

IN Cell Analyzer 2200/2000

User's Reference Manual
Acquisition Software v4.5



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1 Preface

The User Manual describes the *IN Cell Analyzer 2200/2000* system and provides instructions to operate the imager and the software.

All users must read this entire manual to fully understand the safe use of *IN Cell Analyzer 2200/2000*.

1.1 Document audience

The information contained in this manual assumes that you are familiar with the basics of fluorescence microscopy. In addition, an understanding of image processing basics will help you use the system to its full potential. To manage the computer systems, some familiarity with Linux workstations and Windows-based personal computers is helpful.

1.2 Important user information

IMPORTANT!

IN Cell Analyzer 2200/2000 is intended for research use only, and should not be used in any clinical or in vitro procedures for diagnostic purposes.

Safety notices

This manual contains warnings and cautions concerning the safe use of the product. See definitions below.



WARNING! The WARNING symbol and notice highlight instructions that must be followed to avoid personal injury. Do not proceed until all stated conditions are clearly understood and met.



WARNING: LASER RADIATION!

WARNING: LASER RADIATION indicates a hazardous situation which, if not avoided, could result serious injury due to hazardous radiation. It is important not to proceed until all stated conditions are met and clearly understood.



CAUTION! The CAUTION notice highlights instructions that must be followed to avoid damage to the product or other equipment. Do not proceed until all stated conditions are met and clearly understood.



WARNING! This is a Class A product. In a domestic environment, it might cause radio interference, in which case the user might be required to take appropriate measures.



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

Note: A Note is used to indicate information that is Important for trouble-free and optimal use of the product.

CE marking



The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked GE Healthcare 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

CE certification

This product complies with the European directives listed below, by fulfilling corresponding standards. A copy of the Declaration of Conformity is available on request.

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

China RoHS - Declaration of Hazardous Substances



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.

C-tick certification



This symbol indicates that the system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.

Korean Radio Research Agency Notification



This symbol indicates that the product complies with the Korean standard Radio Research Agency Notification Jan 21 2011 to meet the Korea Radio Waves Act 2010 regulation.

Applicant Name: Applied Precision, Inc.

Equipment name: Laboratory Equipment
Basic model name: IN Cell Analyzer
Series model name: 52-852954-000
Registration Number: KCC-REM-1GE-INCellAnalyzer
Country of Origin: U.S.A.

Type of Equipment	User's Guide
A급 기기 (업무용 방송통신기자재) Class A Equipment (Industrial Broadcasting & Communication Equipment)	이 기기는 업무용(A급) 전자파적합기기로서 판 매자 또는 사용자는 이 점을 주의하시기 바라 며, 가정외의 지역에서 사용하는 것을 목적으로 합니다. This equipment is Industrial (Class A) electromagnetic wave suitability equipment and seller or user should take notice of it, and this equipment is to be used in the places except for home.

Recycling



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 an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

Laser requirements

This instrument meets the laser radiation safety requirements specified in CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50 dated July 24, 2007.

Manufacturing information

The table below summarizes the required manufacturing information. For further information, see the EC Declaration of Conformity document.

Requirement	Content
Name and address of manufacturer	GE Healthcare 1040 12th Avenue Northwest Issaquah, Washington 98027 USA

International standards

The *IN Cell Analyzer 2000* has been tested against the following standards:

Standard	Description	Notes
EN 61010-1, IEC 61010-1, CAN/CSA-C22.2 no. 61010-1, UL61010-1	Safety requirements for electrical equipment for measurement, control and laboratory use	EN harmonized to 2006/95/EC
EN 61326-1	EMC emissions and immunity requirements for measurement, control and laboratory use	Harmonized with 2004/108/EC
EN ISO 12100	Safety of machinery – Principles of risk assessment	Harmonized with 2006/42/EC
EN 60825-1 IEC 60825-1	Safety of laser products – Part 1: Equipment classification and requirements	EN harmonized to 2006/95/EC
FCC 47 CFR Part 15	Federal Communications Commission (FCC) title 47 of the Code of Federal Regulations (CFR), Part 15, Radio Frequency Devices	

The *IN Cell Analyzer 2200* has been tested against the following standards:

Standard	Description	Notes
EN 61010-1, IEC 61010-1, CAN/CSA-C22.2 no. 61010-1, UL61010-1	Safety requirements for electrical equipment for measurement, control and laboratory use	EN harmonized to 2006/95/EC
EN 61326-1	EMC emissions and immunity requirements for measurement, control and laboratory use	Harmonized with 2004/108/EC
EN ISO 12100	Safety of machinery – Principles of risk assessment	Harmonized with 2006/42/EC
EN 60825-1 IEC 60825-1	Safety of laser products – Part 1: Equipment classification and requirements	EN harmonized to 2006/95/EC
FCC 47 CFR Part 15	Federal Communications Commission (FCC) title 47 of the Code of Federal Regulations (CFR), Part 15, Radio Frequency Devices	

Regulatory compliance of connected equipment

Any equipment connected to *IN Cell Analyzer 2200/2000* 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.

Directive	Title
2011/65/EU	Restriction of Hazardous Substances (RoHS) Directive
2012/19/EU	Waste Electrical and Electronic Equipment (WEEE) Directive
Regulation (EC) No 1907/2006	Registration, Evaluation, Authorization and restriction of Chemicals (REACH)
ACPEIP	Administration on the Control of Pollution Caused by Electronic Information Products, China Restriction of Hazardous Substances (RoHS)

1 Preface

1.2 Important user information

2 IN Cell Analyzer 2200/2000 overview

This introduction provides an overview of the *IN Cell Analyzer 2200/2000*, its background, the basic architecture of the product, and who to contact with any *IN Cell Analyzer 2200/2000*-related problems or issues.

2.1 What is IN Cell Analyzer 2200/2000?

The *IN Cell Analyzer 2200/2000* is an automated microscope designed for fast, automated imaging and analysis of fixed and live cells. The primary imaging mode is fluorescence microscopy.

Fig 2-1. IN Cell Analyzer 2200/2000 Imaging System



2.1.1 Fluorescence microscopy

In fluorescence microscopy, the sample (for example, a human cell) is stained with one or more dyes that bind to certain structures of interest within the sample. The dyes are designed to fluoresce when illuminated with specific wavelengths of light; the fluorescent light that is emitted is also of a specific wavelength. During imaging, the sample is illuminated by light of a specific wavelength. This causes any dyes with matching excitation profiles to emit light. An objective lens gathers the fluorescent light emitted by the sample, and various optics are used to direct the light towards a very sensitive camera (in the *IN Cell Analyzer 2000* this is a CCD camera, and in the *IN Cell Analyzer 2200* a sCMOS camera). Images taken with this camera can show, for example, the appearance and position of structures in the sample.

2.2 System architecture

2.2.1 Workstation

The workstation is a computer system used for controlling the *IN Cell Analyzer 2200/2000*. The workstation runs software for defining and setting up imaging experiments, conducting experiments, and analyzing the resulting images. All of the *IN Cell Analyzer 2200/2000* system's controls are accessed through the workstation's graphical user interface (GUI). The workstation communicates with the imager through an Ethernet™ connection.



NOTIVE

Any computer used with the equipment shall comply with IEC 60950 and be installed and used according to the manufacturer's instructions.

2.2.2 Imager

The Imager houses all other parts of the *IN Cell Analyzer 2200/2000*, including:

- **Multispectral illumination** - A 200 W metal halide and high pressure sodium discharge lamp is used as the primary light source for the *IN Cell Analyzer 2000*. The lamp is pre-aligned in the factory and uses efficient light guiding technology to direct the light to the sample with 80–90% efficiency. In the *IN Cell Analyzer 2200*, a solid state light source has replaced the lamp as the primary light source.
- **Excitation filters** - Excitation filters excite one fluorophore at a time, chosen to cover standard dye & fluorescent protein excitation ranges.
- **Polychroic QUAD band mirrors** - Direct excitation light up though the objectives toward the sample.
- **Objective turret** - Fitted with four objectives including up to two objectives containing automated spherical aberration collars.
- **Laser Autofocus module** - A laser beam is directed through the objective and is focused on the target. While the Z-axis is moved, a photo sensor monitors the reflected beam to determine the exact position of the target. This information is fed back to the control computer so that subsequent images taken of the sample are in the best plane of focus.
- **Stage** - The sample is placed in a carrier plate that is moved over the optics to image different areas of the sample. The carrier plate rests on a smooth stage plate to ensure consistent motion.
- **Emission filters** - Select particular colors of light to allow the emitted light from only one fluorophore to reach the camera.

- **Camera** - On the *IN Cell Analyzer 2000*, a cooled CCD camera is used to capture the light and create images. On the *IN Cell Analyzer 2200*, a cooled sCMOS camera is used to capture the light and create images.
- **Liquid Handling (optional)** - Incorporates a positional gantry equipped with a single syringe and needle to transfer liquid from a source to its destination. Liquid samples are aspirated from either a sample bottle, or from specified locations on a compound plate, and dispensed in precise quantities into wells in a sample plate. The system supports aspiration and dispensing from 96- and 384-well plates and is easily cleaned using a wash station linked to a bottle of cleaning solution.
- **Control electronics** - The imager contains an on board computer and multiple custom circuit boards to receive commands from the workstation and convert those commands into motion and images.

The imager is enclosed with cover pieces to keep out light and dust. The emission optics are in a positive pressure environment to keep them clean. A filter on the intake ensures only clean air is allowed in this area.

2.3 Configuration options

Four configuration options are available for the *IN Cell Analyzer 2200/2000* Imaging System, in addition to the standard configuration required for the *IN Cell Analyzer 2200/2000* software to operate.

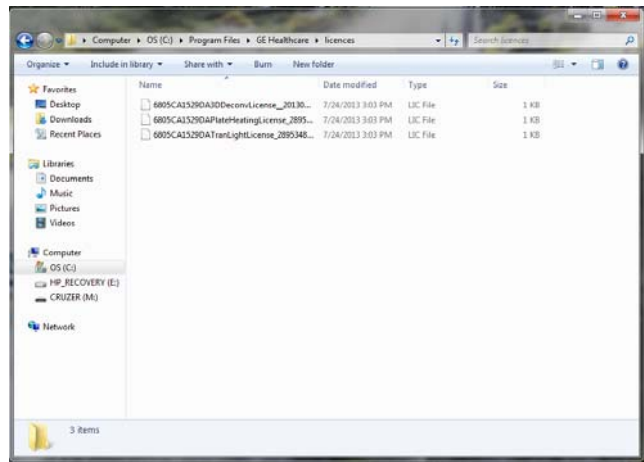
The configuration you choose for your particular system will depend on the applications for which the system will be used. The four options available are:

- **Liquid Handling** - This option allows the system to perform experiments in which liquid samples are aspirated into wells in a sample plate. To determine whether feature is enabled, open the **Protocol Designer**, and look for the **Liquid Handling** card in the card list panel. If it is not listed, the feature is not installed. For more information see *Liquid Handling*.
- **Environmental Control** - This option is required to use the **Plate Heating** feature. To determine whether feature is enabled, click the **Environmental Control** icon on the *Main* toolbar. If the **Turn On** button is disabled on the **Environmental Control** dialog, this feature is not licensed. For more information see *Environmental Control*.
- **3-D Deconvolution** - This option is required to use the **3-D Deconvolution** feature. To determine whether this license is present, open the **Channel Settings** card in the **Protocol Designer**. In the **Image** field dropdown list, the **3-D Deconvolution** option will appear disabled if the feature is not licensed. For more information on the **Protocol Designer**|**Channel Settings** card, see *Channel Settings card*.

- **Transmitted Light** - This option is required for the *Brightfield illumination* feature. To determine whether this license is present, open the **Channel Settings** card in the *Protocol Designer*. In the **Image** field dropdown list, the *Brightfield* option will appear disabled if the feature is not licensed. For more information on the *Protocol Designer|Channel Settings* card, see *Channel Settings card*.

Alternatively, you can, browse to the *licenses* folder:
C:\Program Files\GE Healthcare\elicense Server\Licenses
and check for the existence of the license for a specific feature.

Fig 2-2. License folder

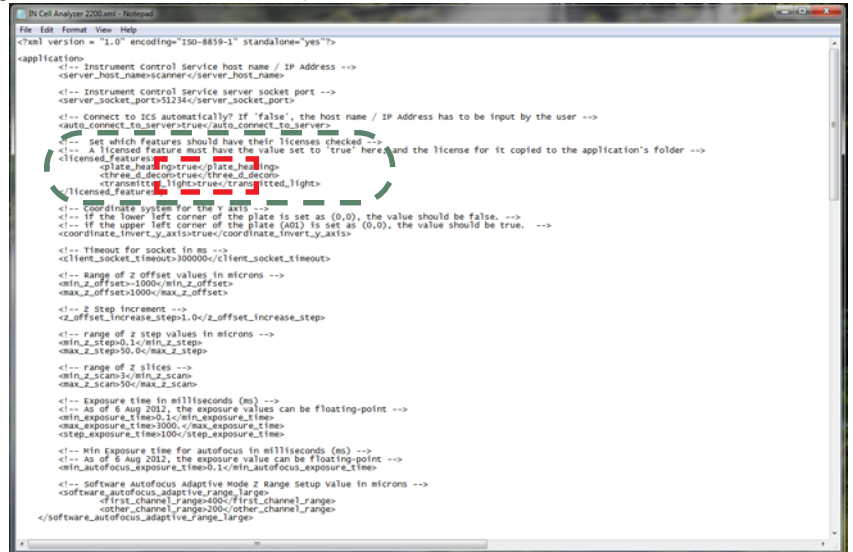


If a license is shown in the folder but is inactive, open the file:

IN Cell Analyzer 2000	C:\Program Files\GE Healthcare\IN Cell Analyzer 2000\config\IN Cell Analyzer 2000.xml
IN Cell Analyzer 2200	C:\Program Files\GE Healthcare\IN Cell Analyzer 2200\config\IN Cell Analyzer 2200.xml

The **config** file must display *true* for each of the optional licenses you have in your license file.

Fig 2-3. License Portion of **config** file.



Note: If **true** is entered into the file for any license you don't actually have available, an error message will display each time you start the software.

2.4 Contacting technical support



WARNING! All repairs should be done by personnel authorized by GE Healthcare. Do not open any covers or replace parts unless specifically stated in instructions.

For *IN Cell Analyzer 2200/2000*-related Issues, please contact you local GE Healthcare Technical Support group.

Regional contact information can be found on the **About** window from the **Help** menu.

2 IN Cell Analyzer 2200/2000 overview

2.4 Contacting technical support

3 IN Cell Analyzer 2200/2000 laser & safety concerns

The *IN Cell Analyzer 2200/2000* imaging system is a Class I laser system. During operation or maintenance, access to laser radiation is limited to levels that are not dangerous. In addition, this radiation is only accessible when the cover door is open. Since imaging is only meant to be performed with the door closed, access to the beam is typically not possible at all. During service, Class 3B radiation is accessible.

IMPORTANT!

Service on the IN Cell Analyzer 2200/2000 should ONLY be performed by GE Healthcare personnel or by persons trained by GE Healthcare specifically for this purpose. Service by other personnel will violate any warranty.

3.1 General safety precautions

Warning statements and an explanation of all warning symbols marked on the equipment are provided in this section.



WARNING! The operator of the IN Cell Analyzer 2200/2000 is assumed to be trained in the correct operation of the instrument and the safety issues.



WARNING! Using controls, making adjustments, or performing procedures other than those specified in the IN Cell Analyzer 2200/2000 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 used for a wide variety of experiments that can utilize potentially hazardous materials. Use of these could cause exposure to biological, chemical and radiation hazards depending on the user's experiments. Users should educate themselves about the samples they are using to avoid these hazards.



WARNING! The IN Cell Analyzer 2200/2000 uses extremely high voltage. Ensure that all power has been shut off prior to opening the lamp housing.



WARNING! Always use protective glasses and other personal protective equipment appropriate with the current application, to ensure personal safety during operation and service.



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



CAUTION! Be aware of potential pinch points when the instrument is powered up. The system can move at any time and could potentially cause injury.



CAUTION! Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.



WARNING! All repairs should be done by personnel authorized by GE Healthcare. Do not open any covers or replace parts unless specifically stated in instructions.

3.2 Laser safety interlock system

The **Laser Safety Interlock Circuit** uses a electromechanical switch located on the top cover and a mechanical switch located on the powered **Sample Access Door** to determine when the system is safe to use either laser illumination or laser autofocus.



WARNING: LASER RADIATION!

This product is a Class 1 laser device and safe under conditions of normal use.

Note: *This feature will not be detected during normal operation or maintenance, and only be encountered by authorized GE Healthcare personnel.*

3.3 IN Cell Analyzer 2200/2000 laser

3.3.1 Laser autofocus

The **Laser Autofocus** module contains a 100 mW, 785 nm diode laser from *Power Technology, Inc.* The laser is continuous wave. Access to the beam path is

prevented by the use of covers and other system parts that shield the operator from the beam.



WARNING: LASER RADIATION!

WARNING: LASER RADIATION indicates a hazardous situation which, if not avoided, could result serious injury due to hazardous radiation. It is important not to proceed until all stated conditions are met and clearly understood.

Note: *IN Cell Analyzer 2000 systems built prior to July 2011 contain a 50 mW, 785 nm diode laser.*

The only access point for the beam during normal operation is through the objective. The powered **Sample Access Door** should always be closed when imaging. However, if the door is open and the **Laser Autofocus** is activated by bypassing the laser switch, the laser beam that comes out is not safe for viewing (Class 3B).

Note: *During normal operation the Laser Switch should never be bypassed! The Laser Autofocus should not be used without the Powered Sample Access Door installed and the Top Cover lowered.*

User-maintainable parts are not in the beam path area. The only access point for the beam during operation or maintenance is through the objective. The cover door should always be closed when imaging.

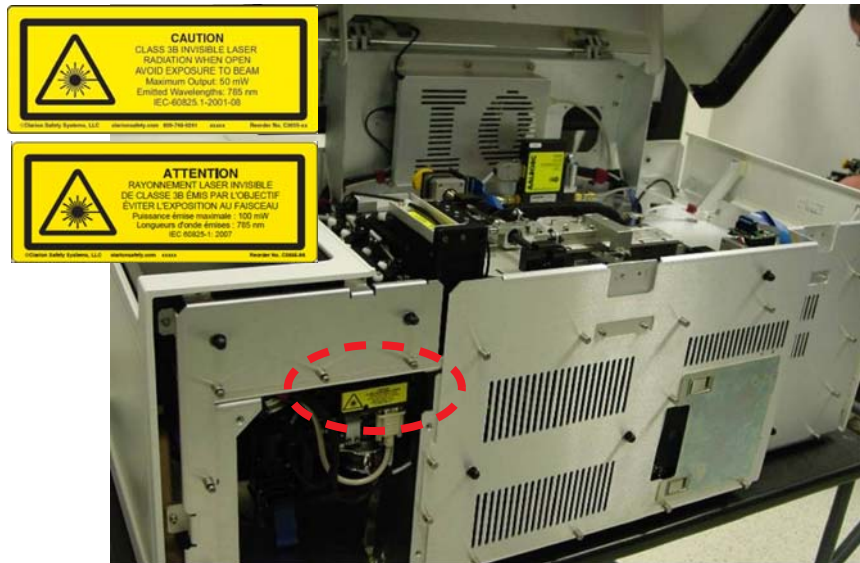
3.4 Safety labeling

This section lists the safety labels attached at various locations to the *IN Cell Analyzer 2200/2000* imaging system.

3.4.1 Laser safety label

The safety label below is attached to the cover of the **Laser Autofocus** module. It is not visible during operation or maintenance, but is visible during service.

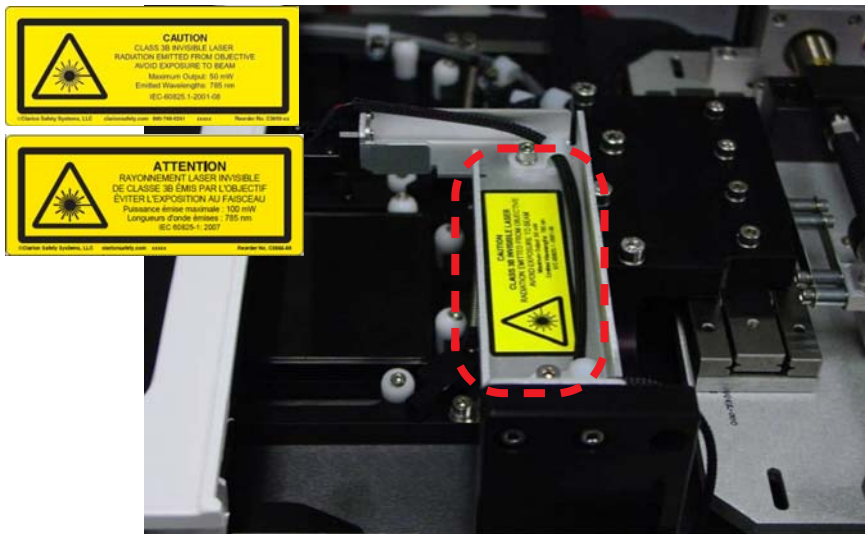
Fig 3-1. IN Cell Analyzer 2200/2000 Panel Safety label and placement.



3.4.2 Objective safety label

The safety label shown below is attached on the stage of the imager, above the objective. It is not visible during operation or maintenance, but is visible during service.

Fig 3-2. IN Cell Analyzer 2200/2000 Objective Safety Label and placement.



3.4.3 Interlocked protective housing warnings

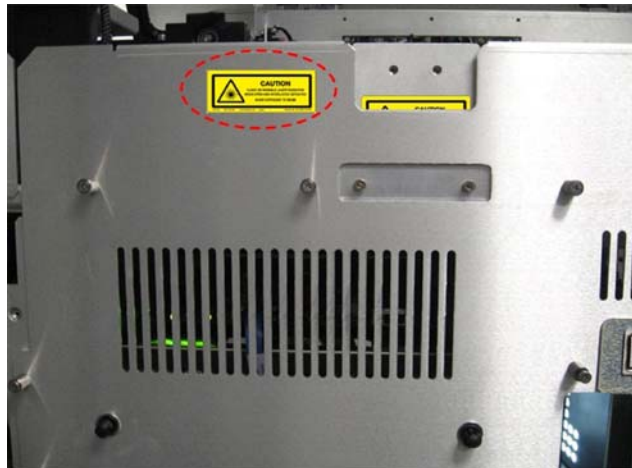
The interlock safety label indicates that the component is part of the **Laser Safety Interlock Circuit**. It is applied at two locations on the *IN Cell Analyzer 2200/2000*. This label is not visible during operation or maintenance, but is visible during service. Use appropriate laser safety precautions when accessing these areas.

Fig 3-3. IN Cell Analyzer 2200/2000 Interlock Safety Label.



The first location for the interlock safety label is on the outside of the front metal chassis cover as shown.

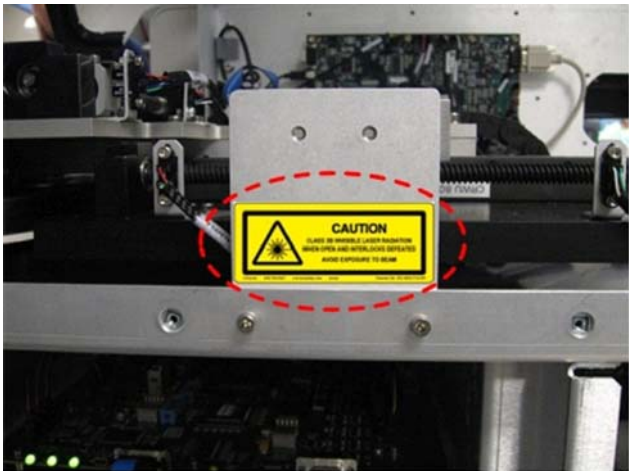
Fig 3-4. IN Cell Analyzer 2200/2000 Interlock Safety Label Placement (Position 1).



The second location for the interlock safety label is attached to the left-front metal tab (behind the metal chassis cover) on the front as shown.

Note: This label is partially covered up when the front metal chassis cover is attached to the system.

Fig 3-5. IN Cell Analyzer 2200/2000 Interlock Safety Label Placement (Position 2).



3.4.4 Instrument identification labeling

This label indicates that the system complies with appropriate CFR and IEC regulations, as well as, containing the system part number, serial number, and manufacturing date.

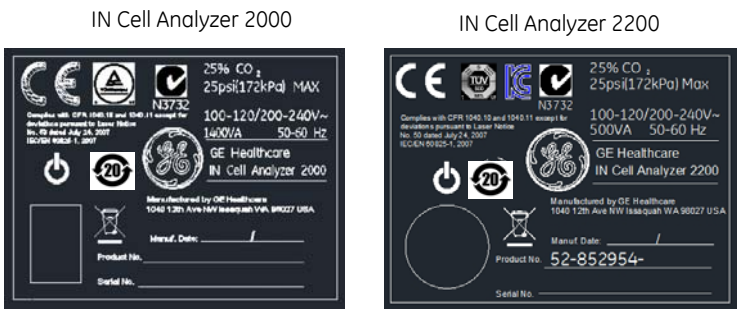


Fig 3-6. I.D. Label placement



3.5 Electrical safety

All the *IN Cell Analyzer 2200/2000* electrical components comply with CE and other applicable standards, such as UL, CSA, and IEC. Follow the electrical safety information in this section to ensure that you are operating the *IN Cell Analyzer 2200/2000* safely.



WARNING! High-voltage electronics may be found in some *IN Cell Analyzer 2200/2000* components.

Under normal operating conditions, you are protected from high voltage.



CAUTION! Before using the *IN Cell Analyzer 2200/2000*, ensure that the system is clean and dry. Any detected problems should be dealt with before the system is started up.



WARNING! The *IN Cell Analyzer 2200/2000* is intended to operate from a power source that does not apply more than 100-120/200-240V (50-60 Hz) between the supply conductors or between either supply conductor and ground. A protective ground connection, by way of the grounding conductor in the power cord, is essential for safe operation.



WARNING! If any liquid is spilled on or around the instrument unplug the instrument immediately and wipe it up, if there is any possibility that some of the liquid may have leaked into the instrument case contact GE Healthcare immediately. **DO NOT PLUG THE INSTRUMENT INTO ANY POWER MAINS**, until the problem is resolved.



WARNING! In case of fire, unplug the instrument. Make sure the rear power connectors are always accessible.



WARNING! Maintain easy 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 and the power cord must always be easy to disconnect.



WARNING! Only use power cords delivered or approved by GE Healthcare.



WARNING! Do not place containers of liquid on top of the instrument. Spilled liquid is a fire and electrical hazard.

3.6 Lamp safety precautions

On the IN Cell Analyzer 2200:

The solid state light source requires no replacement and is not accessible to the user.

On the IN Cell Analyzer 2000:

The lamp assembly module is a replaceable unit. There are three risks associated with handling the lamp assembly: exposure to heat, exposure to ultraviolet light, and exposure to mercury vapor.



WARNING! The lamp contains noxious substances such as mercury. Exposure to mercury is a health hazard. Follow the instructions below to minimize risks associated with the lamp.



WARNING! Oil from fingerprints on the bulb can heat up and cause the bulb to explode when it is turned on, releasing dangerous vapor.

Observe the following safety precautions regarding the *IN Cell Analyzer 2000* system's lamp:

- Never open the lamp access door while the lamp is turned on.
- Do not touch the bulb.
- The bulb used in the primary lamp assembly is a Metal Halide & High Pressure Sodium discharge lamp. Metal Halide discharge lamps can emit ultraviolet radiation that may be harmful to eyes and skin. Metal Halide and High Pressure Sodium discharge lamps should only be operated with the compatible ballast, rated fixture (open/closed, wattage), and socket.
- Never operate the lamp beyond its 2000-hour rated life. The risk of a lamp burst increases with lamp age, temperature, improper operation, and improper handling. Replace the lamp at or before the end of its rated life.

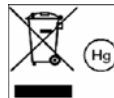
Lamp hour usage can be found within the hardware submenu (*Hardware submenu*).

- Never bump, drop, apply excessive stress, or scratch the lamp. This could cause the lamp to burst!
- Clean any dirt, oil (including fingerprints), or lint from the lamp with alcohol and a lint-free cloth or tissue. Dirt or other contaminants affect light output and may cause the lamp to overheat and/or burst.
- Always power down the system and wait for the lamp to cool before replacing or removing the lamp assembly. Never touch the lamp assembly when it is on, or within 10 minutes after the lamp has been turned off.
- Do not use the lamp in close proximity of paper, cloth or other combustible material that may cause a fire hazard.
- Do not look directly at the operating lamp for any period of time; this may cause serious eye injury.
- The gas discharge lamp used in the *IN Cell Analyzer 2000* system contains mercury.



WARNING! If the bulb should burst the fumes can be toxic. Evacuate the room immediately and remain out of the room for at least 30 minutes. Call GE Healthcare for a replacement bulb assembly.

The following sticker should appear on the *Lamp Assembly* access door:



The lamp contains noxious substances (particularly mercury). Refer to Lamp Disposal in the Lamp Safety section for more information.

3.7 Disposal

3.7.1 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 the manufacturer for information concerning the decommissioning of your equipment.



CAUTION

The IN Cell Analyzer Instrument and Environmental Control Module must not be disposed as unsorted municipal waste and must be collected separately. Please contact GE Healthcare for information concerning the decommissioning of equipment.

3.7.2 Waste materials

Microplates and any used materials in the instrument must be disposed of in the manner prescribed by local standard operating procedures.



CAUTION! Hazardous waste must be specially handled and disposed of properly.

3.7.3 Lamp disposal

On the IN Cell Analyzer 2000:

Since the lamp contains noxious substances, it must be disposed of in Europe as special waste under EWC Code 20 01 21* "Fluorescent tubes and other mercury-containing waste".

The lamp is affected in EU by the scope of WEEE and can be disposed free of charge from private households and small consumers at all communal disposal facilities.

In the United States, refer to <http://www.lamprecycle.org> or call customer support at +1 800 862 5166. In other countries, the relevant national regulations must be followed.

3.8 Radio interference



WARNING! This is a Class A product. In a domestic environment, this product may cause radio interference, in which case, the user may be required to take adequate measures.

3.9 Autofocus

The autofocus mechanism identifies changes in refractive index to locate the exterior (air-solid interface) of the sample support (slide, cover slip, or plate), as well as the interface between the support and the medium surrounding the sample (solid-liquid interface). The image is then acquired at a specified offset relative to the solid-liquid interface.

3.10 Temperature control

The *IN Cell Analyzer 2200/2000* is equipped with a temperature-controlled stage designed to provide an optimal environment for cell plates while inside the instrument. The temperature chamber is a proprietary design. It provides variable temperature control from 5°C above the ambient temperature up to 42°C. The unit

will maintain the cell plate at the set temperature $\pm 1^{\circ}\text{C}$ with minimal evaporation loss, since the plate is enclosed above and below in the temperature environment (with a small aperture above the objective to facilitate liquid addition, when the *Environmental Control* module is not in place).

3.11 Plate and slide formats

The *IN Cell Analyzer 2200/2000* can read either glass or plastic SBS*-standard footprint microplates available from several manufacturers, and ranging from 6-well to 1536-well densities**. A table of plate types with compatible dimensions can be found in *Create a New Slide Configuration*.

* Society for Biomolecular Screening.

** AutoFocus performance may vary with objective and plate type, depending particularly on plate bottom thickness and uniformity. Plate types particularly prone to non-uniformity or extremes of plate-bottom thickness are 6-, 12- and 1536-well formats; all microplates should be validated empirically on the system with the desired assay before use in a screen. When read in conjunction with some objectives, Greiner and Nunc thin-bottomed plates (175 μm and 50 μm base thickness, respectively) with non-standard plate uniformity may present problems with Autofocus.

3.12 Declaration of Hazardous Substance (DoHS)

3.12.1 Introduction

The following product pollution control information is provided according to SJ/T11364-2006 Marking for Control of Pollution caused by Electronic Information Products.

根据 SJ/T11364-2006 《电子信息产品污染控制标识要求》特提供如下有关污染控制方面的信息

3.12.2 Symbols used in pollution control label

电子信息产品污染控制标志说明

Label	Meaning
	This symbol indicates the product contains hazardous materials in excess of the limits established by SJ/T11364-2006 Marking for Control of Pollution caused by Electronic Information Products. The number in the symbol is the Environment-friendly Use Period (EFUP), which indicates the period during which the toxic or hazardous substances or elements contained in electronic information products will not leak or mutate under normal operating conditions so that the use of such electronic information products will not result in any severe environmental pollution, any bodily injury or damage to any assets, the unit of the period is "Year". In order to maintain the declared EFUP, the product shall be operated normally according to the instructions and environmental conditions as defined in the product manual, and periodic maintenance schedules specified in Product Maintenance Procedures shall be followed strictly. Consumables or certain parts may have their own label with an EFUP value less than the product. Periodic replacement of those consumables or parts to maintain the declared EFUP shall be done in accordance with the Product Maintenance Procedures. This product must not be disposed of as unsorted municipal waste, and must be collected separately and handled properly after decommissioning. 该标志表明本产品含有超过电子信息产品中有毒有害物质的限量要求》中限量的有毒有害物质。标志中的数字为本产品的环保使用期，表明本产品在正常使用的条件下，有毒有害物质不会发生外泄或突变，用户使用本产品不会对环境造成严重污染或对其人身、财产造成严重损害的期限，单位为年。 为保证所声明的环保使用期限，应按产品手册中所规定的环境条件 和方法进行正常使用，并严格遵守产品维修手册中规定的期维修和 保养要求。 产品中的消耗件和某些零部件可能有其单独的环保使用期限标志，并且其环保使用期限有可能比整个产品本身的环保使用期限短。应 到期按产品维修程序更换那些消耗件和零部件，以保证所声明的整个产品的环保使用期限。 本产品在使用寿命结束时不可作为普通生活垃圾处理，应被单独收 集妥善处理

3.12.3 List of hazardous substances and their concentrations

产品中有毒有害物质或元素的名称及含量

Indication for each major part if substance exceeds limit

Value	Meaning
O	Indicates that this toxic or hazardous substance contained in all of the homogeneous materials for this part is below the limit requirement in SJ/T11363-2006. 表示该有毒有害物质在该部件所有均质材料中的含量均在标准规定的限量要求以下
X	Indicates that this toxic or hazardous substance contained in at least one of the homogeneous materials used for this part is above the limit requirement in SJ/T11363-2006. - Data listed in the table represents best information available at the time of publication - Applications of hazardous substances in this medical device are required to achieve its intended clinical uses, and/or to provide better protection to human beings and/or to environment, due to lack of reasonably (economically or technically) available substitutes.. 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出标准规定的限量要求 - 此表所列数据为发布时所能获得的最佳信息 - 由于缺少经济上或技术上合理可行的替代物质或方案，此医疗设备运用以上一些有毒有害物质来实现设备的预期临床功能，或给人员或环境提供更好的保护效果。

3.12.4 List of hazardous substances

The IN Cell Analyzer 2000 contains the following substances by component:

	有毒有害物质或元素					
	Hazardous substances' name					
部件名称	铅	汞	镉	六价铬	多溴联苯	多溴二苯醚
Component Name	(Pb)	(Hg)	(Cd)	(Cr6+)	(PBB)	(PBDE)
Printed circuit board assemblies	X	O	O	O	O	O
Arc Lamp assemblies	X	X	O	O	O	O
Power supplies	X	O	O	O	O	O
Camera assembly	X	O	O	O	O	O
Cables	X	O	O	O	O	O
Chassis assembly	O	O	O	X	O	O
Enclosure	O	O	O	O	O	O
Fastners	O	O	O	X	O	O

The *IN Cell Analyzer 2200* contains the following substances by component:

	有毒有害物质或元素					
	Hazardous substances' name					
部件名称	铅	汞	镉	六价铬	多溴联苯	多溴二苯醚
Component Name	(Pb)	(Hg)	(Cd)	(Cr6+)	(PBB)	(PBDE)
Printed circuit board assemblies	X	O	O	O	O	O
Solid State Illumination assembly	O	O	O	O	O	O
Power supplies	O	O	O	O	O	O
Camera assembly	O	O	O	O	O	O
Cables	X	O	O	O	O	O
Chassis assembly	O	O	O	X	O	O
Enclosure	O	O	O	O	O	O
Fastners	O	O	O	X	O	O

4 IN Cell Analyzer 2200/2000 user interface

4.1 Getting started with the IN Cell Analyzer 2200/2000

To start the *IN Cell Analyzer 2200/2000* imaging system, you need to turn on the imaging system workstation and start up the system software.

To turn on the *IN Cell Analyzer 2200/2000* system:

- 1 Turn on the workstation and monitor and log on, if necessary.
- 2 Turn on the imaging system hardware:
 - Depress the system power switch (shown below) once until a click is heard.

Fig 4-1. IN Cell Analyzer instrument power switch



- Wait five minutes to allow the *IN Cell Analyzer 2200/2000* to initialize before starting the system software. During the initialization process three lights will illuminate on the front of the instrument (green, then orange, then red). The green light will display following the opening and closing of the **Stage Access Door**, indicating that initialization is complete.

Note: On early models of the *IN Cell Analyzer 2000* the system power switch is a momentary switch. When you press the switch a second time in exactly the same manner, the imaging system hardware is turned off.

To start the *IN Cell Analyzer 2200/2000* system software:

- **On the *IN Cell Analyzer 2200*:**

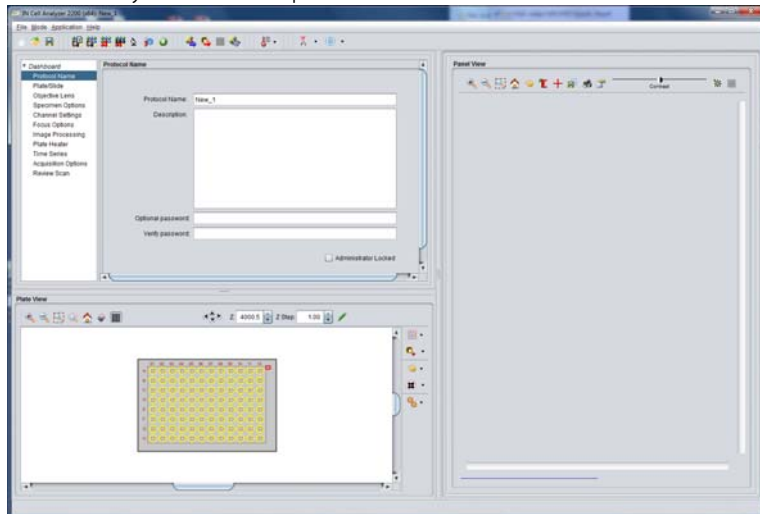
4 IN Cell Analyzer 2200/2000 user interface

4.2 Switching the lamp power on

- **Start | All Programs | GE Healthcare | IN Cell Analyzer 2200**
- Click the desktop icon .
- **On the IN Cell Analyzer 2000:**
 - **Start | All Programs | GE Healthcare | IN Cell Analyzer 2000**
 - Click the desktop icon .

The **Workspace** is displayed. The *Main menu* and *Main toolbar* provide basic navigation and function.

Fig 4-2. IN Cell Analyzer 2200 Workspace



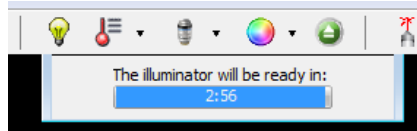
4.2 Switching the lamp power on

On the IN Cell Analyzer 2000 only:

The metal halide lamp needs to be switched on manually prior to use.

- 1 Toggle the **Illuminator**  toolbar icon to turn the lamp on. The icon displays  indicating the lamp is on, and the **Power up** dialog opens. Allow the lamp to warm-up for 3 minutes to the proper intensity.

Fig 4-3. IN Cell Analyzer 2000 lamp dialog



- 2 The lamp will automatically power down to a stand-by mode when the instrument has been idle for 30 minutes or more.

