GE Healthcare
Life Sciences

IN Cell Analyzer 6000

Cell analysis redefined

Acquisition Software v1.0
User Manual

Contents

1 Preface

The User Manual describes the In Cell Analyzer 6000 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 6000.

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 6000 *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.

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.

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.

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

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.

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.

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.

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

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

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.

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Requirement	Content
Name and address of manufacturer	Applied Precision, Inc. A GE Healthcare Company 1040 12th Avenue Northwest Issaquah, Washington 98027 USA

International standards

Standard	Description	Notes
EN 61010-1, IEC 61010-1, CAN/CSA-C22.2 no. 61010-1, UL61010-1 ed 2	Safety requirements for electrical equipment for measurement, control and laboratory use	EN harmonized to 2006/95/EC
EN 61326-1:2006	EMC emissions and immunity requirements for measurement, control and laboratory use	Harmonized with 2004/108/EC
EN ISO 12100:2010	Safety of machinery – Principles of risk assessment	Harmonized with 2006/42/EC
EN 60825-1:2007 ed 2 IEC 60825-1:2007 ed 2	Safety of laser products – Part 1: Equipment classification and requirements	EN harmonized to 2006/95/EC

2 IN Cell Analyzer 6000 Overview

This chapter provides an overview of the IN Cell Analyzer 6000, its background, the basic architecture of the product, and who to contact with any IN Cell Analyzer 6000-related problems or issues.

2.1 What is IN Cell Analyzer 6000?

The IN Cell Analyzer 6000 is an automated microscope designed for fast, automated imaging and analysis of fixed and live cells. The primary imaging mode is fluorescence microscopy.

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 CMOS camera. Images taken with this camera can show, for example, the appearance and position of structures in the sample.

Fig 2-1. IN Cell Analyzer 6000 Imaging System

2.2 System Architecture

2.2.1 Workstation

The Workstation is a computer system used for controlling the IN Cell Analyzer 6000. The Workstation runs software for defining and setting up imaging experiments, conducting experiments, and analyzing the resulting images. All of the IN Cell Analyzer 6000 system's controls are accessed through the Workstation's graphical user interface (GUI). The Workstation communicates with the Imager through an Ethernet connection.

2.2.2 Imager

The Imager houses all other parts of the IN Cell Analyzer 6000, including:

- **Excitation lasers** - select particular colors of light to excite one fluorophore at a time, chosen to cover standard dye & fluorescent protein excitation ranges.
- **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** - Allow particular colors of the emitted light from only one fluorophore to reach the camera. A cooled CMOS camera is used to capture the light and create images.
- **Camera** - A cooled CMOS 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* 6000 Imaging System, in addition to the standard configuration required for the IN Cell Analyzer 6000 software to operate.

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

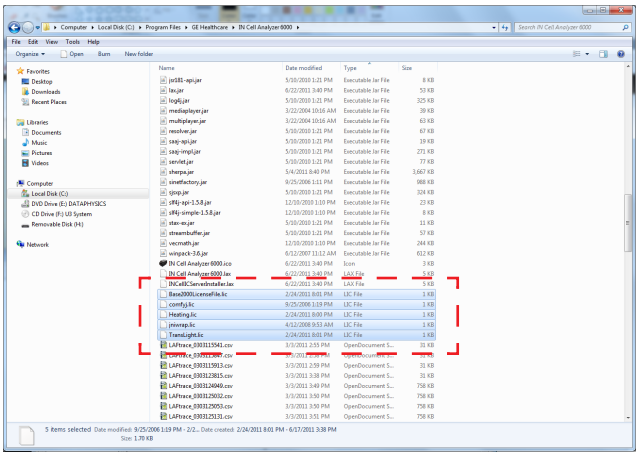
- **Liquid Handling** - This option allows the system to perform experiments in which liquid samples are dispensed into wells in a sample plate. To determine whether feature is enabled, open the ***Protocol Designer***, and look for the ***Liquid Handling*** card in the card list panel. If it is not listed, the feature is not installed. For more information, see *Section 4.3.10*.

- **Environmental Control** - This option is required to use the ***Plate Heating*** feature. To determine whether feature is enabled, click the ***Main toolbar|Environmental Control*** icon. If the ***Turn On*** button is disabled on the ***Environmental Control*** dialog, this feature is not licensed. For more information, see *Section 4.3.11*.

- **Transmitted Light** - This option is required for the ***Brightfield illumination*** feature. To determine whether this feature is enabled, open the ***Wavelength Chooser***. The ***Brightfield*** checkbox will be disabled if the feature is not licensed. For more information on the ***Wavelength Chooser***, see *Section 4.3.7*.

Alternatively, you can, browse to the IN Cell Analyzer folder and open the folder:
`C:\Program Files\GE Healthcare\IN Cell Analyzer 6000\License`

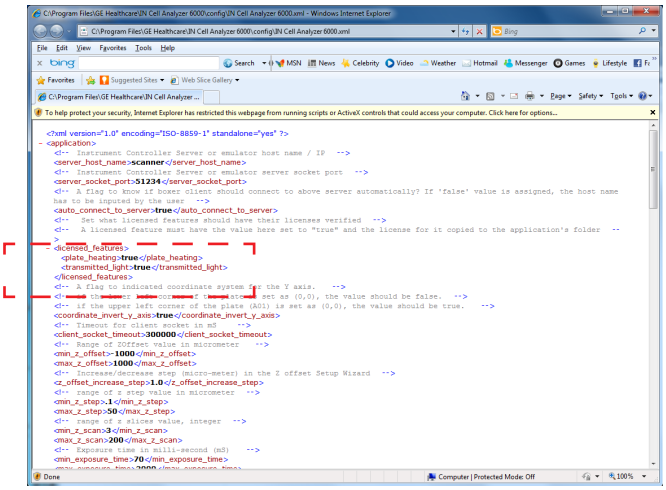
where the individual licenses for the workstation reside.



If a license is shown in the file but is inactive, open the file:
`C:\Program Files\GE Healthcare\IN Cell Analyzer 6000\config\IN Cell Anallyzer 6000.xml`

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

Fig 2-2. 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

For IN Cell Analyzer 6000-related Issues, please contact your local GE Healthcare Technical Support group.

3 IN Cell Analyzer 6000 Lasers and Safety Issues

The IN Cell Analyzer 6000 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 6000 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 6000 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 6000 documentation can result in hazardous exposure to high voltage or moving parts. Exposure to these hazards can cause severe personal injury.

WARNING! The IN Cell Analyzer 6000 uses extremely high voltage. Ensure that all power has been shut off prior to opening the lamp housing.

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

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.

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 6000 Lasers

3.3.1 Laser Illumination

The IN Cell Analyzer 6000 uses five lasers to provide excitation illumination.

Table 3-1. Excitation Laser Wavelengths and Initial Power Output

Wavelength (nm)	Power (mW)
405	100
488	25
561	30
640	50
785 (Autofocus only)	50

The only access point for the beam during normal operation is through the objective. The Powered Sample Access Door is always closed when imaging.

Note: *During Service, when the Laser Switch is bypassed, Class 4 beams are accessible. During normal operation the Laser Switch should never be bypassed!*

3.3.2 Laser Autofocus

The ***Laser Autofocus Module*** contains a 50 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.

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 Laser Safety Labeling

This section lists the safety labels attached at various locations to the IN Cell Analyzer 6000 Imaging system.

3.4.1 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 6000. This label is not visible during operation or maintenance, but is visible during service. Use appropriate Laser Safety precautions when accessing these areas.

Fig 3-1. Laser Safety Interlock warning label.

The interlock safety label can be found:

- under the Filter Cover panel

- On the Front Chassis Skin: one label is partially covered when the front metal chassis cover is attached to the system

3.4.2 Non-interlocked Protective Housing Warnings

The non-interlock safety label indicates that when the component is in place, it is blocking visible and invisible laser radiation. If the component is moved from its installed position, it is possible to be exposed to visible and invisible laser radiation. Components with this label are not part of the interlocked Laser Safety System and are only exposed after removing an interlocked panel.

This label is applied at six locations on the IN Cell Analyzer 6000. This label is not visible during operation or maintenance, but is visible during service. Use appropriate Laser Safety precautions when accessing these areas.

Fig 3-2. Laser Safety Non-interlock warning label.

The non-interlock safety label can be found on the:

- Front Chassis Skin

- Rear Optics Access panel
- Laser Autofocus Module
- left side of the Blink Block Covers
- rear of the Blink Block Covers
- Stage Extension assembly

3.4.3 Aperture Labeling

The Aperture label indicates that laser radiation is emitted from the aperture located directly below the Stage Extension. The Stage Extension prevents laser radiation from being emitted through the objective when the stage is parked. Under normal operating conditions, the Powered Sample Access Cover protects operators from laser radiation during imaging.

Fig 3-3. Laser Safety Aperture waning label

It is applied at one location on the Stage Extension. This label is not visible during operation or maintenance, but is visible during service. Use appropriate Laser Safety precautions when accessing these areas.

3.4.4 Exposure Warning

This label indicates that laser exposure is possible if the fiber optic cable is disconnected.

Fig 3-4. Laser Safety Exposure Avoidance waning label

It is applied at one location on the Laser Chassis, above the fiber optic cable connection. This label is not visible during operation or maintenance, but is visible during service. Use appropriate Laser Safety precautions when accessing these areas.

3.4.5 406nm Laser Warning

The following labels are attached to the 406nm Laser Head by the laser head manufacturer.

Fig 3-5. 406nm Laser Safety label

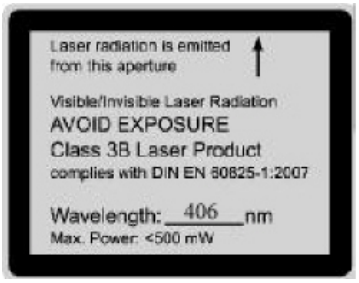
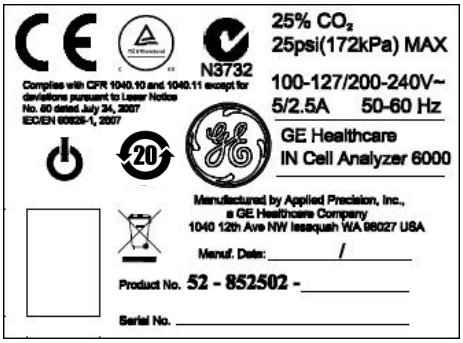


Fig 3-6. 406nm Laser CE compliance label

3.4.6 Instrument Identification Labeling

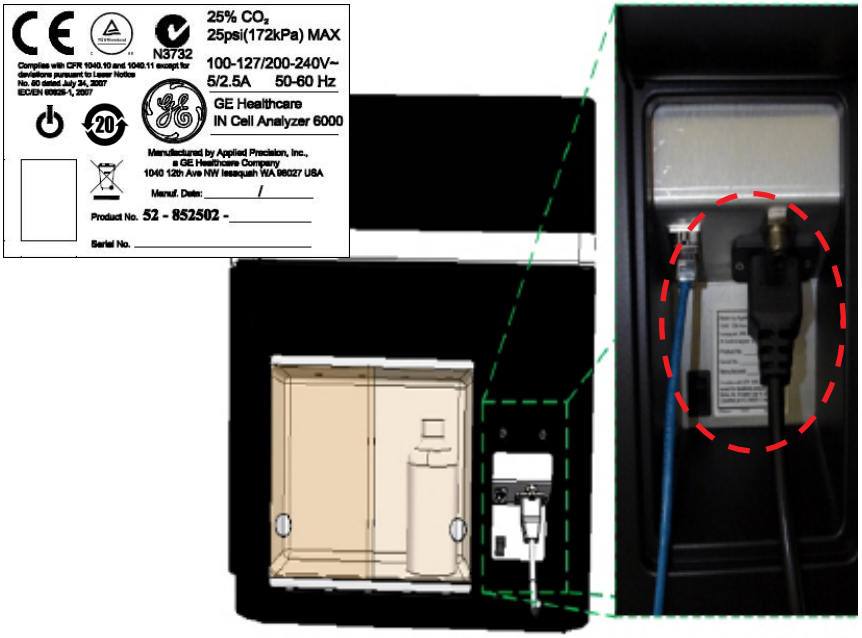
This label indicates that the system complies with appropriate CFR and IEC regulations, as well as, containing the system part number, serial number, and manufacturing date.

Fig 3-7. Instrument Identification label



The safety label is placed on the back of the imager, near the system power switch.

Fig 3-8. Instrument Identification label



3.5 Electrical Safety

All the IN Cell Analyzer 6000 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 6000 safely.

