0											
Н	0											
	0	no imag	es acqu	ired	() par	ially ac	quired		0	acquin	ed
mag We	e II : 🗛 - 1				Field	: 1	- T	ime: [1 🔻] z: [1] []
	A: JAI								-		Autor	Junuasi

Figure 4-36. Image Stack window

2 To view individual images, click on the well location, select the well location from the Well drop-down list, and / or use the navigation keys:

If multiple fields were acquired from the well, select which field to display from the Field drop-down list.

If images were acquired at multiple wavelengths, select which wavelength image to display from the *ë* drop-down list.

3 Manually digitized images may be saved to disk as stand-alone image files in TIFF (*.tif) or proprietary MCID (*.im) format. Individual stack images may also be stored as discrete files in the same manner.

4.7 Procedures after a run

- 1 Close the IN Cell Analyzer 1000 software and shut down the computer.
- 2 Switch off the lamp power supply and then the instrument, Remove all mains cables from the power outlets.
- 3 Microplates and any used materials in the instrument must be disposed of in the manner prescribed by local standard operating procedures. There are no other waste materials resulting from the use of the IN Cell Analyzer 1000.

CAUTION

Hazardous waste must be specially handled and disposed of properly.

4 Operation4.7 Procedures after a run

5 Maintenance

This section provides the user with information on the proper maintenance of the instrument to ensure proper function.

The maintenance methods described below do not require working anywhere near the exposed hazard areas.

5.1 Daily checks and routine maintenance

The following should be checked/performed on a regular/daily basis.

Data Back Up

It is recommended that you back up the data files and programs in the operating computer in accordance with procedures set up by your local data administrator.

Environmental Checks

Check that the room where IN Cell Analyzer 1000 is being used fulfils the environmental requirements listed in *Section Environmental conditions, on page 55*.

Computerized Online Diagnostics

Diagnostic checks of the stage, liquid dispenser, filter wheels, shutter and Auto focus mechanism are accessed from the *Application > Troubleshooting > Self-diagnostic Test* menu. Select the required components to test and click *OK*. The results are saved to file in the Session logs folder of the IN Cell Analyzer 1000 software.

Cleaning the System

Do not try to clean inside any of the component parts of the instrument. Clean off any dust and dirt from the outside of the instrument using a lint free cloth slightly moistened (but not soaked) with 70% ethanol. Wait until all parts are dry.

CAUTION

If any parts of the system should become wet, immediately close down all parts of the system and disconnect the power cables from the mains outlets. Wipe the wet parts with a lens tissue or lint-free cloth.

5.2 Component maintenance

Stuck Charge

High sensitivity CCDs, such as the one used in the IN Cell Analyzer 1000 camera, are sometimes affected by a phenomenon known as a stuck charge in which brighter parts of an image are not completely cleared during the CCD readout process. As a result, these bright areas can appear on subsequent images. To remove a stuck charge, contact the GE Healthcare support team.

Replacement of Lamp in Light Source

The lamp and bulb are not accessible. To replace the light source lamp, contact your local trained super-user or your local GE Healthcare service engineer.

Operating Computer

Please refer to the manual supplied with the operating computer.

6 Troubleshooting

This section contains tips and information for troubleshooting the IN Cell Analyzer 1000. Many problems in instrument loading and cleaning show characteristic baseline and titration abnormalities, and knowing which system is affected can greatly expedite the resolution of the issue. Some problems can easily be corrected by the average user; some may be corrected by the more advanced user, and some require the expertise of a GE Healthcare service technician. The GE Healthcare service department is happy to provide any advice, parts, or service that may be necessary.

6.1 How to get help for IN Cell instruments

Please contact us for any instrument or data analysis questions or issues you may have. For contact information for your local office, please visit: www.gelifesciences.com/ contact, or for IN Cell Analyzer 1000-specific information, visit: www.gelifesciences.com.

Problems that prevent users from operating the instrument require immediate consultation with a GE Healthcare technician. Customers should not attempt to repair the hardware or software unless instructed to do so by a GE Healthcare service representative.

6 Troubleshooting6.1 How to get help for IN Cell instruments

7 Reference information

7.1 Instrument specifications

General specifications

Part	Characteristic	Data	
Camera	Pixel size	6.45 μm × 6.45 μm	
	Field of view at 10 ×	0.603 mm ²	
	Cooling	-30°C	
	Pixel layout	1392 × 1040	
Focus	Method	Confocal laser sensor	
	Focus time	<400 msec.	
	Objective positioner resolution (piezzo Objective resolution)	0.2 µm	
Illumination	Epiflourescent lamp	100 Watt Xenon	
	Transmitted light	LED	
Magnification	4 ×/ 0.20 Plan apo		
	10 ×/0.45 Plan apo		
	20 ×/0.45 ELWD Plan Fluor		
	40 ×/0.60 ELWD Plan Fluor		
Plate positioning	Resolution	± 5 μm	
(XY stage)	Speed(well to well on a 96 well µ-plate	<800 msec.	
Filter selection	Excitation filters	6	
	Emission filters	6	

7 Reference information

7.1 Instrument specifications

Physical specifications

Description	Data
Instrument dimensions	700 × 870 × 685 mm
Service cabinet dimensions	600 × 553 × 670 mm
Environmental control module dimensions	280 × 140 × 185 mm
Instrument weight	125 kg
Service cabinet weight	85 kg
Environmental control module weight	3.5 kg

For operation, the IN Cell Analyzer 1000 with a computer controller requires about 1.2 meters of normal bench space (ca. 70 cm wide).

Electrical Specifications

Characteristic	IN Cell 1000		
Voltage	100 to 240 Volts AC		
Frequency	50 to 60 Hz		
Power	70 Watts		
Output	Secondary/Data connection only		
Protective Earth Terminals	Internal/external marking		

Environmental conditions

Condition	Characteristic	Limits		
Operation	Temperature	15°C to 37°C		
	Humidity	20 to 60% RH		
	Atmospheric Pressure	840 to 1060 mbar		
Storage	Temperature	-25°C to 60°C		
	Humidity	20% to 95% RH		
	Atmospheric Pressure	500 to 1100 mbar		

7.2 Ordering information

For ordering information, visit: www.gelifesciences.com

7 Reference information

7.2 Ordering information

For local office contact information, visit www.gelifesciences.com/contact

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