4.3 Stopping/shutting down the instrument

To stop the *IN Cell Analyzer 2200/2000* acquisition software, follow the steps outlined below:

- 1 (Optional) Switch off the lamp by toggling the **Main toolbar|Lamp** icon, as necessary, on the *IN Cell Analyzer 2000*.
- 2 From the *Main* menu, select **File|Exit**. You will be prompted to save your current protocol file if not already saved.

Note: The **Application|Hardware|Shutdown** option cannot be used when you have stopped the acquisition software from the **File|Exit** option.

- 3 Select either **Yes** or **No** as appropriate. The acquisition software will shutdown.

To shut down the *IN Cell Analyzer 2200/2000* imaging system, follow the steps outlined below:

- 1 Power down the system by:
 - Clicking **Application|Hardware|Shutdown or**
 - Depress the system power switch for one second. After a two minute delay, the system should power down.
- 2 Depress the system power switch again if the system does not shutdown after two minutes.

Note: If the system still does not shutdown, press and hold the power switch to perform a 'hard' system shutdown.

- 3 Switch off the PC and monitor.

4.4 Finding Help information

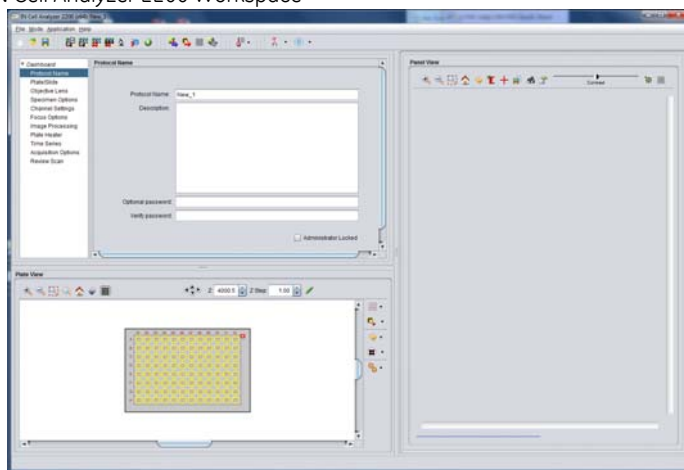
4.4.1 User Guide

The *IN Cell Analyzer 2200/2000* workstation software contains an abridged User Reference Manual. To view or print the document, click the **Main menu|Help** icon. The complete manual is available in print as part of your customer package.

4.5 The Workspace

The *IN Cell Analyzer 2200/2000* acquisition software utilizes a **Workspace** from the predefined system modes can be opened. Additionally, to maximize flexibility, various actions can be performed independently of one of these *modes*. The *Main menu* and *Main toolbar* provide basic navigation and function.

Fig 4-4. IN Cell Analyzer 2200 Workspace



The following sub-sections describe the menu and toolbar functionality.

4.5.1 Main menu

The **Main menu** provides access to most of the features available to define and run acquisition protocols. The menu items are also used in conjunction with the toolbar items to execute tasks.

File menu

From the **File** option, select from the basic operations:

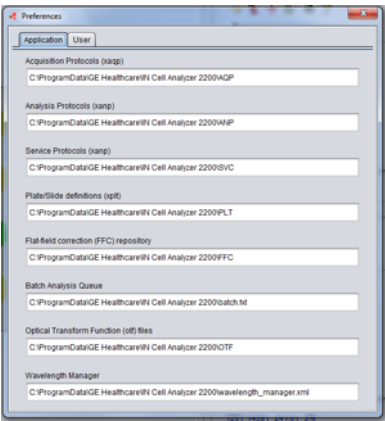
	New	Creates a new acquisition protocol (.xaqp) file.
	Open	Opens an existing acquisition protocol (.xaqp) file.
	Save	Saves an acquisition protocol file with its current file name.
	Save As	Saves the currently active acquisition protocol file with a new name or a new location.
	Preferences	Opens the File Preferences window. see <i>File Preferences window option</i> below.
	Exit	Closes the <i>IN Cell Analyzer 2200/2000</i> system software.

File Preferences window

The **Preferences** window displays both application-based and user-defined settings on two tabs.

- **Application tab** - displays (read-only) the location of the various system folders and files accessed by the *IN Cell Analyzer 2200/2000* application.

Fig 4-5. File Preferences - Application tab.



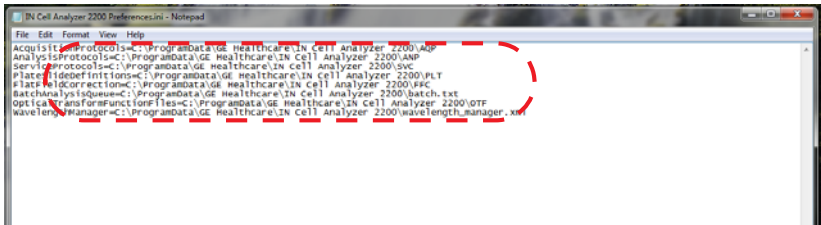
Acquisition Protocol (.xaqp)	Folder where the acquisition protocols are saved for each defined protocol.
Analysis Protocol (.xanp)	Folder containing specialized GE analysis protocol instructions.

Service Protocol (.xanp)	Folder containing specialized GE analysis protocol instructions utilized by GE Healthcare field service personnel.
Plate/Slide Definitions (.xplt)	Folder containing the plate/slide holder configuration information for each plate/slide defined in the system.
Flat Field Correction (FFC) Repository	Folder containing flat field correction data. The FFC data files are stored as .tiff files.
Batch Analysis Queue	Folder containing the Batch Analysis Queue file (*.txt) where the batch information is stored.
Optical Transform Function (.otf)	Folder containing the optical transform function data for each objective supported on your installation.
Wavelength Manager	Folder where the wavelength configuration information generated by the Wavelength Manager is stored in an .xml file.

If necessary, to manually modify the default folder locations, open the file.

IN Cell Analyzer 2000 (XP & Win7 OS respectively)	C:\Program Files\Common Files\GE Healthcare\IN Cell Analyzer 2000\IN Cell Analyzer 2000 Preferences.ini C:\ProgramData\GE Healthcare\IN Cell Analyzer 2200\IN Cell Analyzer 2000 Preferences.ini
IN Cell Analyzer 2200	C:\ProgramData\GE Healthcare\IN Cell Analyzer 2200\IN Cell Analyzer 2200 Preferences.ini

Edit the locations for the application tab parameters as appropriate, and save the file.



- **User tab** - displays, and allows the modification of, the locations to where user data are stored.

Fig 4-6. File Preferences - User tab.

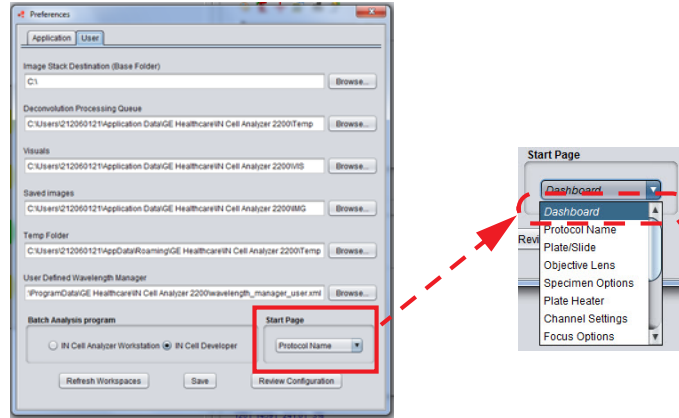


Image Stack Destination (Base Folder)	Displays the location where all of the scan data is stored.
Deconvolution Processing Queue	Shows the location where the Processing Queue file information is stored.
Visuals	Shows the location where the data files are stored for graphic representations displayed in Visuals .
Saved Images	Shows the central location where all of the saved image files are stored.
Temp Folder	Displays the location where temporary files are stored.
User Defined Wavelength Manager	Displays the location where the user defined wavelength configurations are stored.
Batch Analysis program	Mark the radio button to indicate the format of the acquisition results for analysis by <i>IN Cell Developer Toolbox</i> or <i>IN Cell Analyzer Workstation</i> .
Start Page	Select the Protocol Designer card to set as the default start point.

To modify the default locations:

- 1 Click the **Browse** button to the right of the item you want to change.
- 2 From the **Open** dialog presented, navigate to the new location. Click **OK**. The new path name will display in the text field.
- 3 Use the **Refresh Workspace** button to restore the application window settings.

Mode menu

From the **Mode** option, select from:

	Assay Development	Uses the IN Cell Analyzer 2200/2000 in Assay Development mode. Refer to <i>Chapter 5 Assay Development Mode - Designing Experiments</i> for detail.
	Acquisition	Uses the IN Cell Analyzer 2200/2000 in Acquisition mode. Refer to <i>Chapter 7 Acquisition Mode - Conducting Experiments</i> for detail.
	Data Review	Uses the IN Cell Analyzer 2200/2000 in Data Review mode. Refer to <i>Chapter 8 Data Review Mode - Reviewing collected data</i> for detail.
	Analysis	Uses the IN Cell Analyzer 2200/2000 in Review Scan mode. Refer to <i>Chapter 9 Review Scan Mode - focusing on wells of interest</i>
	Service	<u>Do not select this option unless you are an authorized IN Cell Analyzer 2200/2000 Service Representative. Service Mode is designed to be used only by those thoroughly trained on the servicing of the IN Cell Analyzer 2200/2000 imaging system.</u>

Application menu

From the **Application** option, select from:

	Plate/Slide Manager...	Opens the Plate/Slide Manager window. Refer to <i>Plate/Slide Manager</i> below for detail.
	User-defined Wavelength Manager	Opens the Wavelength Manager window. Refer to <i>Wavelength Manager</i> below for detail.
	Start Acquisition Session...	Opens the Start Acquisition Session dialog, from which to run the current protocol or open a saved protocol. Refer to <i>Chapter 7 Acquisition Mode - Conducting Experiments</i> for detail.
	Open Image Stack...	Selects the current image stack in <i>Data Review</i> mode or select a new image stack from the sub-menu.
	Access Protocol...	Allows user to enter a password to access a protected protocol for editing. (<i>IN Cell Compliance Manager feature</i>)
	Hardware	Displays submenu of options; see <i>Hardware submenu</i> in this chapter for detail.
	Robotics...	Opens the Robotics window see <i>Robotics</i> in this chapter for detail.

	Processing Queue	Shows the status of each 3D Deconvolution or Advanced 2D process within a protocol run Refer to <i>Chapter 7 Acquisition Mode - Conducting Experiments</i> for detail.. Shows the status of data export to <i>IN Cell Miner HCM</i>. Refer to <i>IN Cell Miner HCM</i> in this chapter for detail
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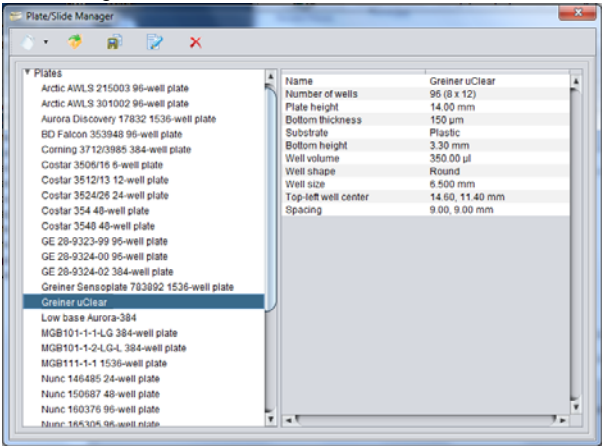
Plate/Slide Manager

The **Plate/Slide Manager** provides the ability to add new plate and slide definitions, and edit the existing plate/slide definitions. The available plate and slide holder types are listed on the left of the window. For a selected plate/slide, the details will display in the panel to the right side of the window.

The **Plate/Slide Manager** is accessed from the **Application|Plate/Slide Manager** menu item.

Alternative tools are displayed when you select a plate or slide name and right-click the mouse. These tools allow you to rename, delete, or edit the file.

Fig 4-7. Plate/Slide Manager window.



The **Plate/Slide Manager** functions are accessed from the provided toolbar.

Button	Function	Description
	New	Creates a new plate/slide configuration file. The Plate or Slide Editor is displayed. Refer to <i>Creating a new plate configuration</i> or <i>Create a new slide configuration</i> for detail.
	Open	Opens an existing plate/slide configuration file. Browse to and select the plate file (*.xplt) you want to open (not in the list).

Button	Function	Description
	Save As	Saves a plate/slide configuration file in a different location or with a different name. Click on the Save As , browse to the desired location and enter a name under which to save the configuration file.
	Edit	Edit an existing plate/slide configuration. The Plate or Slide Editor is displayed. Refer to <i>Creating new plates & slides from existing configurations</i> for detail.
	Delete	Deletes the selected plate or slide configuration.

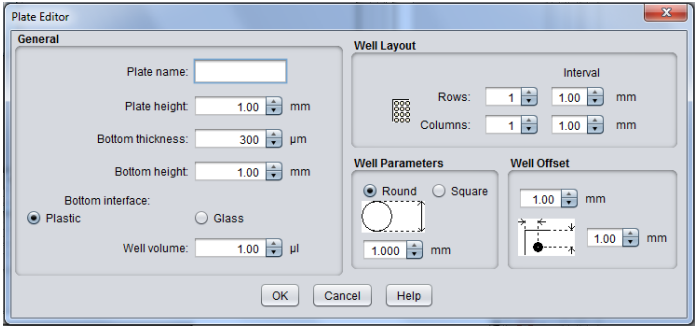
Creating a new plate configuration

The **Plate Editor** is used to specify the spatial parameters of the plate. This information can be found in the specification sheet provided by the plate manufacturer.

To create a plate definition, do one of the following:

- 1 In the *Plate/Slide* list, highlight the **Plates** category name.
- 2 Click the **New Plate** icon on the **Plate/Slide Manager** toolbar. The **Plate Editor** opens.

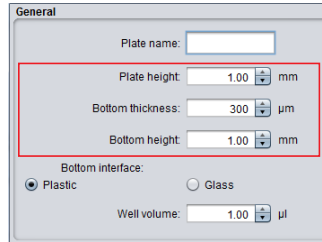
Fig 4-8. Plate Editor Window



- 3 Complete the **General**, **Well Layout**, **Well Parameters** and **Well Offset** sections for the plate.
- *General section:* Assign a name to the plate type, specify the total plate height, Bottom thickness, skirt (or Flange) height, Bottom interface is glass or plastic, and the total volume of each well.

Note: When you are using Laser Autofocus, these measurements must be as

accurate as possible.



- **Well Layout:** Provide the number of rows and columns on the plate, and the Interval (separation space) between adjacent wells. The interval is the distance from the center of one well to the center of an adjacent well.
- **Well Parameters:** Indicate whether the well is round or square in shape, and specify the diameter of the well.
- **Offset:** Specify the distance from the center of the top left well to the top and left edges of the plate.

- 4 When all parameters have been specified, click **OK** to save the definition and close the **Plate Editor**. The plate type is added to the list of available types. Click **OK** to exit the **Plate/Slide Manager**.

Predefined plate types for IN Cell Analyzer 2200/2000

List of the acceptable plates for IN Cell 2200/2000		
	2× / 4× Objective	10× / 20× /40× Objective
6-well plate	Costar 3506/16 Sumilon MS-80060	Costar 3506/16 Sumilon MS-80060
12-well plate	Costar 3512/13	Costar 3512/13
24-well plate	Costar 3524/26 PEWallac 1450-605 Nunc 146485 (replaced by new 142485)	Costar 3524/26 PEWallac 1450-605 Nunc 146485 (replaced by new 142485)
48-well plate	Costar 3548 Nunc 150687	Costar 3548 Nunc 150687
96-well plate	PE ViewPlate-96 6005182 BD Falcon 353948 Arctic AWLS 215003 Arctic AWLS 301002 Greiner µclear 655090 Nunc 165305 Nunc 160376 Nunc 265302	PE ViewPlate-96 6005225 BD Falcon 353948 Arctic AWLS 215003 Arctic AWLS 301002 Greiner µclear 655090 Nunc 165305 Nunc 160376 Nunc 265302

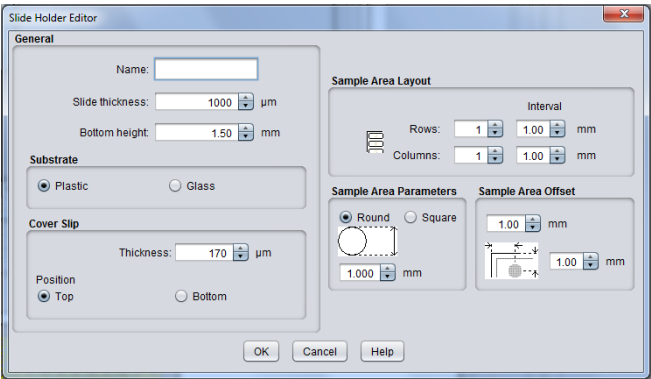
List of the acceptable plates for IN Cell 2200/2000		
384-well plate	MGB101-1-1-LG Corning 3712/3985	MGB101-1-1-LG Corning 3712/3985
1536-well plate	MGB111-1-1	MGB111-1-1 Aurora Discovery 17832 Greiner Sensoplate 783892

Create a new slide configuration

The process of defining slide parameters is similar to that for creating a plate definition described in *Creating a new plate configuration*.

- 1 In the *Plate/Slide* list, highlight the **Slides** category name.
- 2 Click the **New** icon on the **Plate/Slide Manager** toolbar. The **Slide Holder Editor** opens.

Fig 4-9. Slide Holder Editor Dialog Box.



- 3 Complete the **General**, **Cover Slip**, **Sample Area Layout**, **Sample Area Parameters**, and **Sample Area Offset** sections for the slide holder.
- *General*: Assign a name to the slide holder, specify the slide thickness, and the slide bottom height.
 - *Substrate*: Specify whether the slide is glass or plastic.
 - *Cover Slip*: Specify the thickness of the cover slip, and whether it will be positioned on the top or bottom of the slip during acquisition.
 - *Sample Area Layout*: indicates the number of rows and columns on the slide, and the Interval (separation space) between adjacent sample areas. The interval is the distance from the center of sample area to the center of an adjacent sample area.

- *Sample Area Parameters*: Indicates whether the well is round or square in shape, and specify the diameter of the sample area.
 - *Sample Area Offset*: Specify the distance from the center of the top left sample area to the top and left edges of the slide holder.
- 4 When all parameters have been specified, click **OK** to save the definition and close the **Slide Holder Editor**. The slide holder is added to the list of available types. Click **OK** to exit the **Plate/Slide Manager**.

Creating new plates & slides from existing configurations

You may modify an existing plate and slide definitions or you may chose to create a new slide definition using an existing file as a template. Be aware, that when creating a new configuration from an existing one, the fields will contain the parameters of the original file.

- To open an existing plate configuration not already in the list, click the **Open** icon on the **Plate/Slide Manager** toolbar. Then, browse to and select the plate file (*.xpl/t) you want to open.
- To edit the configuration settings for a particular plate, click the **Edit** icon in the **Plate/Slide Manager** toolbar. The **Plate Editor** window is displayed, including all of the settings for the currently selected plate.

Saving & deleting plate/slide configurations

- To save a plate configuration file in a different location or with a different name, click the **Copy** icon on the **Plate/Slide Manager** toolbar. Then, browse to the desired location and enter a name under which to save the plate configuration file.
- To delete a specific plate configuration file, highlight the file name and click the **Delete** icon on the **Plate/Slide Manager** toolbar. A confirmation window is displayed. Click **OK** to delete the file.

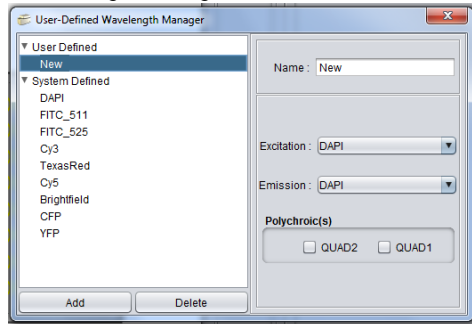
*On the **Plate/Slide Manager** window, alternative tools are displayed when you select a plate or slide name and right-click the mouse. These tools allow you to **Rename**, **Delete**, or **Edit** the file.*

User-defined Wavelength Manager

The **User-defined Wavelength Manager** window provides a method of creating a single-name shortcut for designating filter sets you commonly use.

- 1 From the Applications menu, select **User-defined Wavelength Manager**. The **User-defined Wavelength Manager** dialog opens.

Fig 4-10. User-defined Wavelength Manager window



- 2 In the **Name** field, enter a name (shortcut) for the wavelength definition. An existing wavelength may be selected for modification or to copy as a new filter pair.

Note: Expanding the Pre-defined list at the left side of the window allows you to view the Pre-defined wavelengths. These cannot be modified.

- 3 In the **Excitation** and **Emission** fields, choose the filter pair to use for this wavelength definition.
 - In the **Excitation** field, select an excitation filter from the dropdown.
 - In the **Emission** field, select an emission filter from the dropdown.
- 4 Mark the **Polychroic** checkbox to have the polychroic filter defined with the filter pair. If you want to leave the selection of the polychroic filter flexible, leave the checkbox unmarked. The polychroic must then be selected from the **Protocol Designer|Channel Settings** card.

Note: If the selected polychroic filter is not available on the system when the wavelength is imaged, it will not be used.

Note: You can include a polychroic filter in the shortcut definition; however, if the specified polychroic filter is not available on the system when the shortcut is selected, it will not take effect.

If the checkbox is not activated, the polychroic filter currently active in the system will be used as the default.

- 5 Click **Add** to store the new wavelength definition.
- 6 Repeat Steps 4 and 5 for each filter pair you want to define for this experiment.

Using the Pre-defined Wavelengths

The pre-defined wavelengths allow you to easily access specific channel configurations from the **Add Channels** pop-up from the **Channel Settings** card, **Dashboard Insert Channel** option or **Dashboard** right-click channel list. Selecting the item on the pop-up adds the channel with defined parameters to the protocols list of wavelengths.

Once a channel is used in a protocol it does not retain any reference to the pre-defined wavelength, that is, any changes to a pre-defined wavelength configuration will not affect the protocol.

Note: *If the polychroic setting is changed, the channels tied to the original setting becomes invalid. When an incompatible wavelength/polychroic combination is encountered, the original polychroic setting is appended to the channel name.*

Application>Hardware sub-menu

From the **Hardware** sub-menu, select from:

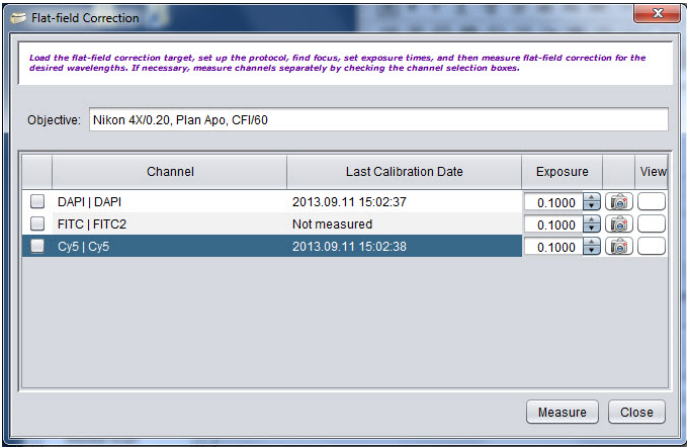
	Eject plate/slide	Click to return the stage to the resting (idle) position and open the plate access door on the top of the instrument to load/unload a plate.
	Measure flat-field correction	Opens the Flat Field Correction window. Refer below for detail.
	Laser autofocus trace	Opens the Laser Autofocus Trace tool. Refer below for detail.
	Illuminator	(IN Cell Analyzer 2000 only) Opens the Lamp dialog from where you can: View information about the lamp age. Reset the lamp age upon replacing the lamp bulb. Note: <i>Do NOT reset the lamp age in the software unless you have physically changed the system lamp.</i>
	Upload instrument log files	
	Shutdown	Click to execute the shutdown sequence, powering down the instrument. Note: <i>It is good practice to unplug the power cable when doing any type of maintenance.</i>

Measure flat-field correction

Clicking **Measure flat-field correction** opens the **Flat Field Correction** window.

The table lists those wavelengths for which flat field correction has been performed and the date of the measurement.

Fig 4-11. Flat Field Correction window



Note: A Flat Field Correction (FFC) Fluorescence Reference Plate will need to be loaded into the IN Cell Analyzer 2200/2000 to complete the flat field correction procedure. The FFC Reference Plate is based on a 96 well PE View plate containing DAPI in well C-3, FITC in well D-3, Cy3 in well E3, and Cy5 in Well F-3. For customizing the reference plate to other dyes, please contact a GE Healthcare application specialist.

The wavelengths displayed are defined by the open acquisition protocol. If a wavelength has not yet been corrected, **Not measured** will display in the **Last Calibration Date** field.

The **Flat Field Correction** interface allows you to set exposures and preview images for each wavelength selected independently of an acquisition protocol.

To perform flat field correction:

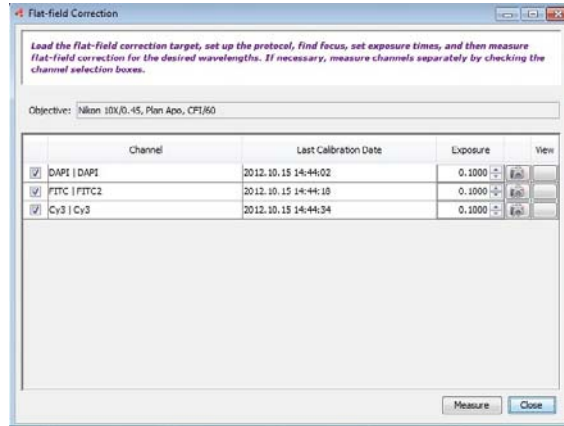
- 1 Select the displayed wavelengths for flat field correction and click the **Measure** button.

Note: Performing flat field correction on a target for which the correction is not available, displays the warning.



Deselect the target and continue by clicking Yes on the dialog. When the process completes, the **Flat Field Correction** window is updated.

Fig 4-12. Flat Field Correction window after clicking Measure



- 2 Click the button the right of a wavelength to display the corrected image in the **Image Explorer** window (see *Image Explorer window*).

Flat field correction files are stored as .tiff files under the FFC directory in the *IN Cell Analyzer 2200/2000* software installation folder. When selected in the protocol, these files will also be placed in the image stack.

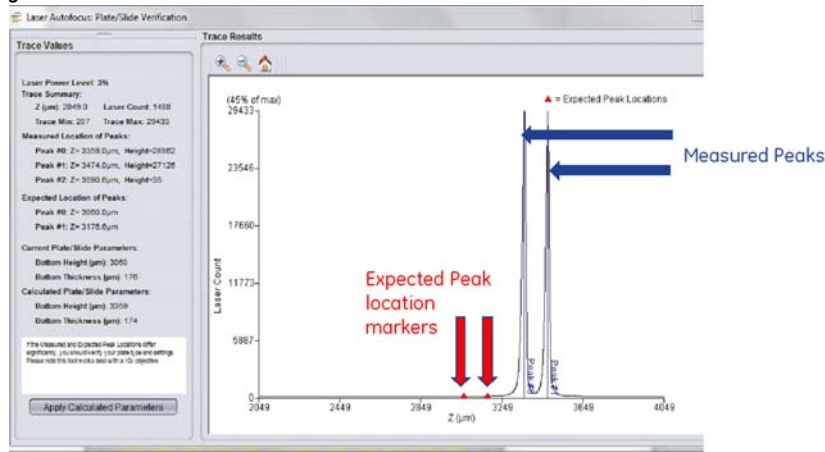
Laser Autofocus Trace

The **Laser Autofocus Trace Tool** window displays the autofocus 'peaks' that indicate the bottom height of the plate and can be used to calculate the bottom thickness. Also displayed are the 'expected' peaks based on the current plate settings.

Trace values are displayed in the Trace Values panel. Manipulating the 'expected' peak markers will update the trace markers real-time. Based on the information provided, the plate/slide settings can be automatically optimized to prevent autofocus errors.

The redesigned LAF tool can be enlarged using the mouse scroll wheel. Additionally, the start and end positions of the scan can be set using the available sliders.

Fig 4-13. Laser Autofocus Trace

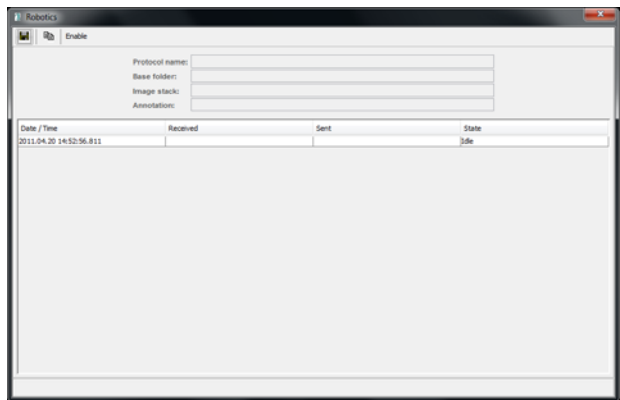


- 1 Click the **Apply Calculated Parameters** button to apply the optimized Laser Autofocus parameters. The **Plate** or **Slide Editor** will open.
- 2 To accept the optimized values, click **OK** on the editor window.

Robotics

Opens the **Robotics** window from where the robotic interface for an acquisition session is defined, and enabled/disabled for use.

Fig 4-14. Robotics window



- 1 In the **Protocol Name**, **Base Folder**, and **Image Stack** fields provide information to define the acquisition.
- 2 Click **Enable** to activate the robotic device.
- 3 Use the **Copy** and **Save** features to save and create additional scenarios.

Consult the information provided by the robotics supplier for more details.

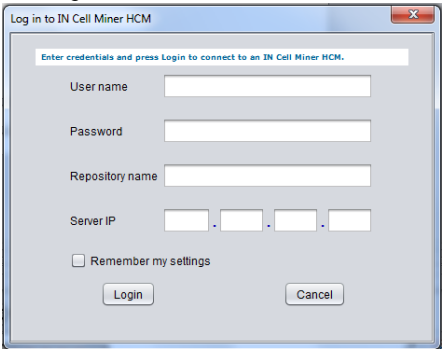
IN Cell Miner HCM

For additional storage capabilities, for storing acquired image stacks, integration with the IN Cell Miner HCM software application is available.

This option opens the **Log in to IN Cell Miner HCM** window where the user credentials, repository name, and server IP address of the Documentum database used by *IN Cell Miner HCM* are supplied.

This information will be used to authenticate the login session when the **Export to IN Cell Miner HCM** checkbox is marked on the **Acquisition Session** window. Refer to the section, *Start the Acquisition* for more detail.

Fig 4-15. IN Cell Miner HCM Login window



Help menu

From the **Help** option, select from:

	IN Cell Analyzer Help	Displays an online version of the <i>IN Cell Analyzer 2200/2000 User's Manual</i> .
	License Agreement	Displays the <i>GE Healthcare Standard Software License Agreement</i> .
	Display Log	Displays a log file for use by GE Healthcare filed service personnel
	About	Displays application information about the <i>IN Cell Analyzer 2200/2000</i> application.

4.5.2 Main toolbar

In addition to the menus, the toolbar provides functionality to perform the tasks necessary for designing and conducting imaging experiments. The toolbar consists of 8 sections.

Table 4-1. IN Cell Analyzer 2200/2000 Main Toolbar

Button	Function	Description
	New	Creates a new acquisition protocol.
	Open	Opens an existing acquisition protocol.
	Save	Saves an acquisition protocol file with its current file name.
	Assay Development Mode	Opens the Assay Development Activity Workspace . See <i>Chapter 5 Assay Development Mode - Designing Experiments</i> for detail
	Acquisition Mode	Opens the Acquisition Activity Workspace . See <i>Chapter 7 Acquisition Mode - Conducting Experiments</i> for detail.
	Data Review Mode	Opens the Data Review Activity Workspace . See <i>Chapter 8 Data Review Mode - Reviewing collected data</i> for details.
	Analysis Mode	Opens the Analysis Activity Workspace . See <i>Chapter 9 Review Scan Mode - focusing on wells of interest</i> for details.
	Microscope Mode	Opens the Microscope mode. See <i>Chapter 10 Microscope Mode - Using IN Cell Analyzer 2200/2000 full-screen</i> for details.
	Service Mode	Designated for use by GE Healthcare Field Service Engineers only.
	Eject Plate/Slide	Moves the stage to the resting position for plate/slide removal.
	Run Protocol	Opens the Acquisition Session window from where to start an image scan. Refer to <i>Chapter 7 Acquisition Mode - Conducting Experiments</i> for details.
	Preview Scan	Previews a selected region of the plate or slide. Refer to <i>Previewing Plate/Slide Regions</i> for details.
	Stop Scan	Halts the currently running acquisition. The icon displays as red when activated.

Button	Function	Description
	Continuous Acquisition	Scans the region of interest continuously acquiring 2D images with the current protocol settings. The Objective and Binning settings can be changed when performing Continuous Acquisition .
	Illuminator	<i>IN Cell Analyzer 2000 only</i> Turns the lamp on/off. The default position is set to <i>OFF</i> .
	Liquid Handling	Enables the Liquid Handling module, if installed. Refer to <i>Liquid Handling</i> in this chapter for details.
	Environmental Control	Enables the Environmental Control module. (turns the Plate Heater feature on and off, sets the target temperature, displays the current temperature, and turns the CO ₂ on or off). Refer to <i>Environmental Control</i> in this chapter for details.
	Laser Autofocus	Activates Laser (hardware) Autofocus . Refer to <i>Focus card</i> for details.
	Software Autofocus	Activates Software Autofocus dropdown list selects available scan regions. Refer to <i>Focus Options card</i> for details.

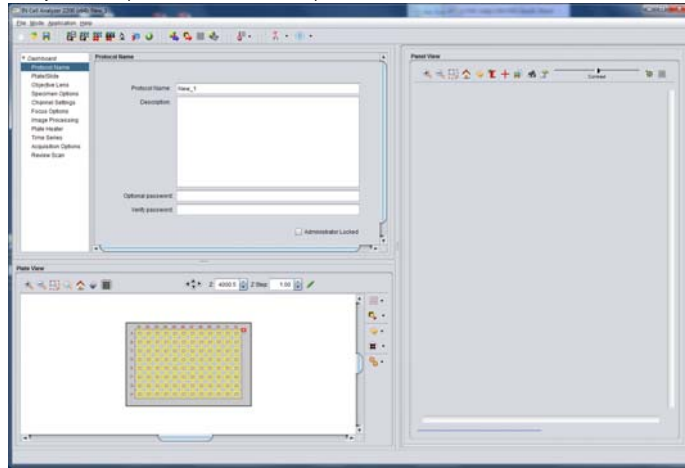
4.6 IN Cell Analyzer 2200/2000 system modes

From the *IN Cell Analyzer 2200/2000* workstation, there are four different modes in which you can view the **Activity Workspace**. The specific type of task you are performing will determine which mode you select at any given time. The system's four operating modes are: *Assay Development Mode*, *Acquisition Mode*, *Data Review Mode*, and *Microscope Mode*.

4.6.1 Assay Development mode

The **Assay Development** mode is for designing an imaging experiment and configuring the appropriate settings for the *IN Cell Analyzer 2200/2000* system. The end result of assay development is a protocol file you can use to run image acquisitions. By default, the *IN Cell Analyzer* system software opens in **Assay Development** mode, or you can select the **Main toolbar|Assay Development** icon. The **Assay Development** workspace displays. Refer to *5 Assay Development Mode - Designing Experiments* for complete detail.

Fig 4-16. Assay Development Mode Workspace.



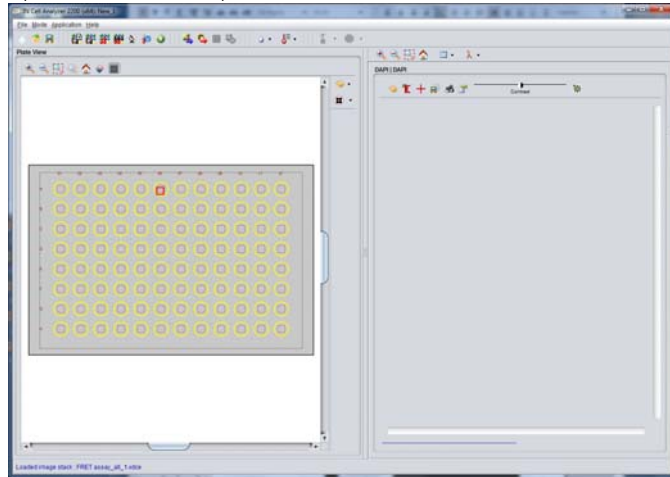
The windows that open are:

- **Protocol Designer** window in the upper left for defining the protocol parameters necessary to perform experiments with the *IN Cell Analyzer 2200/2000* system.
- **Plate View** window in the lower left for viewing the plate and selected regions of interest.
- **Panel View** window in the lower right for previewing images during experiment design and setup.

4.6.2 Acquisition mode

The **Acquisition** mode is for making on-the-fly modifications to previously designed imaging protocols and performing the actual image acquisition. From the **Mode** menu, select **Acquisition** mode, or you can select the **Main toolbar|Acquisition**. The **Acquisition** workspace displays. Refer to *Chapter 7 Acquisition Mode - Conducting Experiments* for complete detail.

Fig 4-17. Acquisition Mode Workspace



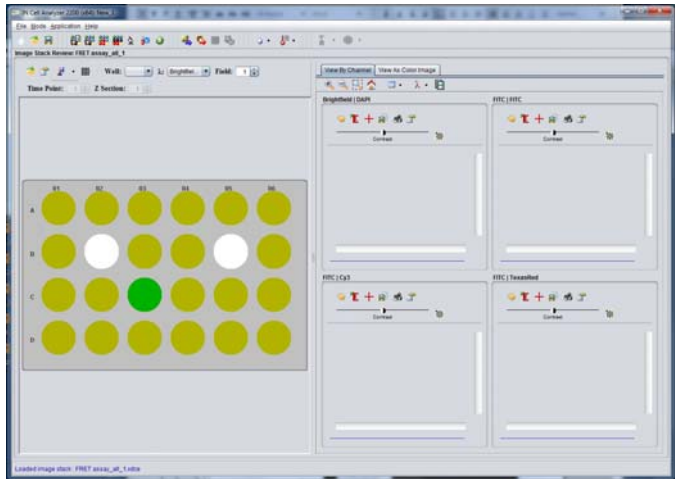
The windows that open are:

- **Plate View** window to the left for viewing and determining plate and sample location.
- **Panel View** window to the right to view for each of the wavelengths defined for an acquisition for a selected well.

4.6.3 Data Review mode

The **Data Review** mode is for browsing through image data to determine specific regions of interest, focus factors, etc. From the **Mode** menu, select **Data Review** mode or you can select the **Main toolbar|Data Review**. The **Data Review** workspace displays. Refer to *Chapter 8 Data Review Mode - Reviewing collected data* for complete detail.

Fig 4-18. Data Review Mode Workspace



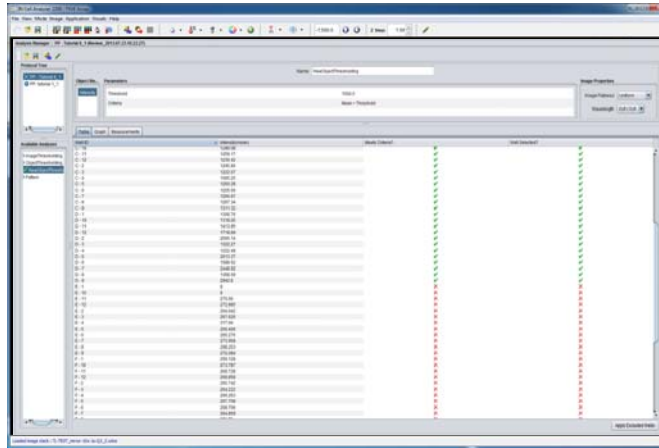
The windows that open are:

- **Image Stack Review** window to the left provides tools for examining the acquired data in relation to the plate wells, wavelengths, fields, time points, and Z-sections defined for the experiment.
- **Panel View** window to the right displays the images acquired for each defined wavelength in the protocol.