WARNING! High-voltage electronics may be found in some IN Cell Analyzer 6000 components.

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

3.6 Disposal

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 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.

3.8 Temperature Control

The IN Cell Analyzer 6000 is equipped with a temperature-controlled stage designed to provide an optimal environment for cell culture microplates while inside the instrument.

The temperature chamber provides variable temperature control from 5°C above the ambient temperature up to 42°C. The unit will maintain the cell culture microplate at the set temperature ± 1°C with minimal evaporation loss, since the plate is enclosed above and below in the temperature environment .

3.9 Plate and Slide Formats

The IN Cell Analyzer 6000 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 Section *4.3.9.4 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.

3.10 Disposal of 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.

3.11 Declaration of Hazardous Substances (DoHS)

3.11.1 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

3.11.2 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。 为保证所申明的环保使用期限，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，以保证所申明的整 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

3.11.3 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。

3.11.4 List of hazardous substances

	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
Laser based Sub-assemblies	X	O	O	O	O	O
Power supplies	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

3.12 Getting Started with the IN Cell Analyzer 6000

To start the IN Cell Analyzer 6000 imaging system, you need to turn on the imaging system workstation and start up the system software.

To turn on the IN Cell Analyzer 6000 system:

1. Turn on the workstation PC and monitor (if necessary). Log on to the system.
2. Turn on the imaging system hardware. Depress the system power switch (shown below) once until a click is heard.
3. Wait five minutes to allow the IN Cell Analyzer 6000 to initialize before starting the system software. During the initialization process three lights will illuminate on the front of the instrument (Green, Yellow, Red). The Green light will display after the instrument plate stage door has opened and closed, indicating that initialization is complete.
4. To start the IN Cell Analyzer 6000 system software by selecting either:
   - ***Start | All Programs|GE Healthcare|IN Cell Analyzer 6000***
   - Click the ***IN Cell Analyzer 6000*** desktop icon.

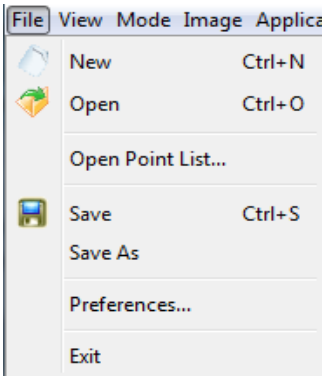
Fig 3-9. IN Cell Analyzer 6000 Power Switch.

Note: *Even though the system power switch appears to be rocker type of switch, it is actually a momentary switch. When you press the switch a second time in exactly the same manner, the imaging system hardware is turned off.*

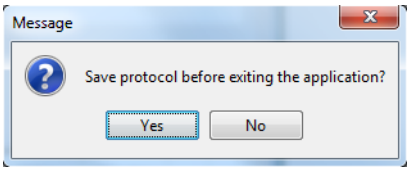
3.13 Stopping / Shutting Down the Instrument

To stop and/or shut down the IN Cell Analyzer 6000 imaging system, do the following:

1 From the ***Main menu***, select ***File|Exit***.



You will be asked to save your open protocol file, if necessary.



2 Select either ***Yes*** or ***No*** as appropriate. The system software will shutdown.

3 Press the system power switch for one second. After a two minute delay, the system should power down.

4 Repeat step 3 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.*

5 Switch off the workstation PC and monitor.

3.14 Finding Help Information

3.14.1 User Guide

The IN Cell Analyzer 6000 Workstation software contains an abridged version of the *IN Cell Anallyzer 6000 User Reference Manual*. To view or print the document, click the ***Main menu|Help*** icon.

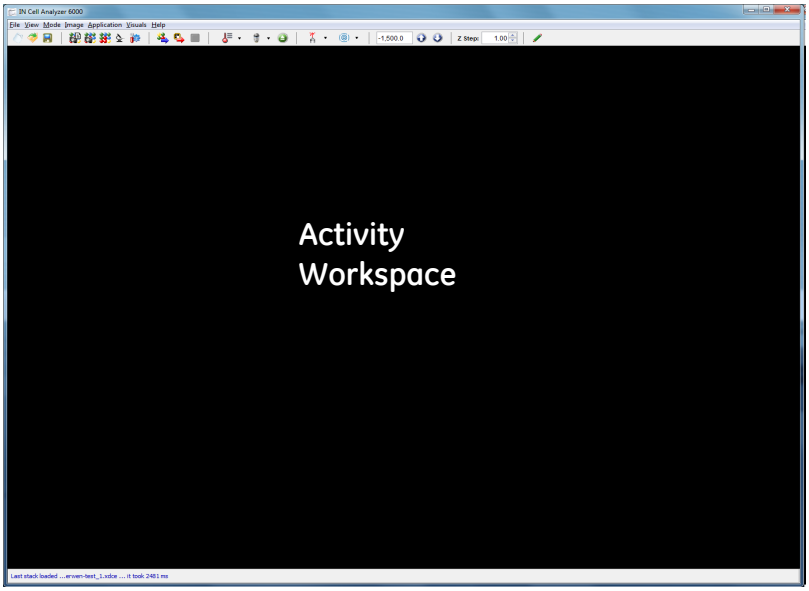
4 Basic IN Cell Analyzer 6000 User Interface

The IN Cell Analyzer 6000 application uses an Activity Workspace from which any of the system modes can be opened; to maximize flexibility various actions can be performed independently of a *mode*.

4.1 The Activity Workspace

On starting the instrument software as described in *Section 3.12 Getting Started with the IN Cell Analyzer 6000*, the ***Activity Workspace*** is displayed. The Main menu and Main toolbar provide basic navigation and function.

Fig 4-1. IN Cell Analyzer 6000 Initial Screen.



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

4.1.1 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.

4.1.1.1 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.
	Open Point List	Opens a point list file to revisit previously selected regions of interest. see *section 4.1.1.1.1 Open Point List* below.
	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 *section 4.1.1.1.2 File Preferences Window* below.
	Exit	Closes the IN Cell Analyzer 6000 system software.

4.1.1.1.1 Open Point List

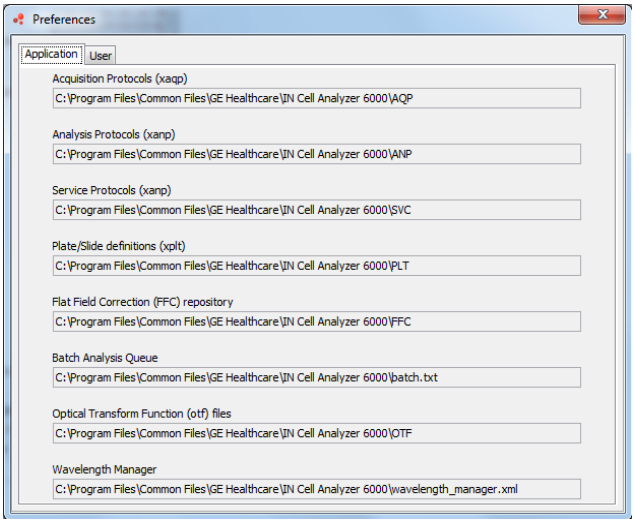
UNDER DEVELOPMENT

4.1.1.1.2 File Preferences Window

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

- ***Application tab***: displays the location of the various system folders and files accessed by the IN Cell Analyzer 6000 application. This tab is a read-only display.

Fig 4-2. File Preferences - Application tab.

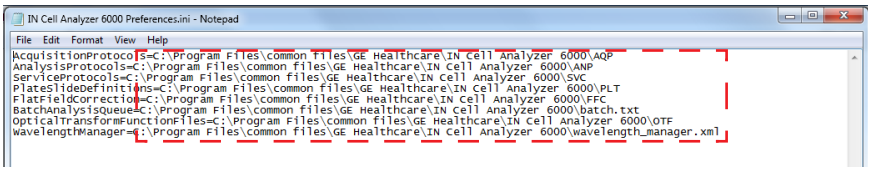


Acquisition Protocol (.xaqp)	Folder where the acquisition protocols are saved for each defined protocol.
Analysis Protocol (.xanp)	Folder containing specialized GE analysis protocol instructions.
Plate/Slide Definitions (.xplt)	Folder containing the plate/slide holder configuration information for each 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.

To modify the default folder locations, open the file.

`C:\Program Files\GE Healthcare\IN Cell Analyzer 6000\IN Cell Analyzer 6000 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 4-3. File Preferences - User tab.

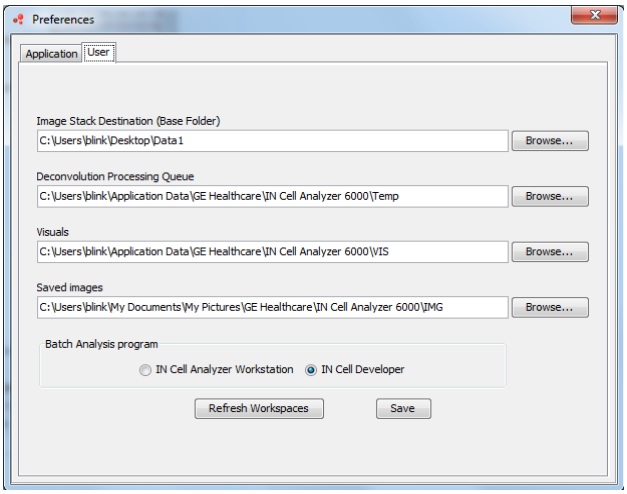


Image Stack Destination (Base Folder)	Displays the location where all of the scan data is stored.
Deconvolution Processing Queue	Shows the location where the Processing Queue file information is stored.
Visuals	Shows the location where the data files are stored for graphic representations displayed in Visuals.
Saved Images	Shows the central location where all of the saved image files are stored.
Wavelength Manager	Displays the location where the wavelength configuration information generated by the Wavelength Manager is stored as an .xml file.

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.

4.1.1.2 View Menu

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

	Protocol Designer	Displays the ***Protocol Designer*** wizard with its current configuration. Refer to *Section 5.2.1 Designing the Experiment - The Protocol Designer* for detail.
	Plate/Slide View	Displays the ***Plate/Slide View***, a graphical representation of the currently selected plate or slide holder. Refer to *Section 4.3.1 Plate/Slide View* for detail.
	Image Preview	Displays the ***Image Preview*** window. Refer to *Section 4.3.3 Image Preview* for detail.
	Image View	Displays the ***Image View*** window. Refer to *Section 4.3.4 Image View* for detail.
	Wavelength Chooser	Displays the ***Wavelength Chooser*** window. Refer to *Section 4.3.7 Wavelength Chooser* for detail.

4.1.1.3 Mode Menu

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

	Assay Development	Uses the IN Cell Analyzer 6000 in ***Assay Development Mode***. Refer to *Chapter 5* for detail.
	Acquisition	Uses the IN Cell Analyzer 6000 in ***Acquisition Mode***. Refer to *Chapter 6* for detail.
	Data Review	Uses the IN Cell Analyzer 6000 in ***Data Review Mode***. Refer to *Chapter 7* for detail.
	Microscope	Uses the IN Cell Analyzer 6000 in ***Microscope Mode***. Refer to *Chapter 8* for detail.
	Service	*Do not select this option unless you are an authorized IN Cell Analyzer 6000 Service Representative. Service Mode is designed to be used only by those thoroughly trained on the servicing of the IN Cell Analyzer 6000 imaging system.*

4.1.1.4 Image Menu

The ***Image*** menu allows you to perform the available actions on the currently active ***Image Preview*** window or the ***Image Explorer*** window. See *Section 4.3.6 Image Explorer (Data Review Mode)* .

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

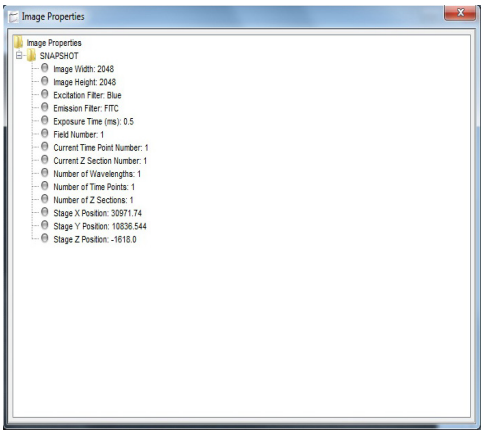
	Save As...	To save the current image to a file.
	Print	To send the current image to a printer.
	Properties...	To display the ***Image Properties*** window for the current image.

4.1.1.4.1 Properties

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

- The **Width** and **Height** of the current image.
- The **Excitation** and **Emission Filters** used to acquire the image.
- The **Exposure Time** used to acquire the image.
- The **Binning** used for the acquisition.
- The **X**, **Y**, and **Z Position** of the system's stage.