4.6.4 Review scan mode

The **Review Scan** mode allows you to automatically run a second protocol on a plate based on pre-defined threshold criteria. From the **Mode** menu, select **Analysis** mode, or you can select the **Main toolbar|Analysis**. The **Review Scan** workspace displays. Refer to *Chapter 9 Review Scan Mode - focusing on wells of interest* for complete detail.

Fig 4-19. Review Scan Mode Workspace



The window that opens is:

- **Analysis Manager** where the threshold criteria are defined to identify the wells of interest.

4.6.5 Microscope mode

The **Microscope** mode is for viewing acquired images using the full screen, with acquisition and display tools available in slide-in/slide-out menus. From the **Mode** menu, select **Microscope** mode, or you can select the **Main toolbar|Microscope**. The full-screen **Microscope** workspace displays. Refer to *Chapter 10 Microscope Mode - Using IN Cell Analyzer 2200/2000 full-screen* for complete detail.

Fig 4-20. Microscope Mode Workspace.



In this mode, acquisition controls provide image acquisition features similar to **Assay Development** mode; pop-up windows provide additional acquisition features; and display controls provide for re-imaging of the displayed image and image magnification, as shown in the overlay above.

4.7 Workspace components

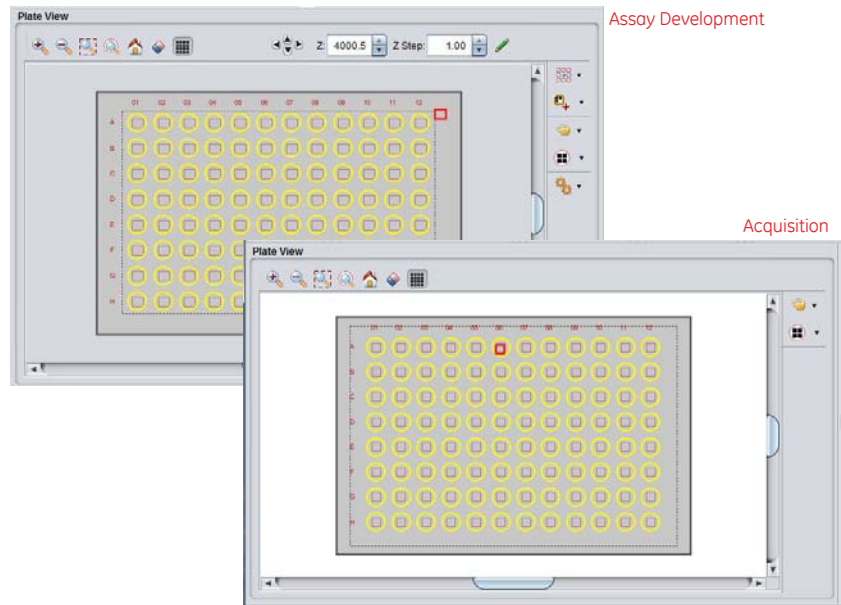
As described in the previous section, the system modes are groupings of dialogs that when used together allow you to perform certain tasks, but may also be used individually by selecting from the Main menu.

4.7.1 Plate View

The **Plate View** window is displayed in the lower left portion of the **Assay Development** or **Acquisition** modes. At the **Plate View** you can:

- Set the alignment of the plate.
- Select imaging position for setting up exposure and focus.
- Select wells for acquisition.
- Set up and define the area of preview scans.
- Set up the fields of view containing particular regions of interest within each of the wells.
- Monitor the progress of the acquisition process by displaying thumbnails images in acquired wells.

Fig 4-21. Panel View window.



To select and deselect wells on the **Plate View**:

- Use **CTRL-Left Mouse** to select single well or click and drag to select multiple wells. When selected, the wells are displayed as yellow in the **Plate View** window.
- Use **CTRL-Shift-Left Mouse** and drag to deselect single or multiple wells. When deselected, the wells are displayed as grey in the **Plate View** window.

Right-click anywhere within the graphical display to open the menu. Utilize the **Plate View** right-click menu to:

- Zoom to location
- Toggle Well Control

Plate View toolbar

The **Plate View** has two toolbars that are described below.

Button	Function	Description
	Zoom in	Enlarges the displayed image. See <i>Image magnification options</i> below for detail.
	Zoom out	Reduces the size of the display. See <i>Image magnification options</i> below for detail.

Button	Function	Description
	Zoom for selection	Zooms to a desired portion of the plate. See <i>Image magnification options</i> below for detail
	Zoom to Well	Zooms to a selected well. See <i>Image magnification options</i> below for detail
	Reset zoom	Resets the display to its original size See <i>Image magnification options</i> below for detail
	Clear Plate View	Clears any Panel View data (thumbnails) from the Plate View window.
	Thumbnails	Toggle to display Well view or Thumbnails view.
	Stage movement	<i>Assay Development mode ONLY</i> Pressing CTRL+arrow key will move the distance between the wells. Pressing CTRL+SHIFT will move 1/10 of a field of view.
	Z position	<i>Assay Development mode ONLY</i> Modify or increment the focus position by the Z step size.
	Z Step	<i>Assay Development mode ONLY</i> Set the Z Step.
	Save to protocol	<i>Assay Development mode ONLY</i> Save the current Z position to the protocol
	Fields to Acquire	<i>Assay Development mode ONLY</i> Opens Setup Fields to Acquire window to define the system's image acquisition on specific regions of interest. Refer to <i>chapter 5 Assay Development Selecting fields of interest</i> for more detail.
	Setup Preview	<i>Assay Development mode ONLY</i> Previews a selected region of the plate or slide. See <i>Set up preview scan area option in chapter 5 Assay Development Selecting fields of interest</i> for detail. Arrow opens a dropdown menu from which to select an exposure time and resolution/binning option.
	Visuals	Displays the Visuals window showing a line graph of the current image. See <i>Adjusting image appearance</i> in this chapter for detail.
	Show Current Well	Displays the Current Well window. Arrow opens a dropdown menu from which to select a location.

	Fine Tune the Well Grid	<i>Assay Development mode <u>ONLY</u></i> allows the positioning of the upper left and right, and lower left corner wells to correct variation in their well/spot layout that cannot be compensated in the X-Y movement of the well layout Refer to <i>chapter 5 Assay Development Selecting fields of interest</i> for more detail
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The following subsections describe common **Plate View** toolbar features.

Image magnification options

A number of magnification controls are available for viewing wells and images. Images are appropriately scaled to fit the size of the display window preventing them from being cropped thereby losing detail. These options are available from a number of dialogs and their operation is equivalent in all:

- **Zoom In/Zoom Out** option - Use the  zoom in /  zoom out options to enlarge or reduce the size of the display, respectively.

When adjusting the magnification in the **Panel View** window all of the image frames are adjusted simultaneously when multiple wavelengths are displayed.

- **Zoom to Selection** option - Use this option to view a desired portion of the plate/image.

- 1 Select  from the toolbar.
- 2 Clicking the left mouse button and

- On the **Plate View**, drag the mouse over the area to zoom on the plate. The display will zoom in to show the details of the selection such as fields to image.
- On the **Panel View**, drag the mouse over the area to zoom in one of the images frames. The selected portion will be enlarged to fill the image frame.

As with zoom in/out, when adjusting the magnification in the **Panel View** and **Panel View** windows all of the image frames are adjusted simultaneously when multiple wavelengths are displayed.

- **Zoom to Well** option - Use this option to view an enlarged image of a specific well:

- 1 Select on a well to view, and click the  icon.
- 2 Place the mouse over the well and click to enlarge you selection.

- **Reset Zoom** option - Click  to reset the display window to its original size and view.

Visuals option

The controls for manipulating image appearance are contained in the **Visuals** operation window. These functions do not affect image data; they only affect the visual appearance of the image as it is displayed in the **Panel View**.

Every pixel in a digital image has a numerical value referred to as the pixel intensity value. On *IN Cell Analyzer 2000*, there are 4096 possible intensity values in a 12-bit image and each is assigned a color (or shade of gray). On *IN Cell Analyzer 2200*, a 16-bit image contains 65,536 possible intensity values. The **Visuals** feature uses a look-up table (LUT) to convert pixel intensities on the displayed image. This image appearance is affected by manipulating the LUT and can be changed by:

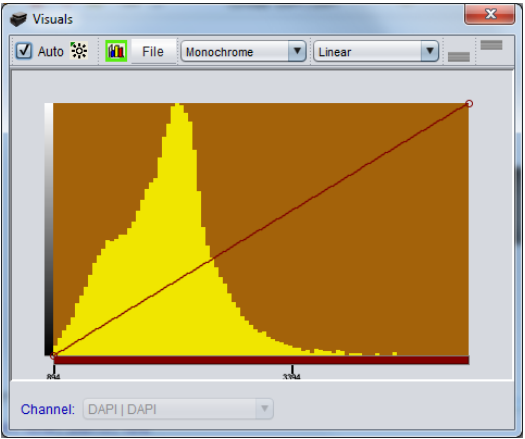
- Selecting a different LUT - A variety of LUTs are available from the Visuals toolbar, and can be applied to any image.
- Changing the LUT mapping function - LUTs are passed through mapping functions, affecting the way in which pixel intensities are displayed. Linear, logarithmic, and exponential mapping functions are included with the *IN Cell Analyzer 2200/2000*.
- Enhancing contrast - Image contrast can be enhanced by mapping all of the available colors / shades onto a smaller range of intensity values. With the total number of colors/shades available to represent a smaller range of intensity values, the visual contrast is enhanced.

The **Visuals** dialog opens displaying a line graph and histogram super-imposed upon each other. The histogram represents the range of intensities of the currently displayed image. You can manipulate the line graph from each end point to scale the image contrast intensity.

The area in the middle of the **Visuals** window displays the mapping function. The **X** axis represents the range of data intensity values, while the **Y** axis represents the range of display pixel values. The default mapping function is linear.

The line that defines the mapping function contains small, circular control points at each end. These control points can be dragged laterally to compress the LUT colors into a smaller range of intensity values. Some mapping functions also contain control point(s) along the length of the curve. These can be dragged to different positions to change the shape of the curve.

Fig 4-22. Visuals window



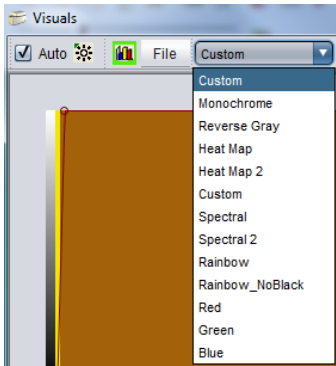
Button	Function	Description
	Auto	Auto-scaling for each newly acquired image is activated by default each time you acquire an image. The contrast scaling (represented by the line graph) is automatically set for optimal image display. When disabled, each newly acquired image uses the contrast scaling indicated by the position of the line graph, while the line graph remains static.
	Auto Contrast	Click to automatically determine the contrast for the currently displayed image. Auto Contrast usually provides the best contrast intensity for viewing the image.
	Histogram	Selected by default, so that when you initially open the Visuals window, the histogram is displayed. Toggle to turn OFF the histogram display.
	File	Open a saved visual configuration from the VIS folder or save the current configuration to the VIS folder.
	Table Selector	Use the dropdown list to select the LUT.
	Mapping Function	Choose a mapping function from the drop-down list to display the colors or shading onto intensity values.

Button	Function	Description
	Above/ Below Contrast Color	Click to define the color to denote the area above the upper contrast threshold, representing pixels with intensities greater than the threshold value. Click to define the color to denote the area above the lower contrast threshold, representing pixels with intensities lower than the threshold value.

To manipulate the image appearance, click the **Visuals** icon from either a plate view or any of the Panel View windows:

- 1 The **Auto Contrast** feature automatically maps the LUT to the actual range of intensity values in which image data lie. This feature is applied by default. To disable this option, unmark the checkbox on the toolbar.
- 2 From the **Table Selector** field (first dropdown field), select a LUT to apply. Each LUT is stored as a visuals file (*.vis).

Fig 4-23. Visuals dialog - Table Selector dropdown list.

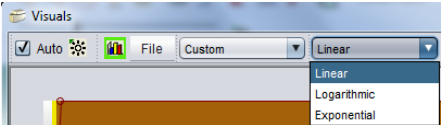


The default LUTs are:

LUT Type	Description
Monochrome	a monotonically increasing grayscale LUT, which provides a reasonably faithful rendition of the original data
Reverse Grey	a monotonically decreasing grayscale LUT, and makes images look as they would in a film negative
Heat Map	
Heat Map 2	

LUT Type	Description
Spectral	displays the gray level image in pseudocolor. To create the LUT, colors are assigned to each gray level
Spectral 2	displays the gray level image in pseudocolor. To create the LUT, colors are assigned to each gray level
Rainbow	displays the gray level image in pseudocolor. To create the LUT, colors are assigned to each gray level
Rainbow_NoBlack	
Red	displays the gray level image in a red pseudocolor, where increasing intensities are assigned to each gray level
Green	displays the gray level image in a green pseudocolor, where increasing intensities are assigned to each gray level
Blue	displays the gray level image in a blue pseudocolor, where increasing intensities are assigned to each gray level

- 3 In the second drop-down list, choose the **Mapping Function** for the contrast scaling.

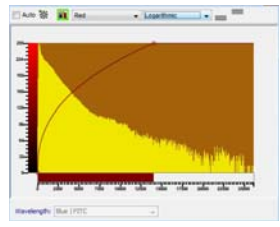
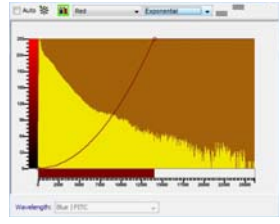


- 4 Use the graph in the **Visuals** window to customize the selected **Mapping Function**. These mathematical functions map the colors or shading onto the intensity values as described below.

A screenshot of the 'Visuals' window showing a graph of the 'Linear' mapping function. The graph plots intensity values on the x-axis (ranging from 0 to 255) against a color scale on the y-axis. A diagonal line represents the linear mapping from input intensity to output brightness. The background of the graph area is filled with a color gradient corresponding to the mapping.

Linear

intensity values are mapped on a linear scale. The signal input (pixel intensity) is directly proportional to the signal output (screen brightness). This is the default function.

	Logarithmic Intensity values are mapped using a logarithmic scale, giving a high degree of differentiation at lower pixel intensities.
	Exponential Intensity values are mapped using an exponential scale, giving greater differentiation at higher pixel intensities.

Using the Custom option

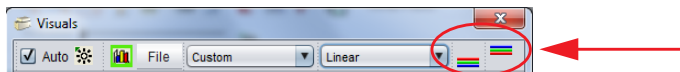
Contrast enhancement maps the available LUT colors/shades onto a smaller range of intensity values. Thus, across this smaller range of intensities, more shades of gray are used, and visual contrast, within the range, is enhanced.

By default, the contrast range spans the entire intensity range of the system. The contrast range is adjusted by dragging the small, circular control points at the ends of the line that defines the LUT function in the **Visuals** window. When the mouse cursor is moved to a control point at either end of the line, the cursor changes into a double-headed white arrow.

To change the contrast range, drag the control points. Because the control points can be dragged to any position, you can easily set the contrast bounds to include the data range in the specimen. You can also drag a contrast range across the **X**-axis, by moving the mouse cursor onto the contrast range. The cursor becomes a double-headed black arrow that can drag the contrast range to a new region of the **X**-axis.

To establish this sharper contrast:

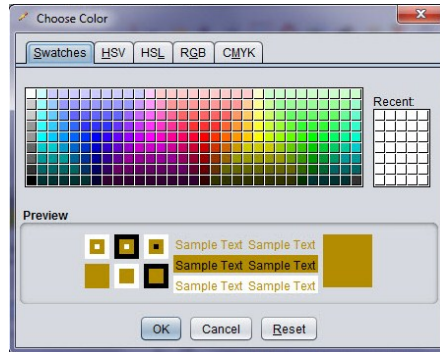
- 1 Select **Custom** from the **Table Selector** dropdown. The **Below Contrast Color** and **Above Contrast Color** toolbar items are enabled.



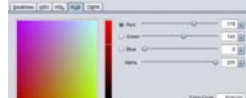
Note: You must select **Custom** in the **Look-up Table Selector** drop-down list for the **Above** and **Below Contrast** color options to be available.

- 2 Click the **Below Contrast Color** icon to open the **Choose Below Contrast Color** window.

Fig 4-24. Choose Below Contrast Color window

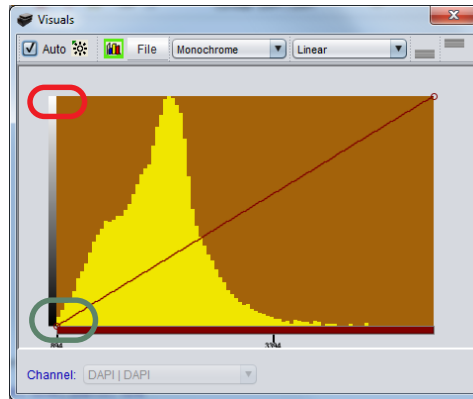


- 3 Select a color, from the palette, to indicate the segments of an image that fall below the specified threshold. The palette offers 5 options to select a color:

from the Swatches tab, click on a color swatch square 	in the HSL tab, select a color & refine by clicking in the color frame 	from the RGB tab, use the sliders to define the values 
in the HSV tab, select a color & refine by clicking in the color frame 	in the CYMK tab, select a color & refine by clicking in the color frame 	

- 4 Click the **Above Contrast Color** icon to open the **Choose Above Contrast Color** window. This window is similar to the **Below Contrast Color** window described above.
- 5 Select a color, from the palette, to indicate the segments of an image that fall below the specified threshold as described above.
- 6 Once the **Above** and **Below Contrast** colors have been set, they display at along the **Y-axis** with the **Above** color to the top and the **Below** color to the bottom.

Fig 4-25. Above & Below Contrast Colors display on Y-axis.



In this example, **Red** was chosen for the Above Contrast color and **Green** was chosen for the Below Contrast color.

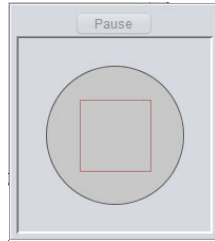
The image appearance can be adjusted by clicking the upper or lower endpoints of the Y-axis graph line and dragging across the **X**-axis to achieve the desired appearance.

- Moving the upper endpoint from right to left, increases the number of high-intensity pixels in the displayed image. Press and hold left mouse button to adjust upper line graph endpoints.
- Moving the lower endpoint from left to right, increases the number of low-intensity pixels in the displayed image. Press and hold left mouse button to adjust lower line graph endpoints.
- Adjust the X-axis scale by clicking the X-axis scaling bar and dragging the left or right edges to each the necessary scaling. Press and hold left mouse button to adjustment the scale.

Show current well option

Clicking the **Show current well** icon opens a window to display the currently selected well, in the acquisition. The window can be used to monitor the image acquisition process in real-time. As field images are acquired, the acquired field is marked in black. The current field being acquired is marked as red. Clicking the **Pause** button will halt the acquisition.

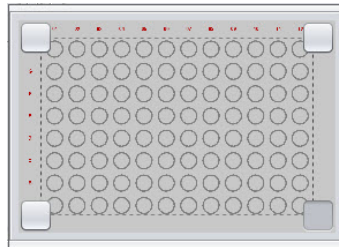
Fig 4-26. Current Well window.



To select a well to mark as the current well:

- 1 Click the arrow to the right of the **Select Well** icon. The **Set Current Well Display Location** dialog opens. Mark the well to set it as the current location.

Fig 4-27. Set Current Well display location.



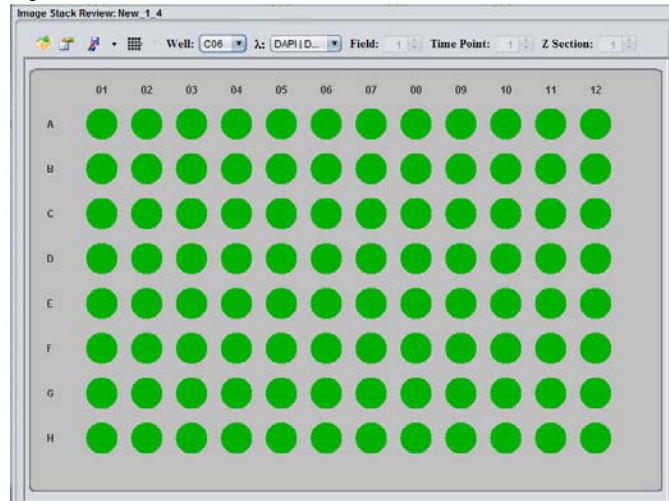
The gray box in the image above indicates that the **Current Well** window will be oriented in the top left corner of the **Plate View** window. Close the window to continue. The **Current Well** window displays.

4.7.2 Image Stack Review

The **Image Stack Review** window provides access to the acquired wells, and fields, for the experiment and opens in **Data Review** mode.

The graphical plate/slide display can be toggled to display the wells to be imaged and their completion status, or a thumbnail image of the wells imaged.

Fig 4-28. Image Stack Review window.

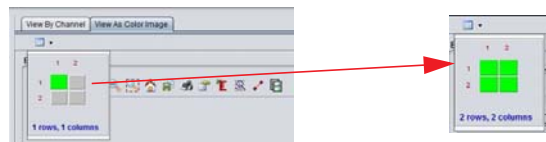


To select and deselect wells on the *Image Stack Review*:

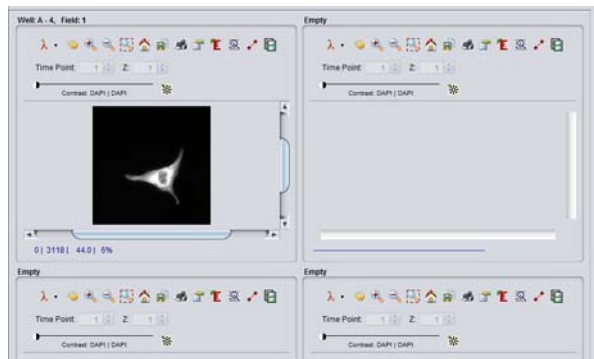
- Double click on well to select. Image will appear in **Panel View** window. Up to four wells can be viewed at a time on the **View as Color Image** tab.

To display multiple images:

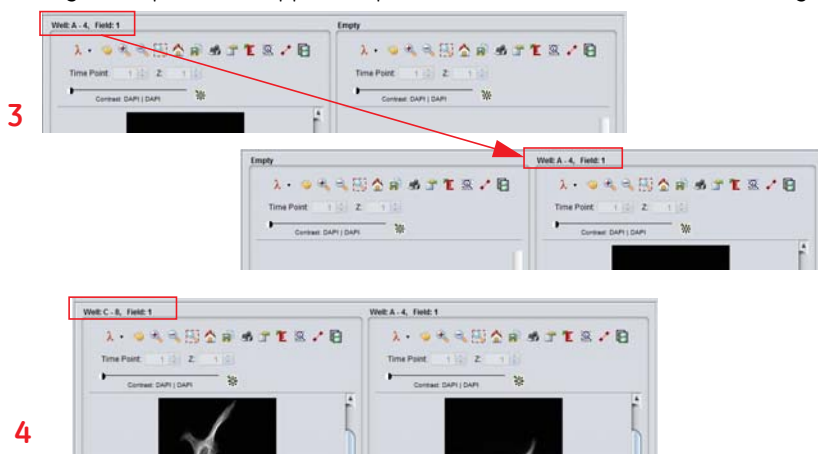
- 1 On the **View as Color Image** tab, drag the mouse cursor over the Image layout to set up to four image panes.



- 2 On the *Image Stack Review*, double-click on a well to view the image. The image will display in the upper-left pane, by default.



- 3 Move the image to one of the other panes by placing the mouse cursor over the title area (i.e., well location) and drag over the title area of another pane.
- 4 From the **Image Stack Review**, double-click on another well to display. The new image will open in the upper-left pane from where we moved the first image.

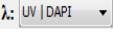
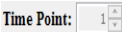


- 5 Repeat steps 3 and 4 to add additional images to fill the four display panes, as necessary.

Image stack review toolbar

The toolbar provide the ability to manipulate the data acquired by selecting from various attributes. Additionally, you may generate fused images from this window.

Item	Function	Description
	Open Image Stack file (*.xdce)	Displays the Open Image Stack File window. Browse to the location of the image stack file (.xdce). See <i>Properties option</i> below for details.
	Properties	Opens the Image Stack Properties window, listing the acquisition protocol attributes.
	Save Fused image/ Define Fused Image	Saves fused images for the selected wells, at the selected field and time point. See <i>Chapter 8 Data Review Mode Fused image</i> below for details. Use the dropdown list to select the images to fuse.
	Display Type	Select from the heat maps to view image intensity metadata. For more detail see <i>Chapter 8 Data Review Mode</i> .

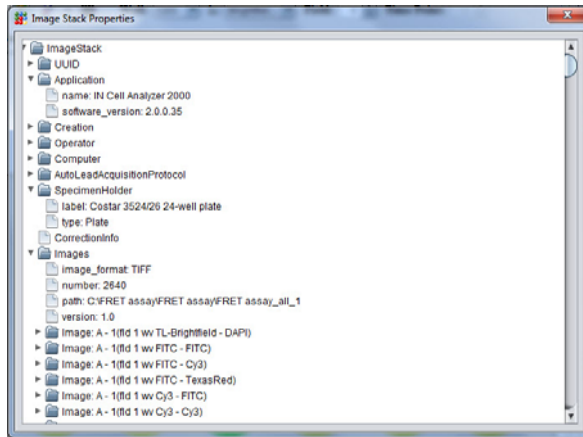
Item	Function	Description
	Well	Use the drop-down list in the Well Number field to select the well you want to examine. The data for the chosen well is opened in the Panel View window.
	Wavelength	Use the drop-down list in the Wavelength field to select the wavelength pair you want to examine. To confirm that the wavelength was used in the acquisition protocol, the plate view will update with a status that will help to determine if any data exists for the chosen wavelength. Color Status  OK, image acquired  Can't find data  No image acquired
	Field	Use the arrow keys in the Field portion of the toolbar to select the specific field you want to examine.
	Time Point	
	Z Section	Use the arrow keys in the Z Section field to select the specific Z Section you want to examine.
	Thumbnails	Click the Thumbnails button to toggle between Well view and Thumbnails view.

The features unique to the window are explained in the following subsections.

Image stack properties

Select the icon to open the **Image Stack Properties** window is displayed as shown.

Fig 4-29. Image Stack Properties window



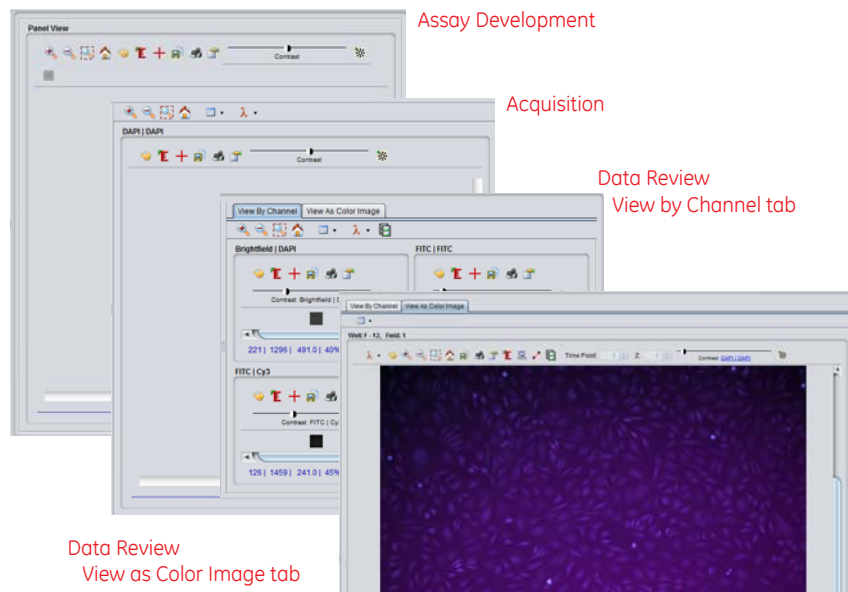
- Click on the **ImageStack** folder to expand the folder and view the detailed information available.
- Expand any portion of the **Image Stack Properties** by clicking on the “+” sign next to the folder name.
- Collapse any expanded portions of the **Image Stack Properties** window by clicking on the “-” sign next to the folder name.
- Explore the various headers and their contents within the **Image Stack Properties** window to become familiar with the information available.
- To see the properties of each image, first expand the Images folder, and then expand the individual Image folders within for specific information.

4.7.3 Panel View

The **Panel View** can display single or multiple images that can be used in:

- **Assay Development** - assist in defining the acquisition protocol to preview images in selected wells.
- **Acquisition** - to monitor the progress of image acquisition. Each of the wavelengths will display in a separate frame, and can be configured to display up to 13 images simultaneously; can also be configured to display multiple single-wavelength images.
- **Data Review** - allows for the exploration of specific wells in detail; collect information such as distance between objects, wavelength intensity across the image, and relative object size. An added feature is the option to generate a slideshow 'movie' from a Z-sectioned image stack.

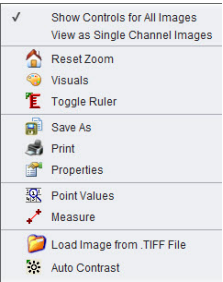
Fig 4-30. Panel View window in Assay Development Mode.



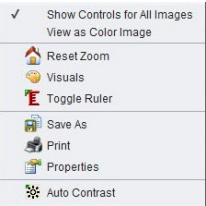
Note: Right-clicking anywhere on the image in the **Panel View** opens a menu providing quick access to some of the most commonly used Panel View features.



Assay Development & Acquisition modes



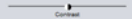
Data Review View by Channel tab



Data Review View as Color Image tab

Panel View toolbar

The toolbar includes the operations that can be performed on an image. A number of common features are available across the Panel View in all modes.

Button	Function	Description
	Zoom in	Enlarges the displayed image. Refer to <i>Image magnification options</i> in this chapter for detail.
	Zoom out	Reduces the size of the display. Refer to <i>Image magnification options</i> in this chapter for detail.
	Zoom to selection	Used to view a desired portion of the image. Refer to <i>Image magnification options</i> in this chapter for detail.
	Reset zoom	Resets the display to it's original size. Refer to <i>Image magnification options</i> in this chapter for detail.
	Visuals	Opens the Visuals window. Refer to <i>Adjusting image appearance</i> in this chapter for detail.
	Ruler	Used to provide reference when displaying an image. see <i>Measurement (Ruler) feature</i> below for detail.
	Crosshairs	Click to enable the crosshair
	Save as	Opens a Save Image As a JPEG file in the IMG folder
	Print	Opens a Print dialog from where you can print the image to a physical printer or file.
	Properties	Opens the Image Properties window
	Contrast	Contrast adjustment - sets the pixel density at which the displayed intensity will saturate for the specified wavelength. Right-click the hyperlink below the slider bar to select the wavelength to adjust.
	Auto-contrast	Toggle ON/OFF to automatically set the saturation point determined from the image intensity statistics
<i>Assay Development mode <u>ONLY</u></i>		
	Apply Flat-field Correction	Enabled only if a flat-field correction has been already measured.

Button	Function	Description
<i>Acquisition mode <u>ONLY</u></i>		
	Panel View Layout	Sets up the display in the Panel View . Refer to <i>Panel View layout in chapter 7 Acquisition</i> for detail.
	Wavelength Selection	Displays the Wavelength window. Refer to <i>Wavelength selection in chapter 6 Acquisition</i> for detail.
<i>Data Review View by Channel <u>ONLY</u></i>		
	Movies	Used to generate movies from multiple images, such as time series or Z-stack acquisitions See <i>Microscope Mode - Using IN Cell Analyzer 2200/2000 full-screen</i> for detail.
<i>Data Review View as Color Image <u>ONLY</u></i>		
	Point Values	Used to determine wavelength intensity values at points within an image. Refer to <i>Point values in chapter 7 Data Review</i> for detail.
	Measure	Used to calculate distances between various points within the image to determine exact locations of regions of interest. Refer to <i>Measure option in chapter 7 Data Review</i> for detail.
	Movies	Used to generate movies from multiple images, such as time series or Z-stack acquisitions See <i>Microscope Mode - Using IN Cell Analyzer 2200/2000 full-screen</i> for detail.
Time Point: 	Time Point Selection	Used to select a time point to view the corresponding images.
Z: 	Z Section Selection	Used to select a Z-section plane to view the corresponding images.

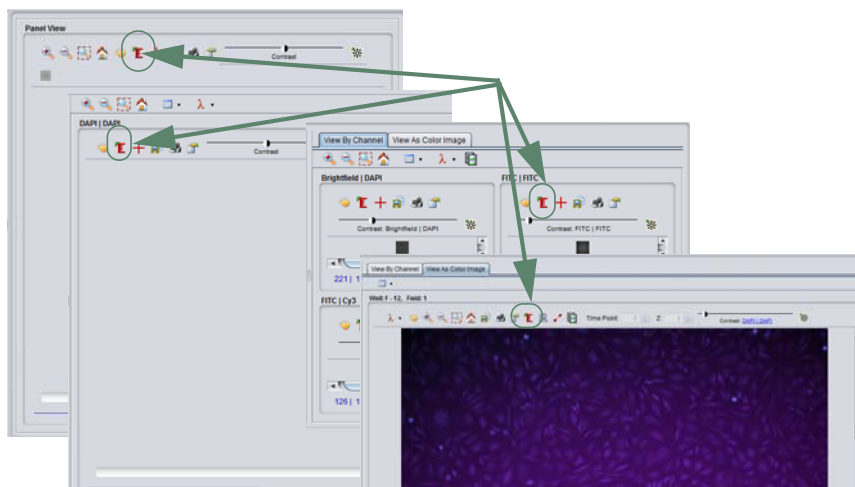
Measurement (Ruler) feature

The **Ruler** feature provides a tool to measure the relative size of an object within an image. To enable the **Ruler** feature:

- 1 Click the **Ruler** icon to display a 'ruler' in the lower left corner of the viewing area of the Panel Viewing window. When the Panel View consists of multiple frames, the ruler will display for each frame. (Only one frame is shown in **Panel View**).

- 2 To move the ruler about the image, click and drag to the area you want to measure.
- 3 Once you have placed the ruler, place the mouse cursor at either end of the ruler, and click-drag to enlarge or reduce the ruler to measure the object.

Fig 4-31. Measurement feature locations



Crosshairs

The following functionality is available when the  displays:

- Click anywhere within the field of view to center the **XY** stage on that point.

Note: Centering will work without the crosshairs enabled.

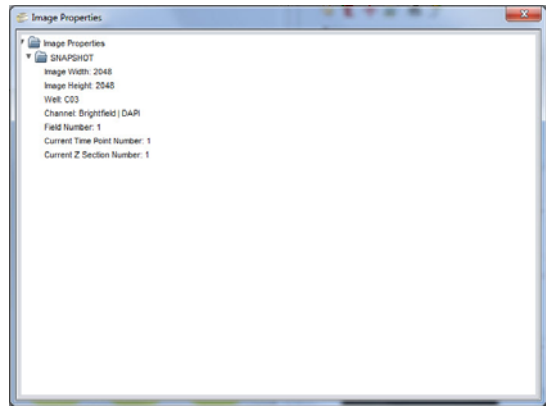
- Right-click within the image to center the view and zoom in.
- **SHIFT** right-click within the image to center the view and zoom out.
- Roll mouse wheel to zoom in or out on the image.

Image properties

Display the acquisition details of the image currently selected, such as:

- The width and height of the image
- The excitation and emission filters used to acquire the image
- The exposure time used to acquire the image
- The binning used to acquire the image
- The XYZ position of the instrument stage

Fig 4-32. IN Cell Analyzer 2200 Image Properties



Status bar

The status bar provides information about the image and acquisition settings and is formatted as described below:

138 | 4095 | 228.0 | 8%

Min / Max / Mean / % Optimum X: ###, Y: ###, GL: ##	
Min:	The minimum intensity value within the image.
Max:	The maximum intensity value within the image.
Mean:	The mean intensity value within the image.
% Optimum:	The percent of optimum exposure for the image.

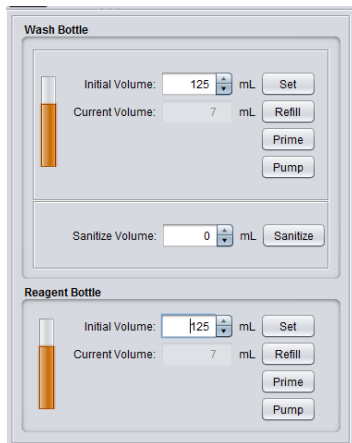
4.7.4 Liquid Handling

If installed, the *IN Cell Analyzer 2200/2000* has a liquid handling system for dispensing or aspirating a reagent from a bottle or 96- and 384-well compound plates and for cleaning the system via a wash station. Controls for the **Liquid Handling** module can be found on the *Main* toolbar, and in the **Protocol Designer**. Use the **Protocol Designer** to define the liquid handling events. Use the **Liquid Handling** dialog to track, and dispense liquid volumes used, and wash the system.

Note: For instructions on setting up **Liquid Handling** from within the **Protocol Designer**, see *Liquid Handling card* in Chapter 5.

To start the **Liquid Handling** system:

- 1 Click on the **Liquid Handling** toolbar icon. The **Liquid Handling** dialog opens.



- 2 In the *Wash Bottle* section,;
 - a. Enter the **Initial Volume** of wash solution (in mL) in the bottle. Click the **Set** button initialize the system. The Wash bottle has a maximum capacity of 250 mL.
 - b. Install the Wash bottle by connecting to the Wash tubing, and click the **Prime** button.
 - c. Click the **Pump** button to fill the dispense tube with wash solution.

For example, when you switch from running experiments with the reagent bottle to using a compound plate, you can use the **Pump** button to fill the dispense tube with wash solution. Conversely, when you switch from running experiments with compound plates to using the reagent bottle, use the **Pump** button in the Reagent bottle section to fill the dispense tube with wash solution.

- d. When it becomes necessary to refill the reagent bottle, to the standard 250 mL, click the **Refill** button. The system will clear the connecting tubing of any fluid so the reagent bottle can be removed.
- 3 In the *Reagent Bottle* section:
 - a. Enter the **Initial Volume** of reagent solution (in mL) in the bottle. Click the **Set** button initialize the system. The reagent bottle has a maximum capacity of 250 mL.
 - b. Install the reagent bottle by connecting to the reagent tubing, and click the

Prime button.

When you want to run experiments using the reagent bottle, typical priming of the system is achieved by priming with wash solution, and then priming with reagent solution.

- c. Click the **Pump** button to fill the dispense tube with reagent solution.

For example, when you switch from running experiments with the reagent bottle to using a compound plate, you can use the **Pump** button to fill the dispense tube with reagent solution. Conversely, when you switch from running experiments with compound plates to using the reagent bottle, use the **Pump** button in the reagent bottle section to fill the dispense tube with reagent.

- d. When it becomes necessary to refill the reagent bottle, to the standard 250 mL, click the **Refill** button. The system will clear the connecting tubing of any fluid.

Note: *When there is insufficient fluid volume to complete an operation, the system will no longer allow liquid handling operations.*

- 4 To sanitize the Liquid Handling system:

- a. Click the **Sanitize** button. Wash solution is pumped from the bottle to the needle. The system will prompt whether you want to 'air dry' the tubing.

Air drying the tubing requires that you remove the end of the tube from the wash bottle. Click **OK** at the prompt to proceed. Air is now pushed through the Liquid Handling system from the wash bottle to the needle.

See *Liquid Handling card* in Chapter 5 for detail on completing the **Protocol Designer**|**Liquid Handling** card.

4.7.5 Environmental Control

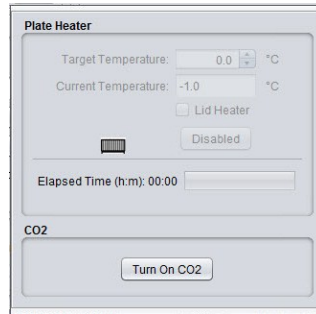
The **Environmental Control** dialog is used to control plate heating and the delivery of humidified CO₂ on systems enabled with modules for temperature and environmental control.

Important!

The Environmental Control dialog box may appear even if the system is not equipped with temperature or environmental control modules. Check with a system administrator if you are unsure of the unit's specifications.

From the main toolbar, click the **Environmental Control** icon. The **Environmental Control** window is displayed.

Fig 4-33. Environmental Control dialog



- *Plate Heater* - To enable the plate heater, use the arrow keys to specify the *Target Temperature* (°C). Click the **Turn On** button, and allow the plate heater to warm to the target temperature. The *Current Temperature* will increment as the plate heater warms, so you can use it as an indicator to determine when the plate chamber has researched the target temperature.
- *CO₂ humidifier* - To enable the humidifier, click the **Turn On CO₂** button. Allow the CO₂ humidifier to run for a minimum of at least 1 hour before beginning any experiments. Verify CO₂ humidifier operation by confirming slow bubble production in the humidifier bottle and bubbles moving through the tubing at approximately one bubble every three seconds.

Refer to 11.2 before turning on the humidifier.

4 IN Cell Analyzer 2200/2000 user interface

4.7 Workspace components

5 Assay Development mode - designing experiments

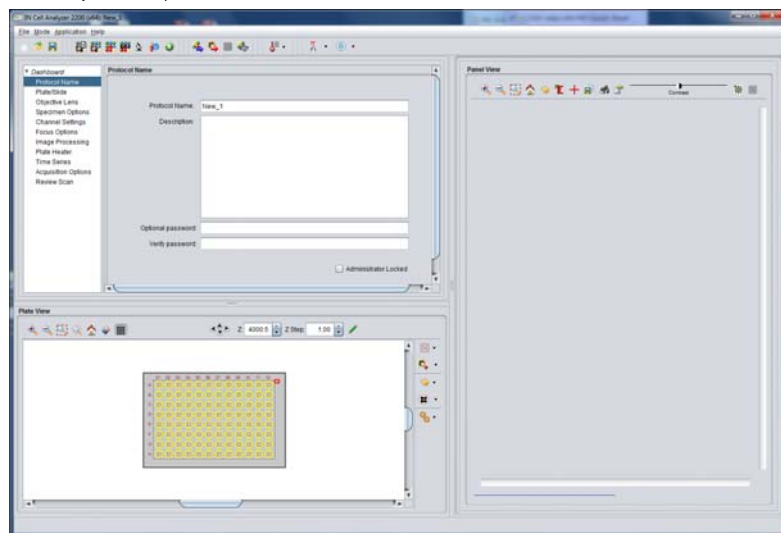
In general, an experiment is a series of imaging tasks. The experimental design defines the acquisition process and resulting images. The acquisition protocol establishes the plates and wells to be imaged, wavelengths and exposure times to use, and other parameters.

When the predefined protocol is launched, the system works without user input to complete the experiment, which can last from seconds to days.

To begin designing an experiment, open **Assay Development** by clicking the *Main* toolbar **Assay Development** icon.

The **Plate View**, the **Protocol Designer**, and the **Panel View** all open as the Assay Development workspace.

Fig 5-1. Assay Development mode



5.1 Loading a sample

To begin designing your protocol, a microplate should be loaded so you can preview the acquisition results. The plate access door is allowed to open only when the plate carrier is in the load/unload position.

To load a plate into the *IN Cell Analyzer 2200/2000*:

- 1 Click the *Main* toolbar **Eject** icon. The access door on the top left of the instrument slides open.

Fig 5-2. Plate Access Door.



- 2 Place the plate into the plate carrier. The plate should be firmly seated.

Fig 5-3. Plate Carrier.



- 3 To close the access door, click on a well location within the **Plate View** window. The plate is automatically positioned for imaging.

5.2 Establishing protocol settings

The steps to designing a successful experiment include:

- Define wavelengths, imaging modalities, and exposure times.
- Define your focus strategy for the experiment.
- Determine the general location of the region of interest within the sample you want to image.

- Define the wells and field locations to acquire.

The settings you choose in this operation will determine the images, and the quality of those acquired images.

5.3 Designing the experiment - the Protocol Designer

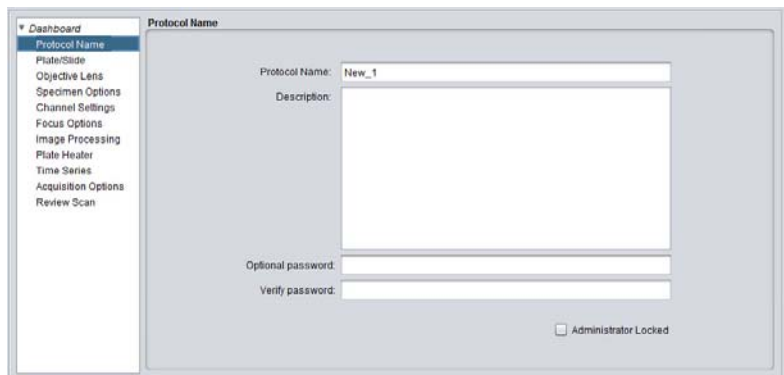
The **Protocol Designer** is used to define each of the parameters of an imaging experiment, and is located at the upper-left of the *Assay Development* workspace.

The Protocol Designer wizard allows you to define your protocol. Progression through the Protocol Designer can be sequential or random.

5.3.1 Protocol Name card

On opening the **Protocol Designer**, the **Description** card and **Protocol Name** window displays.

Fig 5-4. Protocol Name card



- 1 Enter a name for the protocol in the **Protocol Name** field,

Note: The special characters */*, **, and *|* are not supported, and should not be used as part of the protocol name.

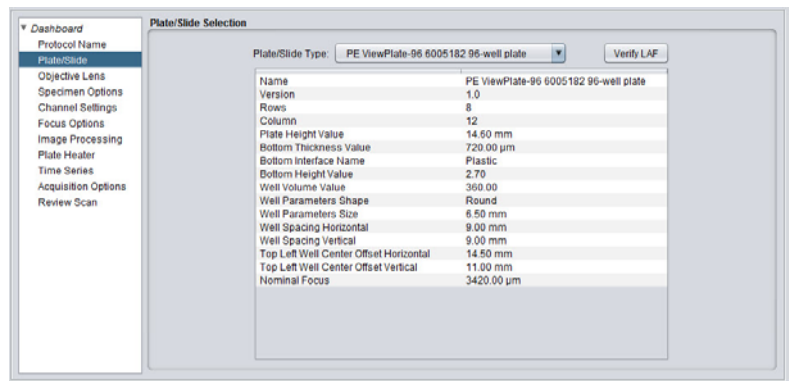
- 2 In the free-form text entry **Description** field, document the particular protocol and its purpose. For example, you may want to include the date, the name of the person designing the protocol, and details about the experiments performed.
- 3 Optional: Use the **Password** field to enter a password for this protocol. Verify the password in the **Verify Password** field.

- 4 *Optional:* If using *IN Cell Analyzer 2000* with *IN Cell Compliance Manager*, use the **Locked** checkbox to prevent the protocol from being edited.

5.3.2 Plate/slide card

Clicking the **Plate/Slide** card option opens the **Plate/Slide Selection** window.

Fig 5-5. Plate/Slide card



- 1 Mark either the **Plate** or **Slide** radio button, and select a plate or slide holder from the drop-down list. The grid below the drop-down will display the characteristics of your selection.

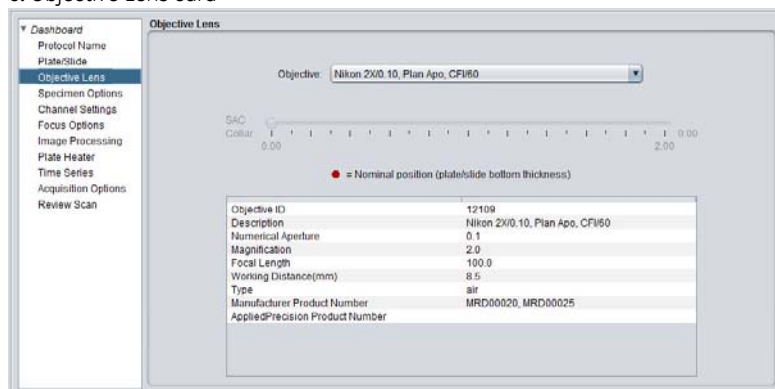
Note: To define a new plate/slide holder type, if you cannot locate the type you need, use the **Plate/Slide Manager**, accessed from the **Main menu|Applications |Plate/Slide Manager**. See *Plate/Slide Manager*.

- 2 Click the **Verify LAF** button to display a laser trace based on the plate type selected along with the expected peaks. Clicking the **Apply Calculated Parameters** button will automatically update the plate parameters to the system calculated values.

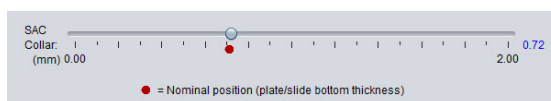
5.3.3 Objective Lens card

Clicking the **Objective Lens** card option opens the **Objective Lens** window.

Fig 5-6. Objective Lens card



- 1 From the **Objective** field, select an appropriate objective type from the drop-down list. The grid below the drop-down will display the details of the selected objective.
- 2 If the selected objective is equipped with a **SAC collar** (Spherical Aberration Correction), the **SAC Collar** slider is enabled. If necessary, the **SAC collar** can be adjusted by moving the slider left or right, to the value of the plate bottom thickness, and minimize any observed effects of spherical aberration.



Objectives available for IN Cell Analyzer 2200/2000

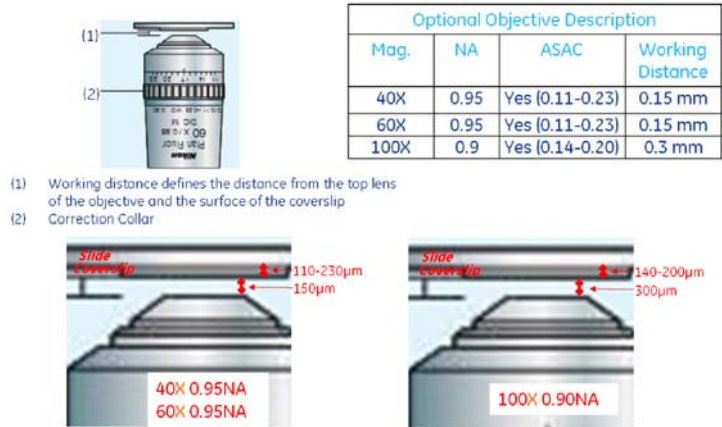
A range of standard and high NA lenses are available for the *IN Cell Analyzer 2200/2000* as shown in the tables below. The ASAC range determines the device (plate/slide) that can be used with these lenses, for example the 60 × 0.7 NA objective can be used with devices of plate bottom thickness of 0.1–1.3 mm (100–1300 µm).

Standard Objective Description			
Mag.	NA	SAC	Working Distance
10×	.45	No	4 mm
20×	.45	Yes	8.1–7 mm

Optional Objective Description			
Mag.	NA	SAC	Working Distance
2×	.1	No	8.5 mm
4×	.2	No	15.7 mm
20×	.75	No	1 mm
40×	.95	Yes	0.14 mm
40×	.6	Yes	3.7–2.7 mm
60×	.95	Yes	0.15 mm
60×	.7	Yes	2.1–1.5 mm
100×	.9	Yes	0.3 mm

High NA lenses are only compatible with thin plates and coverslips. The thickness is defined by the ASAC range; 100 x 140-200 μ m and 40x/60x 110-230 μ m. From this distance you then only have the working distance to travel before the objective touches the bottom of the plate/coverslip – maximum distance 100 x = 300 μ m and 40 x/60 x = 150 μ m. Flat substrates such as the coverslip or thin glass plates are ideally suited to use with this category of lenses.

Fig 5-7. Objectives.

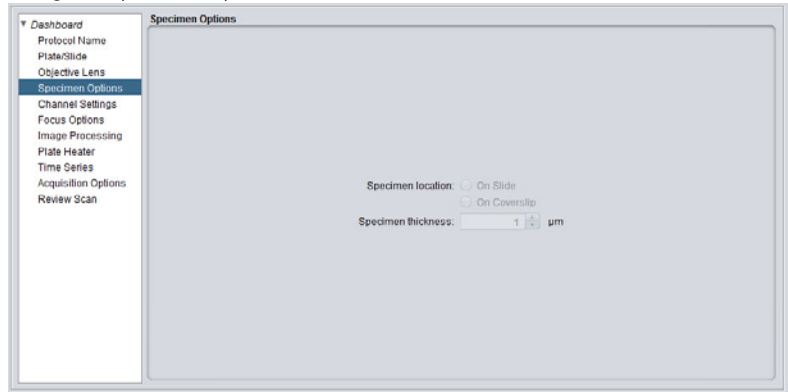


5.3.4 Specimen Options card

Note: Parameter selection on this card is enabled when the slide type is selected from the **Plate/Slide Selection** card.

Clicking the **Specimen Options** card option opens the **Specimen Options** window.

Fig 5-8. Specimen Options card



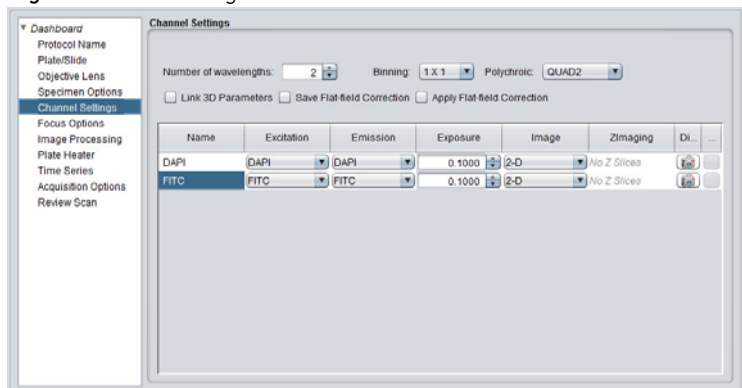
Note: The options on the **Specimen Options** window are active only if you selected to use a slide in the **Plate/Slide** window.

- 1 In the **Specimen location** field, mark either the **On Slide** or **On Cover Slip** radio button to indicate the location your specimen.
- 2 Enter the thickness of the specimen into the **Specimen thickness** field. You may enter a value or use the arrow keys.

5.3.5 Channel Settings card

Clicking the **Channel Settings** card option opens the **Channel Settings** window.

Fig 5-9. Channel Settings card

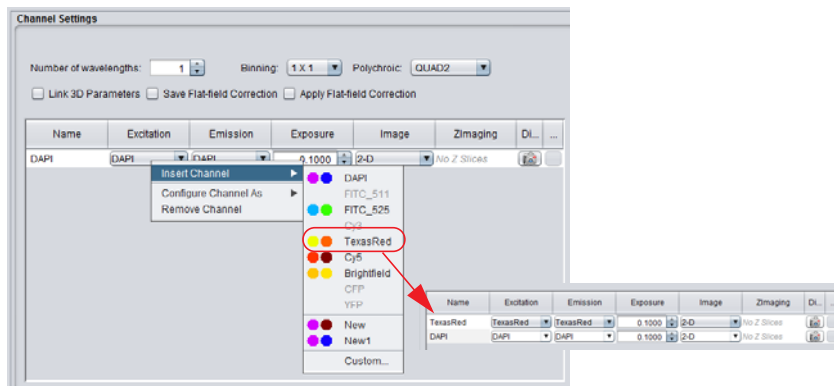


- 1 Set the number of wavelengths that will be used in the **Number of wavelengths** field. The grid will update to provide a row for each wavelength to be defined for the protocol.

5 Assay Development mode - designing experiments

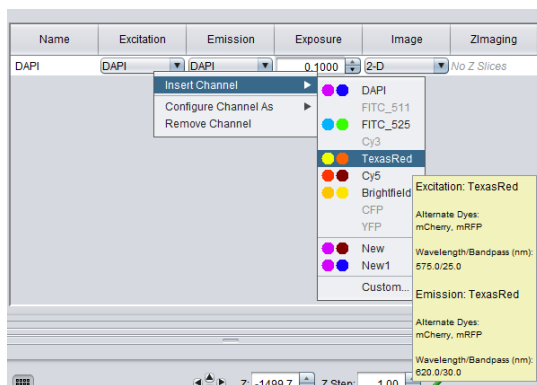
5.3 Designing the experiment - the Protocol Designer

Alternatively, additional wavelengths can be added by selecting Insert Channel from the right-click menu and selecting a wavelength from the Channels list.



The order of the channels selected can be done by the drag and drop method. Right-click on the channel to move and while holding the mouse button drag the channel to the desired location.

To view the details of a wavelength, hover the mouse near the wavelength name in the Channel list.

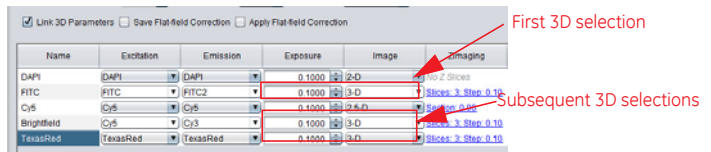


To define new wavelength pairs (shortcuts), select **Custom** from the Channel list (described below) or open the **User-defined Wavelength Manager** window. Refer to *Chapter 4, Applications menu, User-defined Wavelength Manager*.

- 2 Set the polychroic filter for this experiment, mark the **Polychroic** checkbox and select from the drop-down list if the default setting needs to be changed.
- 3 Confirm that the **Link 3D Parameters** checkbox is marked.

Mark the **Link 3D Parameters** checkbox if you want to link the selected 3D parameters. With the **Link 3D Parameters** checkbox marked, the parameters selected for the **Z Imaging** of the first 3-D image selection are replicated in all subsequent 3-D image selections.

Fig 5-10. Link 3D parameters .

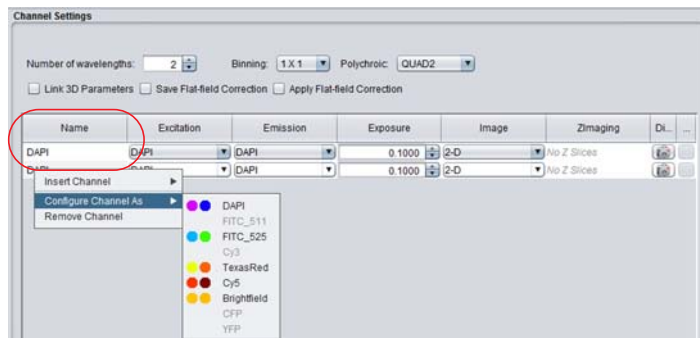


- 4 Mark the **Save Flat Field Correction** checkbox to save the flat field correction files as part of the experimental data.
- 5 Mark the **Apply Flat Field Correction** checkbox to apply the flat field correction to the experimental data.

Define the wavelengths to be used in the experiment in the grid. Wavelength definitions can be added 'on-the-fly' by selecting **Custom** from the list.

- 1 To change the wavelength from DAPI to another wavelength click on the channel name and choose **Configure channel as** from menu and select from the Channels list. The pre-defined and user-defined wavelengths are included in the dropdown list.

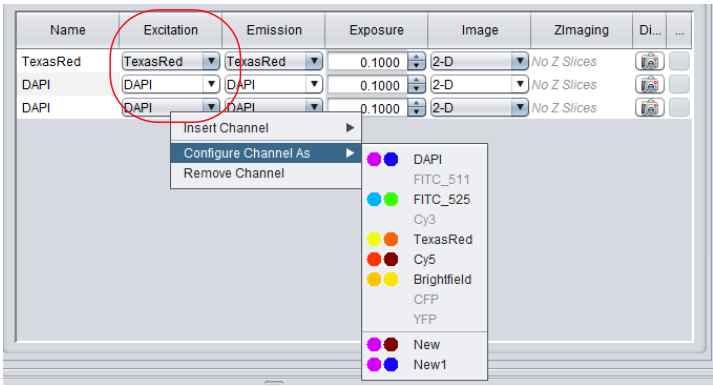
Fig 5-11. Channel Settings card - select wavelength



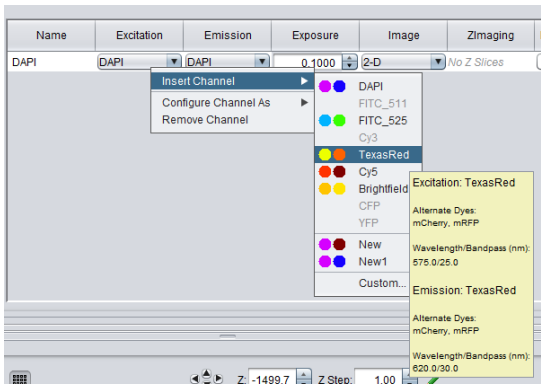
Note: The wavelength name can be changed by double-clicking in the name field and typing the new name in the field. The Channel list will not be updated

Name	Excitation
TexasRed	TexasRed
DAPI	DAPI
FITC	FITC

Alternatively, the **Excitation** and **Emission** filters can be set independently by click on the **Excitation** and **Emission** names and choosing **Configure channel as** from menu and select from the Channels list.



To view the details of a wavelength, hover the mouse near the wavelength name in the Channel list.



- 2 Select the imaging modality, for each wavelength, from the **Image** field drop-down list.

Number of wavelengths: 3 Binning: 1 X 1 Polychroic: QUAD2

☐ Link 3D Parameters ☐ Save Flat-field Correction ☐ Apply Flat-field Correction

Name	Excitation	Emission	Exposure	Image	ZImaging	Di...
TexasRed	TexasRed	TexasRed	0.1000	2-D	No Z Slices	
DAPI	DAPI	DAPI	0.1000	2-D	No Z Slices	
DAPI	DAPI	DAPI	0.1000	2-D	No Z Slices	

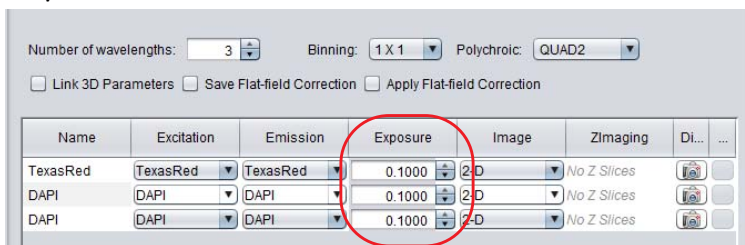
Type	Description
2-D	The system acquires a standard two-dimensional image. A single .tiff file is generated.
2-D Deconvolution	The system acquires a deconvolved two-dimensional image. A single .tiff image is generated.
2.5-D	The system uses the camera's CCD/sCMOS chip to integrate the signal over the specified Z section, and then deconvolves the result for a pseudo 3-D projection. A single .tiff file is generated.
Adv 2D Deconvolution	The system deconvolves a complete three-slice Z stack and then saves only the middle slice. A single .tiff file is generated.
Phase Contrast	The system generates a phase map from three images: one in-focus, one under-focus, and one over-focus. The two out-of-focus positions must be of equal displacement from the in-focus position. This is handled automatically by the system. Using this phase map and the current intensity data received, the system reconstructs the wavelengths returned and generates a single .tiff file for the image.
DIC	The system utilizes a technique known as Differential Interference Contrast microscopy, in which polarizers and prisms shear and recombine the light beam, which passes through the sample. This technique provides a method for enhancing phase contrast in the specimen. A pseudo-3D shadow relief image is acquired when using DIC. The system generates a single .tiff file for the image.
3-D	The system acquires a standard three-dimensional image (Z stack) and generates a .tiff file for each Z slice.
3-D Deconvolution	The system acquires a standard three-dimensional image (Z stack), deconvolves the result, and generates a .tiff file for each Z slice.

Note: If the **3-D Deconvolution** option is unavailable, you may not have a license for using the 3-D Deconvolution feature. See Configuration

options for more information.

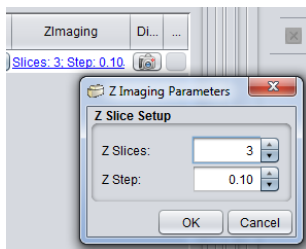
Note: If you open a protocol that specifies **3-D Deconvolution** image mode for one or more wavelengths, **2-D** image mode will be substituted for the wavelength(s).

- 3 Set the exposure time for each wavelengths in the experiment in the **Exposure** field.



Name	Excitation	Emission	Exposure	Image	Zimaging	Di...
TexasRed	TexasRed	TexasRed	0.1000	2-D	No Z Slices	
DAPI	DAPI	DAPI	0.1000	2-D	No Z Slices	
DAPI	DAPI	DAPI	0.1000	2-D	No Z Slices	

- 4 In the **Z Imaging** field, click on a set of parameters for a wavelength. The **Z Imaging Parameters** dialog opens.



Z Imaging Parameters

Z Slice Setup

Z Slices: 3

Z Step: 0.10

OK Cancel

Note: The **Z Section** field is only available if 2.5-D is selected for that wavelength's imaging type.

Note: The **Z Slices** and **Z Step** fields are only available if you selected 3-D or 3-D Deconvolution for that wavelength's imaging type.

- 5 Click **OK** complete and close the dialog.

Previewing the settings - Digitize

Use the **Digitize** button  on the **Channel Settings** card to view a 2D image from the selected wavelength, using all of the settings currently configured in the **Protocol Designer**. The resulting image is displayed in the **Panel View** window. The **Digitize** button will take an image at the current Z-position shown in the **Z position** box on the **Main** toolbar. When you initially click on a well to select an imaging position the objectives are in the default home position, -1500. To capture an image

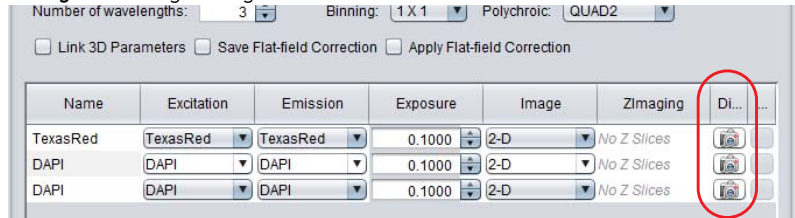
of your biology in focus, energize the laser autofocus  to give coarse focus, then software autofocus  to give fine focus.

Note: On IN Cell Analyzer 2000, if the **Illuminator** is not turned ON when attempting to **Digitize**, a Lamp warning will display. Turn ON the illuminator from the **Toolbar**|**Illuminator** button and wait for the lamp to energize.

At any point from the **Channel Settings** card, you can sample for the selected wavelength, and the resulting image is displayed in the **Panel View** window.

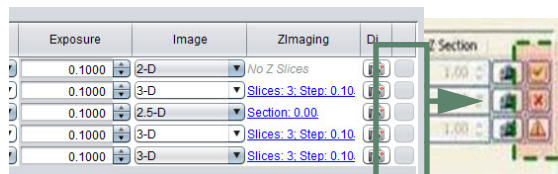
- 1 Click  to the right of each row in the **Wavelength** grid to view a 2D image of the selected wavelength. The image is generated using the settings currently configured in the **Protocol Designer**. The resulting image is displayed in the **Panel View** window.

Fig 5-12. Using the Digitize button.



Note: Selecting the **Digitize** button in the **Panel View** window will not generate an image based on the current protocol. Rather, this button generates an image based on the current settings.

- 2 On digitizing a wavelength, the **Flat Field Correction** status will update and display in the last column of the grid.

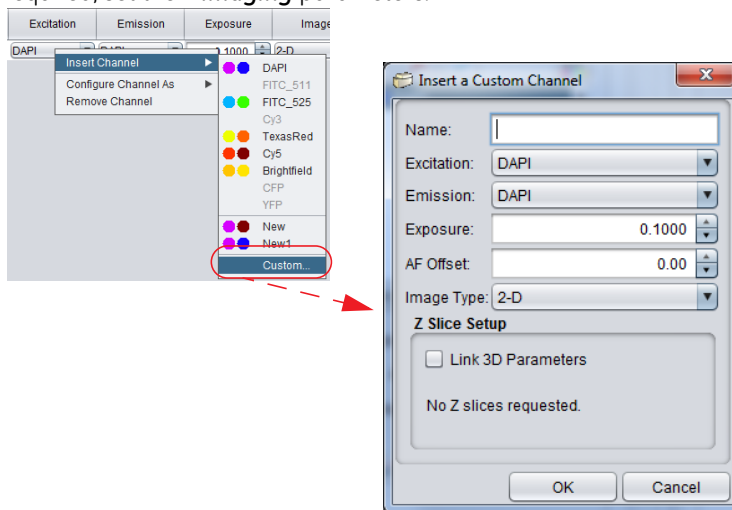


Status	Description	
	Calibrated	Fresh flat field correction files exist for this wavelength.
	Un-calibrated	Flat field correction files do not exist for this wavelength.
	Stale	You should consider refreshing the flat field correction files for this wavelength before running the experiment.

Custom option

Channels can be defined 'on-the-fly' using the **Custom** option on the *Channel* list or the right-click menu.

The **Insert a Custom Channel** dialog opens. Name the channel, select the **Excitation** and **Emission** filters, set the **Exposure**, **AF Offset**, and **Imaging Mode**. If required, set the **Z Imaging** parameters.



5.3.6 Focus Options card

Multiple focus methods are available on the *IN Cell Analyzer 2200/2000*. The method you choose will be dependent on the experimental sample, the imaging modality selected, and the types of images to be acquired. The Focus window options change as you select different types of focus strategies.

- Perform Laser Autofocus only – automatically finds the interface between the air and the glass surface underneath the slide or cover slip or solid/liquid interface of a microplate. However, this may not be the optimal plane of focus for imaging the samples. The hardware autofocus offset uses a software algorithm to finely focus your image, taking you to a slightly different plane of focus, which could be a few microns above or below the calculated position.
- Perform Software Autofocus only – uses software algorithms to automatically find the Z-axis position with the sharpest focus. This software-based autofocus feature can be used instead of, or combined with, the laser-guided system. It will add considerable time to the image acquisition process.
- Perform Laser Autofocus in combination with Software Autofocus – performs the laser autofocus operation to find the solid/liquid interface, then performs a software autofocus operation using that plane as a reference. In this mode, the software autofocus settings are grayed out (predetermined).

Clicking the **Focus Options** card option opens the **Focus Options** window.

Fig 5-13. Focus Options card (all options enabled)

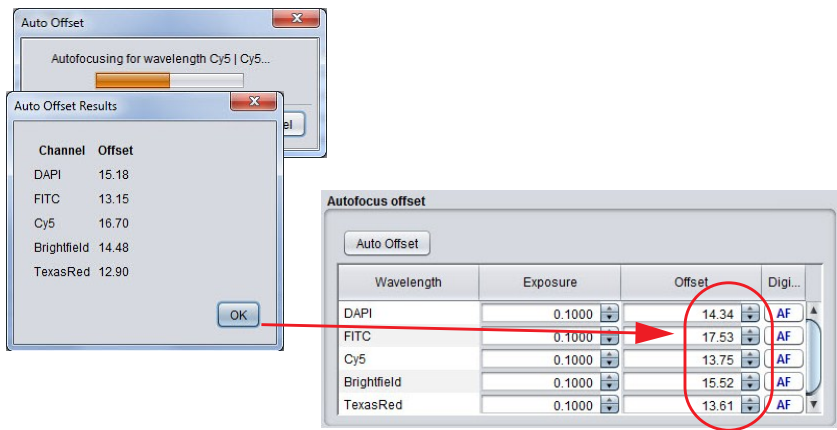
Hardware Autofocus search

- 1 In the *Hardware Autofocus* section, use the **Laser Autofocus** checkbox to enable the Laser Autofocus feature. The **Auto Offset** button, in the *Focus Offset* section, will be enabled.

- 2 In the *Autofocus Offset* section, the **Auto Offset** button is only available if you selected to use the **Laser Autofocus** without using the **Software Autofocus**. Use this button to have the system automatically set the autofocus offsets for this experiment. When you click the **Auto Offset** button, an auto-focusing status window is displayed.

The **Auto Offset Results** window displays. Click **OK** to update the **Autofocus Offset** panel with the offset for each selected wavelength pair, its exposure time, and its offset.

Fig 5-14. Auto Offset dialog.



Note: When Laser Autofocus is selected, all wavelengths are set to Digitize with the Autofocus displayed in the far-right column.

When *Hardware Autofocus* is selected, clicking the icon will take you directly to the focus position (found interface + the offset position).

This is extremely useful when checking exposures times; entering a new exposure time in the exposure box and then pressing the **AF** icon for that wavelength will display an in-focus image with the new exposure settings applied in the **Panel View** window.

The returned offset value can manually be edited by inserting a new offset into the box, and pressing **Return** or **Enter**, or by using the up/down arrows. A new image using these settings can be visualized in the **Panel View** window by pressing the **AF** button.

Software Autofocus search

The *Software Autofocus Search* feature uses software algorithms to automatically find the Z-axis position with the sharpest focus for each acquired image. The maximum search distance, relative to the current stage position is specified. The Initial step setting specifies the gross increment for the acquisition of an initial test image set. Once the best test image is found, the *IN Cell Analyzer 2200/2000* searches between adjacent focal planes until the specified accuracy level is reached.

Note: Ensure all offsets are set to zero, otherwise, when the SWAF is performed the offset will cause the image to be out of focus.

- 1 In the *Software Autofocus* section, mark the **Use Software Autofocus** checkbox to enable the software autofocus features.

Fig 5-15. Software Autofocus.

- 2 Choose the autofocus increments to use for this experiment. Note that using higher increments will decrease the experiment's duration. In the Mode section, select either the **Adaptive** or **Static** mode by marking the radio button. Make your selection based on the following:

Adaptive Mode	For each Z-step, autofocus starts from the previous in-focus point. The drop-down list next to the Adaptive radio button indicates the search range from the previously discovered in-focus point. <i>Large</i> specifies a 400 µm search range, <i>Medium</i> specifies a 200 µm search range, and <i>Small</i> specifies a 100 µm search range. Selecting a larger search range will adapt to more curvature in the plate bottom.
Static Mode	For each Z-step, autofocus uses the number in the Initial Focus field as a starting point, and then uses the number indicated in the Static drop-down as a search range. Static mode is usually the fastest software autofocus mode and works well for a relatively flat target, such as a slide.

- 3 Use the next radio buttons to indicate whether you want the selected software autofocus settings to operate on the **First wavelength only** or on **Every wavelength** in the experiment.
 - When you use *Software Autofocus* only (without using *Laser Autofocus*) and you select the **First wavelength only** radio button, the *Focus Offset* table displays the **AF** icon in the far-right column only in the first row of the *Autofocus Offset* table. The  button is displayed for the remaining rows.

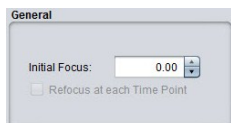
Wavelength	Exposure	Offset	Digi...
DAPI	0.1000	15.82	AF
FITC	0.1000	15.12	
Cy5	0.1000	15.35	
Brightfield	0.1000	15.09	
TexasRed	0.1000	13.71	

- If you have selected the **Every wavelength** radio button, the *Focus Offset* table displays  in all rows of the far-right column.

Since the hardware and software focusing features function in tandem, you can enable both the **Laser Autofocus** and the **Use Software Autofocus** checkboxes simultaneously.

- 1 Set the **Initial Focus** field to match the initial focal plane for this experiment.

Fig 5-16. Initial Focus



Note: For **Software Autofocus** to work effectively the biological sample needs to be within the Software Autofocus range (see Table below).

For example starting from home position -1500 μm , energize **Laser Autofocus** to give coarse focus and then **Software Autofocus** to give fine focus. Alternatively, manually enter the nominal focus position (this can be found in the plate description) or manually adjust the Z focus position using the up/down arrows and then finely focus using the **Software Autofocus**.

- 2 Use the  ,  buttons in the table to preview the result of the offset for the wavelength using all of the settings currently configured in the **Protocol Designer**. The number in the **Initial Focus** field and the indicated offset are used for focus.

The resulting image is displayed in the **Panel View** window. Based on this image, you can manually adjust the exposure time and offset for the wavelength as necessary.

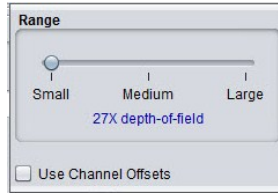
Determining the optimal focus settings

To generate the highest quality images from the *IN Cell Analyzer 2200/2000* system, it is important that you use all of the information available to attain the best focus possible. This section outlines one method for determining optimal focus. For additional information on setting up focus strategies, refer to the beginning of this section (*Focus Options card*).

To determine optimal focus:

- 1 From the *Main* toolbar, click the **Software Autofocus** icon to perform the software autofocus. To refine the autofocus, click the arrow to the right of the icon associated with the software autofocus to select a **Software Autofocus Range** from the dialog.

Fig 5-17. Software Autofocus Range slider.



The scale is defined as follows

Small	The sample should be within 100 µm for Software Autofocus to be effective.
Medium	The sample should be within 200 µm for Software Autofocus to be effective.
Large	The sample should be within 400 µm for Software Autofocus to be effective.

- 2 From the **Protocol Designer** window, select the **Focus** card.
- 3 Set the **Initial Focus** field to match the initial focus for this experiment.
- 4 In the **Mode** section, select either the **Adaptive** radio button, and small, medium, or large for the focus range. Or select **Static** and indicate the size of the search range by entering a value in the field or using the arrow keys.
- 5 In the **Software Autofocus** section, use the radio buttons to indicate whether you want the selected software autofocus settings to operate on the **First wavelength only** or on **Every wavelength** in the experiment. Note that selecting **First wavelength only** will decrease the experiment's duration.

Choose the fields and/or wells in which to autofocus. Note that using higher increments in these fields will limit the number of autofocus tasks and decrease the experiment's duration.

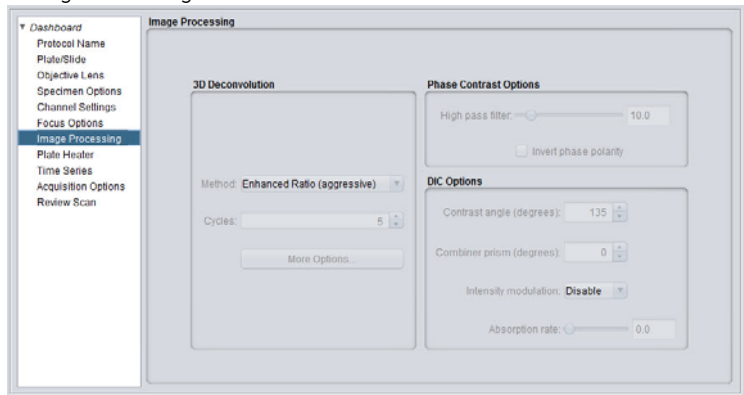
- 6 Click the  icon on the **Main** toolbar to save the settings in the currently active protocol file.

Note: If the currently active objective is equipped with an SAC Collar, the SAC Slider settings are also saved.

5.3.7 Image Processing card

Clicking the **Image Processing** card option opens the **Image Processing** window.

Fig 5-18. Image Processing card .



This window is divided into three processing-related sections:

3D Deconvolution	Used to determine the method of deconvolution, the number of deconvolution cycles, and various other deconvolution options for the protocol.
Phase Contrast Options	Used to determine the level of high-pass filtering and whether or not to invert the phase polarity for the protocol.
DIC Options	Used to determine the contrast angle, the angle for the combiner prism, and the level (if any) of modulation intensity you want to use.