Fig 4-4. Image Properties.



4.1.1.5 Application menu

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

	Plate/Slide Manager...	Opens the ***Plate/SlideManager*** window. Refer to *Section 4.3.11 Environmental Control* 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 *Chapter 6* for detail.
	Open Image Stack...	Selects the current Image Stack or select a new image stack from the sub-menu.
	Unlock Protocol...	Allows user to enter a password to access a protected protocol for editing.
	Hardware	Displays submenu of options; see *section 4.1.1.5.1 Hardware Sub-menu* below.
	Robotics...	Opens the ***Robotics*** window see *section 4.1.1.5.3 Robotics* below.
	Processing Queue	Shows the status of data export to *IN Cell Miner HCM*. Refer to *Section 4.1.1.5.2* below for detail.

4.1.1.5.1 Hardware Sub-menu

- Eject Device ...

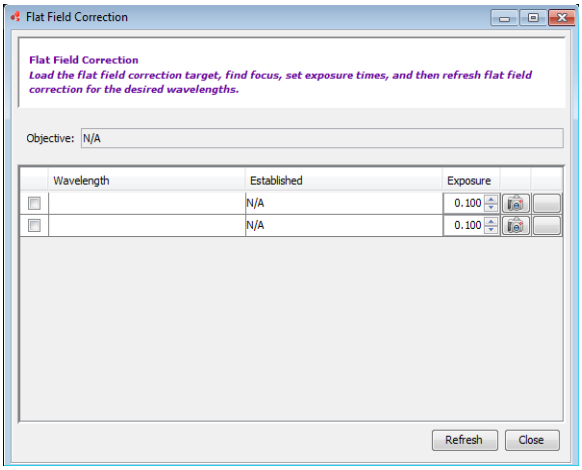
  Click the ***Eject*** icon to return the stage (holding a plate or slide) to the resting (idle) position and open the plate access door on the top of the IN Cell Analyzer 6000.

- Refresh Flat Field ...

  Clicking ***Refresh Flat Field*** opens the ***Flat Field Correction*** window.

  The table lists those wavelengths for which flat field correction has been performed and the date of the correction. If a wavelength is displayed in red, it does not match the wavelength in the Acquisition Protocol. If the date displays in red, a new flat field correction should be established before acquiring images.

Fig 4-5. Flat Field Correction window



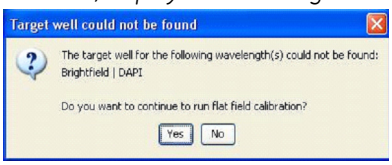
Note: *A Flat Field Correction (FFC) Fluorescence Reference Plate will need to be loaded into the IN Cell Analyzer 6000 to complete the Flat Field Correction procedure. The FFC Reference Plate is based on a 96 well PE Viewplate containing DAPI in well C-3, FITC in well D-3, and Cy5 in Well F-3. For customizing the reference plate to other dyes, please contact a GE applications specialist.*

The wavelengths displayed are defined by the open acquisition protocol. If a wavelength has not yet been corrected, N/A will display in the ***Established*** field.

To perform flat field correction:

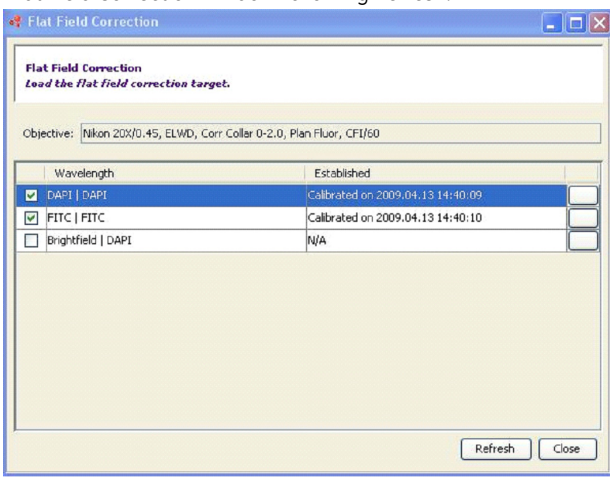
1 Select the displayed wavelengths for Flat Field Correction and click the ***Refresh*** button.

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 4-6. Flat Field Correction window following Refresh.



2 Click the button the right of a wavelength to display the corrected image in the ***Image Explorer*** window (see *Section 4.3.6 Image Explorer (Data Review Mode)*).

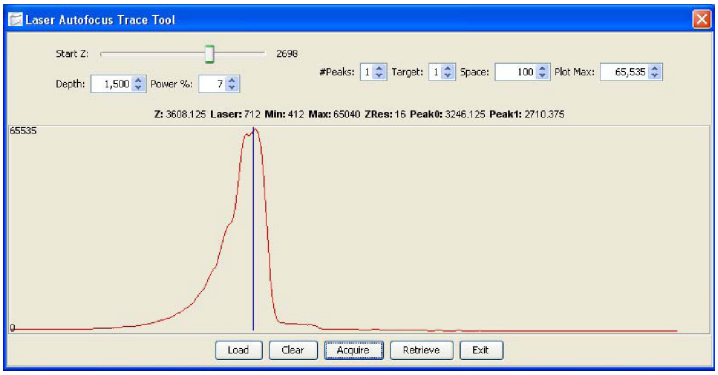
Flat Field Correction files are stored as **tiff* files under the FFC directory in the same location where the IN Cell Analyzer 6000 software was installed. When selected in the protocol, these files will also be placed in the image stack.

- Laser Autofocus Trace ...

The ***Laser Autofocus Trace*** tool can be used to aid in determining the accuracy of the Laser Autofocus. The laser-guided Hardware Autofocus system automatically finds the interface between the liquid in the well and the plastic/glass surface of the well. However, this may not be the optimal plane of focus for imaging cells within the plate.

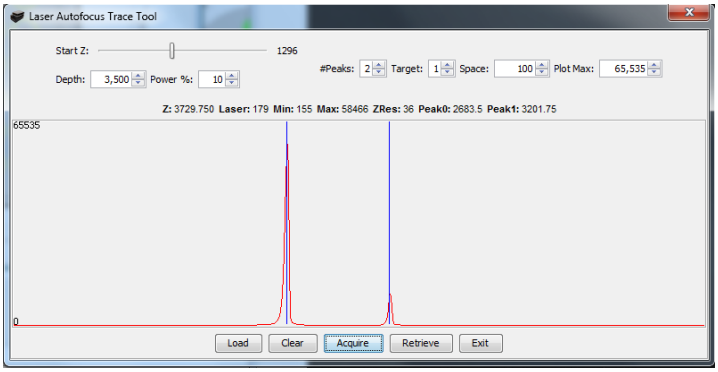
For slides and for most of the lower powered objectives, a single-peak algorithm is used. In the ***Autofocus Trace Tool*** display, the peak is indicated with a blue line (above) and represents the point at which the laser light enters the coverslip or slide bottom.

Fig 4-7. Laser Autofocus Trace for low power objectives and slides.



For objectives 10X or above and when using a thicker plastic plate bottom (> 500µm), a two-peak algorithm is used. The center point of each of the two peaks is indicated in the tool with a blue line.

Fig 4-8. Laser Autofocus Trace for 10X or higher objectives and plastic-bottom plate .



The first large peak displayed in the tool represents the point at which the laser light enters the plate bottom. The second, smaller peak represents the point at which the laser light enters the media containing the sample.

The dialog allows you to adjust several parameters, load saved traces, and acquire new trace information based on modification made to the Laser Autofocus settings.

To use the Laser Autofocus Trace:

1 Click within a well, and set ***Start Z*** at the start position of the scan.

2 Next set depth as the distance of scan.

3 Press ***Acquire*** and ***Retrieve*** to obtain the reflectance peaks associated with your plate/slide assembly.

4 Change ***Plot Max*** to change the upper limit of the visualized scan.

- Refresh Aperture Calibration

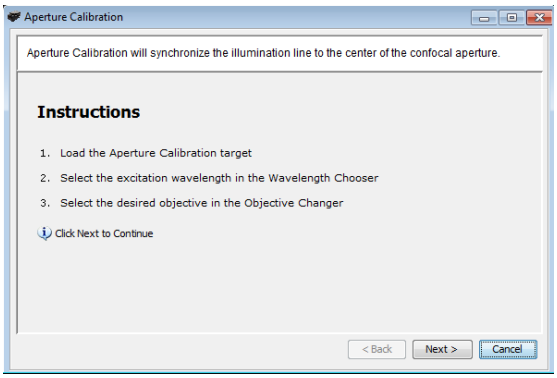
This option is used to align the laser line with the camera to ensure the illumination overlaps the readout of the camera during image acquisition.

1 Load the Fluorescence Reference plate (GE Healthcare part #29-0033-69) provided with the *IN Cell Analyzer 6000* system. Refer to *Section 5.1 Loading a Sample* for detail.

2 Select ***Refresh Aperture Calibration*** from the Hardware menu. The ***Aperture Calibration*** wizard opens.

3 Follow the steps outlined, and click ***Calibrate*** to perform the alignment.

Fig 4-9. Refresh Aperture Calibration wizard.

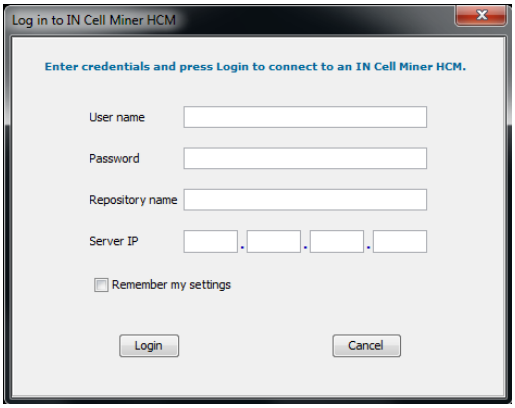


4.1.1.5.2 IN Cell Miner HCM

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 *section 6.3.3 Start the Acquisition* for more detail.

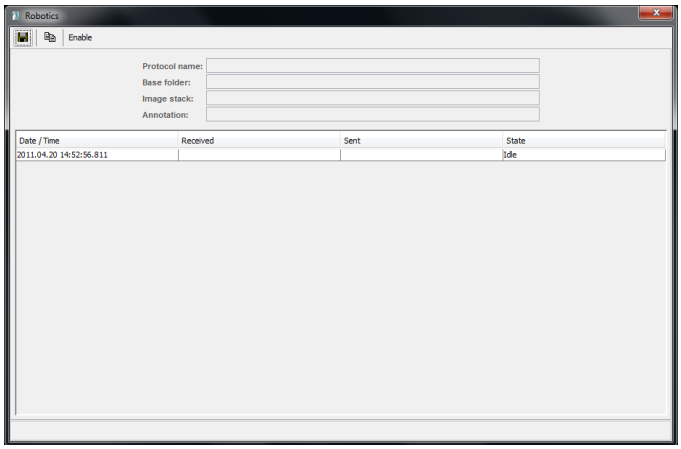
Fig 4-10. IN Cell Miner HCM Login window



4.1.1.5.3 Robotics

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

Fig 4-11. Robotics window.



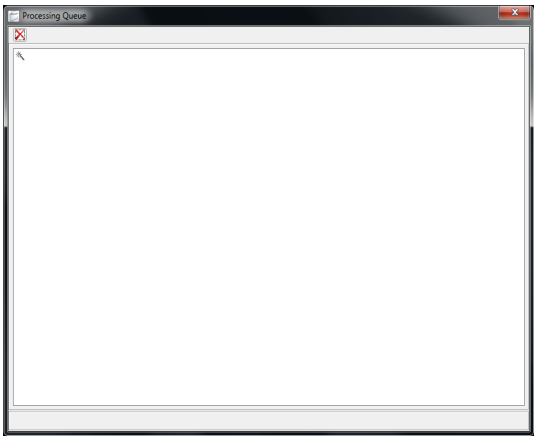
1. In the ***Protocol Name***, ***Base Folder***, and ***Image Stack*** fields provide information to define the acquisition.
2. Click ***Enable*** to activate the robotic device.
3. Use the ***Copy*** and ***Save*** features to save and create additional scenarios.

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

4.1.1.5.4 Processing Queue

To monitor the export of data to the IN Cell Miner HCM application, select ***Processing Queue*** from the ***Main menu|Application menu.*** When selected, the ***Processing Queue*** window is displayed.

Fig 4-12. Processing Queue window.



The icon to the left of a table entry indicates that the entry is currently being processed. At successful completion of the process for an entry, a green checkmark is displayed next to the entry. To skip an entry and not perform the export, 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 the export process completely, highlight the image stack file name 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.