3D - Deconvolution

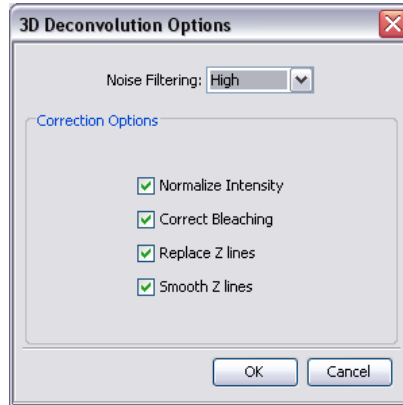
- 1 Use the drop-down list in the **Method** field to select the type of 3-D Deconvolution process (if any) to use for this experiment. The options are:

Ratio (conservative)	Is a more conservative algorithm than Enhanced and converges more reliably on a solution. Images with punctuate fluorescence deconvolve better using the Ratio method.
Enhanced (aggressive)	Provides faster convergence.
Additive	Is tolerant of data with a low signal-to-noise ratio.
Enhanced Additive	Is similar to, but faster than Additive. Enhanced Additive is faster because it requires fewer deconvolution iterations (typically ten or less).

- 2 In the **Number of Cycles** field, enter the number of deconvolution cycles.

- 3 Click the **More Options** button to open the **3D Deconvolution Options** window.

Fig 5-19. 3D Deconvolution options



- a. In the **Noise Filtering** field, use the drop-down list to select *High*, *Medium*, or *Low* noise filtering.
- b. In the *Correction Options* section, mark the checkboxes for the correction options to be used for this experiment. The available options are:

Normalize Intensity	Control whether to use intensity normalization for image correction.
Correct Bleaching	Attempts to correct photo-bleaching, which occurs when the fluorescent dyes lose their emission intensity as they are exposed to illumination. Certain dyes are more susceptible to photo-bleaching than others, and other potential for excessive photo-bleaching is increased with increased exposure time.
Replace Z Lines	Use the Z-line correction designed to detect CCD defects by searching for unusual intensity profiles along the Z-axis. For example, a "dead" CCD element appears as a straight, dark line along the Z-axis, and a "hot" CCD element appears as a straight, bright line along the Z-axis. Voxels identified as Z-line errors are replaced with the average of their neighbors.
Smooth Z Lines	Smooth the stack along the Z-axis.

- 4 When you are satisfied with the selections in the **3D Deconvolution Options** window, click **OK**.

Phase Contrast Options

- 1 Use the slider to select the level of **High-pass filtering** for this experiment.
- 2 If you want to invert the phase polarity, activate the **Invert phase polarity** checkbox.

DIC Options

- 1 Use the up/down arrows to indicate the degree of the **Contrast Angle**.
- 2 In the **Combiner prism** field, use the up/down arrows to select the degree of the angle of the combiner prism.
- 3 In the **Intensity modulation** field, select from the drop-down list:

Modulation Type	Description
Disable	Amplitude data is not included in the processed image.
Standard	Amplitude data is included in the processed image.
Advanced	Absorption effects are included in the image.

If **Advanced** is selected as the intensity modulation, use the slider in the **Absorption rate** field to indicate the desired level of absorption.

5.3.8 Plate heater card

Clicking the **Plate Heater** card option opens the **Plate Heater** window.

Fig 5-20. Plate Heater card



- 1 Check the **Use Plate Heating** checkbox to enable the plate heating unit.

Note: Unmarking the **Plate Heater** checkbox will disable both the plate and lid

heaters.

- 2 Mark the **Lid Heater** checkbox to switch on the lid heater.

Note: This option is only enabled when the Plate Heater is enabled.

Note: This option is only used with Environmental Control.

- 3 Set the target temperature (°C) in the **Specify Target Temperature** field by either entering a value in the field or using the arrow keys.

Note: The plate heating unit must be allowed to reach the Target Temperature specified.

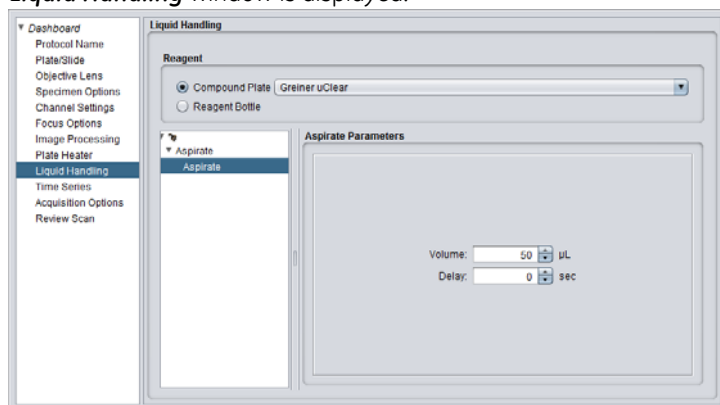
Note: Allowable temperature range is 5°C above ambient temp to 42°C.

5.3.9 Liquid Handling card

Note: If the **Liquid Handling** card is unavailable, this optional feature is not installed on your system. See Configuration options for more information.

Use the **Liquid Handling** card to define a sequence of events that occurs at a particular time point (referred to as a *block*). No two blocks can occur at the same time point.

- 1 In the **Protocol Designer** window, click on the **Liquid Handling** card. The **Liquid Handling** window is displayed.

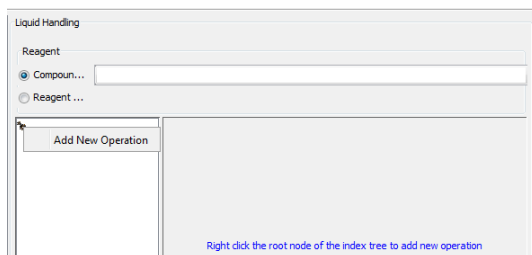


- 2 In the **Reagent** section, mark the radio button for either:

- **Compound Plate** - select the type of compound plate you will be using for this experiment from the dropdown list.
- **Reagent Bottle**

Note: The plate size must be the same for both the sample and compound plates.

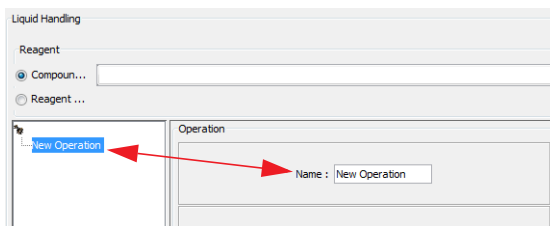
- 3 Place the mouse cursor on the root node  in the panel below the Reagent section.
- 4 Click the right mouse button, and then click **Add New Operation**.



Note: Each operation you define will be available when you set up time points on the **Time Series** card.

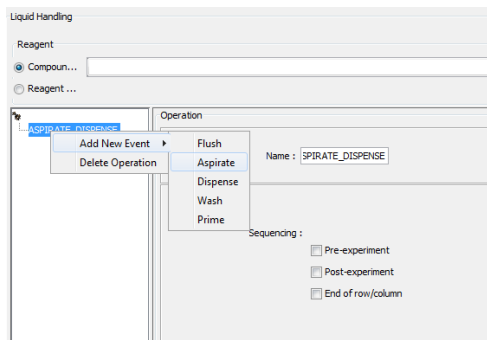
- 5 An operation node is added to the tree. By default it is labeled **New Operation**. The **Operation** panel displays.
- 6 Enter a name for the operation/task in the **Name** field. This name will appear in the tree.

To remove an operation/task from the tree, right-click on the operation name, and select **Delete Operation** from the context menu.



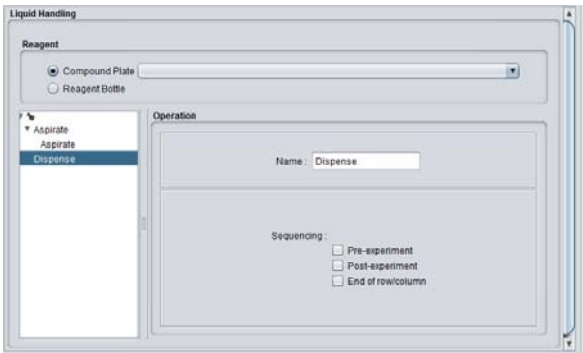
- 7 To add the events to occur for the operation/task:
 - Highlight the operation in the tree, and right-click the mouse.
 - Select **Add New Event** from the context menu, and select an event to add

from the dropdown list.



Aspirate	Draws up the specified volume of solution from the compound plate. (see <i>Event parameters</i>).
Dispense	Dispenses the specified volume of solution into the specified well of the sample plate. (see <i>Event parameters</i>).
Flush	Sends 300 µl of wash solution through the needle causing an internal flush, flowing a volume of fluid from the pump to the needle. There are no other parameters to enter on this page.
Prime	Fills all the tubing and syringe needle with 1 ml of wash or the reagent solution. There are no other parameters to enter on this page.
Pump	Flows a volume of wash or the reagent fluid from the bottle to the pump to the needle as defined in the protocol by the user.
Wash	Washes the needle tip internally and externally. There are no other parameters to enter on this page. (see Step 11(c)).
Sanitize	Flows a volume of wash fluid from the wash bottle to the needle (including the wash volume set in the Liquid Handling dialog). From the Sanitize action, an Air dry wash tubing option is available. • Selecting this option prompts you to disconnect the tubing from the wash bottle. • A volume of air is pumped through the tubing between the wash bottle the needle to evacuate any residual fluid. The volume of air displaced is greater than that of the combined tubing between the wash bottle and the needle.

- 8 In the **Sequencing** section of the **Operation** panel, select from the three sequencing options:

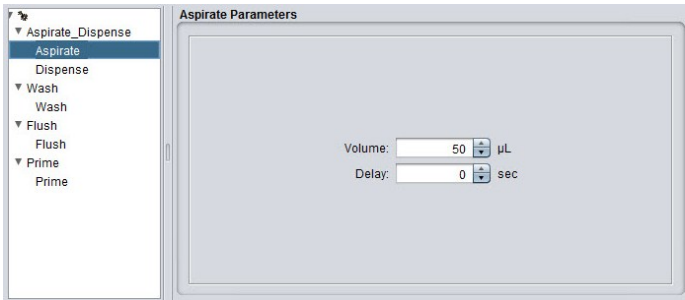


Pre-experiment	The operation occurs before the experiment (first image is acquired) starts and happens only once.
Post-experiment	The specified operation occurs after the experiment has completed (last image has been acquired).
End of row/ column	The operation occurs at the end of the row or column on the plate.

- 9 To add more operations, repeat from Step 3.
- 10 Refer to *Event parameters* for instruction on completing the **Aspirate**, **Dispense**, and **Wash** parameter options.

Event parameters

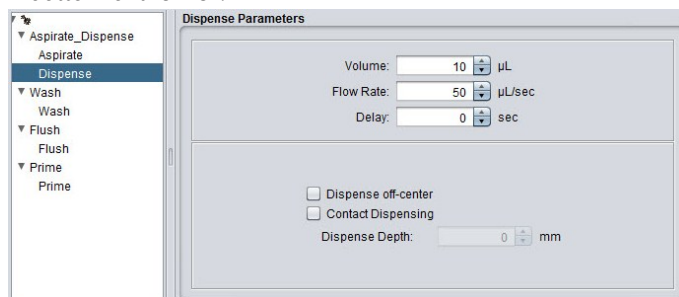
Aspirate Parameters



- 1 In the **Volume** field, enter the volume (in µL) to be aspirated from the reagent bottle or compound plate. The volume range is 10 - 100 µl dispensed in 10 µl increments.
- 2 In the **Delay** field, enter the time (in secs) that the needle remains in the well to allow more viscous solutions to be completely aspirated.

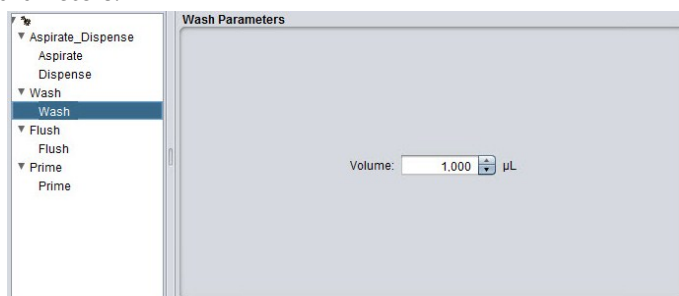
Dispense Parameters

Note: The needle will not dispense from a depth closer than 1mm from the bottom of the well.



- 1 In the **Volume** field, enter the volume (in µL) to be dispensed into the sample well. The volume range is 10 - 100 µL dispensed in 10 µL increments.
- 2 In the **Flow Rate** field, enter the amount of liquid (in µL) to be dispensed per second.
- 3 In the **Delay** field, enter the time (in secs) that the needle remains in the well to allow more viscous solutions to be completely dispensed.
- 4 The pre-set, normal dispensing depth places the needle at the midpoint of the well. To have the needle dispense:
 - Closer to the edge of the well, mark the **Dispense off-center** checkbox.
 - At a specific depth, mark the **Contact Dispensing** checkbox, and enter a value in the **Dispense Depth** field. This will cause the needle to drive into the selected well to a dispensing depth just above the well bottom.

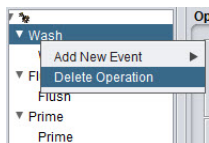
Wash Parameters.



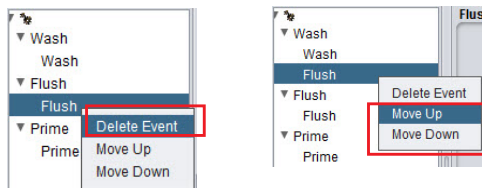
- 1 In the **Volume** field, enter the volume of wash fluid (in µL) to use for each wash event.

Modifying the index tree operations and events

- 1 To remove operations from the index tree, right-click on the operation name, and select **Delete Operation** from the context menu.



- 2 To remove an event from the index tree or to reorder an event within the tree, right-click the mouse on the event and select the desired function. You can **Delete** the event entirely, or select **Move Up** or **Move Down** to change where the event will fall within the sequence.



5.3.10 Time Series card

The **Time Series** card is used to specify the time intervals between 1) image acquisition and 2) liquid handling operations. There should be sufficient time between image acquisitions to enable any liquid handling procedure to be completed.

Note: When adding liquid handling procedures, the liquid handling operations **MUST BE** defined before the time series can be specified (see Liquid Handling card).

Clicking the **Time Series** card option opens the **Time Series** window.

Fig 5-21. Time Series card

- 1 Mark the **Acquire time series** checkbox to enable the **Time Series** card options. Column headings will appear for each defined wavelength in the time point table.

Note: There is an option on the **Focus Options** card to re-focus between timepoints. Ensure this option is checked when using multi-well mode with more than one timepoint or the images acquired for the second timepoint will be out of focus. Do not select this option for single-well mode as it will add extra time and is not required.

If the experiment does not require a time series, do not mark the **Acquire time series** checkbox, and proceed to the next card.

- 2 Enter the **Starting Time Point** for the experiment where the unit of measure is seconds.

Note: The first time point is usually 0.0, but may also be set to any time point within the range of the experiment.

- 3 Provide the **Time Interval** if you plan to add a series of time points. This is the amount of time the system will wait between each defined event, and is calculated in seconds.

Note: Timepoints can be inserted between existing timepoints in the series.

- 4 Specify the **Number of Time Points** to be included in the series.

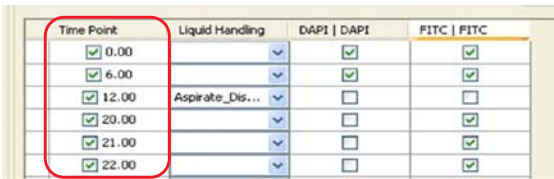
Enable the **Incubate between timepoints** checkbox when running long time courses using live cell assays to return the plate to the load position (optimal incubation conditions) between timepoints.

Note: Do not use if running fast kinetic imaging.

- 5 Click the **Add** button. Rows are added to the table for each time point in the defined series.

Note: *Note If the more than two or three wavelengths are specified in the **Channel Settings** card for the experiment, it is recommended that you enlarge **Protocol Designer|Time Series** window to view the entire table.*

- 6 In the **Mode** field, select the type of time series to be executed from the dropdown list. There are two modes available:
- Single well - used for short experimental time periods. In this mode, the *IN Cell Analyzer 2200/2000* captures the complete time series from one well before moving to the next well.
 - Multi-well - useful when considering assays over a longer time period. In this mode, the *IN Cell Analyzer 2200/2000* calculates the optimum order for the specified operations to minimize the time taken. For example, this may result in dispensing operations taking place in several wells before the instrument returns to the first well to acquire images.
 - As fast as possible - used for single well fast kinetic assays or on *IN Cell Analyzer 2200* to acquire multiple images/second. In this mode, you can only define the number of timepoints and at which timepoint a Liquid Handling and/or imaging event. is required. You may not set the time intervals.
- 7 In the time series table:
- a. Under the **Time Point** column, unmark the checkbox, to the left of each time point to deselect and exclude them from your experiment.



Time Point	Liquid Handling	DAPI DAPI	FITC FITC
☒ 0.00		☒	☒
☒ 6.00		☒	☒
☒ 12.00	Aspirate_Dis...	☐	☐
☒ 20.00		☐	☒
☒ 21.00		☐	☒
☒ 22.00		☐	☒

- b. In the **Liquid Handling** column, select a liquid handling instruction for the time point you require the operation to occur from the dropdown list. If the **Liquid Handling** option is not installed on the instrument, the fields

in this column will appear disabled.

Time Point	Liquid Handling	DAPI DAPI	FITC FITC
☒ 0.00	Aspirate	☐	☐
☒ 7.00		☒	☒
☒ 11.50		☒	☒
☒ 16.50	dispense	☐	☐
☒ 20.00		☐	☒
☒ 21.00		☐	☒

Note: The liquid handling instructions are defined on the **Protocol Designer| Liquid Handling** card.

- c. For each timepoint in the time series, unmark the checkboxes for each wavelength you do not want to image. By default, all the wavelengths are selected and need to be deselected if not required for a certain timepoint.

To select multiple, contiguous rows use the **SHIFT+Left Mouse** combination, or to select specific rows use the **CTRL+Left Mouse** combination.

Time Point	Liquid Handling	DAPI DAPI	FITC FITC
☒ 0.00		☒	☒
☒ 4.50	Dispense	☐	☐
☒ 6.50		☐	☒
☒ 7.50		☐	☒
☒ 8.50		☐	☒
☒ 9.50		☐	☒
☒ 10.50		☐	☒
☒ 11.50		☐	☒
☒ 12.50		☐	☒
☒ 13.50		☐	☒
☒ 14.50		☐	☒
☒ 15.50		☐	☒
☒ 16.50		☒	☒
☒ 21.50		☒	☒

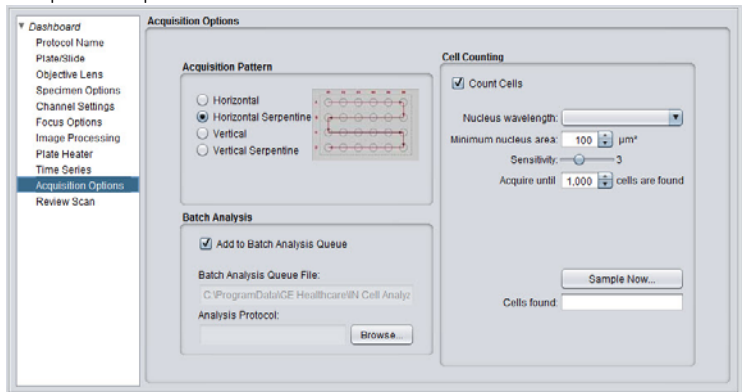
- 8 To delete any timepoint from the series, highlight the row to delete and click **Delete**. To delete multiple, contiguous rows use the **SHIFT+Left Mouse** combination, or to delete specific rows use the **CTRL+Left Mouse** combination.

5.3.11 Acquisition Options card

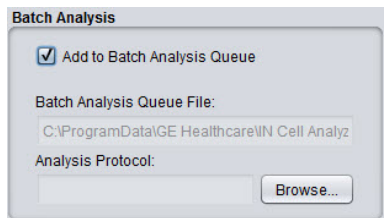
Use the **Acquisition Options** card to define how the target plate will be acquired. You can also setup the **Cell Counting** feature which will acquire the set number of images to match the criteria.

Clicking the **Acquisition Options** card option opens the **Acquisition Options** window.

Fig 5-22. Acquisition Options card

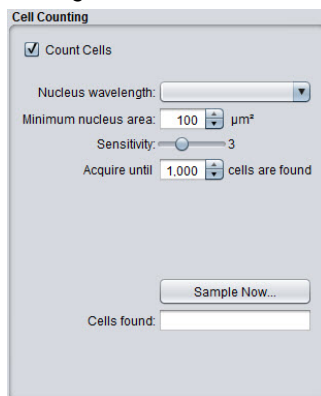


- 1 In the *Acquisition Pattern* section, mark the radio button of the acquisition pattern to use when traversing the plate wells. Selecting an option will show the acquisition pattern in graphical form.
- 2 In the *Batch Analysis* section, mark the **Add to Batch Analysis Queue** checkbox to add the experiment defined by this protocol to the batch analysis queue. The request will be added to the batch queue file specified in the **Batch Analysis Queue File** field. Click the **Browse** button to select a different location to save the analysis protocol file (.xanp).



Note: It is important to select the *IN Cell Developer Toolbox* or *IN Cell Analyzer Workstation* to ensure that the output of the batch analysis is formatted properly.

- 3 In the *Cell Counting* section, mark the **Count Cells** checkbox to enable the cell counting feature.

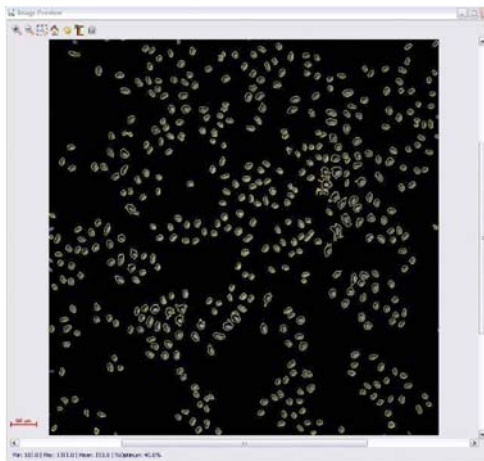


The screenshot shows a dialog box titled "Cell Counting". It contains the following elements:

- A checked checkbox labeled "Count Cells".
- A "Nucleus wavelength:" dropdown menu.
- A "Minimum nucleus area:" field with a value of "100" and a unit of "µm²".
- A "Sensitivity:" slider set to "3".
- An "Acquire until" field with a value of "1,000" and the text "cells are found".
- A "Sample Now..." button.
- A "Cells found:" field at the bottom.

- In the **Nucleus Wavelength** field, select a wavelength from the dropdown list.
 - Select the **Minimum Nucleus Area** from the dropdown list, or enter a value in the **Minimum Nucleus Area** field.
 - Use the **Sensitivity** slider to optimize nucleus identification.
 - In the **Acquire Until** field, enter a value, or use the arrow keys, to set the upper limit for the number of cells acquire.
- 4 Click **Sample Now** to sample the cell counting parameters and preview the results in the **Panel View** window. The **Cells found** field will update with the number of cells found in the sampled image.

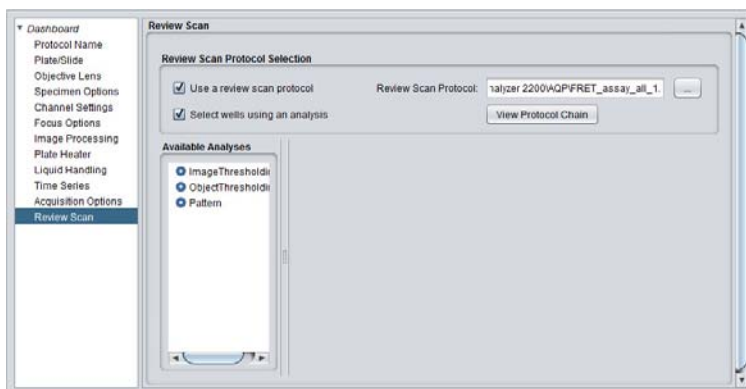
Fig 5-23. Cell Counting results in Panel View Window.



5.3.12 Review Scan card

Use the **Review Scan** card to select a protocol for a follow-on scan on wells meeting a certain criteria. Clicking the **Review Scan** option opens the **Review Scan** card.

Fig 5-24. Review Scan card



- 1 Mark **Use a review scan protocol** and select a protocol to use for the follow-up scan on the selected wells. Use the **Review Scan Protocol** field **Browse** button to navigate to the protocol location.

Note: The follow-up protocol should use the same plate selected as the current protocol.

- 2 Mark **Select wells using an analysis** to have the wells selected as defined by the analysis parameters.

- 3 Select any of the available analyses from the **Available Analyses** pane. Measures, criteria, image flatness and wavelength can then be set.

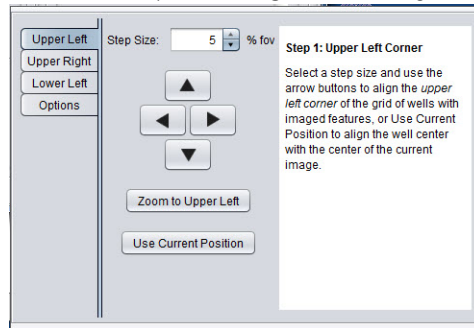
5.4 Aligning the plate

The **Fine Tune** option allows the positioning of the upper left and right, and lower left corner wells required of this additional angular control, as necessary.

Large format plates (e.g., 384 well and larger) and custom arrays may show variation in their well/spot layout that cannot be compensated in the X-Y movement of the well layout.

To perform the plate map alignment:

- 1 Click the **Fine Tune** icon to open the **Alignment** dialog.



- 2 Navigating through Steps 1-3 align the plate.
 - Adjust the upper left corner to align the plate in the X and Y orientations.
 - Next, align the upper right corner of the plate to adjust well spacing and rotate the plate around the upper left corner.
 - Lastly, align the lower left corner to adjust the spacing of the wells and rotate the plate around the upper left corner as well.
- 3 On each dialog, set the percent rotation in the **Step size** field. This is the movement about the FOV for each mouse click.

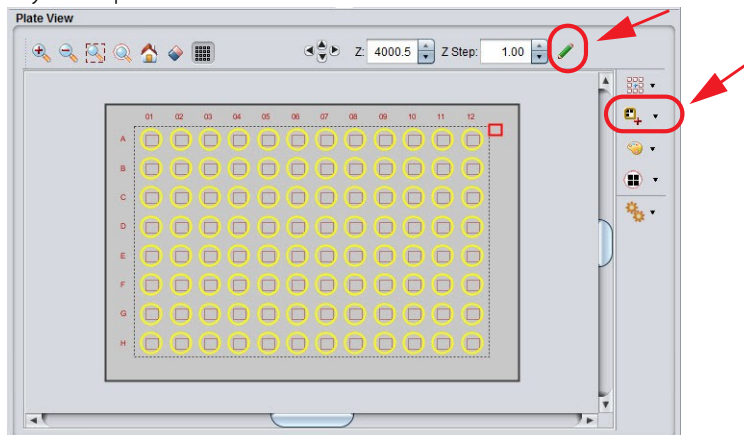
Clicking the **Zoom to Upper Left** button will enlarge the upper left well for clear visualization. Clicking **Use Current Position** will align the well center with the center of the current image.

Note: This alignment is saved independently of the protocol and plate map, and is loaded each time a protocol is opened.

5.5 Previewing plate/slide regions

In image processing, the desirable areas of the sample plate are typically those that contain cells at a desired level of confluence. In many cases, consistent well-to-well growth patterns are observed. For example, compound additions may tend to ablate cells in the center of the wells more readily than at the edges. To help identify such patterns to locate the best area for imaging, use the **Preview Scan** feature to view a selected region of the plate or slide with the current protocol before initiating an acquisition run (i.e., preview a user-selectable region of the plate or slide to test the settings in your protocol). This feature is useful when designing your protocol to explore the output of your settings before finalizing them.

Fig 5-25. Assay Development Plate View



Set up preview scan area

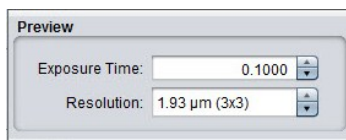
The **Preview Scan** feature allows a low resolution view of a user-defined region of the plate/slide, providing the ability to pre-scan for sample position and refine the position of the fields-of-view to acquire the best possible images.

To perform a preview scan:

- 1 Select a well on the plate to be imaged.
- 2 On the **Protocol Designer|Objective Lens** card, select a low magnification objective to view a larger area of the sample.
- 3 Refocus, if necessary.
- 4 In the **Plate View** window, click the well in which you are interested to take a snapshot. The image will display in the **Panel View** window. Use this image to determine the best possible targets for imaging.

- 5 Clicking the arrow to the right of the **Preview Scan** icon opens the **Set Up Preview Scan** window. From here, set the **Exposure Time** and **Resolution** for the preview.

Fig 5-26. Setup Preview Scan



- Use the arrow keys to set the exposure time for the scan.
 - Select the binning for the scan from the dropdown list.
- 6 Close **Set up Preview** window. Hold down the left mouse button and drag across the **Plate View**, a brown box outline defines the region for previewing.
 - 7 Click on the **Preview Scan** icon in the main toolbar to start. Images will populate the box.

Note: On the IN Cell Analyzer 2000, higher binning levels combine pixels in the image, so exposure time should be lowered as binning is increased.

- 8 If the resulting images are out of focus, use the **Z** and **Z Step** fields to improve the clarity.

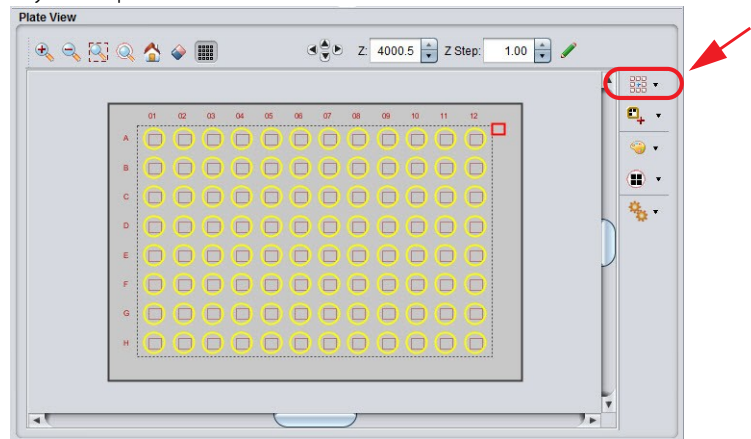
Note: If you are using Phase Contrast or DIC imaging, however, you'll need to set the exposure times in the **Channel Settings** card and Digitize each wavelength to preview your images.

- 9 Use the Save to protocol icon  to save the settings to the protocol.

5.6 Selecting fields of interest

You can acquire multiple fields of view within each well of a plate. The **Fields to Acquire** allows you to position the defined fields over your region of interest where you can set up fields of view for the wells in your selected plate, for image acquisition on specific regions of interest within the sample. The software gives you flexible control over positioning, so that fields of view can be grouped together or distributed throughout the well.

Fig 5-27. Assay Development Plate View

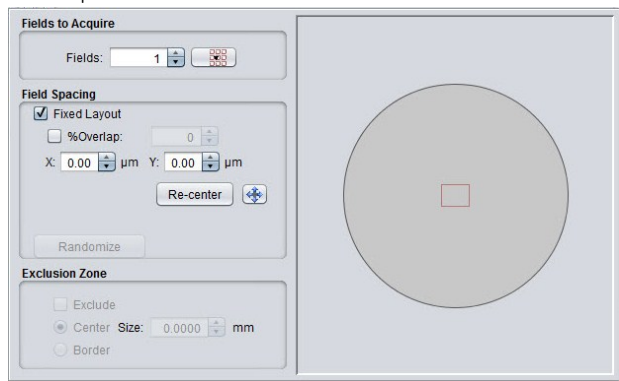


Fields to acquire option

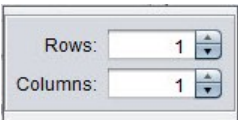
To define the fields for acquisition:

- 1 Click the **Fields to acquire** icon on the **Plate View** toolbar. The **Fields to Acquire** window opens.

Fig 5-28. Fields to Acquire



- 2 In the **Fields to Acquire** section, use the arrow keys to select the number of fields to acquire, or click the **Setup rows/columns** icon to set up fields of view in a specific number of rows and columns.



- 3 In the **Field Spacing** section, choose between a fixed or random layout.

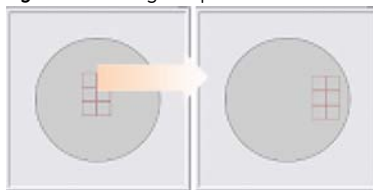
- Mark the **Fixed Layout** checkbox to define the distance between the fields.
 - a. Use the **X:** and **Y:** field arrow keys (or enter a value in the text box) to specify the vertical and horizontal location of the fields. The well graphic on the right side displays your selections.

Fig 5-29. Set up field spacing.



- b. If overlapping images are required to accommodate image stitching, marking the **% Overlap** checkbox allows you to specify the amount of overlap. Image stitching can be particularly useful when relatively large features (for example, neurites) are likely to extend across field boundaries. Generally, the more separate objects there are in the image, the greater degree of overlap will be required in order to ensure accurate alignment at the boundaries to be stitched.
- c. The location of the fields to be acquired within the well can be adjusted by clicking the **Filed mover**  button and using click-and-drag to move the group of fields to the desired location.

Fig 5-30. Moving the position of the fields.



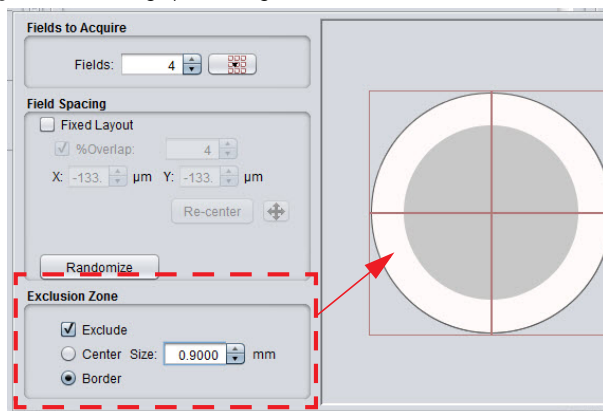
- d. To return the grouping to its original centered position, click the **Re-center** button.

Note: You can view the fields as they move over the preview scan in the **Plate View** window.

- 4 Clicking the **Randomize** button will set the acquisition pattern randomly. The fields in the **Exclusion Zone** section will also be enabled.
- 5 Use the **Exclusion Zone** section to define the regions of the well to be excluded:

- Mark the **Exclude** checkbox to enable the exclusion options. If you unmark the option your settings will be retained.
- Select either the **Center** or **Border** radio button to exclude either a portion of the center or a portion of the edge of the well.
- Specify the size of exclusion area in the **Size** field. Clicking **Randomize** a second time will activate the exclusion zone as shown below.

Fig 5-31. Excluding specific regions of the well.



- 6 When you are satisfied with the settings, close the **Set Up Fields to Acquire** window.

5.7 Choosing wavelengths

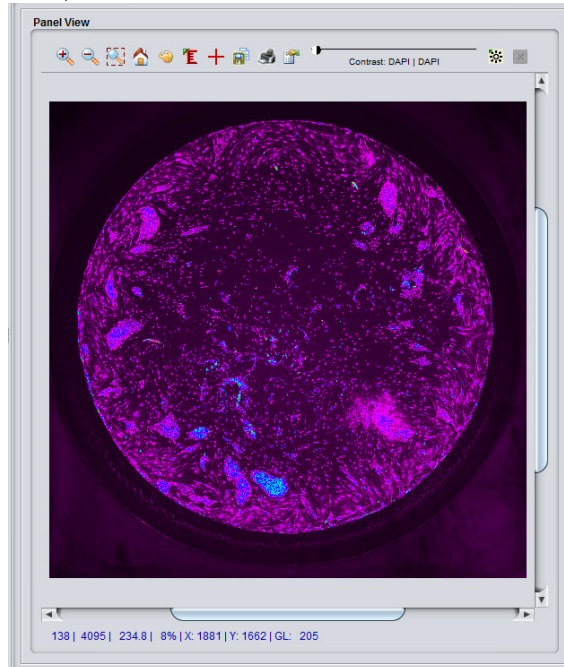
During the protocol design process, use the **Protocol Designer|Channel Settings** card to help define the wavelength settings or monitor the wavelength settings during image acquisition in the Acquisition Workspace. Changes to the wavelength settings can be included in the protocol by clicking **Save to protocol** . The settings will display on the **Protocol Designer|Channel Settings** card.

Note: Higher binning levels combine pixels in the image, so exposure time may need to be lowered as binning is increased.

5.8 Previewing the image

In the right-hand portion of the **Assay Development** workspace, the **Panel View** window is displayed. Images acquired using **Preview Scan** or **Protocol Designer** will display in this window. Click on a well in the **Plate View** window, and then click the **Digitize** button to acquire the image based on the current protocol settings. Refer to the *Panel View* for detail.

Fig 5-32. Assay Development Panel View



5.9 Saving the protocol

After completing the **Protocol Designer**, you will want to save the protocol.

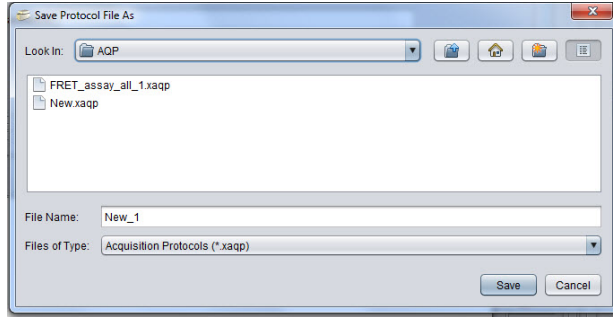
- Save the protocol for future use.
- Save a protocol with a different name (as when modifying any protocol settings).

Save a protocol without running the acquisition

- 1 Choose **File|Save** from the *Main* menu bar, or click the  icon on the *Main* toolbar.

Save a protocol using another name

- 1 Choose **File|Save As** from the *Main* menu bar. The **Save Protocol File** window opens.



- 2 Browse to the desired location and enter a name for the protocol file (.xaqp).
- 3 Click **Save**.

5.10 Editing protocols

Protocol Designer

The **Protocol Designer** allows previously created protocols to be modified. From the *Main* menu, click **Open**, or the **Open** toolbar, select a protocol from the **Open Protocol File** dialog to display the **Protocol Designer** with the selected protocol settings. Go to **Model|Assay Development** to open the **Protocol Designer** window.

- 1 From the left-hand panel of the **Protocol Designer**, select the card that you want to modify from the list, and double-click to display the card.
- 2 Make your changes as necessary.
- 3 Repeat steps 1 and 2 as necessary.
- 4 On completion, save your changes by choosing **File|Save As** from the *Main* menu bar.

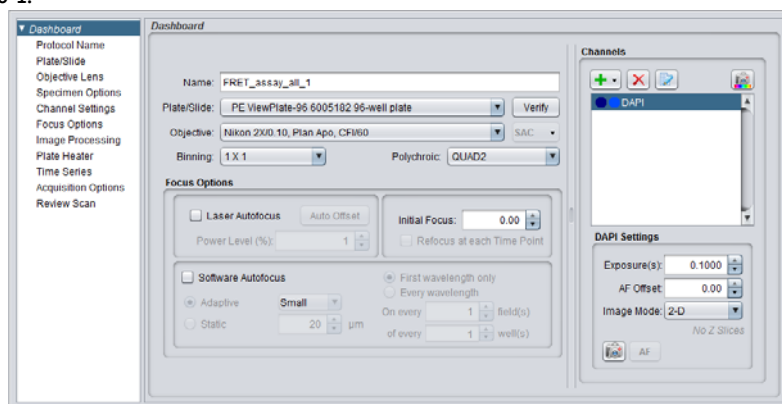
The Dashboard

The Dashboard can be used to modify protocols by providing access to the most commonly used parameters. Refer to *Chapter 6 Using the Assay Development Dashboard*.

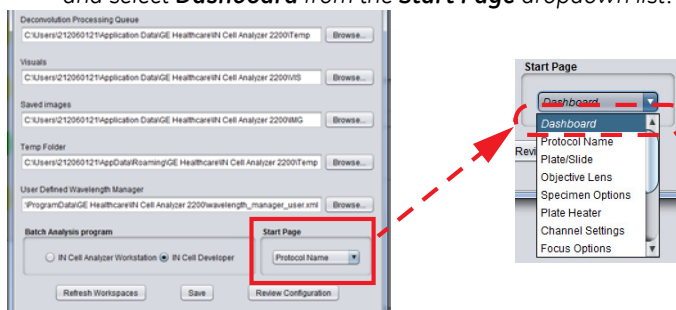
6 Using the Assay Development Dashboard

Dashboard can be used by intermediate and advanced users of the *IN Cell Analyzer 2200/2000* system to create and modify settings of the currently open protocol. It represents the most commonly used settings in a protocol allowing the user to modify them. Changes will be updated in the *Protocol Designer* on the corresponding cards. Future snapshots/acquisition runs will use these settings.

Fig 6-1.



Note: Dashboard can be set as the default start page in the **Protocol Designer** from **File|Preferences**. On the **Preferences** window, click the **User** tab and select **Dashboard** from the **Start Page** dropdown list.



6.1 Creating the protocol

General

In the *General* section, change or update the protocol name, sample holder and optics information. These fields correspond to the **Protocol Name**, **Plate/Slide**, **Objective Lens**, and **Channel Settings** cards in the **Protocol Designer**.

The screenshot shows a 'General' settings window with the following fields and values:

- Name: FRET_assay_all_1
- Plate/Slide: PE ViewPlate-96 6005182 96-well plate (with a Verify button)
- Objective: Nikon 2X/0.10, Plan Apo, CFI/60 (with a SAC dropdown)
- Binning: 1 X 1
- Polychroic: QUAD2

- **Name** - Provide a name for your experiment.
- **Plate/Slide** - Choose a plate from the drop-down menu. Click the **Verify** button to use the LAF to get plate/slide information about the nominal focus position (Z) at the solid/liquid interface.
- **Objective** - Choose an objective from drop-down menu.
- **Binning** - Select binning options from drop down menu.
- **Polychroic** - Select polychroic from drop down menu.

Focus options

In the *Focus Options* section, manipulate the Hardware Autofocus and Software Autofocus settings. These items will be enabled when the checkboxes are marked on the **Protocol Designer** **Focus Options** card.

The screenshot shows the 'Focus Options' dialog box with the following settings:

- Laser Autofocus**: ☐ (disabled), **Auto Offset** button, **Power Level (%)**: 1
- Initial Focus**: 0.00
- Software Autofocus**: ☐ (disabled)
- Adaptive**: ☒ (selected), **Static**: ☐ (disabled)
- Small**: 20 μm
- First wavelength only**: ☒ (selected), **Every wavelength**: ☐ (disabled)
- On every**: 1 field(s)
- of every**: 1 well(s)
- Refocus at each Time Point**: ☐ (disabled)

Clicking the **Auto Offset** button will calculate the *Hardware Autofocus Offset*. A completion dialog will display the channels and their offset settings in the **Focus Options** card **Offset** table. When the HWAFF Offset calculation is requested, a progress window displays and on completion a **Channel Offset Results** dialog

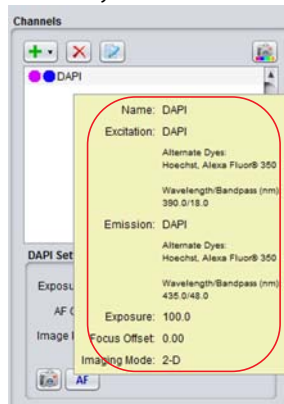
opens. Review the offsets for the wavelengths and click **OK** to apply the settings (visible on the **Protocol Designer|Focus Options** card).



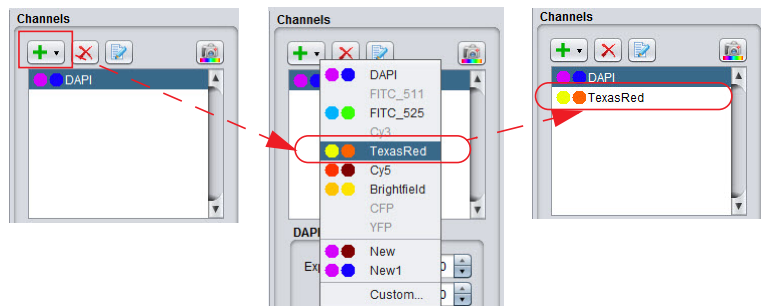
Channels

In the *Channels* section, a number of actions are available. As on the **Protocol Designer|Channel Settings** and **Focus Options** cards, wavelengths can be added/edited/removed from the protocol and their parameters modified.

- **Channels** – Hovering the mouse over a wavelength pair opens the information window where you can view *Excitation/Emission* channel information.



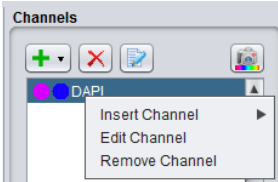
Add channels appropriate to your experiment by clicking the **Add Channel** icon. Modify or remove channels from the Channels list using the toolbar buttons.



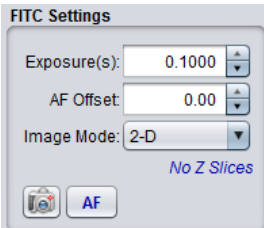
The following actions are available:

	Add new channel	Opens the channels list. New channel becomes the current channel when added to the protocol. A new image is acquired and Panel View/Plate View thumbnail are updated. Selecting Custom open the Insert Custom Channel dialog
	Remove current channel	Removes the selected channel from the protocol. A new image is acquired using the channel now in the current location
	Edit current channel	Opens the Edit Custom Channel dialog for the selected channel
	Acquire color image	Acquires all available wavelengths in the protocol and generates a fused image that displays in the Data Review Panel View View as Color Image tab

Alternatively, the right-click menu can also be utilized directly from the Channels list to perform these functions.

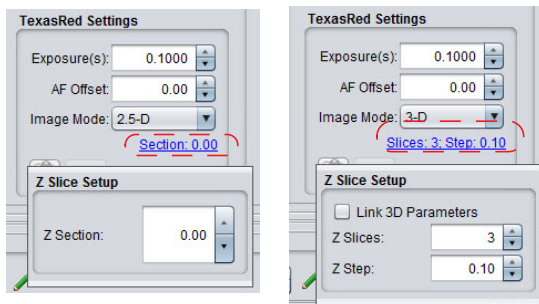


- Use the **Custom** option on the Channels list to create custom channels with the desired Excitation-Emission combination. See *Creating/editing channels* below.
- **Channel Settings** - Click on each of the channels in the list to display it's **Exposure**, **AF Offset**, and **Imaging Mode** settings. Use the dropdown lists to modify the settings.



When 2.5-D, 3-D or 3-D Deconvolution are selected as the **Imaging Mode**, the **Z**

Slices Setup dialog can be opened by clicking on the **Slices** hyperlink.



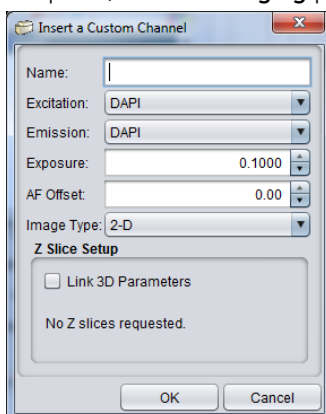
Double-clicking on a channel name will perform an autofocus and snap an image. Single-clicking on a channel will capture an image without applying autofocus.

After optimizing exposure times for all channels, get an offset value for each wavelength by clicking **Auto offset** in the **Focus Options** panel. Single-click on a channel name (e.g., Texas Red) and click **AF** to see focus with offset added. Repeat to optimize the focus in other wells.

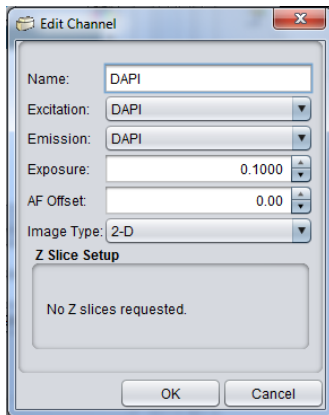
Creating/editing channels

As on the **Protocol Designer|Channel Settings** card, channels can be defined 'on-the-fly', using the **Custom** option on the **Channel** list. In addition, existing channel definitions can be modified by selecting the **Edit** option. These features are available from both the toolbar and right-click menu.

Selecting the **Custom** option opens the **Insert a Custom Channel** dialog. Name the channel, select the **Excitation** and **Emission** filters, set the **Exposure**, **AF Offset**, and **Imaging Mode**. If required, set the **Z Imaging** parameters.

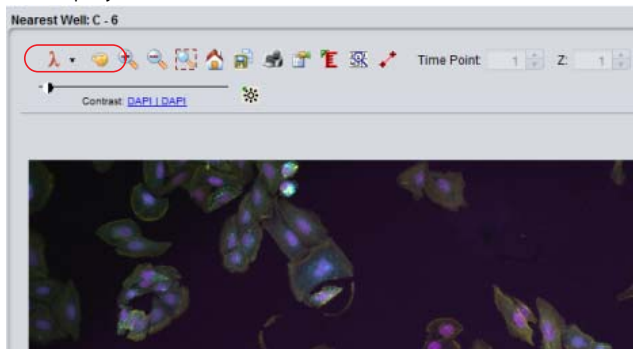


Selecting the **Edit** option opens the **Edit Channel** dialog. Modify the parameter settings as necessary.



Acquiring a color image

- 1 Click the **Acquire color image**  button to generate a colored (fused) image that will display in the **Panel View** window.



- 2 In the **Panel View** window, click the **Wavelength** toolbar icon to change the color options and the **Visuals** toolbar icon to adjust the contrast for each wavelength.
- 3 To save the colored image, click on  icon. The image is saved as a **JPEG** file.

6.2 Saving the protocol

After completing the protocol, you will want to save it.

- Choose **File|Save** from the *Main* menu bar, or click the  icon on the *Main* toolbar.

- Choose **File|Save As** from the *Main* menu bar. The **Save Protocol File** window opens. Browse to the desired location and enter a name for the protocol file (.xaqp). Click **Save**.

6 Using the Assay Development Dashboard

6.2 Saving the protocol

7 Acquisition mode - conducting experiments

The purpose of conducting experiments is to collect data on the samples being imaged. The user has previously defined the necessary parameters of the experiment using the **Protocol Designer**. In general, an experiment is a series of imaging tasks. When the pre-defined experiment is launched, the *IN Cell Analyzer 2200/2000* system works without user input to complete the experiment which can last from seconds to days.

7.1 Load the sample

To begin designing your protocol a microplate should be loaded so you can preview the acquisition results. The plate access door is allowed to open only when the plate carrier is in the load/unload position.

To load a plate into the *IN Cell Analyzer 2200/2000*:

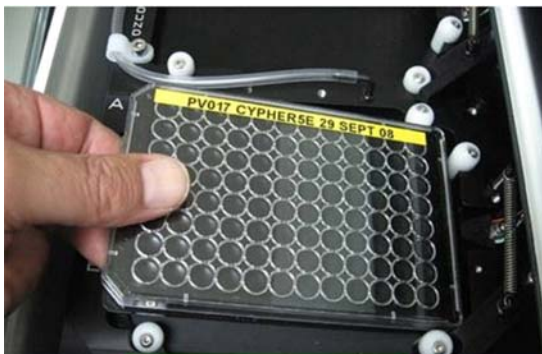
- 1 Click the **Eject** icon on the *Main* toolbar. The access door on the top of the system slides open.

Fig 7-1. Plate Access Door.



- 2 Place the plate into the plate carrier. The plate should be firmly seated.

Fig 7-2. Plate carrier.



- 3 To close the access door, click on a well location from the **Plate View** window. The plate is automatically positioned for imaging.

7.2 Opening a protocol

After the sample is loaded, open the acquisition protocol for the experiment. If an acquisition protocol has not already been designed for this experiment, refer to *Designing the Experiment - The Protocol Designer* for instruction on creating a protocol.

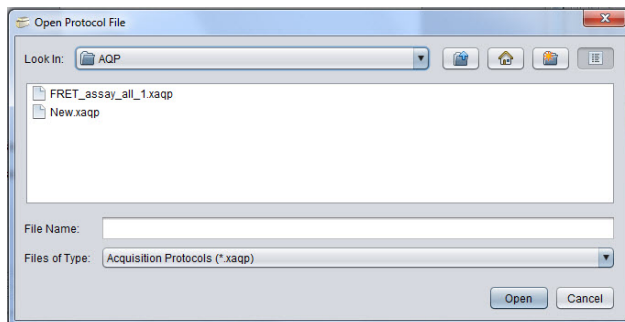
- 1 Open the acquisition protocol file from either:

- **Main menu|File|Open**
- Clicking the **Open** icon on the **Main** toolbar.

Note: If starting the acquisition from the currently open protocol, click **Run Protocol**. The plate from which images are to be acquired must be loaded.

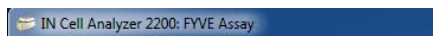
- 2 Select the protocol you want to use from the **Open Protocol File** dialog.

Fig 7-3. Open protocol File



- 3 Click **Open**. The selected acquisition protocol is loaded into the system and the protocol file name is displayed in the top left corner of the *IN Cell Analyzer 2200/2000* main window.

Fig 7-4. Protocol name displaying in title bar



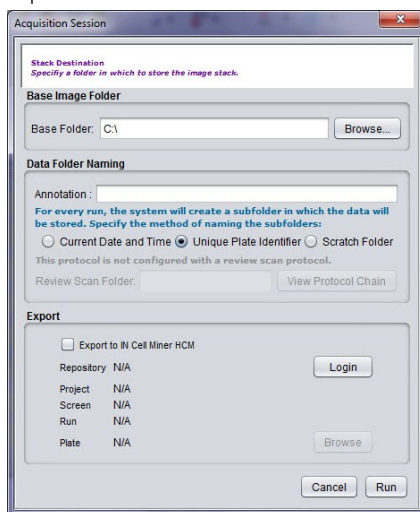
- 4 Review the parameters of the acquisition protocol in the **Protocol Designer**. Make any necessary adjustments.
- 5 If changes are made to the existing protocol, you may want to save it under a new name, using **File|Save As** from the *Main* menu.

7.3 Run the acquisition

To start an acquisition:

- Click the **Run protocol** toolbar icon. The **Acquisition Session** window is displayed.

Fig 7-5. Acquisition Session



- 1 In the *Base Image Folder* section, click the **Browse** button and navigate to the base image folder location. This is the folder for all of the images you acquire using the *IN Cell Analyzer 2200/2000* system.

- 2 In the *Data Folder Naming* section, select a method for naming subfolders for the images to be acquired.

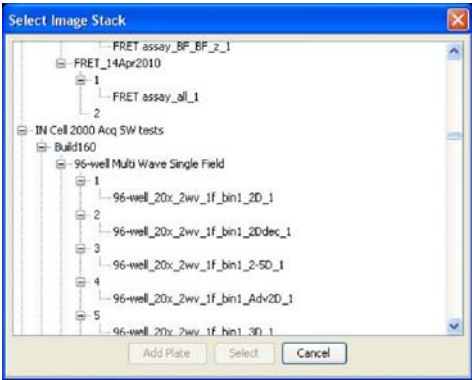
Current Date and Time	Appends the folder name with the current date and time of the experiment.
Unique Plate Identifier	Appends the folder name with the name of the current plate.
Scratch Folder	Creates a scratch folder in which to store test images.

- 3 In the *Export* section, mark the **Export to IN Cell Miner** checkbox.
- Click the **Switch** button to open the Log in to **IN Cell Miner HCM** dialog (refer to section 4.1.1.5.2). Enter the user credentials, repository name and server IP address.



Note: The *IN Cell Miner HCM* software application must be purchased and the user must have a valid *IN Cell Miner HCM* user account.

- Click **Login** to log in to the **IN Cell Miner HCM** application. Return to the **Acquisition Session** dialog.
- Click **Browse** from the **Acquisition Session** window to select the target project within **IN Cell Miner** where the acquired images are to be stored.

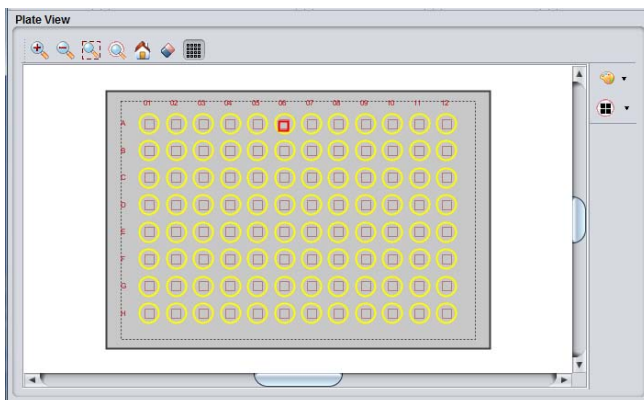


- 4 Click the **Run** button to start the acquisition session.

Monitor the acquisition session

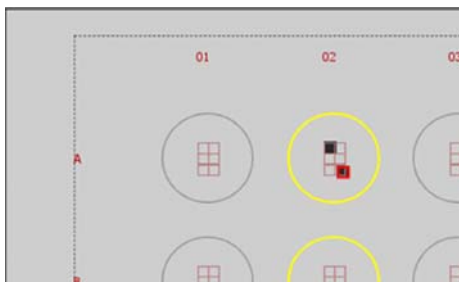
In the upper-left portion of the Activity Workspace use the Plate View window to monitor the acquisition process. Use the toolbar options to manipulate the wells. Refer to *Plate View* for detail on the toolbar options.

Fig 7-6. Plate View



While the acquisition progresses, each acquired field is denoted by a black square, whereas the field currently being acquired is outlined in red, as shown below.

Fig 7-7. Plate View (enlarged)



The processing queue

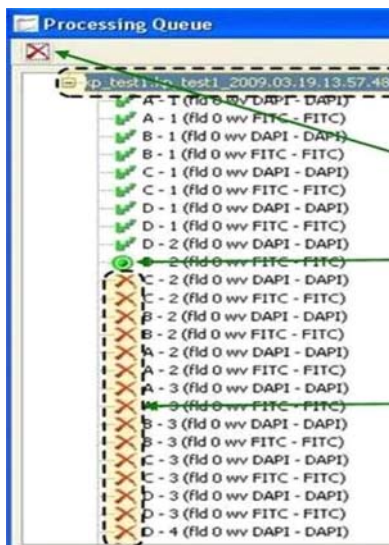
Either during an acquisition session or afterward, you can select **Main menu|Application menu|Processing Queue** to view the status of each 3D Deconvolution or Advanced 2D Deconvolution process within a protocol run. When selected, the **Processing Queue** window is displayed.

Note: The location of the 3D Deconvolution queue can be found on the **File|Preferences User** tab.

If the data from your recent scan are not available for viewing in **Data Review** mode, check the **Processing Queue** to determine whether a previous scan is currently being processed. Note that processing for any previous acquisition must

finish completely or be stopped from the **Processing Queue** before a subsequent acquisition's processing can begin.

Fig 7-8. Processing Queue.



In the **Processing Queue** window, the  icon indicates that the well/wavelength entry is currently being processed. When the process is successfully completed for the entry, a green checkmark  is displayed next to the entry. To skip the processing of any of the listed entries, highlight the entry and select  from the **Processing Queue** toolbar. A red  is placed next to the entry indicating that the entry will be skipped.

To stop and cancel acquisition processing, highlight the file name of the acquisition currently processing in the **Processing Queue** window. Click  on the **Processing Queue** toolbar. The remaining entries in the queue are marked with the indicator and processing is stopped as soon as the current entry has finished processing.

Monitor the Wavelength Settings

While the wavelength choices cannot be changed during acquisition, it can be useful to track the image acquisition from select wavelengths. This information can then be used to modify the protocol. The settings will display on the **Protocol Designer|Channel Settings** card.

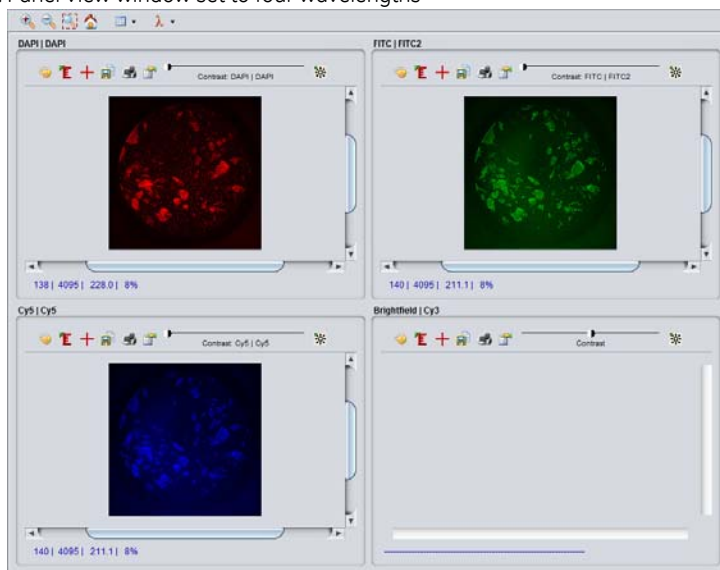
Viewing Acquired Images

The **Panel View** window allows you to view images that are acquired. Each of the wavelengths defined for an acquisition (up to a maximum of 13 simultaneously) is displayed in a separate frame for the field being acquired. You can configure the

Panel View window to display as few or as many single-wavelength images as necessary for your experiments.

Use this window to define how each wavelength is displayed and to monitor the progress of image acquisitions. Refer to *Chapter 4 Panel View* for detail on the common toolbar options. Toolbar features specific to *Acquisition Panel View* are described below.

Fig 7-9. Panel View window set to four wavelengths

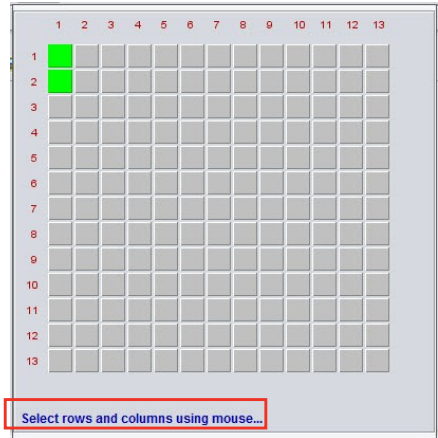


Panel View layout

The **Panel View Layout** tool is used to define the number of images to display in the Panel View window.

- 1 Click the **Panel View Layout** icon to open the **Panel Viewer Layout Setup** window.
- 2 Holding down the left mouse button, drag over the number of images to display in the window. The status bar displays the dimensions of the viewing area.

Fig 7-10. Panel View Layout Setup defining a 1x2 grid

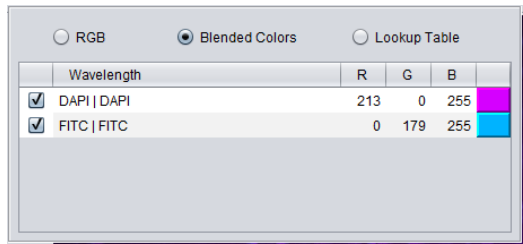


Wavelength selection

To define the wavelengths that you want to display in the **Panel View** window:

- 1 Click the **Wavelength Selection** icon on the toolbar. The **Wavelengths** window opens.

Fig 7-11. Wavelengths window.



- 2 Mark and unmark the checkboxes of the wavelengths to display in the **Panel View** window.
- 3 Open dropdowns in the **Visuals** column to set user defined colors or a LUT for each wavelength in the view. These settings will not be saved.

8 Data Review mode - reviewing collected data

The **Data Review** mode allows you to review acquired image stacks and their associated data.

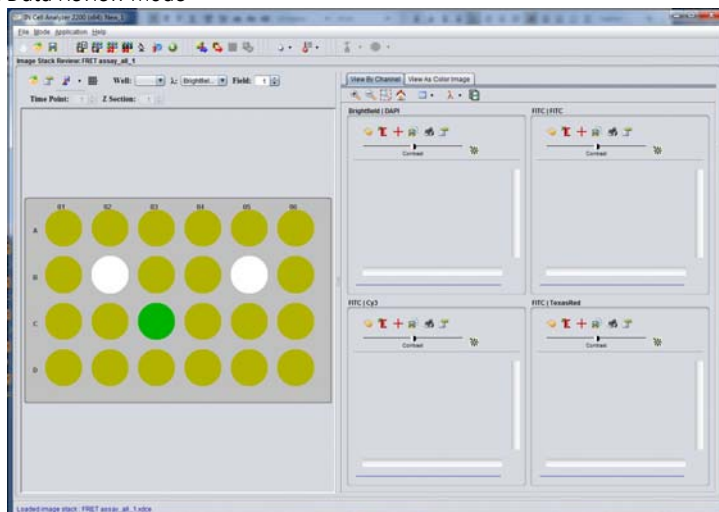
Note: If the data from your recent scan do not appear to be available for viewing in Data Review Mode, check the Processing Queue from the Main Menu (Application|Processing Queue) to determine whether a previous acquisition is currently being processed. Processing for any previous acquisition must finish completely or be stopped from the Processing Queue (see the Processing Queue section) before a subsequent acquisition's processing can begin.

8.1 Viewing acquired images

After acquisition, **Data Review** mode will open automatically. To view previously acquired data, open Data Review mode by either:

- Choose **Mode|Data Review** from the *Main* menu bar.
- Click the **Data Review** icon on the *Main* toolbar.

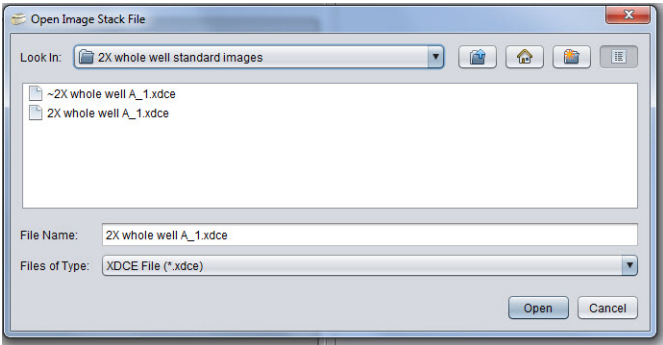
Fig 8-1. Data Review mode



8.1.1 Opening an Image Stack (.xdce)

- 1 From the **Image Stack Review** window toolbar, click the **Open** icon. The **Open Image Stack File** window is displayed.

Fig 8-2. Open Image Stack File Window.



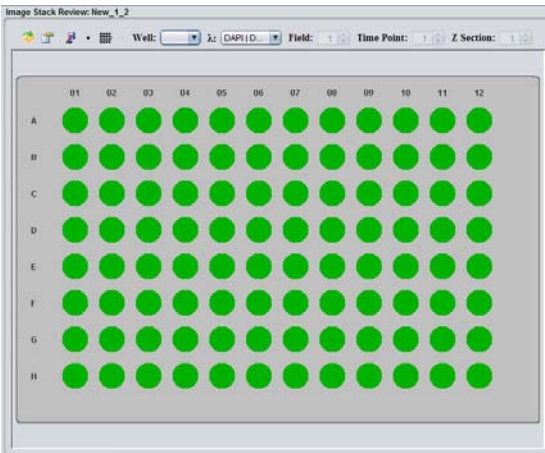
- 2 Browse to the location of the image stack file (.xdce) and select **Open**. When the image stack file is finished loading, a status message is displayed at the status bar of the *IN Cell Analyzer 2200/2000* application window. It represents the name of the image stack that has been opened.

Loaded image stack : New_1_2.xdce

8.1.2 Image Stack Review window

The **Image Stack Review** window is designed to show you the acquired image data in relation to the plate, wells, and fields defined for the experiment. The common toolbar feature are described in *Chapter 4, Image Stack Review*. Features specific to the **Image Stack Review** window are described below.

Fig 8-3. Image Stack Review window



Fused image

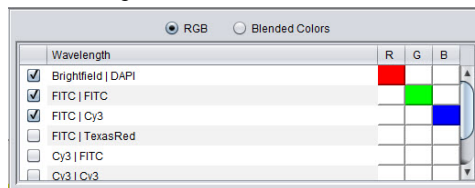
Fused images are created from multi-wavelength assays. Image fusion allows color composite images to be created from individual, colored wavelength images.

For example, if an image shows both a blue nuclear and a green cytoplasmic counter-stain (such as Hoechst 33342 and Green Fluorescent Protein (GFP)), using image fusion a blue LUT can be assigned to the Hoechst image and a green LUT to the GFP image. The two images are then fused together into a single, 213-bit color image with green cytoplasm and blue nuclei.

To define the fused image:

- 1 Select the image to fuse by selecting a well in the **Image Stack Review** window.
- 2 Click the arrow to the right of the **Fused Image** icon. The **Fused Image Definition** window opens.

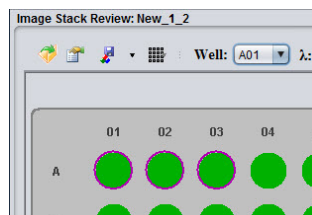
Fig 8-4. Fused Image Definition Window.



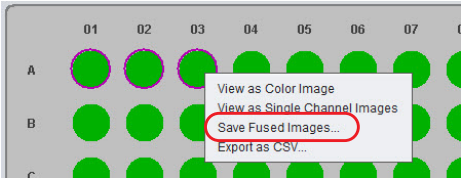
- 3 Select the type of fused image to create:
 - **RGB** has a three wavelength maximum.
 - **Blended Colors** has a five wavelength maximum.
- 4 Mark the checkboxes of the wavelengths to use for the fused image definition.

Next, generate and save the fused image. Once the wavelengths to be included in the fused image are defined:

- 1 On the **Image Stack Review** window, select the well(s) for which to save as a fused image.
- 2 Use **CTRL-click** to select the wells to fuse. They will be marked as shown below.



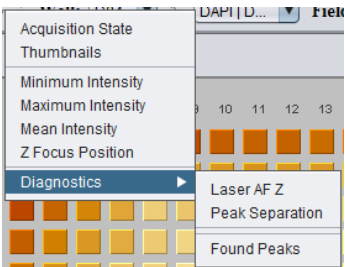
- 3 Click the **Fused Images** icon on the **Image Stack Review** toolbar. The fused images for the selected wells, are generated. Alternatively, fused images can be generated using the right-click context menu and selecting **Save Fused Images**.



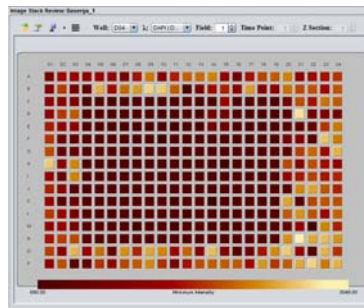
Note: Fused images are always saved for the middle Z slice for 3D stacks.

Display type

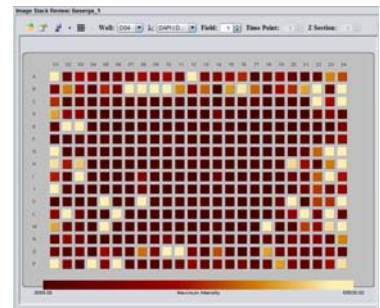
In addition to the standard Acquisition state and Thumbnail views, new diagnostic tools can be used to review an image stack and it's associated autofocus (intensity) metadata. These complement the standard image display whereby the output can be viewed as heat maps of whole image intensity, Z focus position, laser AF peak separation and found peaks.



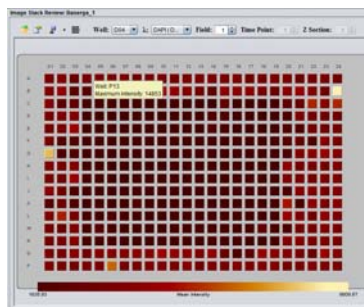
Minimum, maximum, and mean intensity heat maps are available that provide a graphical view of the acquired image intensity.



Minimum Intensity

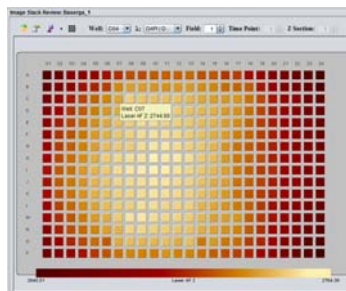


Maximum Intensity

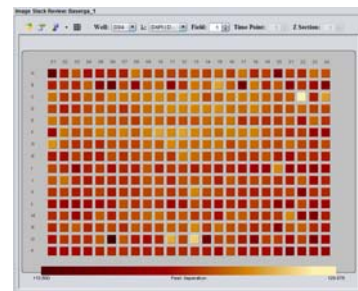


Mean Intensity

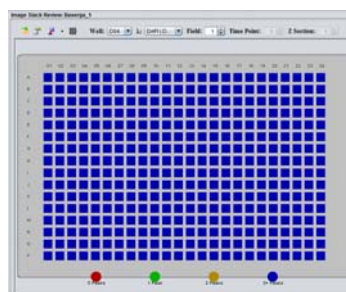
The Diagnostic tools provide yet more interrogation of the output.



Z Focus Position



Peak separation



Found peaks

8.1.3 Panel View

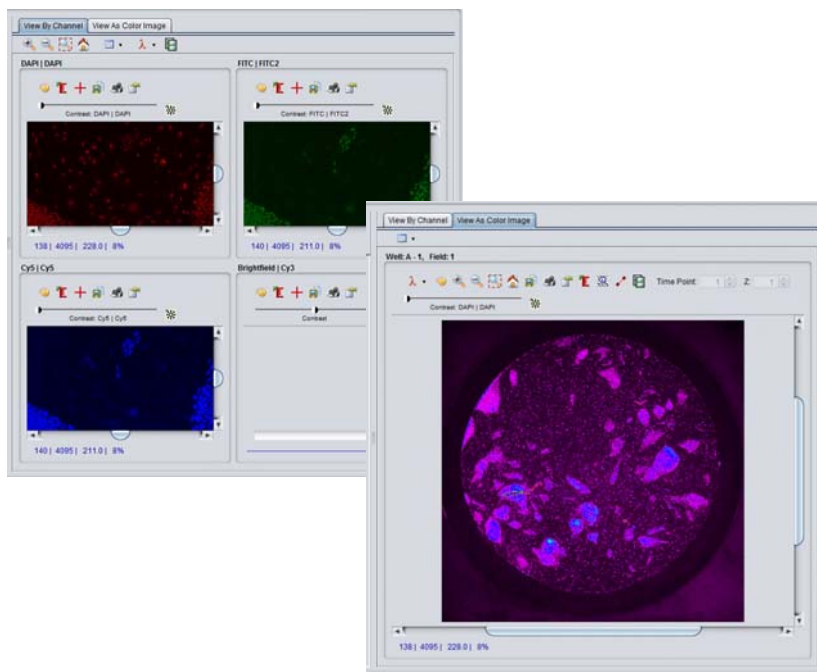
The **Data Review Panel View** window shows the acquired image data from each of the defined wavelengths or can show a fused (combined wavelength) image. For common feature descriptions refer to *Chapter 4, Panel View*.

Specific information about fields or wells can be displayed.

Use the features in the **View as Color Image** tab to manipulate the fused image, measure distances between points, and generate movies from Z stacks.

In the **View by Wavelength** tab, when multiple views are set up in the **Panel View** window, each of the views will contain individual Visuals and Ruler tools.

Fig 8-5. Data Review Panel View tabs



Point values

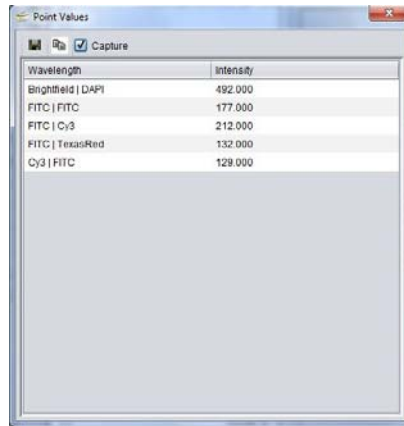
(Capturing wavelength intensities of points within an Image)

An object within an image can be identified by determining the relative density of it's surrounding area. By calculating the wavelength intensities of a series of points within the image, the **Point Value** feature captures the intensity values for selected 'points' and formulates a representation of a 3D image.

To capture a series of wavelength intensities within the image:

- 1 Click the **Point Values** icon to open the **Point Values** window.

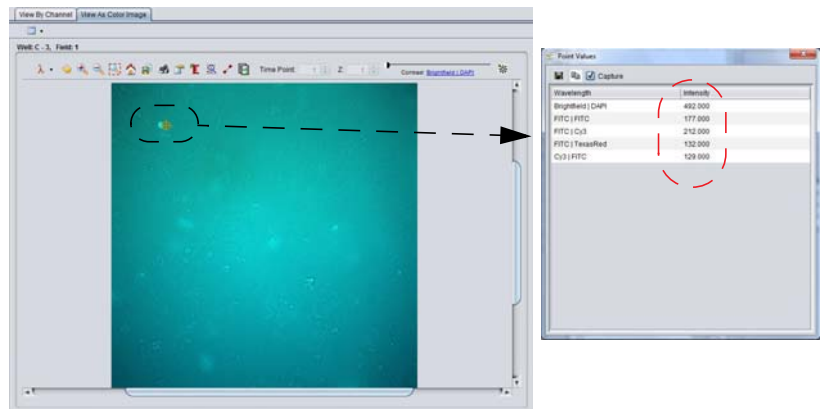
Fig 8-6. Point Values window.



The screenshot shows the 'Point Values' window with a 'Capture' checkbox checked. Below it is a table with two columns: 'Wavelength' and 'Intensity'.

Wavelength	Intensity
Brightfield DAPI	492.000
FITC FITC	177.000
FITC Cy3	212.000
FITC TexasRed	132.000
Cy3 FITC	129.000

- 2 Mark the **Capture** checkbox to enable the collection of the wavelength intensities for selected points.
- 3 Click the mouse cursor on the point of interest within the image (the cursor changes to an orange reticle). The wavelength and corresponding intensity values are reported in the **Point Values** window.



- 4 On completing your investigation, you can click the **Save** icon to save the values to *.CSV file for future use.

Measure option

(Calculating distances between points in an image)

The measurement of distances between points within the image can be valuable for determining the location of objects in an image or the distance or location of objects relative to one another. The **Measure Distance** feature uses various

methods to calculate distance between points. The dialog lists **XYZ** coordinates, the distance from point to point, and the total distance measured.