4.1.1.6 Visuals menu

The ***Visuals*** menu is used in conjunction with the Visuals feature to open existing, or save new *.vis* files. From the ***Visuals*** option, select from:

	Open	Browse to and open an existing Visual (*.vis) file.
	Save As	Save an existing Visual (*.vis) file with a new name or directory location.

4.1.1.7 Help menu

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

	IN Cell Analyzer 6000 Help	Displays an online version of the *IN Cell Analyzer 6000 User's Manual*.
	License Agreement	Displays the *GE Healthcare Standard Software License Agreement*.
	About	Displays application information about the IN Cell Analyzer 6000 application.

4.1.2 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 segments.

Table 4-1. IN Cell Analyzer 6000 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 IN Cell Analyzer 6000 Activity Workspace in Assay Development Mode. See *Chapter 5* for detail
	Acquisition Mode	Opens the IN Cell Analyzer 6000 Activity Workspace in Acquisition Mode. See *Chapter 6* for detailS.
	Data Review Mode	Opens the IN Cell Analyzer 6000 Activity Workspace in Data Review Mode. See *Chapter 7* for details.
	Microscope Mode	Opens the IN Cell Analyzer 6000 in Microscope Mode. See *Chapter 8* for details.
	Service Mode	Designated for use by GE Healthcare Field Service Engineers only.

Button	Function	Description
	Run Protocol	Start Acquisition Session... opens the Acquisition Session window from where to start an image scan. Refer to *Chapter 6* for details.
	Preview Scan	Previews a selected region of the plate or slide. Refer to *Section 4.3.1 Plate/Slide Viewl* and *Section 5.2.2.1 Set up preview scan:* for details.
	Stop Scan	Halts the currently running acquisition. The icon displays as red when activated.
	Liquid Handling	Enables the Liquid Handling module, if installed. Refer to *Section 4.3.10 Liquid Handling* 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). Refer to *Section 4.3.11 Environmental Control* for details.
	Objective Changer	Opens the Objective Changer window. Refer to *Section 4.3.8 Objective Changer* for details.
	Eject Plate/ Slide	Moves the stage to the resting position for plate/slide removal.
	Laser Autofocus	Activates Laser (Hardware) Autofocus Refer to *Section 5.2.1.6 Focus card* for details.
	Software Autofocus	Activates Software Autofocus; dropdown list selects available powers Refer to *Section section 5.2.1.6 Focus card* for details.
-1,500.0	**Z Position**	View current position in the Z-stack, -1500 is the default position at rest.
	Increase Focus Position	Ascend through the Z-stack from the current position.
	Decrease Focus Position	Descend through the Z-stack from the current position.

Button	Function	Description
Z Step: 1.00	**Z Step**	Defines distance of the Z step which will be used to ascend or descend through the Z-axis using the up/down arrows.
	Save to Protocol	Saves the Z position displayed in the Z position window to the protocol.

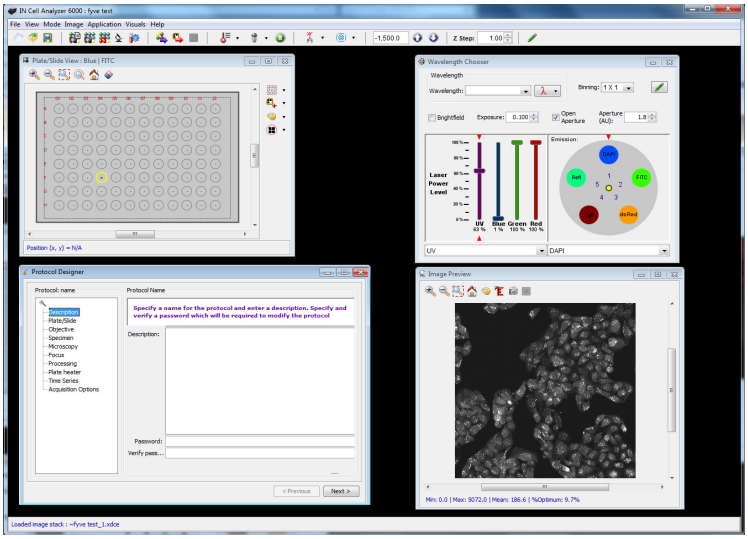
4.2 IN Cell Analyzer 6000 System Modes

From the IN Cell Analyzer 6000 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*.

4.2.1 Assay Development Mode

The Assay Development mode is for designing an imaging experiment and configuring the appropriate settings for the IN Cell Analyzer 6000 system. The end result of assay development is a protocol file you can use to run image acquisitions. From the ***Main menu|Mode menu*** select ***Assay Development***, or the ***Main toolbar|Assay Development*** icon . The ***Assay Development Workspace*** displays. Refer to *Chapter 5* for complete detail.

Fig 4-13. Assay Development Mode Workspace.



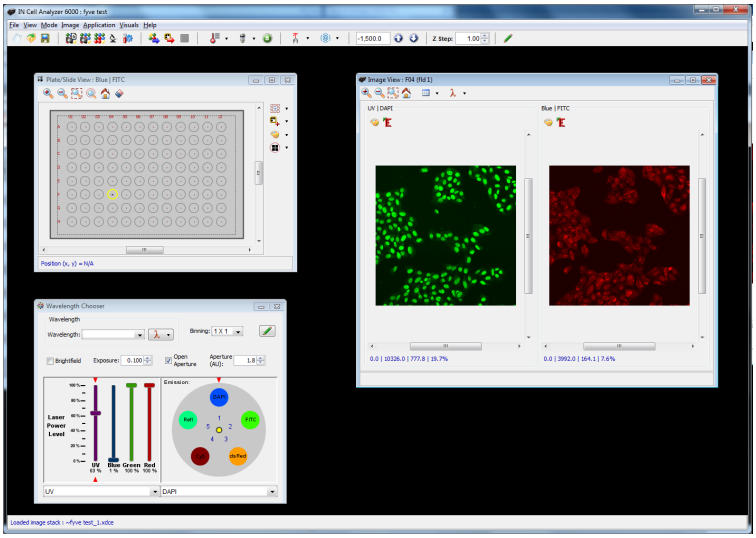
The windows that open are:

- ***Plate/Slide View*** window in the upper left for viewing the plate and selected regions of interest.
- ***Wavelength Chooser*** window in the upper right for viewing and selecting wavelengths pairs, and for monitoring current filter configurations.
- ***Protocol Designer*** window in the lower left for defining the protocol parameters necessary to perform experiments with the IN Cell Analyzer 6000 system.
- ***Image Preview*** window in the lower right for previewing images during experiment design and setup.

4.2.2 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 ***Main menu|Mode menu***, select ***Acquisition***, or the ***Main toolbar,|Acquisition*** icon . The ***Acquisition Workspace*** displays. Refer to *Chapter 6* for complete detail.

Fig 4-14. Acquisition Mode Workspace



The windows that open are:

- ***Plate/Slide View*** window in the upper left for viewing and determining plate and sample location.
- ***Wavelength Chooser*** window in the lower left for monitoring current filter

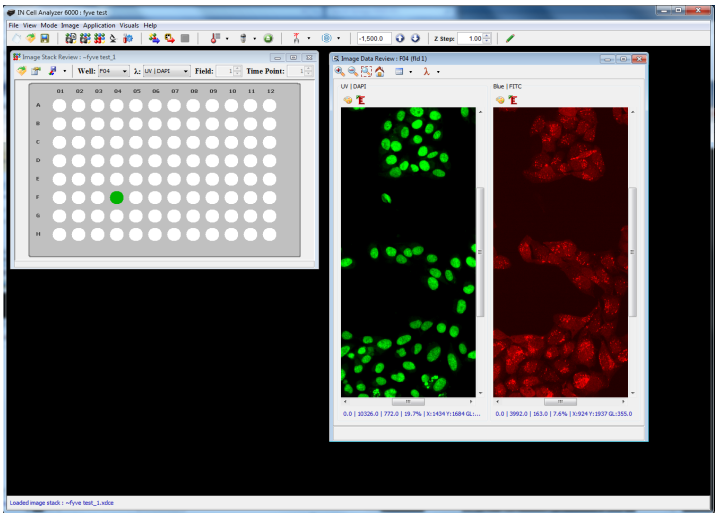
configurations.

- ***Image View*** window to the right to view for each of the wavelengths defined for an acquisition for a selected well.

4.2.3 Data Review Mode

The Data Review mode is for browsing through image data to determine specific regions of interest, focus factors, etc. From the ***Main menu|Mode menu***, select ***Data Review***, or the ***Main toolbar|Data Review*** icon . The ***Data Review Workspace*** displays. Refer to *Chapter 7* for complete detail.

Fig 4-15. Data Review Mode Workspace.



The windows that open are:

- ***Image Stack Review*** window (shown in Thumbnail view) 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.

  The ***Image Explorer*** window is also accessed from the Image Stack Review.

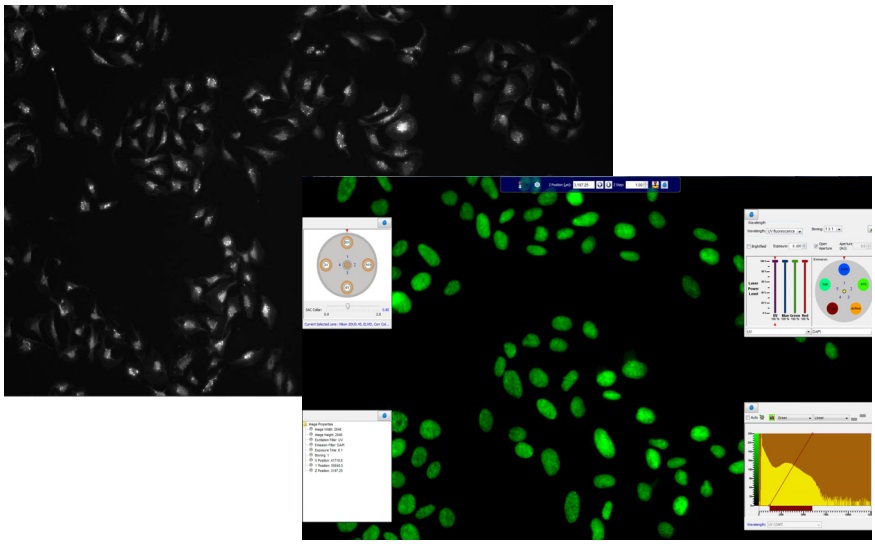
- ***Image Data Review*** window to the right displays the images acquired for each defined wavelength in the protocol.

4.2.4 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 ***Main menu|Mode menu***, select ***Microscope***, or the ***Main toolbar|Microscope*** icon .

The full-screen ***Microscope Workspace*** displays. Refer to *Chapter 8* for complete detail.

Fig 4-16. 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.

4.3 Activity 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.

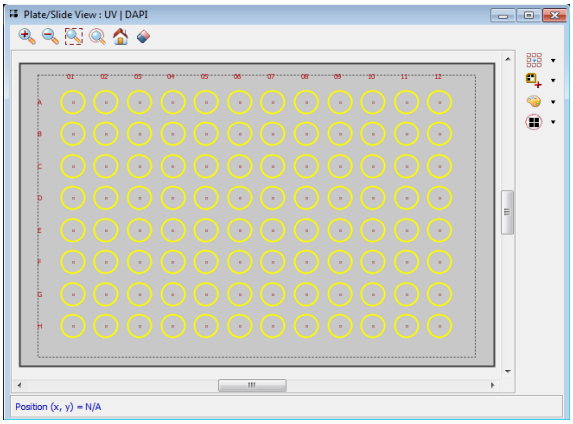
4.3.1 Plate/Slide View

The ***Plate/Slide View*** window is displayed in the upper left portion of the Assay Development or Acquisition Modes. It may also be opened independently by clicking the ***View menu|Plate/Slide View*** item. The ***Plate/Slide View*** consists of 3 sections: *toolbar, graphical display,* and *status bar.*

- 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 4-17. Plate/Slide View window.



To select and deselect wells on the Plate/Slide 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/ Slide 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/Slide View*** window.

4.3.1.1 Plate/Slide View Toolbar

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

Button	Function	Description
	Zoom in	Enlarges the displayed image. See *section 4.3.1.1.3 Image magnification* .
	Zoom out	Reduces the size of the display. See *section 4.3.1.1.3 Image magnification* .
	Zoom for selection	Zooms to a desired portion of the plate. See *section 4.3.1.1.3 Image magnification.*
	Zoom to Well	Zooms to a selected well. See *section 4.3.1.1.3 Image magnification.*

Button	Function	Description
	Reset zoom	Resets the display to its original size See *section 4.3.1.1.3 Image magnification.*
	Clear Plate/ Slide View	Clears any image preview data (thumbnails) from the ***Plate/Slide View*** window.