Fig 8-7. Measure tool with a Multiple Segment measurement

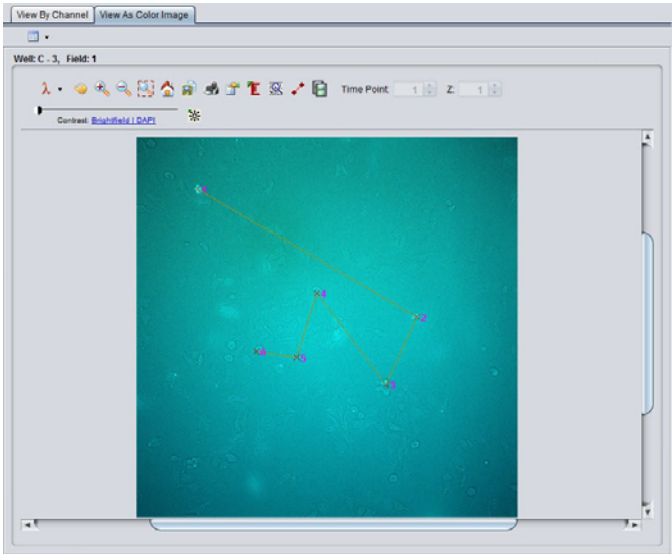


Fig 8-8. Measure Distance window with mutple point distance calculations for 6 points

Line	Point	X	Y	Z	Distance	Total Dist
1	1	338	282	1		
1	2	1594	970	1	504.7527	504.7527
2	3	1341	1331	1	146.5547	651.3074
3	4	967	841	1	220.0764	879.3838
4	5	859	1186	1	133.7586	1013.1424
5	6	642	1152	1	81.2096	1094.4120

Button	Function	Description
	Save Data to File	Saves the calculated measurements to a CSV file.
Method: Standard two-point ▾	Measurement Method	Used to select the a measurement method from the dropdown list.

Button	Function	Description
	Line Color	Used to change the color of the lines used to show the plotting of the distances. See <i>Line Color</i> below.
	Crosshair color	Used to change the color of the begin/end markers on the image. See <i>Crosshairs</i> below.
	Font Selection/ Font color	Used to change the text style of the point labels. Used to change the text color of the of the point labels. See <i>Font</i> below.
	Hide Labels	Click to view or hide the measurement labels.
	Hide	Click to view the image with or without all label, point, and line data.

Calculating Distances Between Points in an Image

- 1 Click the **Measure** icon on the **Image Explorer** toolbar. A blank **Measure Distance** window opens.
- 2 From the **Method** dropdown list, select a measurement method. The measurement methods available are:

Standard two-point	Provides measurement data between two specified points. After selecting two points, the next point selected starts a new measurement.
Single reference	Provides measurement data between two points, with the start point remaining stationary.
Leap frog	Provides measurement data between two points, with the end point of the initial measurement as the start point for the subsequent measurement.
Multiple segment	Provides continuous measurement data as you select point after point contiguously within the image.

- 3 To collect your measurement begin/end points, click the mouse cursor on the point of interest in the image. The data is displayed in the **Measure Distance** window-Line #, Point#, X-Y coordinate, and the distance between points.
- 4 Values may be removed from the calculations by either:
 - Select individual lines of data and click the **Delete** button to remove the selected line from the **Measure Distance** window and from the image.

8 Data Review mode - reviewing collected data

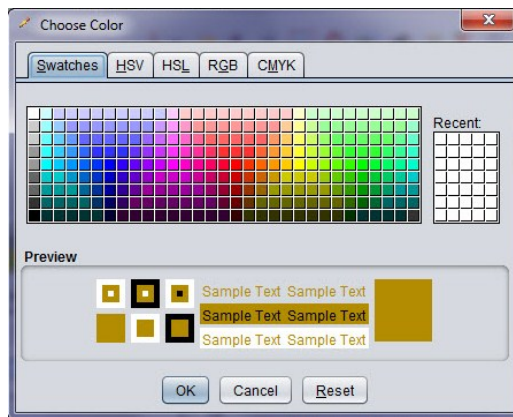
8.1 Viewing acquired images

- Click the **Delete All** button to clear all the data in the window and from the displayed image.
- 5 The collected values can be saved by clicking the **Save** button. The data is saved to a CSV file.

Customizing the point/measure descriptors

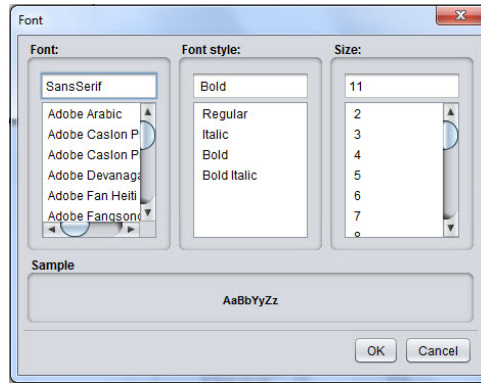
- **Line Color** option - Click the **Line Color** icon to change the color of the connecting lines used to denote a measurement on the image. From the **Choose Color** window, select a color from the palette, using any of the tabs to define the color, as described previously in *Visuals option*.

Fig 8-9. Choose Color window.



- **Crosshair Color** option - Click the **Crosshairs** icon to change the color of the crosshair start/end markers for the measurements. On the **Choose Color** window select a color for the crosshair markers as described above.
- **Font** option - Click the **Font Selection** icon to change the label text style used to identify the measurements on the image. On the **Font** window, select the font, font style, and size to use for the label text. Click **OK** to accept the change and close the dialog.

Fig 8-10. Font Window



Clicking the arrow to the right of the **Font** icon opens the **Choose Color** window from where you can select a color for the label text as described above under *Line Color*.

8 Data Review mode - reviewing collected data

8.1 Viewing acquired images

9 Review Scan mode - focusing on wells of interest

Review Scan mode allows you to scan a plate with more than one protocol. The Review Scan feature can be used to acquire valuable data, for example:

- the first protocol could use a low magnification objective, and the second protocol could use a high magnification objective

To help make the Review Scan feature even more useful, it is also possible to use image analysis between scans to automatically select the wells that should be scanned by the second protocol.

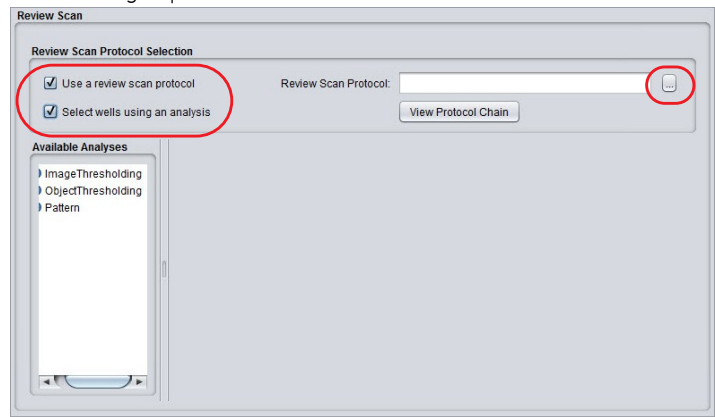
In some cases, the plate may contain control wells that need to be rescanned regardless of the analysis results. To define control wells, open the right-click menu over the appropriate well(s) in the **Plate View** and select the **Control Well** option.

Create the analysis protocol

- 1 Create the analysis protocol for the follow-on high magnification scan (e.g., 10x objective).
- 2 Create a protocol for the first-pass scan to be used to define the thresholding.
- 3 On the **Protocol Designer|Review Scan** card for the *first-pass* scan protocol, check **Use a review scan protocol** and **Select wells using an analysis**. The **Available Analysis** list is enabled.

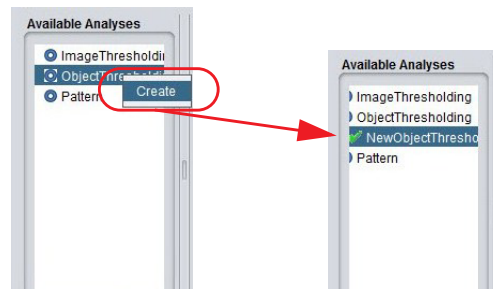
Click the **Browse** button to the right of the **Review Scan Protocol** field to select the Review Scan protocol to use.

Fig 9-1. Protocol Designer|Review Scan card

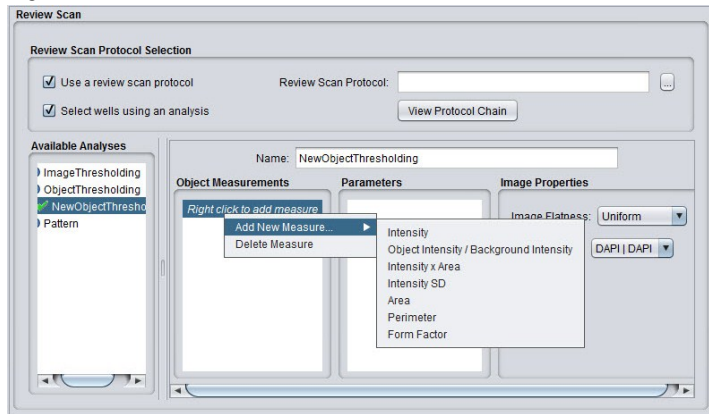


Note: The user may choose to select only **Use a review scan protocol** and not check **Select wells using an analysis**. In this case, all the wells scanned in the first-pass protocol will be selected for review scan with the follow-on protocol. This allows, for example, for automated imaging of all wells with a combination of objectives.

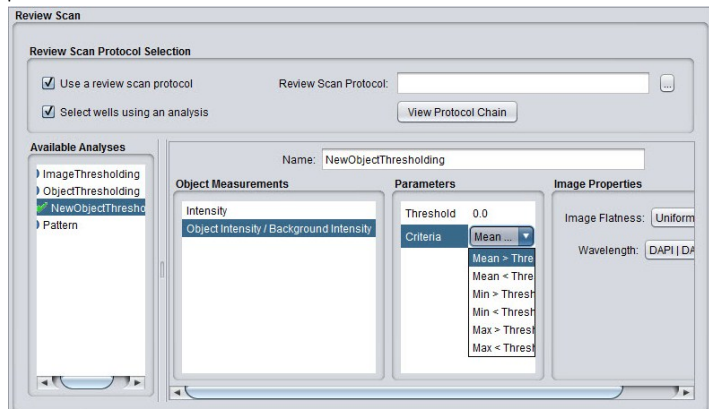
- 4 Right click on an entry under **Available Analysis** to create a new threshold measure.



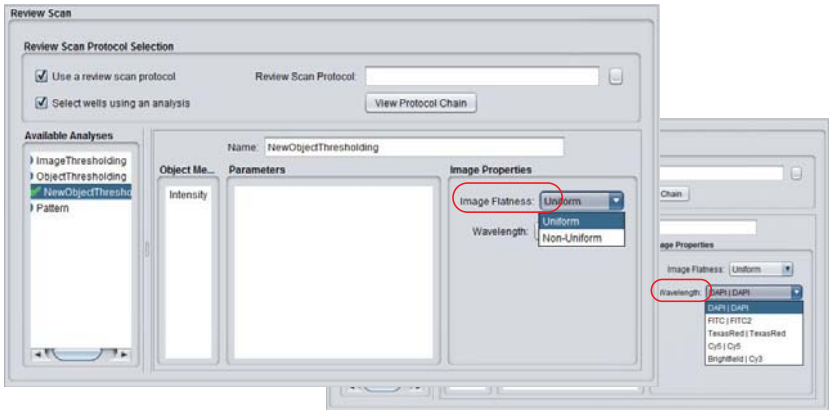
- 5 The **Object Measurements** table opens. Select **Add New Measurements** from the right-click menu and select a measure to add from the list.



- 6 Under **Parameters**, set the criteria by which to calculate the threshold from the dropdown list.



- 7 In the **Image Properties** pane, select a wavelength from the **Wavelength** dropdown list. From the **Image Flatness** dropdown select either *Uniform* or *Non-uniform*.



Note: Select **Non-uniform** for image flatness if there are large intensity variations in the image or a large number of bright spots in the image.

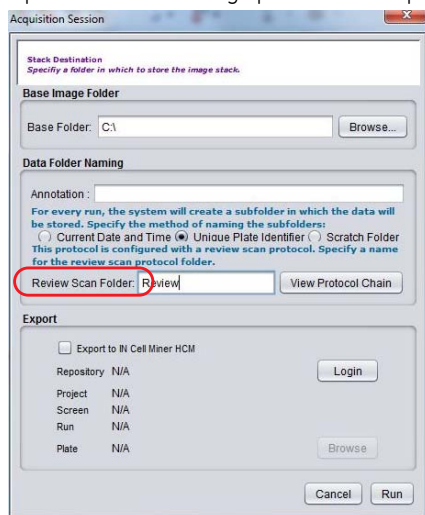
- 8 Navigate to the **Focus Options** card and select wells to image and click **Digitize**. Record the segmentation values from the **Panel View**.



- 9 Return to the **Review Scan** card and enter a threshold value based on the value sampled.
- 10 Use the **Fields to Acquire** option on the **Plate View**.

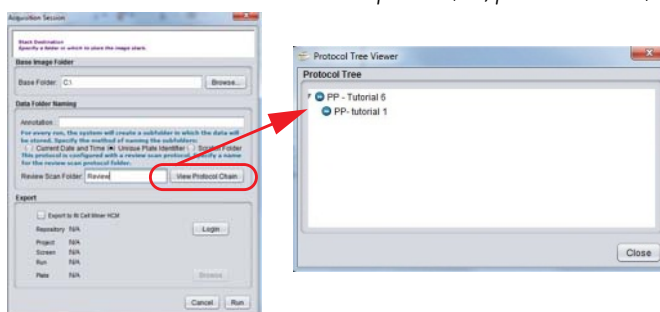
- 11 Save the protocol and click the **Run protocol** toolbar icon. On the **Acquisition Session** window, specify a **Review Scan** folder where all data (including the Review Scan output) is to be saved under the **Base Folder** location.

Fig 9-2. Acquisition Session dialog opens to run the protocol



When the protocol is run, the instrument performs the first scan (e.g., with a 4x objective) saves all these images, then automatically re-scans with the follow-on protocol, imaging only those wells exceeded the user-defined threshold in the first pass.

Note: Click the **View Protocol Chain** button to view all the protocols that will be run in the Review Scan sequence (i.e., protocol chain).

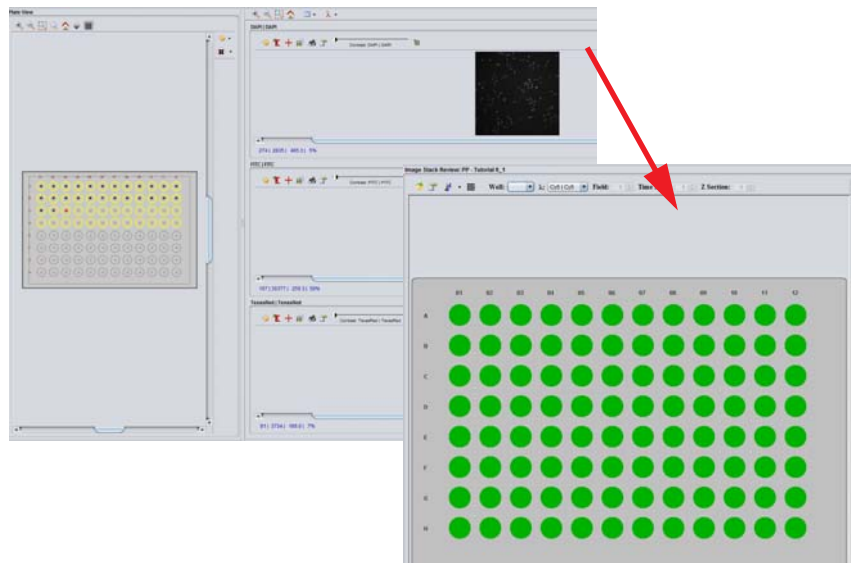


Viewing the review scan results

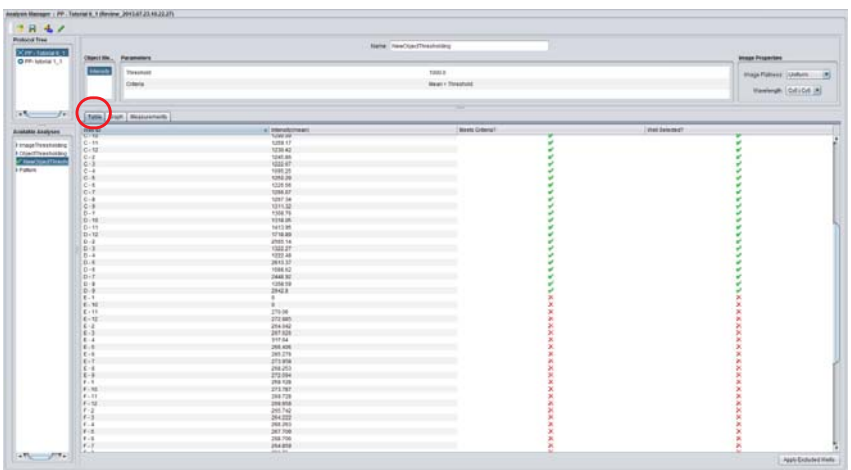
Upon completion of the image analysis, wells that meet the threshold criteria display in the **Plate View** window. The plate is now scanned with the review scan (high magnification) protocol.

9 Review Scan mode - focusing on wells of interest

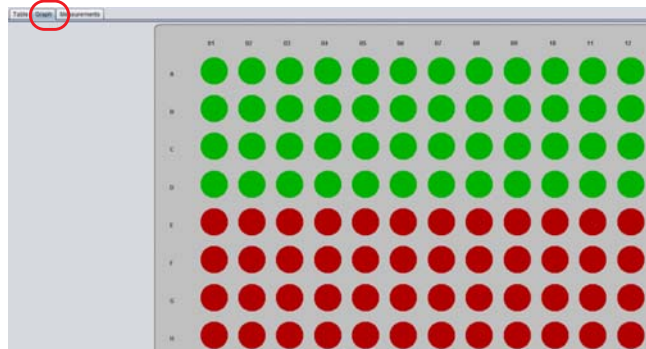
- 1 When the review scan run completes, the results of the first scan (low magnification) display in **Data Review** mode. Double-click on individual wells to review data



- 2 Click the **Analysis mode** toolbar icon to open the **Analysis Manager**. The **Analysis Manager** has three display modes: *Table*, *Graph* and *Measurement* view.
 - Table tab - the wells selected for review scan and measure values are displayed. The data table can be exported as a CSV file for further analysis. Right-click anywhere within the data table and select **Export as CSV**.



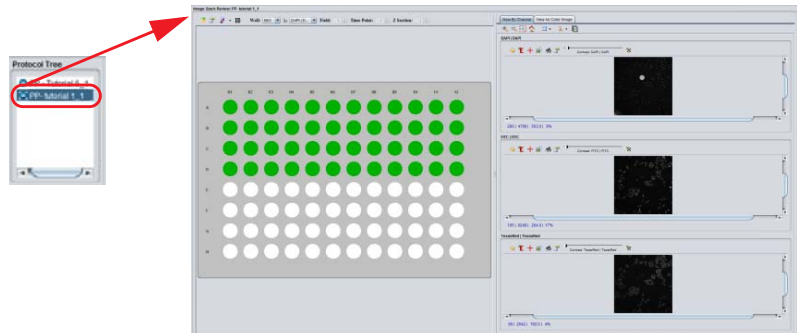
- Graph tab - displays a plate map showing the wells with values that meet the object threshold criteria (greater than the user-defined threshold (green) or lower than the user defined threshold (red)).



- Measurements tab - view the wells meeting the parameters of the threshold criteria. Click on the measures in the **Object Measure** panel to show the wells associated with that measure.



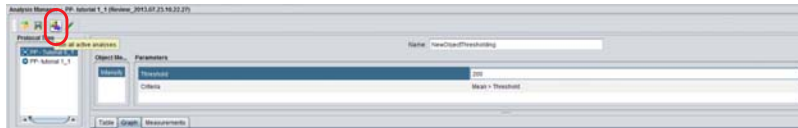
- To view the review scan protocol data, select the review scan protocol from the **Protocol Tree** panel in the upper left of the **Analysis Manager** window and then select the **Data Review** mode toolbar icon.



Changing threshold values

Threshold values and criteria, as well as the addition of new measures for well selection can be done post-acquisition. The new set of selected wells can then be rescanned.

- 1 To change threshold value, open **Analysis mode** from the main toolbar. Double-click on the threshold value and enter the new value. Click on the **Run analyses** icon on the **Analysis Manager** toolbar to reanalyze.

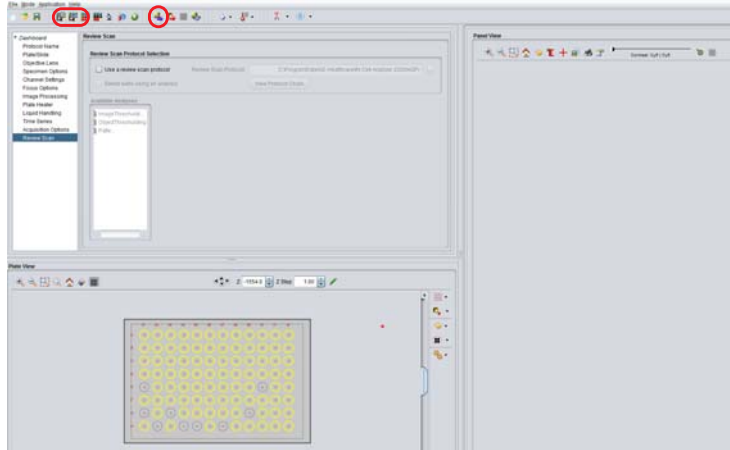


After re-analysis, the wells selected using the new threshold criteria display on the **Graph** tab.



- 2 Click the **Apply excluded wells** button to apply the change and rescan the wells with the review scan (high magnification) protocol. Verify that the wells to be excluded are applied to the correct protocol and click **Yes** on the **Apply Excluded Wells** dialog.

- 3 Open either **Acquisition** or **Assay Development** mode. The new set of wells displays in the **Plate View** window. Click the toolbar **Run protocol** icon to rescan.



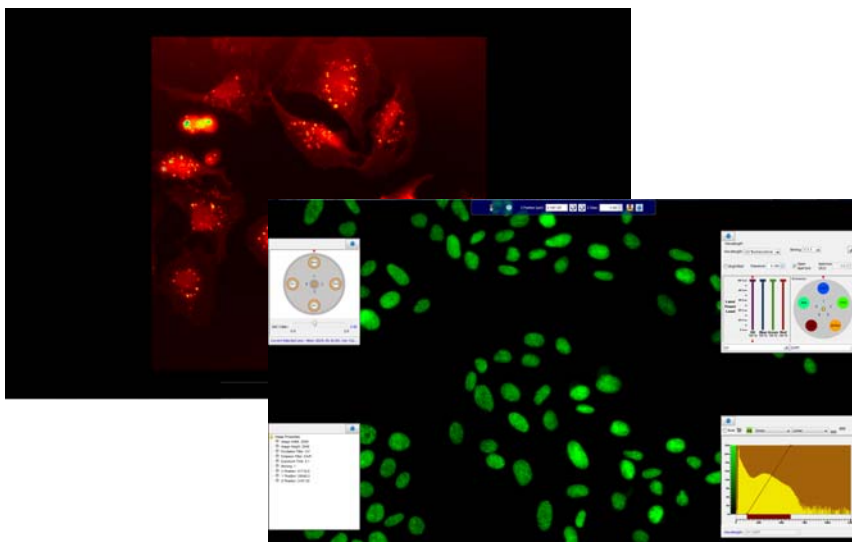
Note: The changed values do not automatically re-scan the plate. The new threshold values can be transferred to the saved follow-on protocol by clicking the Save to protocol  icon.

10 Microscope mode - using IN Cell Analyzer 2200/2000 full-screen

When you switch the *IN Cell Analyzer 2200/2000* system software to **Microscope** mode, you can view images using the full screen. The tools used for defining image acquisition and controlling the display are contained in toolbars hidden from view until you move the mouse to select them. Alternatively, there are a number of keyboard shortcuts available for use while in Microscope Mode.

From the *Main* toolbar, click the **Microscope**  icon. The **Microscope Mode** screen is displayed using your entire display area.

Fig 10-1. Microscope Mode



10.1 Microscope mode display toolbar

From the **Microscope** mode screen, move the mouse to the bottom edge of the screen to view the **Display** toolbar. The toolbar pops up from the bottom of the viewing area.



10 Microscope mode - using IN Cell Analyzer 2200/2000 full-screen

10.2 Microscope mode acquisition toolbar

The tools necessary to adjust the displayed image or exit **Microscope** mode are available from the **Display** toolbar.

Button	Function
	Enables the checkbox to show images at 100% zoom instead of full-screen.
	The slider increases and decreases the image zoom.
	Exit Full-screen mode (Esc).

10.2 Microscope mode acquisition toolbar

From the **Microscope** mode screen, move the mouse to the top edge of the screen to view the **Acquisition** toolbar. The toolbar drops down from the top of the viewing area.

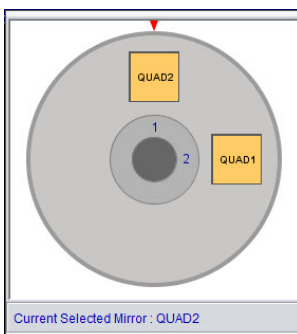


Button	Function
	Opens the Polychroic Changer window. Refer to the section <i>Polychroic Changer</i> below.
	Perform Laser Autofocus. Click  to save the setting to the current protocol.
	Perform S/W AutoFocus.
	Z Position (in μm).
	Increase Z Position by Z Step.
	Decrease Z Position by Z Step.
	Z Step.
	Joy stick - navigate about the image;
	Pin button - toggle to pin and unpin the Acquisition toolbar.

Polychroic Changer

- 1 Click the **Polychroic Changer** icon to open the **Polychroic Changer** window. The active polychroic filter is centered at the top of the window, below the red arrow.

Fig 10-2. Polychroic Changer.



- 2 To change the active filter, select the alternate filter and single-click over it to move it to the active position.

Note: Double-clicking on either polychroic location on the graphic results in no action.

Accessing hidden tools

Pressing the **spacebar** in **Microscope** mode, when viewing an image, converts the standard mouse pointer to a red *reticle*  making available the following functionality:

Mouse right-click	At any point within the image to center the view at the click position and zoom in.
Mouse SHIFT right-click	At any point within the image to center the view at the click position and zoom out.
PAGE UP (or NUMPAD - 9)	Moves the Z position upward by the specified Z step (coarse focus).
PAGE DOWN (or NUMPAD - 3)	Moves the Z position downward by the specified Z step (coarse focus).
SHIFT PAGE UP (or SHIFT NUMPAD - 9)	Moves the Z position upward by the specified Z step (fine focus).
SHIFT PAGE DOWN (or SHIFT NUMPAD - 3)	Moves the Z position downward by the specified Z step (fine focus).
Mouse Wheel	Zooms the Panel View in or out.

CTRL Mouse Wheel	Zooms the image Z position by the specified Z step (coarse focus).
CTRL-SHIFT Mouse Wheel	Zooms the image Z position by one tenth of the specified Z step (fine focus).

10.3 Image viewing tools

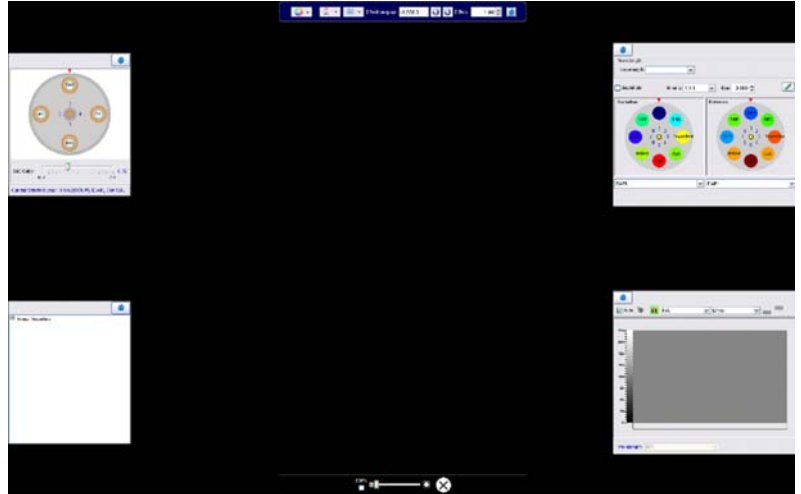
Four additional imaging tools are accessible directly from the screen by hovering at the left and right edges. Each can be 'pinned' so as to allow you to use the feature.

- 1 Move the mouse to the right or left edge of the screen. The following acquisition tools will pop-up:

Tool	Reference
Objective Changer	Move the mouse to the upper-left edge of the screen; currently active objective is at the top of the window denoted by red arrow. Refer to <i>Objective Changer</i> below for more detail.
Image Properties	Move the mouse to the lower- left edge of the screen; Image File Information window provides data for the selected image file. Refer to <i>Properties option</i> for more detail.
Configure Wavelength	Move the mouse to the upper-right edge of the screen; the currently active wavelengths are at the top of the window denoted by red arrows. Refer to <i>Wavelength Chooser</i> below for more detail.
Visuals	Move the mouse to the lower-right edge of the screen; histogram represents the various intensities of the selected wavelength; manipulate the line graph from either end point to scale the image contrast intensity. Refer to <i>Chapter 4 Visuals option</i> for more detail.

- 2 On any window, click the **Pin**  icon to keep the window open. The icon changes to . Clicking the icon again unpins the window, allowing it to close when the mouse is moved away.

Fig 10-3. Microscope Tools windows.

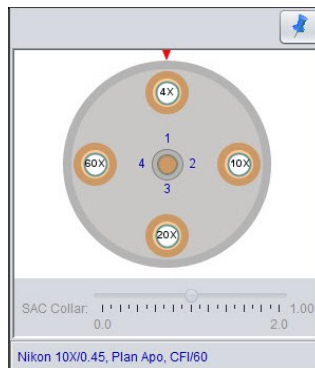


Objective Changer

Use the **Objective Changer** to change the active objective to explore areas of interest with different magnification.

- 1 Place the cursor at the upper left margin of the display. This action displays the **Objective Changer** window. Click the **Pin** button to keep the window open, if desired.

Fig 10-4. Objective Changer.



- 2 The active objective is centered at the top of the window, directly below the red arrow. Place the mouse cursor over your selection. Single-click on an objective in the graphic. The instrument will physically rotate the turret to change the objective.

Note: Double-clicking on an objective in the graphic will result in no action.

3 Use the **SAC collar** slider to adjust the spherical aberration correction.

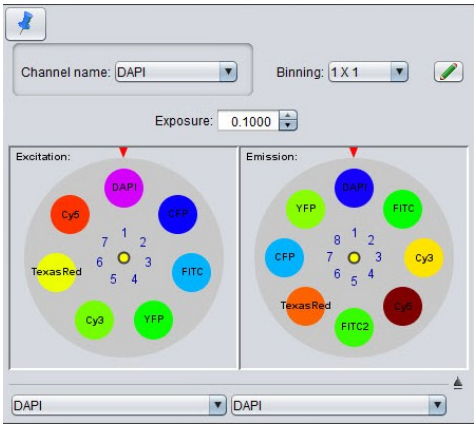
Note: The **SAC Collar** slider will only be enabled when the active objective is equipped with a SAC collar.

Wavelength Chooser

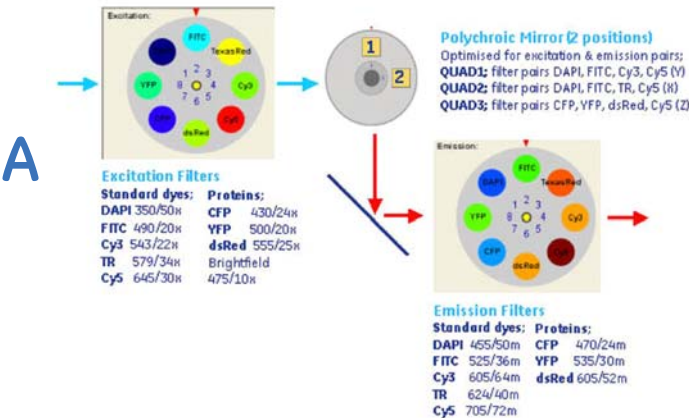
Use the **Wavelength Chooser** to explore the available wavelength pair settings before applying them to the protocol.

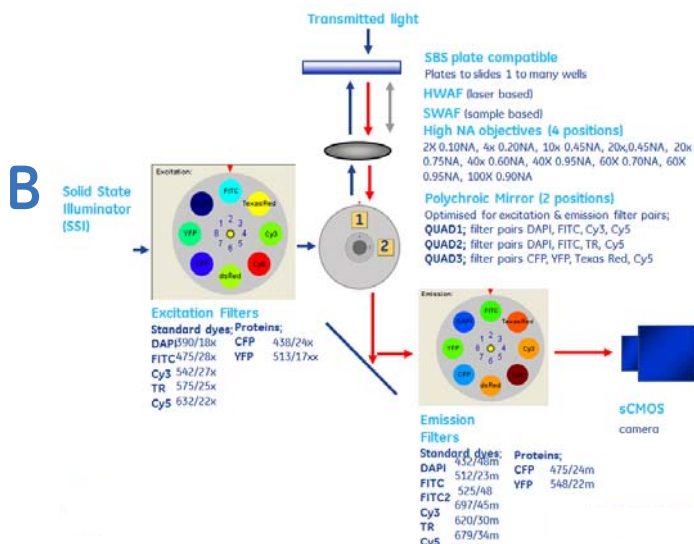
- 1 Place the cursor at the upper left margin of the display. This action displays the **Wavelength Chooser** window. Click the **Pin** button to keep the window open, if desired.

Fig 10-5. Wavelength Chooser



Note: Hovering the mouse pointer over one of the colored filter icons in **Wavelength Chooser** displays about that filter. The available filters ((A) IN Cell Analyzer 2000 and (B) IN Cell Analyzer 2200) are shown in the diagrams below.





- 2 In the **Wavelength** group, select a filter pair from the **Wavelength** dropdown list. If the filter pair needed for your experiment does not appearing the list, a new wavelength definition can be defined. Clicking the ☒ icon will save the current settings.

Alternatively, you can manipulate the **Wavelength Chooser** settings manually by setting the **Excitation** or **Emission** filter positions by either:

- Double-clicking on the filter name in the graphic.
- Selecting from the associated dropdown lists.

Note: On IN Cell Analyzer 2000, when using brightfield illumination, the DAPI emission type will be selected automatically; however, you can manually change to a different emission filter by double-clicking on the filter wheel graphic.

Note: On IN Cell Analyzer 2200, when using brightfield illumination, the Cy3 emission type will be selected automatically; however, you can manually change to a different emission filter by double-clicking on the filter wheel graphic.

- 3 In the **Binning** field, use the dropdown to set the image resolution.

On IN Cell Analyzer 2000, bin settings range from 1x1 (no binning; full resolution) to 4x4 (combines signal from a 4x4 block of pixels).

On IN Cell Analyzer 2200, bin settings range from 1x1 (no binning; full resolution)

to 16x16 (combines signal from a 16x16 block of pixels).

Note: On IN Cell Analyzer 2000, by combining signals from multiple pixels, binning decreases resolution, but can increase sensitivity so you may need to reduce the exposure time.

Shortcut keys

In addition, several keyboard shortcuts keys are available on both the numeric keypad and function keys:

Numeric Keypad:	
1	Decrease the Z step value.
2	Move the stage Y position down by one Field of View (FOV)
3	Move the stage Z position down one Z step.
4	Move the stage X position left by one FOV.
5	N/A
6	Move the stage X position right by one FOV.
7	Increase the Z step value.
8	Move the stage Y position up by one FOV.
9	Move the stage Z position up one Z step.
/	Synchronize both filters to the Excitation filter and rotate the filters backwards (CCW).
*	Synchronize both filters to the Excitation filter and rotate the filters forward (CW).

Additional Shortcut Keys:	
Esc	Exit Microscope Mode (full-screen mode).
Spacebar	Take a snapshot of the currently active image.
F5	Perform Laser Autofocus.
F6	Select Software Autofocus Large selection (400 µm range).
F7	Select Software Autofocus Medium selection (200 µm range).
F8	Select Software Autofocus Small selection (100 µm range).

11 Creating an image slideshow

This feature is available from the **Data Review mode only**

Note: Movies cannot be saved if the selected well contains more than 100 timepoints.

11.1 Generating movies

From either of the **Data Review Panel View** tabs you can generate movies from multiple images. This feature is most useful for viewing time series, and serial Z-sections.

Note: Selecting **Movie** from the **View by Channel** tab will automatically switch you to the **View as Color Image** tab.

- 1 From the **Panel View** toolbar, click the **Movie**  icon. The **Play Movie** window opens.

Fig 11-1. Play Movie Window.



- 2 In the **Range** section:
 - For a time (T) series, set the [T] slider for the T_{min} and T_{max} frames for the movie. You can also use the [Increment] field arrow keys to set the series interval.

- For a Z section series, set the [**Z**] slider for the *Z min* and *Z max* frames for the movie. Set the desired frame increment using the up/down arrows in corresponding the [**Increment**] field.
- 3 In the **Play Control** section of the window, select one of the control options by marking its radio button:

Swing	The movie plays forward and back continuously between the <i>Z min</i> and <i>Z max</i> frames. until you click the Stop button.
F-Loop	The movie plays forward from <i>Z min</i> to <i>Z max</i> once and then stops.
B-Loop	The movie plays backward from <i>Z max</i> to <i>Z min</i> once and then stops.
None	The movie plays forward, starting from <i>Z min</i> and ending on <i>Z max</i> , and then repeats the process continuously until you click the Stop button.

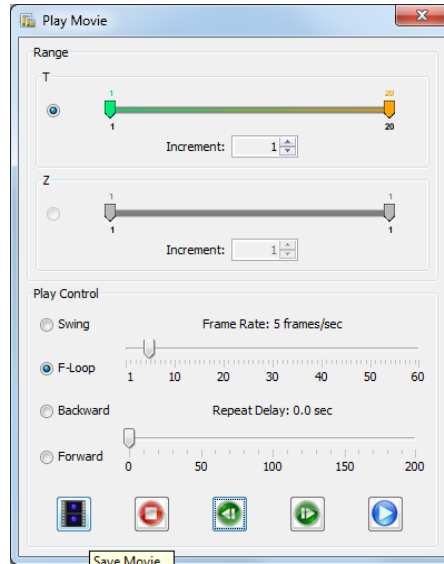
- 4 Adjust the **Frame Rate** slider for the speed at which you want the movie to run.
- 5 Adjust the **Repeat Delay** slider to adjust the amount of delay between replays.
- 6 The control buttons at the bottom of the **Play Movie** window function as follows:

Button	Description
	Play the currently active Z stack as a movie.
	Stop the movie.
	Rewind - Step the movie backward one image at a time.
	Forward - Step the movie forward on image at a time.
	Save Movie - the Save Movie window is displayed.

To save a recorded movie, click the **Save** button. The file will be saved as <format> in the <folder>.

Adjust the **Z Range**, **Z Increment**, **Compression Quality**, and **Frame Rate** as desired. Then click **Save** to save the movie.

Fig 11-2. Save Movie Window when Save Movie icon is clicked.



11 Creating an image slideshow

11.1 Generating movies

12 Environmental Control

The **Environmental Control** module delivers humidified CO₂ and is designed to work in conjunction with variable temperature control to enable maintenance and growth of living samples over extended time periods. The **Environmental Control** module consists of a CO₂ supply line, a regulated humidifier unit, and a purpose-built lid designed to sit on top of a standard microplate sealed with a Breathe-Easy® membrane. The humidifier is connected by tubing to the environmental control chamber, the user-supplied carbon dioxide source (regulated by a gas flow controller) and a waste bottle.

The system is designed to use pre-mixed 25% CO₂. Regulated CO₂ to the gas flow controller should be at 25 psi. The controller is rated for a maximum inlet pressure of 1.7 bar (~20 psi or 138 kPa). The water bottle is rated for a maximum of 2.1 bar (~30 psi).

12.1 Using the Environmental Control module

The supplied carbon dioxide gas (25% mixture recommended) is humidified by a water bottle. The water bottle should be filled with about 100 ml of water. Make sure the water level is at 100 ml before each use. Carbon dioxide is fed into the water bottle from the gas controller. It is recommended that the water bottle be refilled when the level reaches ~75 ml, and refreshed if the instrument has been idle (not in use) for 30 days.