Button	Function	Description
	Setup Preview	Previews a selected region of the plate or slide. See *Section 4.3.1.1.1 Set up preview scan area.* Arrow opens a dropdown menu from which to select an exposure time and resolution/binning option.
	Fields to Acquire	Opens ***Fields to Acquire*** window to define the system's image acquisition on specific regions of interest.
	Visuals	Displays the ***Visuals*** window showing a line graph of the current image. See *section 4.3.1.1.4 Adjusting image appearance.*
	Show Current Well	Displays the ***Current Well*** window. Arrow opens a dropdown menu from which to select a location.

The following subsections describe the ***Plate/Slide View*** toolbar features.

4.3.1.1.1 Set up preview scan area

A preview scan allows you to view a selected region of the plate or slide with the current protocol before initiating an acquisition run. This feature is useful when designing your protocol to explore the output of your settings before finalizing them. See *Section 5.2.3 Choosing the Wavelengths.*

4.3.1.1.2 Fields to acquire

Opens the ***Fields to Acquire*** dialog 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. See *Section 5.2.3 Choosing the Wavelengths.*

4.3.1.1.3 Image magnification

A number of magnification controls are available for viewing wells and images. These options are available from a number of dialogs and their operation is equivalent in all:

- Zoom In / Zoom Out

  Use the ***Zoom In*** and ***Zoom Out*** options to enlarge or reduce the size of the

display, respectively.

When adjusting the magnification in the ***Image View*** and ***Image Data Review*** windows all of the image frames are adjusted simultaneously when multiple wavelengths are displayed.

- Zoom to Selection

  Use this option to view a desired portion of the plate/image.

  1. Select ***Zoom to Selection*** 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 ***Image 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 ***Image View*** and ***Image Data Review*** windows all of the image frames are adjusted simultaneously when multiple wavelengths are displayed.

- Zoom to Well

  Use this option to view an enlarged image of a specific well:

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

- Reset Zoom

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

4.3.1.1.4 Adjusting image appearance

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

Every pixel in a digital image has a numerical value referred to as the pixel intensity value. The *IN Cell Analyzer 6000* produces 16-bit images where 65,536 grey level intensity values are possible. The Visuals feature uses a look-up table (LUT) to

convert pixel intensities ion 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 6000.
- 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 4-18. Standard Visuals Line Graph and Histogram.

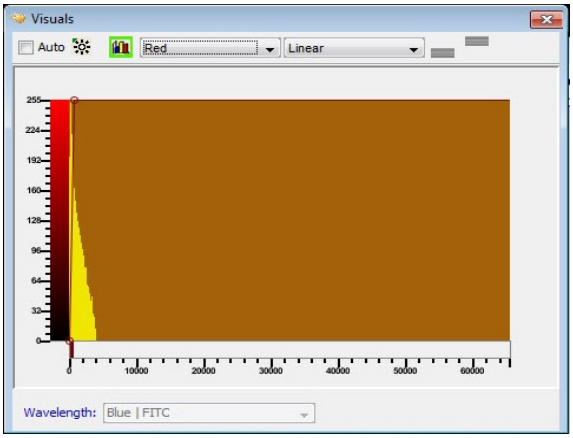


Table 4-2. Visuals Window toolbar

Button	Function	Description
Auto	**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.
Custom	**Table Selector**	Use the dropdown list to select the LUT.
Linear	**Mapping Function**	Choose a mapping function from the drop-down list to display the colors or shading onto intensity values.
	Above or Below Contrast Color	Click to define the color to denote the area above the upper contrast threshold, or above the lower contrast threshold, representing pixels with intensities greater than the threshold value.

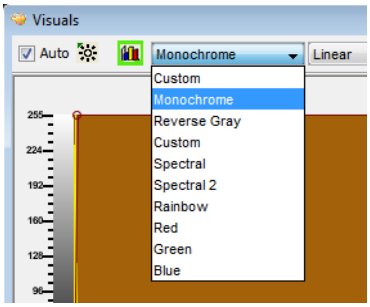
4.3.1.1.5 Manipulating the Appearance

To manipulate the image appearance, click the ***Visuals*** icon from either a plate view or any of the image 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)*.

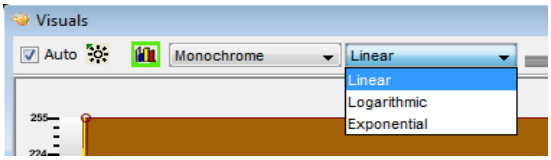
Fig 4-19. Visuals dialog - Table Selector dropdown list.



The default LUTs are:

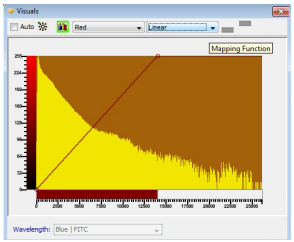
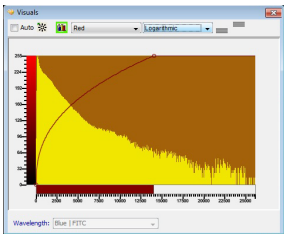
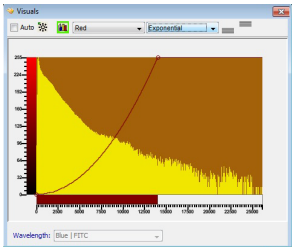
- **Monochrome** - This monochrome LUT is a monotonically increasing grayscale, which provides a reasonably faithful rendition of the original data.
- **Reverse Grey** - The reverse LUT is a monotonically decreasing grayscale, and makes images look as they would in a film negative.
- **Custom** - This LUT allows for user-defined colors to be assigned to each gray level.
- **Spectral** - This LUT displays the gray level image in pseudocolor. To create the LUT, colors are assigned to each gray level.
- **Spectral 2** - This LUT displays the gray level image in pseudocolor. To create the LUT, colors are assigned to each gray level.
- **Rainbow** - This LUT displays the gray level image in pseudocolor. To create the LUT, colors are assigned to each gray level.
- **Red** - This LUT displays the gray level image in a red pseudocolor, where increasing intensities are assigned to each gray level.
- **Green** - This LUT displays the gray level image in a green pseudocolor, where increasing intensities are assigned to each gray level.
- **Blue** - This LUT 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.

Table 4-3. Description and Examples of the mapping functions.

	**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 Settings

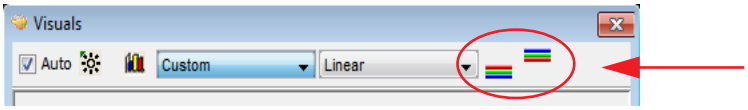
Contrast enhancement maps the available LUT colors/shades onto a smaller range of intensity values. Thus, across this smaller range of intensities, more shades of gray are used, and visual contrast, within the range, is enhanced.

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:

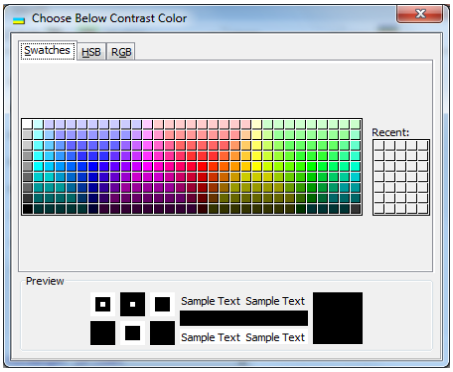
1 Select ***Custom*** from the ***Table Selector*** dropdown. The ***Below Contrast Color*** and ***Above Contrast Color*** toolbar items are enabled.



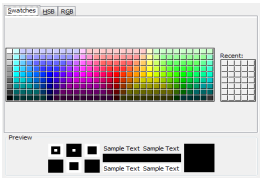
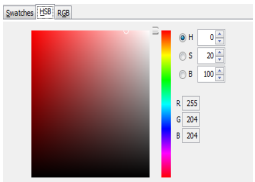
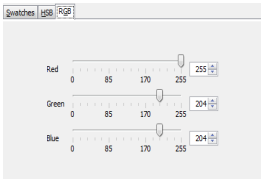
Note: *You must select Custom in the Look-up Table Selector drop-down list for the [Above] and [Below] Contrast Color options to be available.*

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

Fig 4-20. Choose Below Contrast Color window

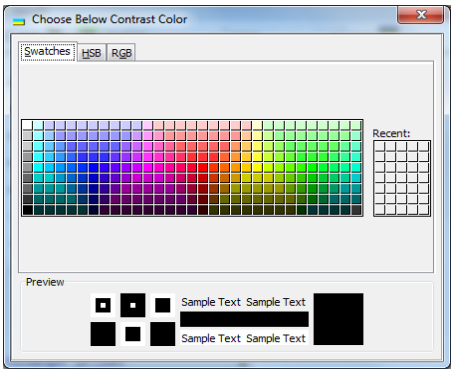


3 Select a color, from the palette, to indicate the segments of an image that fall below the specified threshold. The palette offers 3 options to select a color:

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

4 Click the ***Above Contrast Color***  icon to open the ***Choose Above Contrast Color*** window.

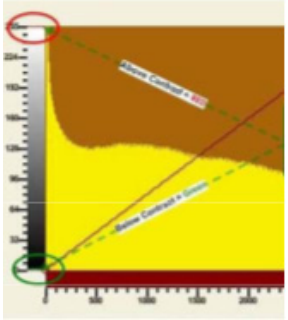
Fig 4-21. Choose Above Contrast Color window.



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, the selected colors are displayed at the top and bottom of the graph's **Y**-axis.

Fig 4-22. Above & Below Contrast Colors display on Y-axis.

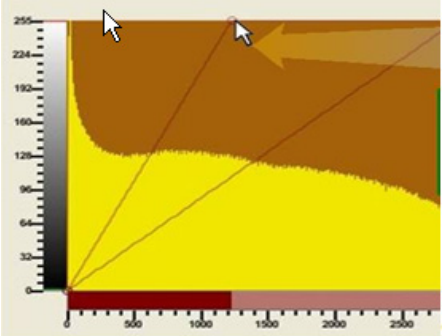


In this example, Red was chosen for the Above Contrast color and Green was chosen for the Below Contrast color.

The image appearance can be adjusted by selecting either the upper or lower endpoint of the line graph and clicking the left mouse button to drag the endpoint across the **X**-axis.

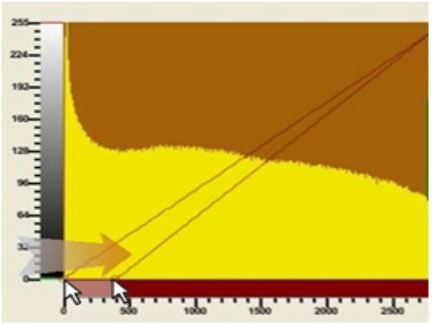
7 Moving the upper line graph endpoints from right to left increases the number of high-intensity pixels in the displayed image.

Fig 4-23. Press and hold left mouse button to adjust upper line graph endpoints.



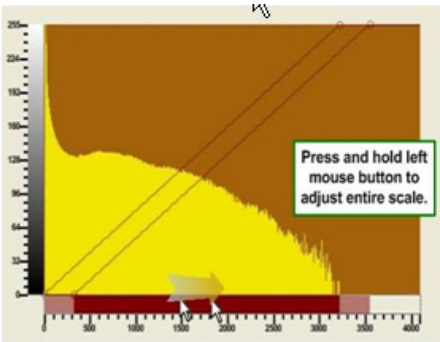
8 Moving the lower line graph endpoint from left to right increases the number of low intensity pixels in the displayed image.

Fig 4-24. Press and hold left mouse button to adjust lower line graph endpoints.



The **X**-axis scale may also be adjusted by clicking the left mouse button and dragging the left / right edges of the scale bar.

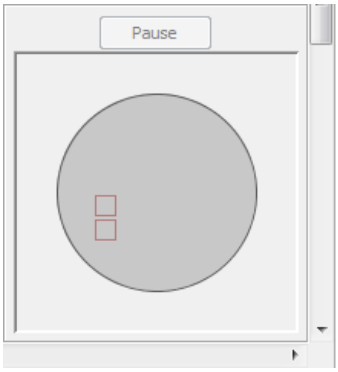
Fig 4-25. Press and hold left mouse button to adjustent the scale.



4.3.1.1.6 Show current well

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.