In order to deliver 5% CO₂ to the read chamber, the **Environmental Control** module has been validated for use with microplates that have been pre-incubated at 37°C, and with the plate heater pre-equilibrated to the same temperature. The *IN Cell Analyzer 2200/2000* system should be allowed 60 minutes to come to thermal equilibrium. Insert the microplate directly from an incubator into the *IN Cell Analyzer 2200/2000* system after it has come to thermal equilibrium.

To use the **Environmental Control** module:

- 1 Supply 25% CO₂ at 25 psi to the instrument.
- 2 Turn on the plate heater, lid heater, and CO₂ from the **Environmental Control** window by selecting the **Environmental Control** toolbar icon.
- 3 Insert a dummy plate and cover with the **Environmental Control** chamber lid (with the CO₂ tubing and lid heater electrical connector attached). Allow the system to equilibrate for 60 minutes.
- 4 Dock stage by clicking the **Eject** icon on the toolbar. The plate access door opens.

12 Environmental Control

12.1 Using the Environmental Control module

- 5 Insert a pre-incubated microplate containing live cells sealed with a Breatheasy membrane (no lid).
- 6 Install the lid of the **Environmental Control** module on top of the microplate.
- 7 Attach the tubing of the **Environmental Control** module to the gas flow tubing connector and connect the lid heater electrical connector.
- 8 Bring the plate into the system. Click **OK**.
- 9 Run the protocol.

Prior to installation of the Environmental Control Module at a customer site, GE Healthcare requires the customer to take responsibility for the following:

- 1 All items in the *Environmental Control Module Ancillary Parts List* (Table 1) must be supplied prior to installation.
- 2 The provision of a pressured gas supply will most likely be controlled by local Environmental Health Service (EHS) policies at the setup site.
- 3 Correct setup and installation of the gas supply with guidance from your EHS representative.
- 4 Operation of the module requires CO₂ delivery to the **Environmental Control** module gas control unit at 25 psi from a 25% CO₂ premix cylinder (25% CO₂ premixed in air).
- 5 A dual stage regulator (Table 1) is required to set the 25 psi output pressure from the cylinder to the **Environmental Control** module gas control unit.
- 6 The dual stage regulator requires an outlet fitting (Table 1), a 1/4" FPT fitting with a barbed end to fit 4 mm internal diameter tubing (supplied), which fits directly into the back of the **Environmental Control** module gas control unit.
- 7 The 4 mm tubing supplied for the **Environmental Control** module gas control unit is approximately 1 m therefore the gas cylinder needs to be positioned no more than this away from the *IN Cell Analyzer 2200/2000*.

Environmental Control module- Ancillary parts list

Material	Description	Supplier	Code	Alternative suppliers	Quantity
Breathe-Easy	Breathable plate sealing film	Diversified Biotechnology	BEM-1	SIGMA(part: Z380059)	1 box (100)
25% CO₂ pre-mix	CO ₂ supply	Any supplier		Any other supplier	1 cylinder, any size
Dual stage regulator for industrial air tank	590 CGA inlet, 0-50 PSI outlet pressure, 0-4000 psi inlet pressure, 1/4" MPT Port	VWR	55850-498	Any other supplier	1
Outlet fitting for regulator	1/4" FPT fitting with barbed end, to fit 4 mm ID tubing	McMaster-Carr	5346K82	Any other supplier	1

12.2 Removing & replacing the CO₂ humidifier

The fluid storage for the CO₂ humidifier is on the side of the *IN Cell Analyzer 2200/2000* system farthest from the access door (outlined in green below).

Fig 12-1. CO₂ compartment access door.



12 Environmental Control

12.2 Removing & replacing the CO₂ humidifier

The operator slides a door open to access the fluid storage, removes the tubing from the fluid storage bottle, and refills the bottle.

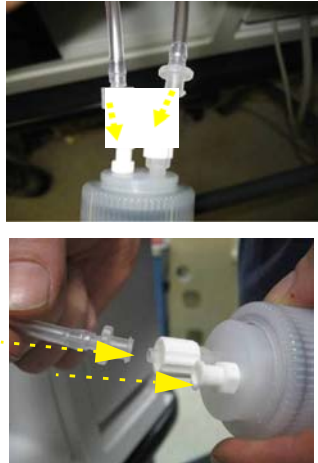


Note: The laser is not visible during this procedure because the laser optical path is not accessible from this location.

Removing the CO₂ humidifier bottle

1 Slide open the Plexiglas doors on the fluid storage access area. Gently lift out the CO₂ humidifier bottle.	
2 Unscrew the tubing from both caps on the humidifier bottle.	A close-up photograph showing a person's hand unscrewing a clear plastic tube from a white, ribbed cap of a humidifier bottle. A yellow curved arrow indicates the direction of rotation.
3 Disconnect the two tubes from the bottle. Note: The two tubing ends will only fit onto their appropriate connectors on the humidifier bottle. The two tubes are not interchangeable.	A close-up photograph showing two clear plastic tubes being inserted into the white, ribbed cap of a humidifier bottle. Yellow dashed arrows point to the specific connectors for each tube, indicating they are not interchangeable.

Reconnecting the humidifier bottle

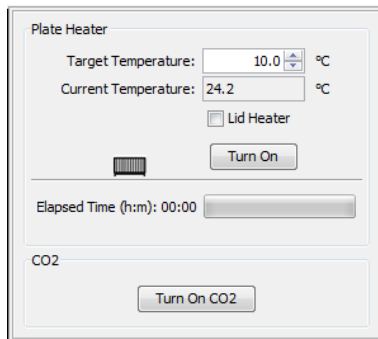
1 Reconnect the two tubes to the humidifier bottle. Note: The two tubing ends will only fit onto their appropriate connectors on the humidifier bottle. The two tubes are not interchangeable.	
2 Screw the tubing cap firmly into the humidifier bottle.	
3 Replace the bottle in the fluid storage access area.	
4 Slide the Plexiglas doors closed on the fluid storage access area.	

12 Environmental Control

12.2 Removing & replacing the CO₂ humidifier

Once the bottle is reconnected, click on the **Environmental Control** main toolbar icon. The **Environmental Control** window is displayed.

Fig 12-2. Environmental Control dialog



- 1 Click the **Turn On CO₂** button near the bottom of the window.
- 2 Allow the CO₂ humidifier to run for a minimum of 10 minutes before beginning any experiments.
- 3 Verify CO₂ humidifier operation by confirming slow bubble production in the humidifier bottle and bubbles moving through the tubing at approximately one bubble every three seconds.

13 Basic system maintenance

The *IN Cell Analyzer 2200/2000* system requires some basic maintenance that an operator can perform. This chapter includes these maintenance activities. The tasks described in this chapter should be undertaken only by more experienced users. Special training may be required by your facility before you complete these actions.



WARNING! Some of these tasks involve potential exposure to dangerous levels of laser radiation. These risks can be minimized to a safe level by the customer adhering to all safety recommendations in this manual.

13.1 Removing and replacing the air filter

It is necessary to perform this procedure at least every six months (more often if the system is operating in a dirty environment). This will ensure the free movement of air through the system to keep the optics and camera area cool and clean. In this procedure, a portion of the cover is removed and the air filter is removed, cleaned, and reinstalled.

Note: *Even though part of the cover is removed during this process, the laser is not visible during this procedure because that optical path is not accessible.*

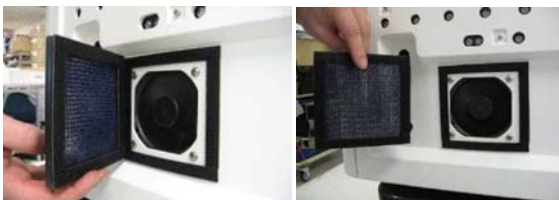
Removing the air filter

Note: *Even though part of the cover is removed during this process, the laser is not visible during this procedure because that optical path is not accessible.*

- 1 Remove the *IN Cell Analyzer 2200/2000* system's left side cover as shown. The cover is held in place with pressure pins that release when pulled.



- 2 Remove the air filter from the *IN Cell Analyzer 2200/2000* system's air intake as shown. The air filter is held in place with heavy Velcro and can be removed easily.



3 Clean out the air filter mesh using a vacuum cleaner. Be sure to get the mesh as clean as possible.	
4 Return the air filter to its original position on the <i>IN Cell Analyzer 2200/2000</i> system and press to make sure it is firmly seated.	
5 Return the left side panel to its original position and press firmly until you feel the pressure pins lock into place.	

Resume using the IN Cell Analyzer 2200/2000 system as normal.

13.2 Removing & replacing the lamp assembly

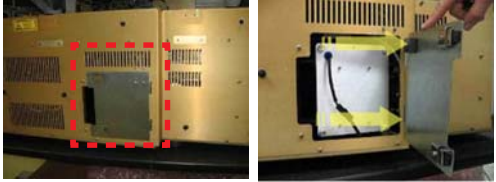
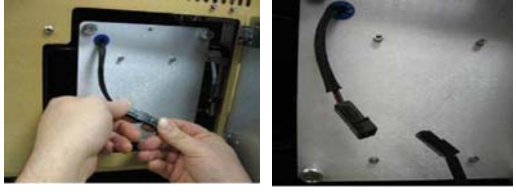
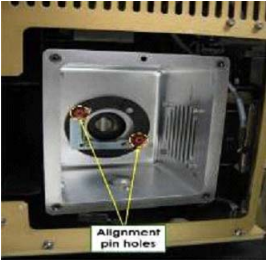
On the IN Cell Analyzer 2000 only

This step is occasionally necessary when the lamp burns out or is too dim to perform properly. A portion of the cover is removed and the old lamp assembly is removed using thumb screws. A new lamp assembly is installed in its place.

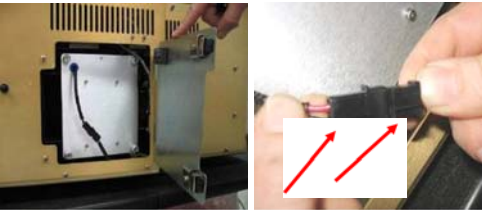
Removing the lamp assembly

Note: *Even though part of the cover is removed during this process, the laser is not visible during this procedure because that optical path is not accessible.*

1 Remove the <i>IN Cell Analyzer 2000</i> system's front cover as shown. The cover is held in place with pressure pins that release when pulled.	
---	---

2 Unlatch and open the lamp module access door.	
3 Disconnect the lamp housing power cable as shown.	
4 Loosen the thumbscrews on all four corners of the lamp housing assembly.	
5 Remove lamp housing as shown.	
6 Inspect the lamp housing bay to make sure it is free of dust or dirt.	

Replacing the lamp assembly

1 Insert the new <i>IN Cell Analyzer 2000</i> lamp assembly into the lamp housing bay using the Alignment Pins on the housing as a guide (see previous figures). Then tighten the thumbscrews on all four corners.	
2 Re-connect the lamp housing power cable. Note: The lamp housing power cable connectors are keyed for a particular orientation.	
3 Close and latch the lamp assembly door as shown.	
4 Return the <i>IN Cell Analyzer 2000</i> system's front panel to its original position and press firmly until you feel the pressure pins lock into place. Tapping each of the pressure pin locations with the palm of your hand can sometimes be the best method for snapping the pins into place.	

13.3 Daily checks & 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 the *IN Cell Analyzer 2200/2000* is being used meets the environmental requirements listed in the *GE Healthcare Site Preparation Guide* which will be provided by the Field Service Engineer prior to installation.

13.4 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.

13.5 IN Cell Analyzer 2200/2000 instrument

If one of the components in an *IN Cell Analyzer 2200/2000* installation does not work, follow the course of action below:

Switch off the unit that is not working using the mains power switch.

- 1 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.
- 2 Check that the mains power cable is connected correctly and that the plugs are firmly located in the sockets.
- 3 Test the unit again by switching it on.
- 4 If the unit still does not work, contact your local GE Healthcare service engineer.

13.6 Operating computer

Please refer to the manual supplied with the operating computer.

13 Basic system maintenance

13.6 Operating computer

Appendix A Acquiring flat field correction reference images

A.1 Introduction

This protocol describes the procedure for making a flat field correction (FFC) reference plate and generating FFC reference images which can be then used to provide on-the-fly FFC when an acquisition protocol is run with the *IN Cell Analyzer 2200/2000*. The protocol is optimized for use of a 96-well reference plate, and the following points apply:

- 1 Any type of 96-well plate can be used for the reference plate, providing that the *IN Cell Analyzer 2200/2000* plate manager has a correct plate map for the plate type used.
- 2 The FFC images acquired from the 96-well reference plate can be used for on-the-fly correction of any images acquired under the same imaging conditions (objective, filters and polychroic) from samples in the same or different plate types (e.g., 96-well plate-derived FFC images to correct sample images taken in 24-well or 384-well plates).
- 3 The protocol has been optimized using the large camera format of the *IN Cell Analyzer 2000*.
- 4 FFC reference images have to be acquired for each choice of filter combination (ie wavelength) objective lens and polychroic (quad).
- 5 The FFC procedure is not validated for on-the-fly whole-well image FFC when the well wall is present in the image (e.g., 2x objective images of 96-well plates, 4x objective images of 384-well plate or 10x objective images of 1536-well plates).
- 6 FFC reference images would need to be refreshed under the following conditions:
 - After a service call which has resulted in a change of any of the optical components (e.g., lamp, filters, camera)
 - Detectable degradation of the FFC performance
 - Detectable degradation of the lamp output

If none of the above criteria are met the FFC reference images may be used despite observing the “stale image” software flag in the protocol editor. The required FFC refresh rate for an instrument operating under stable conditions is under investigation.

A.2 Procedure

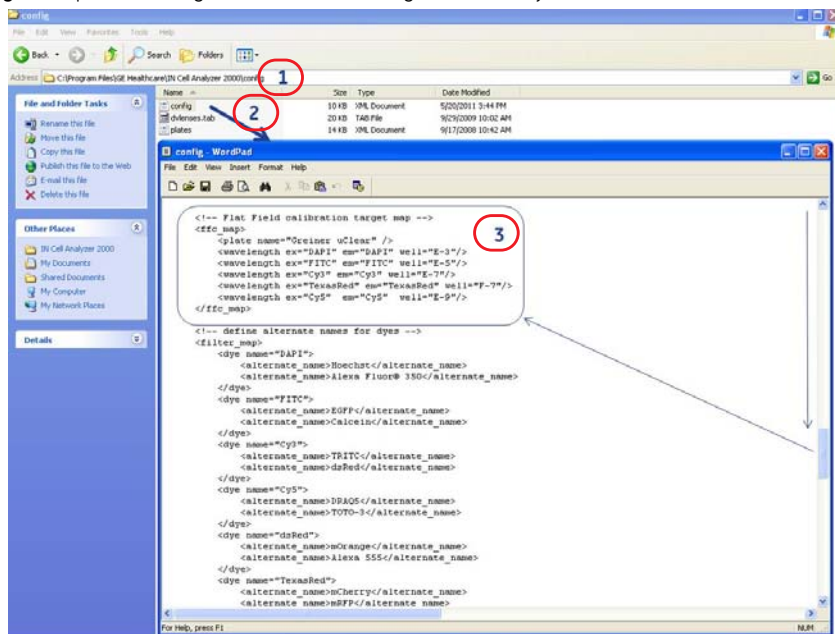
A.2.1 Editing the *config* file

1 The FFC plate parameters can be found under:

IN Cell Analyzer 2000 (XP and Win7 OS respectively)	<code>C:\Program Files\Common Files\GE Healthcare\IN Cell Analyzer 2000\IN Cell Analyzer 2000 Preferences.ini</code> <code>C:\ProgramData\GE Healthcare\IN Cell Analyzer 2200\IN Cell Analyzer 2000 Preferences.ini</code>
IN Cell Analyzer 2200	<code>C:\ProgramData\GE Healthcare\IN Cell Analyzer 2200\IN Cell Analyzer 2200 Preferences.ini</code>

- 2 Open the *config* file in Wordpad or Notepad and find the entry for **Flat Field Calibration Target Map**. It is essential that the FFC plate parameters match the layout of the correction plate being used. Each well must be mapped to the fluorophore being used in that location, and the plate name must exactly match the name used in plate manager.
- 3 If the *config* file needs to be edited, type in the required changes for FFC parameters. Additional lines can be added for extra wavelengths. The names of the fluorophores must exactly match the names used in the acquisition protocol editor.

Fig A-1. Open the config file and edit the settings as necessary



- 4 Save any changes to the *config* file,
- 5 Close and re-open the acquisition software.

It is not necessary to re-initialize the instrument.

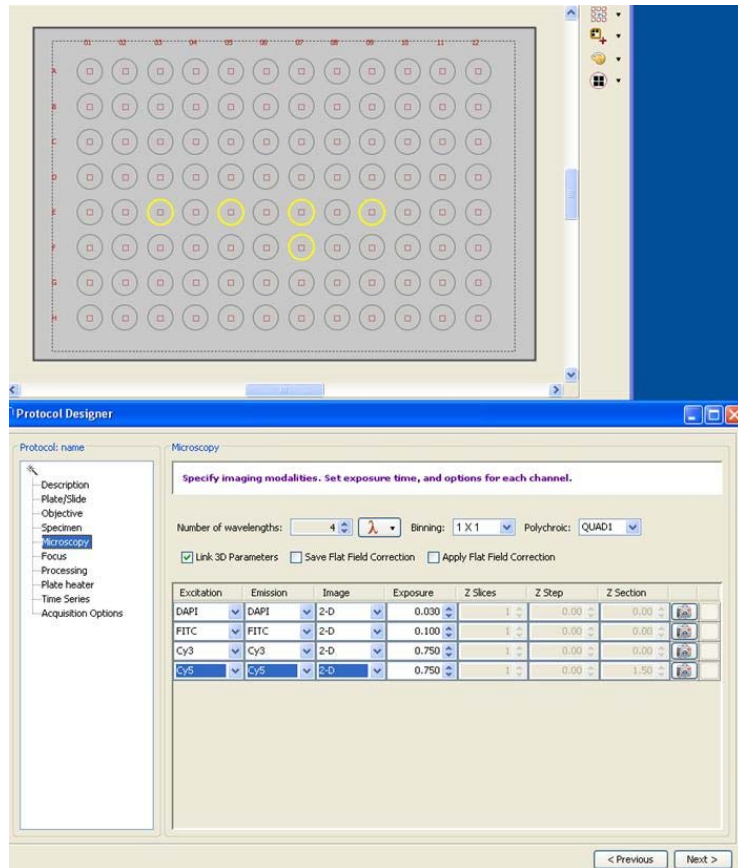
A.2.2 Creating an acquisition protocol for the FFC plate

Load the FFC plate onto the plate stage and in **Assay Development Mode** of instrument operation, create a new acquisition protocol, and modify the plate map to match the FFC plate.

On the **Microscopy** card of the **Protocol Designer**, select the wavelengths to be acquired, set camera binning = 1x1 and select the appropriate polychroic (Quad). The image acquisition mode should be 2D.

Note: Do not use deconvolution image settings for acquisition.

Fig A-2. Select the wavelengths

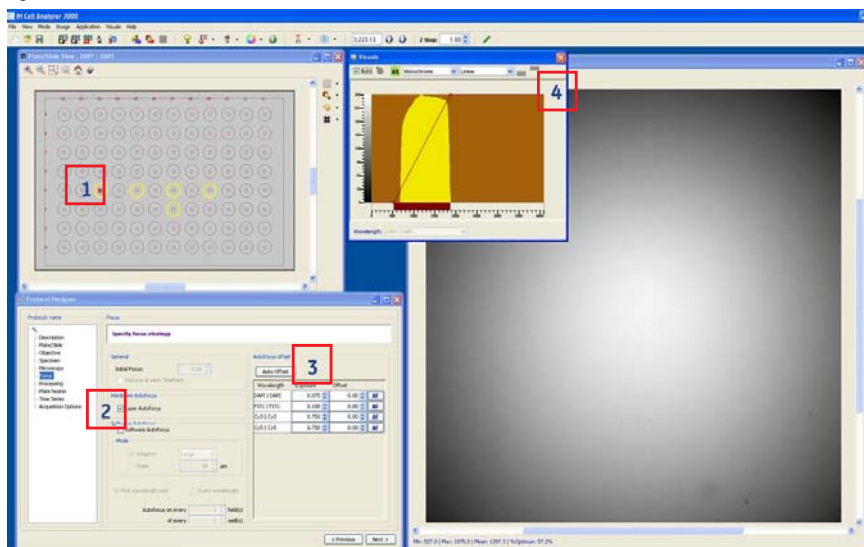


- 1 On the **Focus** card of the **Protocol Designer**, click on the well containing the DAPI fluorophore.
- 2 Check the **Laser Autofocus** box.
- 3 Click on **AF** next to the DAPI wavelength – an image will appear in the **Preview** window.
- 4 On the menu bar of the **Panel View** window, click the **Visuals** icon to open the image display histogram and view with auto-contrast (**Auto** checkbox) checked. Modify the exposure time (3) until the pixel values (yellow display on graph) fall in the mid-range of the X axis. It is very important not to under- or over-expose the acquisition for each fluorophore. Typical exposure times are <100msec – 400msec per fluorophore.

Note: On the IN Cell Analyzer 2000 the suggested range is between 1000 levels

to 3000 levels (12-bit camera). On the IN Cell Analyzer 2200 the suggested range is between 16000 levels to 48000 levels (16-bit camera).

Fig A-3.



- 5 Repeat steps 2-4 for each fluorophore moving the acquisition field to the appropriate well each time.
- 6 *Optional:* Save the FFC acquisition protocol(s) for future use when FFC refresh is needed. The user may choose to save the following protocols, specific for each objective available in the instrument, for example:
 - a) 4x objective - DAPI/FITC/Cy3/Cy5 using Quad1 and 4x objective - DAPI/FITC/TexasRed/Cy5 using Quad 2
 - b) 10x objective - DAPI/FITC/Cy3/Cy5 using Quad1 and 10x objective - DAPI/FITC/TexasRed/Cy5 using Quad 2
 - c) 20x objective - DAPI/FITC/Cy3/Cy5 using Quad1 and 20x objective - DAPI/FITC/TexasRed/Cy5 using Quad 2
 - d) 40x objective - DAPI/FITC/Cy3/Cy5 using Quad1 and 40x objective - DAPI/FITC/TexasRed/Cy5 using Quad 2..

A.2.3 Creating and saving the FFC reference images

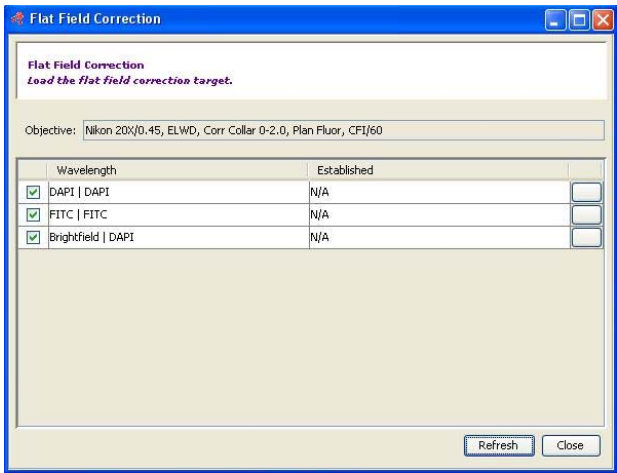
On the *Main Menu* bar select **Applications|Hardware** to display the following submenu:

Fig A-4. Application|Hardware submenu



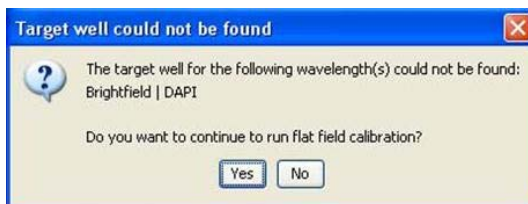
From the submenu, select **Refresh Flat Field** to display the **Flat Field Correction** window.

Fig A-5. Flat Field Correction window



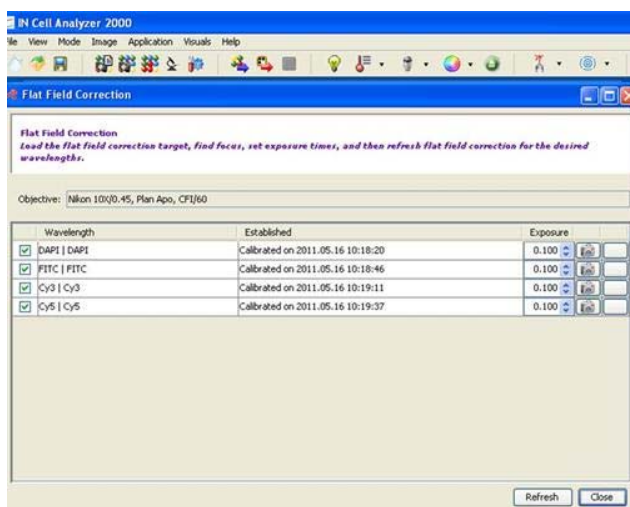
The wavelengths displayed in this window are from the active acquisition protocol. If a wavelength has not yet been corrected, **N/A** is displayed in the **Established** field. Select the displayed wavelengths for flat field correction and click the **Refresh** button

If FFC is performed on a target for which the correction is not available (i.e., not designated in the *config* file) the following is displayed.



Click **Yes** to run the flat field calibration without including the designated target wavelength. When the process is complete, the **Flat Field Correction** window is displayed:

Fig A-6.



The camera icon at the right of each wavelength can be clicked to display the corrected image in the **Image Explorer** window.

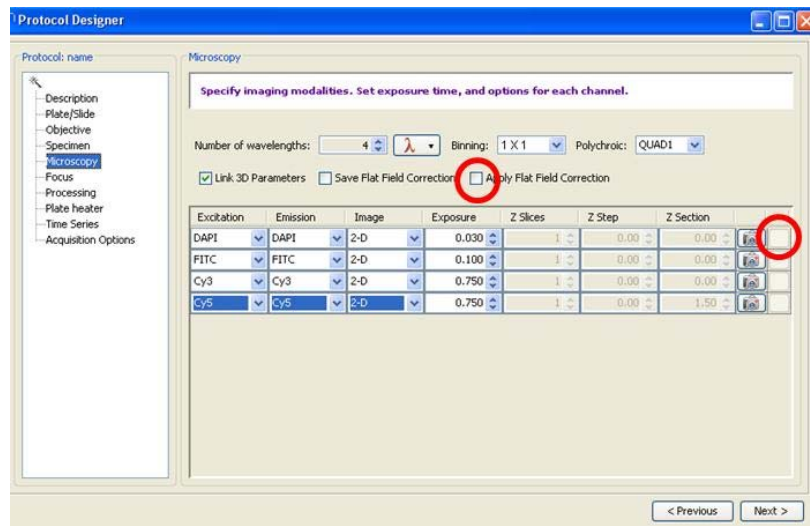
Note: The exposure times displayed in the **Flat Field Correction** window are not used to generate the FFC images when **Refresh** is selected, rather the exposure times in the active acquisition protocol are used.

A.2.4 Checking flat field correction

To run an acquisition protocol using on-the-fly FFC check the **Apply Flat Field Correction** box in the **Microscopy** card of the **Protocol Designer**. If a reference image is available for use a green tick will show next to the camera icon. This may also show as an exclamation mark for “stale” reference images. FFC will still be

applied under this circumstance (see introduction). If no FFC reference image is available (i.e., not designated in the *config* file) a red cross is displayed. None of these displays disable the use of the acquisition protocol.

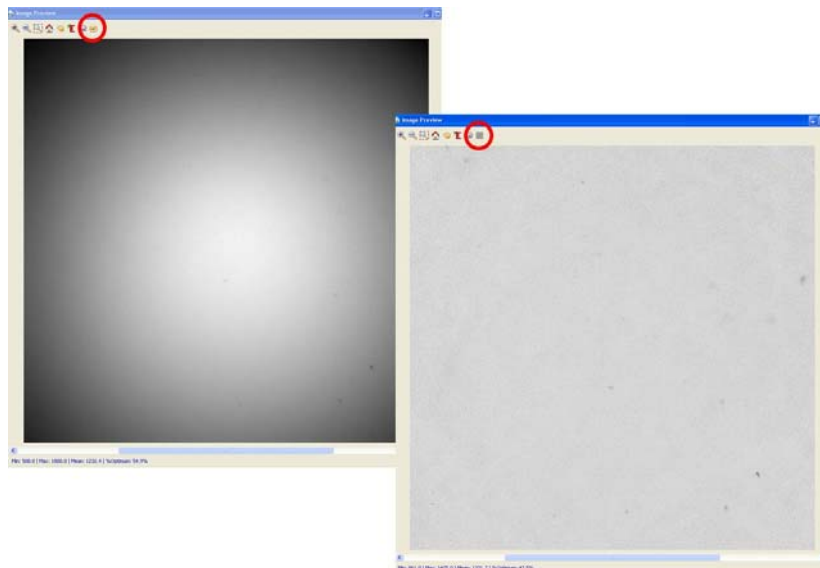
Fig A-7.



Note the **Panel View** window will not show a flat field corrected image unless the **Digitize** icon is clicked.

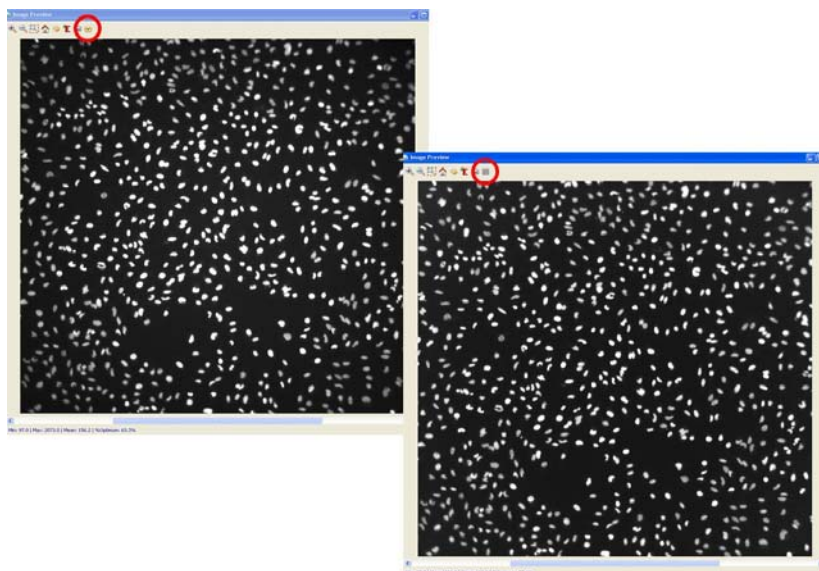
The example below shows before flat field correction from the DAPI well of the reference plate, and after clicking on the **Digitize** icon (circled).

Fig A-8.



The example below shows before flat field correction from images of nuclei labeled with DAPI, and after clicking the **Digitize** icon (circled).

Fig A-9.



A.2.5 Refreshing flat field correction

When the FFC needs to be refreshed (see section 1 - Introduction), re-load the FFC calibration plate, re-open the FFC acquisition protocol (or create a new one if required) and repeat the procedure outlined in section 2.3. The **Refresh** flat field correction command will use the current active protocol, so it is important the FFC acquisition protocol is open. It is recommended to re-focus and check the exposure times required for the calibration plate each time this procedure is performed.

A.3 Solution preparation

A.3.1 Procedures for preparing standard fluorescence samples for FFC reference plates

The following protocol describes the dyes (fluorophores), and methods used for preparing solution based reference fluorescent samples for flat field correction (FFC) for *IN Cell Analyzer 2200/2000*.

Materials and suppliers

The following material are needed for preparing the reference samples.

Materials	Vendor's name and Cat #
Fluorescein sodium salt	Sigma F6377-500G
Cascade Blue® hydrazide	Invitrogen Catalog Number C-687
Rhodamine 6G chloride	Invitrogen Catalog Number R-634
Alexa Fluor® 647 hydrazide, tris(triethylammonium) salt.	Invitrogen Catalog Number A-20502
UCAR Polyphobe™ 106 HE	www.specialchem4coatings.com
Glycerol	Sigma G5516-500ML
Sulfuric acid	Sigma 258105-500ML
Ammonium hydroxide	Sigma 221228-2L-A
96 well micro title plate	

Methods

For DAPI channel FFC

Prepare a 5 µg/ml concentration Cascade Blue® hydrazide solution

- 1 Dissolve 10mg of dye in 200 ml deionized water to make 50µg/ml stock solution, and transfer the stock solution in the brown glass bottle and keep in the refrigerator (4°C).
- 2 Dilute the stock solution 10 times to make 5µg/ml dye solution
- 3 Transfer certain amount of 5µg/ml solution to the micro-plate and seal it. For example, for 96-Packard View plates, 200ul stock should be used.

For FITC channel FFC

Prepare a .5 µg/ml concentration Fluorescein solution

- 1 Dissolve 0.100g of dye in 200 ml deionized water to make 0.5mg/ml stock solution, and transfer the stock solution in the brown glass bottle and keep in the refrigerator (4°C).
- 2 Dilute the stock solution 100 times to make 5 µg/ml dye solution
- 3 Transfer certain amount of 5 µg/ml dye solution to the micro-plate and seal it. For example, for 96-Packard View plates, 200ul stock should be used.

For Cy3 channel FFC

Prepare 0.25 µg/ml concentration Rhodamine 6G chloride solution

- 1 Dissolve 0.010g of dye in 200ml deionized water to make 50µg/ml stock solution, and transfer the stock solution in the brown glass bottle and keep in the refrigerator (4°C).
- 2 Dilute the stock solution 200 times to make 0.25 µg/ml dye solution.
- 3 Transfer certain amount of 0.25 µg/ml solution to the micro-plate and seal it. For example, for 96-Packard View plates, 200ul stock should be used.

For Texas Red channel

Prepare 5 µg/ml concentration Rhodamine 6G chloride solution

- 1 Dissolve 0.010g of dye in 200ml deionized water to make 50 µg/ml stock solution, and transfer the stock solution in the brown glass bottle and keep in the refrigerator (4°C).
- 2 Dilute the stock solution 10 times to make 50 µg/ml dye solution.

A Acquiring flat field correction reference images

A.3 Solution preparation

- 3 Transfer certain amount of 5 µg/ml solution to the micro-plate and seal it.
For example, for 96-Packard View plates, 200ul stock should be used.

For Cy5 channel FFC

Prepare a 0.5 µg/mL concentration Alexa Fluor[®] 647 hydrazide, tris(triethylammonium) salt. solution

- 1 Dissolve 0.1mg dye in 20ml deionized water to make 5 µg/ml stock solution, and transfer the stock solution in the brown glass bottle and keep in the refrigerator (4°C).
- 2 Dilute the stock solution 10 times to make 0.5 µg/ml dye solution.
- 3 Transfer certain amount of 0.5 µg/ml stock solution to the micro-plate and seal it. For example, for 96-Packard View plates, 200ul stock should be used.

To seal the micro-plate:

- 1 Seal each plate with a Microseal F Foil adhesive sealing foil (cat# MSF-1001 MJ Research, Inc).
- 2 Label each plate.

Note: *The sealed reference plate should be good for use for up to 6 months. The dye stock solutions should be good for use for up to 3 years if they are kept in refrigerator.*

Appendix B Site requirements

Shipping container

The IN Cell Analyzer 2200/2000 is shipped in a container with the following dimensions:

134.5 x 80.6 x 86.4 cm (53 x 31.75 x 34 in) (W x H x D), with a total shipping weight of 187 kg (413 lbs).

The IN Cell Analyzer 2200/2000 weighs 100 kg (221 lbs).

System foot print

Space and tools needed to uncrate the system:

Floor space 2.4 x 1.2 m (8 x 4 ft)

Tools needed

- 13 mm (1/2 in) wrench or equivalent
- Knife, or equivalent, to remove stretch film

Moving the IN Cell Analyzer 2200/2000

Doors, corridors, and elevators should have a minimum width of 91 cm (36 in) and a minimum height of 2 m (6.5 ft) to allow transport of the system to its installation location.

Lifting requirements

A forklift or pallet jack capable of carrying up to 200 kg (440 lbs) is required when moving the system while in its shipping crate.

At least four people are required to lift the instrument.

Power requirements

Supply voltage:	100-120/200-240V, 5A/2.5A 50-60 Hz
Maximum power consumption:	1400 VAC (for IN Cell Analyzer 2000) 500VA (for IN Cell Analyzer 2200)
Overvoltage category:	Cat II

Equipment noise level

IN Cell Analyzer 2000 ~58dB

IN Cell Analyzer 2200 ~58dB

Environmental Control module requirements

May be used at altitudes up to 2000 m

CO₂ supply 25% CO₂, externally regulated:
25 psi (1.7 bar). The 25% CO₂ is mixed with Air

Connection 6 mm (1/4 in) OD nylon or polyurethane tubing
The customer needs to supply the 6mm tubing
from the instrument to the regulator- in addition
to the tank and regulator

Service access requirements

During normal operation, the Instrument requires a minimum of 10 cm (4 in) of clearance to the rear. There must be adequate clearance on the right of the system to access and change bottles in the Bottle Holder panel. There must be adequate clearance on the left side of the system to remove the Filter Cover Panel and service the filter.

To allow service and maintenance access, up to 1 m (3 ft) of free space around the system is required.

The *IN Cell Analyzer 2200/2000* dimensions are 112.8 x 63.5 x 59.2 cm (44.4 x 25 x 23.3 in) (W x H x D) (footprint: W x D)

Temperature requirements

The following are valid for elevations up to 2000 m:

Ambient temperature 15°C to 33°C (59°F to 91.4°F)

Relative humidity 20% to 80%, noncondensing

Operating environment Pollution Degree 2

Transport/Storage requirements

Ambient temperature -40°C to 60°C (-40°F to 140°F)

Relative humidity 10% to 95%, noncondensing

Facility connections

- One RJ-45 for network connection

- Three AC ports (1 for the instrument, 1 for the PC, 1 for the monitor)
- 6 mm (1/4 in) OD nylon or polyurethane tubing to CO₂ supply

Internet access

Internet access is required to activate Licensing, also for remote access - Bio InSite™.

An appropriate length CAT5 cable is required to connect the Workstation to the local facility network.

GE, imagination at work and GE monogram are trademarks of General Electric Company.

Cascade Blue and Alexa Fluor are trademarks of Invitrogen Life Science.

UCAR Polyphobe is a trademark of Union Carbide Corp. (UCAR Emulsion Systems)

Packard ViewPlate is a trademark of Packard Instrument Company (Packard Bio-Science)

Microseal is a trademark of Bio-Rad Laboratories, Life Science Research.

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Notice to purchaser

The IN Cell Analyzer 2000 and IN Cell Analyzer 2200 systems are for research purposes only. It is not approved for diagnosis of disease in humans or animals.

IN Cell Analyzer system and In Cell Investigator software

The IN Cell Analyzer system and the In Cell Investigator software are sold under use license from Cellomics Inc. under US patent numbers US 5989835, 6365367, 6416959, 6573039, 6620591, 6671624, 6716588, 6727071, 6759206, 6875578, 6902883, 6917884, 6970789, 6986993, 7060445, 7085765, 7117098, 7160687, 7235373, 7476510; Canadian patent numbers CA 2282658, 2328194, 2362117, 2381344; Australian patent number AU 730100; European patent numbers EP 0983498, 1095277, 1155304, 1203214, 1348124, 1368689; Japanese patent numbers JP 3466568, 3576491, 3683591, 4011936 and equivalent patents and patent applications in other countries.

License agreement

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GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Bio-Sciences AB
Björkgatan 30 751 84, Uppsala, Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

For local office contact information, visit:

www.gelifesciences.com/incell

GE Healthcare UK Ltd
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA, UK

www.gelifesciences.com



imagination at work