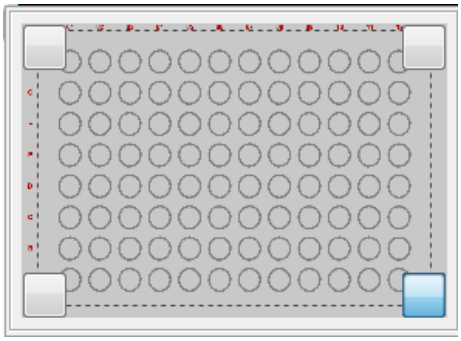
Fig 4-26. Current Well window.



To select a well to mark as the Current Well:

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

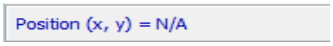
Fig 4-27. Set Current Well display location.



The blue box in the image above indicates that the ***Current Well*** window will be oriented in the lower right corner of the ***Plate/Slide View*** window. Close the window to continue. The ***Current Well*** window displays.

4.3.1.2 Status bar

The ***Plate/Slide View Status Bar*** displays the **X** and **Y** coordinates for the current camera position on the plate.

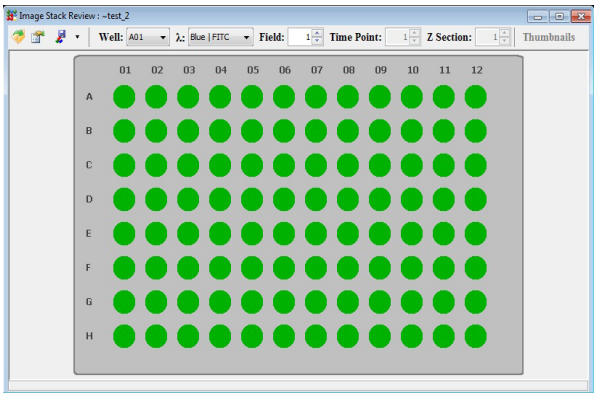


4.3.2 Image Stack Review

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.

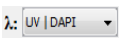


To select and deselect wells on the ***Image Stack Review***:

- Double-click on a well to select. Image will appear in ***Image Data Review*** window. *Note:* Only one well at a time can be viewed.

4.3.2.1 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).
	**Properties**	Opens the ***Image Properties*** window, listing the acquisition protocol attributes.
	**Save Fused image**	Saves fused images for the selected wells, at the selected field and time point. See *section 4.3.2.1.1 Properties*. Clicking the arow opens a dropdown list to select the images to fuse.
	**Well Number**	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 ***Image Data Review*** window.
	**Wavelength**	Use the drop-down list in the ***Wavelength*** field to select the wavelength pair you want to examine.

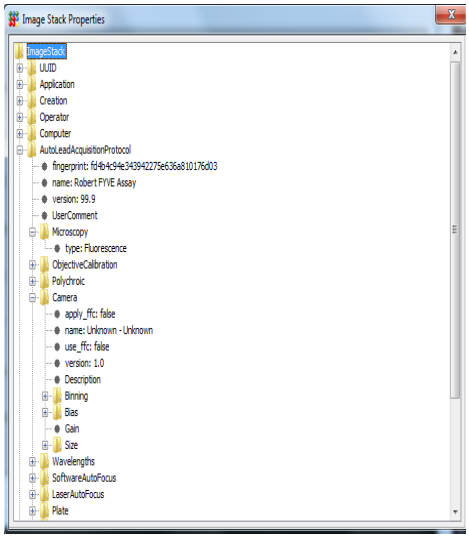
Item	Function	Description
Field: 1	**Field Number**	Use the arrow keys in the ***Field*** portion of the toolbar to select the specific field you want to examine.
Time Point: 1	**Time Point**	Use the arrow keys in the ***Time Point*** field to select the specific time point you want to examine.
Z Section: 1	**Z Section Number**	Use the arrow keys in the ***Z Section*** field to select the specific Z Section you want to examine.
Thumbnails	**Thumbnail view**	Click the ***Thumbnails*** button to toggle between Well view and Thumbnails view .

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

4.3.2.1.1 Properties

Select the ***Properties*** icon. 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*** window 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.

4.3.2.1.2 Fused images

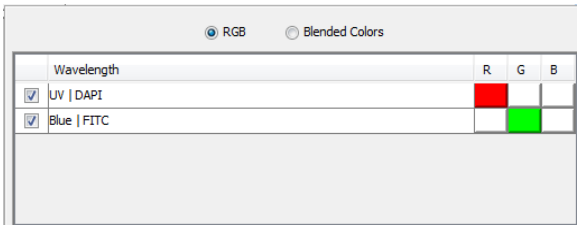
Fused images are created from multi-wavelength assays. *Image fusion* allows color composite images to be created from individual, colored wavelength images.

For example, if an image shows both a blue nuclear and a green cytoplasmic counter-stain (i.e., 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.

- Defining the Fused Image

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

Fig 4-30. Fused Image Definition Window.



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

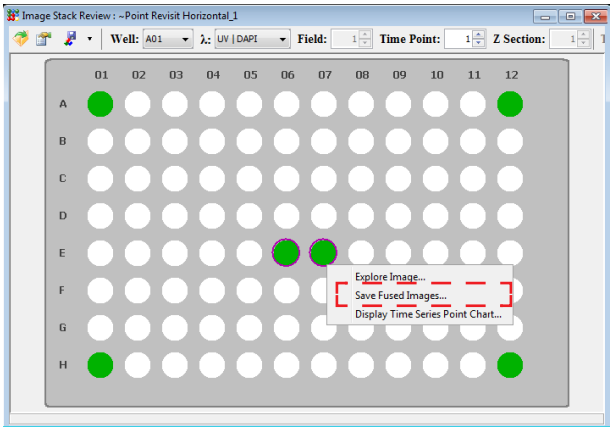
- Generating / Saving the Fused Image

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 the ***CTRL-click*** combination to select the wells to fuse. They will be marked with red borders.

3 Click the 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.*

4.3.2.1.3 View a wavelength

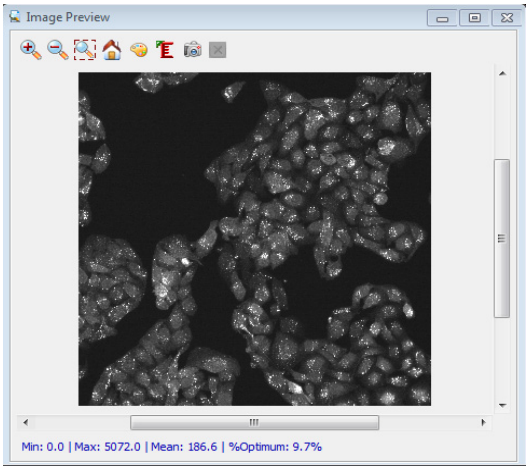
To view images acquired for a given wavelength, select from the ***Wavelength*** field dropdown list. To confirm that the wavelength was used in the acquisition protocol, the plate view will update with a status that will help to determine if any data exists for the chosen wavelength.

Color	Status
	OK, image acquired.
	Can't find data.
	No image acquired.

4.3.3 Image Preview

The ***Image Preview*** window is used during Assay Development in defining the acquisition protocol. Located in the upper-right of the workspace, this window displays a single preview image of the well.

Fig 4-31. Image Preview window in Assay Development Mode.



4.3.3.1 Image preview toolbar

The toolbar includes the operations that can be performed on an image. A number of common features have been described previously in *Section 4.3.1.1 Plate/Slide View Toolbar*.

Button	Function	Description
	Zoom in	Enlarges the displayed image. Refer to *section 4.3.1.1.3 Image magnification.*
	Zoom out	Reduces the size of the display. Refer to *section 4.3.1.1.3 Image magnification.*
	Zoom to selection	Used to view a desired portion of the image. Refer to *section 4.3.1.1.3 Image magnification.*
	Reset zoom	Resets the display to it's original size. Refer to *Section 4.3.1.1.3 Image magnification.*
	Visuals	Opens the ***Visuals*** window. Refer to *Section 4.3.1.1.4 Adjusting image appearance.*
	Ruler	Used to provide reference when displaying an image. see *Section 4.3.3.1.1 Ruler feature.*
	Digitize	See *Section 4.3.3.1.2 Digitize feature.*

4.3.3.1.1 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 image viewing window. When the image view consists of multiple frames, the ruler will display for each frame.

Fig 4-32. Image Preview window

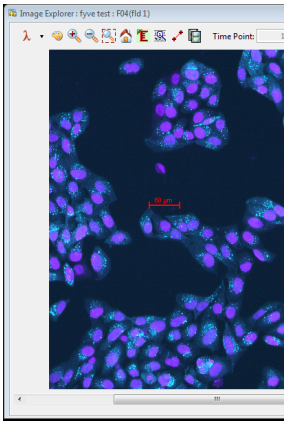
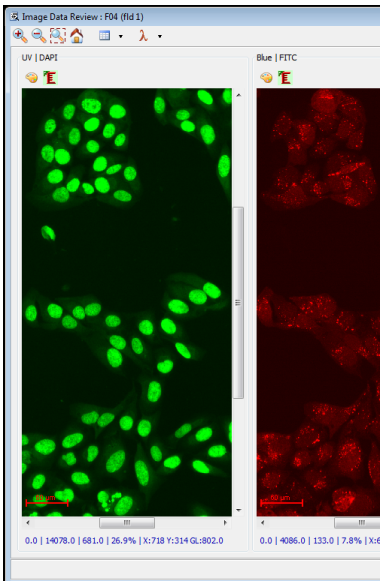


Image View window



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.

4.3.3.1.2 Digitize feature

Use the ***Digitize*** to refresh/view 2D images in the ***Image View*** windows for the current protocol settings. These settings may come from:

- The wavelength, binning, and exposure settings in the***Wavelength Chooser*** window.
- The camera position in the ***Plate/Slide View*** window.
- The currently selected objective in the ***Objective Changer*** window.

4.3.3.2 Additional Image Preview Functionality

Within the ***Image Preview*** window, the mouse cursor appears as a red reticle . The following functionality is available when the reticle displays:

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

- ***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.

4.3.3.3 Status bar

The ***Status Bar*** provides information about the image and acquisition settings and is formatted as described below:

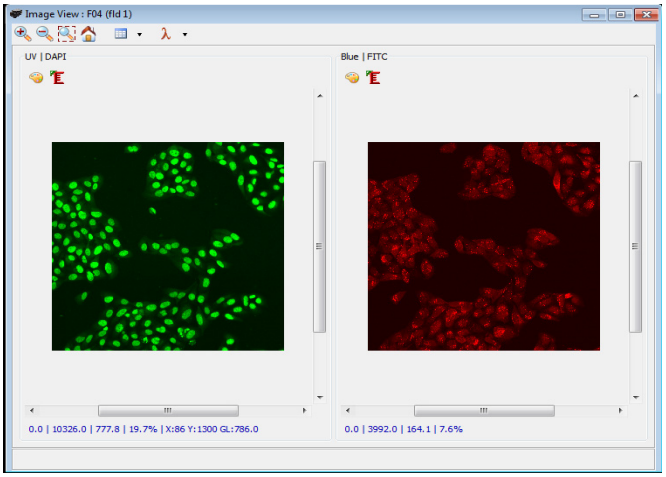
Min: 0.0 | Max: 5072.0 | Mean: 186.6 | %Optimum: 9.7%

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.
The Image View Cursor Coordinates after the straight slash (|) indicate the current cursor position and pixel intensity within the window	
X: ###	The X coordinate for the current location of the cursor.
Y: ###	The Y coordinate for the current location of the cursor.
GL: ## **(Grey Level)**	The pixel intensity for the current location of the cursor.

4.3.4 Image View

The ***Image View*** window opens with Acquisition mode and can be used to monitor the progress of image acquisitions. The ***Image View*** window provides a separate view for each of the wavelengths defined for an acquisition (up to a maximum of 13 simultaneously). You can also configure the ***Image View*** window to display as few or as many single-wavelength images as necessary for your experiments.

Fig 4-33. Image View window in Acquisition Mode.



The ***Image View Status Bar*** provides information about the image and acquisition settings, as described in *section 4.3.3.3 Status bar*.

4.3.4.1 Image view toolbar

The toolbar contains the operations that can be performed on the images displayed in the ***Image Viewer***. A number of common features have been described previously in *section 4.3.1.1 Plate/Slide View Toolbar*.

Button	Function	Description
	Zoom in	Enlarges the displayed image. Refer to *section 4.3.1.1.3 Image magnification.*
	Zoom out	Reduces the size of the display. Refer to *section 4.3.1.1.3 Image magnification.*
	Zoom to selection	Displays a desired portion of the image. Refer to *section 4.3.1.1.3 Image magnification.*
	Reset zoom	Resets the display to it's original size. Refer to *section 4.3.1.1.3 Image magnification.*
	Image View Layout	Sets up the display in the ***Image Viewer***. Refer to *section 4.3.4.1.1 Image View Layout.*
	Wavelength Selection	Displays the ***Wavelengths*** window. Refer to *section 4.3.4.1.2 Wavelength Selection.*
Wavelength toolbar		

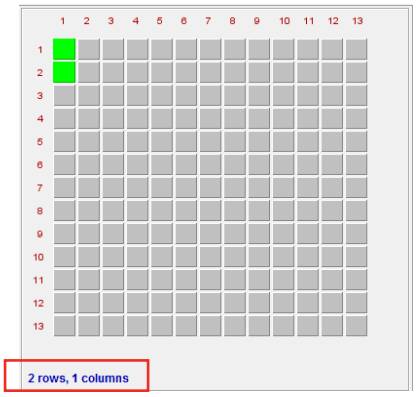
Button	Function	Description
	Visuals	Opens the ***Visuals*** window. Refer to *section 4.3.1.1.4 Adjusting image appearance.*
	Ruler	Opens a tool to measure the relative size of an object within an image. Refer to to *section 4.3.3.1.1 Ruler feature.*

4.3.4.1.1 Image View Layout

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

1 Click the icon to open the ***Image Viewer Layout*** window.

Fig 4-34. Image View Layout Setup window.



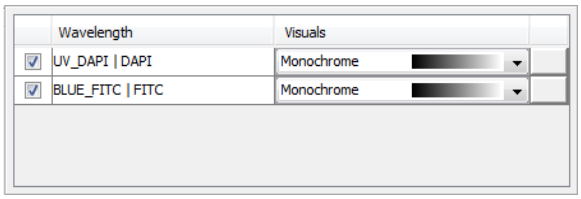
2 Holding down the left mouse button, drag over the number of images to display in the window. The ***Status Bar*** displays the dimensions of the viewing area.

4.3.4.1.2 Wavelength Selection

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

1 Click the item on the toolbar. The ***Wavelengths*** window opens.

Fig 4-35. Wavelengths window.



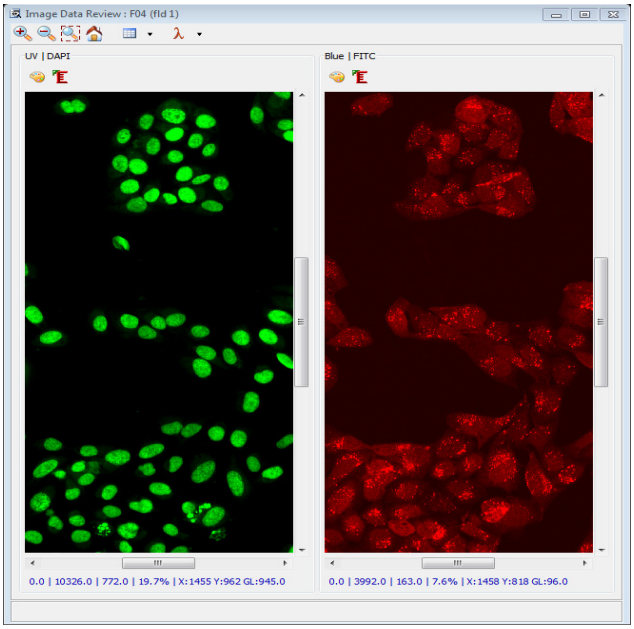
2 Mark and unmark the checkboxes of the wavelengths to display in the ***Image View*** window.

3 Open the dropdown lists under the ***Visuals*** column to set user defined colors or a LUT for each wavelength in the view. These settings will not be saved.

4.3.5 Image Data Review

Similar to the ***Image View*** window, the ***Image Data Review*** window can be used to view and manipulate acquired images. Each of the wavelengths will display in it's own frame, and the image view can be configured to display up to 13 images simultaneously.

Fig 4-36. Image Data Reviewer.



The ***Image Data Review Status Bar*** provides information about the image as described in *section 4.3.3.3 Status bar*.

4.3.5.1 Image Data Reviewer Toolbar

The toolbar provides the operations that can be performed on the images in the ***Image Data Reviewer***. A number of common features have been described previously in *section 4.3.1.1 Plate/Slide View Toolbar*.

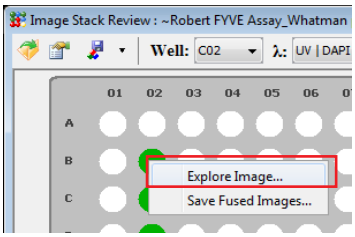
Button	Function	Description
	Zoom in	Enlarges the displayed image. Refer to *section 4.3.1.1.3 Image magnification.*
	Zoom out	Reduces the size of the display. Refer to *section 4.3.1.1.3 Image magnification.*
	Zoom to selection	Displays a desired portion of the image. Refer to *section 4.3.1.1.3 Image magnification.*
	Reset zoom	The display to it's original size. Refer to *section 4.3.1.1.3 Image magnification.*
	Image View Layout	Displays the ***Image Viewer Layout*** window. Refer to *section 4.3.4.1.1 Image View Layout.*
	Wavelength	Displays the ***Wavelength*** window is displayed. Refer to *section 4.3.4.1.2 Wavelength Selection.*
Wavelength toolbar		
	Visuals	Opens the ***Visuals*** window. Refer to *section 4.3.1.1.4 Adjusting image appearance.*
	Ruler	Opens a tool to measure the relative size of an object within an image. Refer to *section 4.3.3.1.1 Ruler feature.*

4.3.6 Image Explorer (Data Review Mode)

The ***Image Explorer*** window, accessed via the ***Image Stack Review*** window, allows you to explore specific wells in detail, and 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.

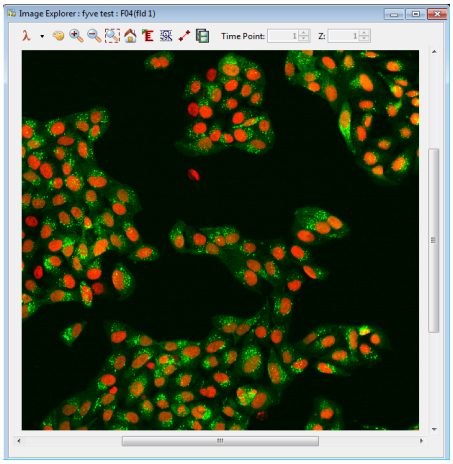
To open the ***Image Explorer***:

1 From the ***Image Stack Review*** window, right-click on any of the wells and select ***Explore Image*** from the following pop-up menu.



The ***Image Explorer*** window is displayed.

Fig 4-37. Image Explorer Window.



4.3.6.1 Image Explorer Toolbar

The toolbar operations on the ***Image Explorer*** allow the following activities. A number of common features have been described previously in *section 4.3.1.1 Plate/Slide View Toolbar*.

Button	Function	Description
λ	**Wavelength**	Displays the ***Wavelength*** window. Refer to *section 4.3.4.1.2 Wavelength Selection.*
	Visuals	Opens the ***Visuals*** window. Refer to *section 4.3.1.1.4 Adjusting image appearance.*
	Zoom in	Enlarges the displayed image. Refer to *section 4.3.1.1.3 Image magnification.*
	Zoom out	Reduces the size of the display. Refer to *section 4.3.1.1.3 Image magnification.*

Button	Function	Description
	Zoom to Selection	Displays a desired portion of the image. Refer to *section 4.3.1.1.3 Image magnification.*
	Reset zoom	Resets the display to it's original size. Refer to *section 4.3.1.1.3 Image magnification.*
	Display Ruler	Enables the ***Ruler*** feature. Refer to *section 4.3.3.1.1 Ruler feature.*
	Point Values	Used to determine wavelength intensity values at points within an image, opens the ***Point Values*** window. Refer to *section 4.3.6.1.1 Point Values: Capturing Wavelength Intensities of Points within an Image.*
	Measure	Used to calculate distances between various points within the image to determine exact locations of regions of interest. The ***Measures*** window opens. Refer to *section 4.3.6.1.2 Measure: Calculating Distances Between Points in an Image.*
	Movies	Opens the ***Movies*** window used to generate movies from multiple images, such as time series or Z-stack acquisitions See *Chapter 9 Creating an Image Slideshow* for detail.
Time Point: 1	**Time Point Selection**	Used to select a time point to view the corresponding images.
Z: 1	**Z Section Selection**	Used to select a Z-section plane to view the corresponding images.

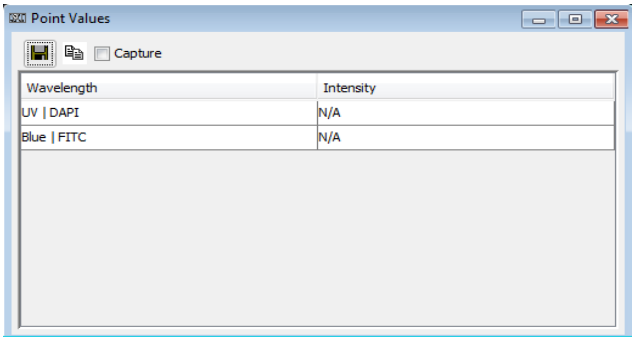
4.3.6.1.1 Point Values: Capturing Wavelength Intensities of Points within an Image

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:

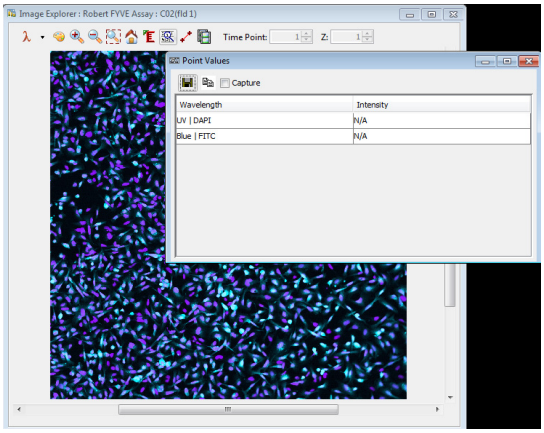
1 Click on the ***Point Values*** icon to open the ***Point Values*** window.

Fig 4-38. Point Values window



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 wavelength and corresponding intensity values are reported in the ***Point Values*** window.

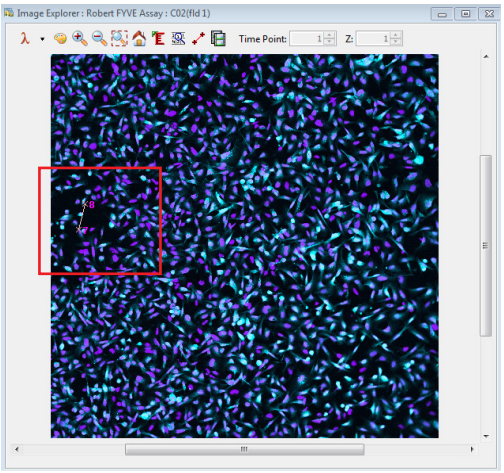


4 On completing your investigation, you can click the ***Save*** icon on the toolbar to save the values to **.CSV* file for future use.

4.3.6.1.2 Measure: 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 form point to point, and the total distance measured.

Fig 4-39. Image Explorer Window Measure tool with a Multiple Segment measurement.



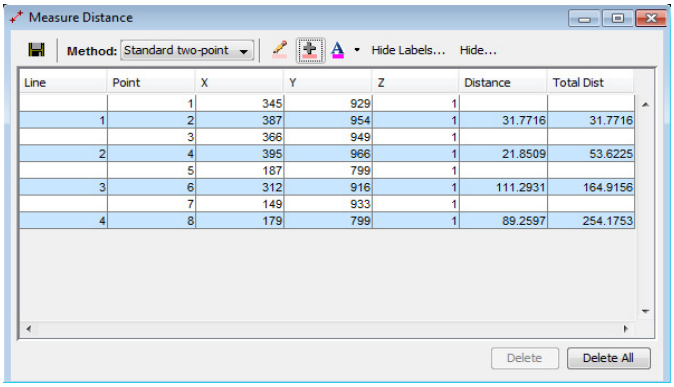
The operations on the ***Measure Distance*** dialog are available from the toolbar.

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.
	Line Color	Opens the ***Line Color*** window to change the color of the lines used to show the plotting of the distances. See *Line Color* section below.
	Crosshair Color	Opens the ***Crosshair Color*** window to change the color of the begin/end markers on the image. See *Crosshairs* section below.
	Font	Opens the ***Font*** window to change the text style of the point labels. Used to change the text color of the of the point labels. See *Font* section below.
	Hide Labels	Click to toggle to view or hide the measurement labels.
	Hide	Click to toggle to view the image with or without all label, point, and line data.

To calculate distance between objects in an image:

1 Click the ***Distance*** icon on the ***Image Explorer*** toolbar. A blank ***Measure Distance*** window opens.

Fig 4-40. Measure Distance Window populated with standard 2-point distance calculations for 4 points.



Line	Point	X	Y	Z	Distance	Total Dist
	1	345	929	1		
1	2	387	954	1	31.7716	31.7716
	3	366	949	1		
2	4	395	966	1	21.8509	53.6225
	5	187	799	1		
3	6	312	916	1	111.2931	164.9156
	7	149	933	1		
4	8	179	799	1	89.2597	254.1753

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 are recorded.

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.
- Click the ***Delete All*** button to clear all the data in the window and from the displayed image.

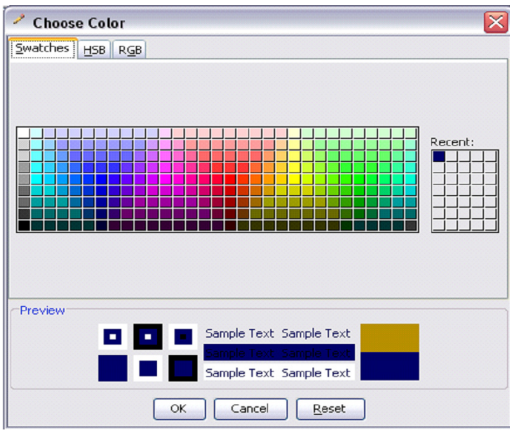
5 The collected values can be saved by clicking the ***Save*** icon on the toolbar. The data is saved to a *CSV* file.

The markers and lines used on the image to plot the distances can be customized to your preference.

- Line Color

  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 *section 4.3.1.1.4 Adjusting image appearance*.

Fig 4-41. Choose Color window.



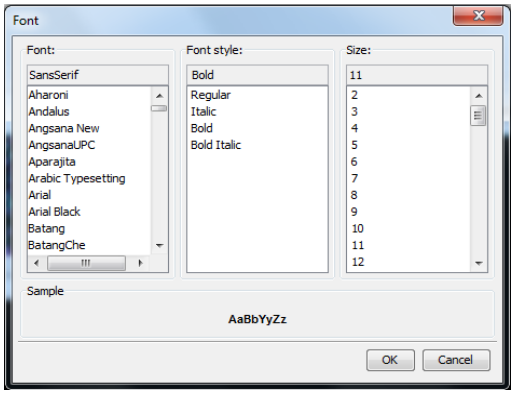
- Crosshair Color

  Click the ***Crosshair Color*** 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

  Click the ***Font*** 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 4-42. Font Window



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

4.3.7 Wavelength Chooser

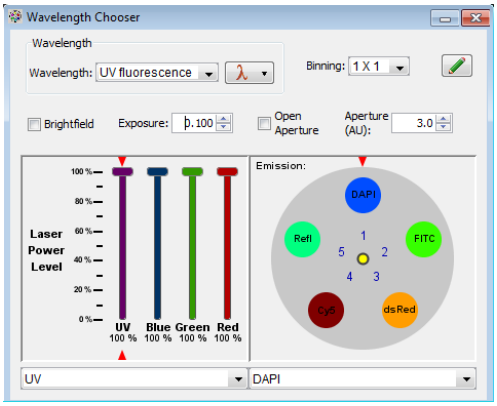
The ***Wavelength Chooser*** provides a graphical display of the available wavelength filter pairs. You may also use this window to explore the use of these settings before applying them to the protocol.

The ***Wavelength Chooser*** window opens in:

- Assay Development Mode to define the wavelength filter settings.
- Acquisition Mode to monitor the wavelength filter being used during an acquisition.

Note: *If the **Brightfield** checkbox is unavailable, you may not have a license for using the Transmitted Light feature. See Section 2.3 Configuration Options.*

Fig 4-43. Wavelength Chooser.



4.3.7.1 Using the Wavelength Chooser

The ***Wavelength Chooser*** dialog can be used to define, modify, or view the filter definitions used by protocols on IN Cell Analyzer 6000.

Note: *Wavelength pairs may also be defined during the creation of your protocol on the Microscopy card.*

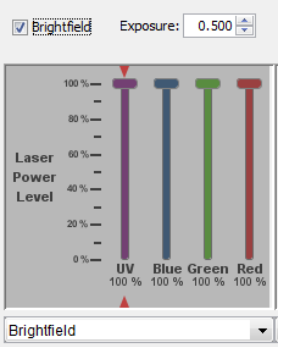
1 In the *Wavelength* panel, select a filter from the ***Wavelength*** dropdown list. If the filter needed for your experiment does not appearing the list, a new wavelength definition can be defined as described in *section 4.3.7.2 Defining New Wavelength Pairs - Wavelength Definition*.

Alternatively, you can manipulate the ***Wavelength Chooser*** settings manually:

- Set the ***Excitation*** wavelengths by either:
  - Move the sliders up/down for each of the four lasers in the graphic.
  - Selecting from the associated dropdown lists.
- Set the ***Emission*** filter positions by either:
  - Double-clicking on the filter name in the graphic.
  - Selecting from the associated dropdown lists.
- To enable the use of the Transmitted Light feature (i.e., Brightfield illumination), mark the ***Brightfield*** checkbox. The *Excitation filter selector* is disabled.

Note: *When using Brightfield illumination, the DAPI Emission type will be selected*

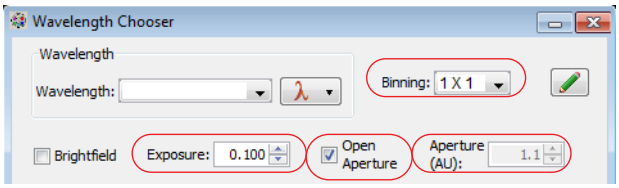
automatically; however, you can manually change to a different Emission filter by double-clicking on the filter wheel graphic.



2 In the ***Binning*** field, use the dropdown to set the image resolution. By combining signals from multiple pixels, binning decreases resolution, but can increase sensitivity and imaging speed. Bin settings range from 1x1 (no binning; full resolution) to 8x8 (combines signal from a 8x8 block of pixels). The higher the bin setting, the lower the resolution.

3 In the ***Exposure*** field, use the arrow keys to set the image exposure time.

Fig 4-44. Setting the Binning and Exposure time.



4 Mark the ***Open Aperature*** checkbox to disable confocality, and open the aperture to it's widest setting (wide-field imaging similar to that of the IN Cell Analyzer 2000 2D mode).

5 In the ***Aperture (AU)*** field, use the arrow keys to select the size of the aperture. This field is disabled when the ***Open Aperature*** checkbox is marked.

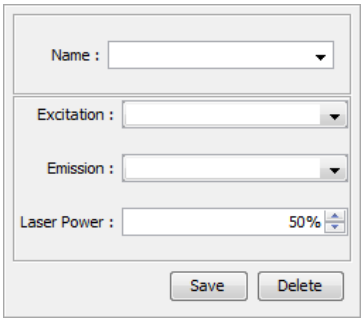
Alternatively, the exposure time can be adjusted when using the ***Preview Scan*** feature, See *Section 5.2.2.1 Set up preview scan:* for detail or in the microscope or focus cards of the protocol designer (see *Section 5.2.1.6 Focus card* for details).

4.3.7.2 Defining New Wavelength Pairs - Wavelength Definition

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

1 Click on the ***Wavelength Definition*** icon in the *Wavelength* group. The ***Wavelength Definition*** dialog opens.

Fig 4-45. Wavelength Definition Window.



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

3 In the ***Excitation*** and ***Emission*** fields, choose the filter pair to use for this wavelength definition.

- In the ***Excitation*** field, select an excitation laser from the dropdown.
- In the ***Emission*** field, select an emission filter from the dropdown.

4 In the ***Laser Power*** field, use the arrow keys to set the percent age of the laser power that is applied to the sample being acquired.

5 Click ***Save*** to store the new wavelength definition.

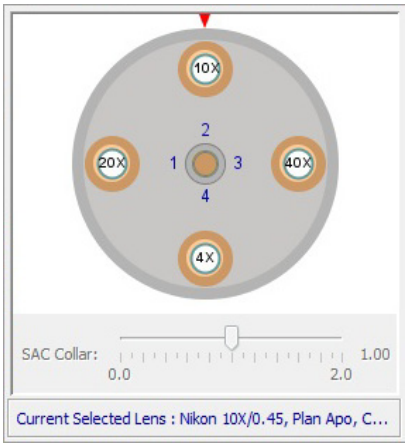
6 Repeat Steps 2 through 7 for each filter you want to define for this experiment.

4.3.8 Objective Changer

The ***Objective Changer*** window can be utilized to investigate regions of interest during the protocol design phase, or to explore images that contain areas of interest. You can also click on the ***Main Toolbar|Objective Changer*** icon to change the active objective. This action displays the ***Objective Changer*** window.

1 To change the active objective, open the ***Objective Changer*** window.. The active objective is centered at the top of the window, directly below the red arrow.

Fig 4-46. Objective Changer.



2 To change the active objective, place the mouse cursor over your selection. Double-click on an objective in the graphic. The instrument will physically rotate the turret to change the objective.

3 Use the ***SAC collar*** slider in either the ***Protocol Designer*** or the ***Objective Changer*** 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.*

4 Once selected, to enable the new objective, click the ***Software Autofocus*** toolbar icon ,or click on a well in the plate view.

Note: *To effect the objective change to a protocol, you must manually edit the* ***Protocol Designer|Objective*** *card.*

4.3.8.1.1 Objectives Available for IN Cell Analyzer 6000

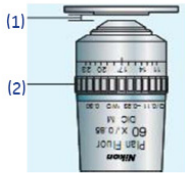
A range of standard and high NA lenses are available for the IN Cell Analyzer 6000 as shown in the tables below. The ASAC range determines the device (plate/slide) that can be used with these lenses.

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
40×	.6	Yes	3.7–2.7 mm
60×	.95	Yes	0.15 mm

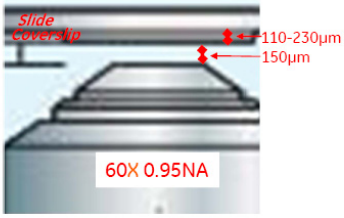
High NA lenses are only compatible with thin plates and coverslips. The thickness is defined by the ASAC range; 60x 110-230 µm. From this distance you then only have the working distance to travel before the objective touches the bottom of the plate/coverslip – maximum distance 60 x = 150 µm. Flat substrates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

Fig 4-47. Objectives.



Optional Objective Description			
Mag.	NA	ASAC	Working Distance
60X	0.95	Yes (0.11-0.23)	0.15 mm

(1) Working distance defines the distance from the top lens of the objective and the surface of the coverslip
(2) Correction Collar



4.3.9 Plate/Slide Manager

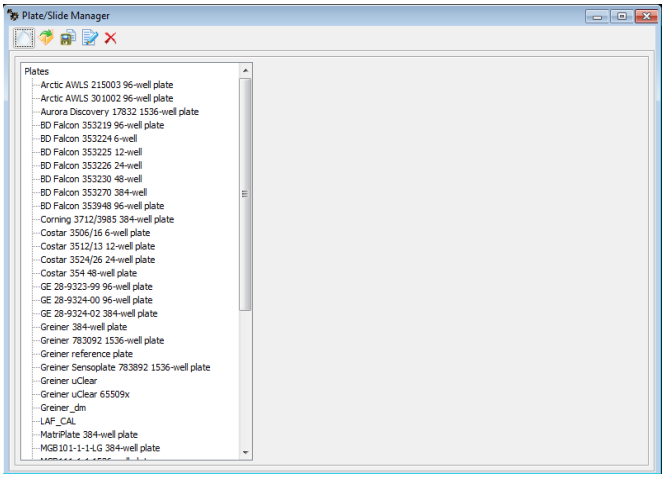
The ***Plate/Slide Manager*** provides the ability to add new plate and slide definitions, and edit the existing plate/slide definitions. The available plate and slide holder types are listed on the left of the window. For a selected plate/slide, the details will display in the panel to the right side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

The Plate/Slide Manager is accessed by:

- Selecting the ***Open Plate Manager*** option from the option screen that appears when you enter Assay Development mode; or

- Opening the *Application* menu and select the ***Plate Manager***.

Fig 4-48. Plate / Slide Manager Window.



4.3.9.1 Plate/Slide Manager Toolbar

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.

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

Button	Function	Description
	New	Creates a new plate/slide configuration file. The ***Plate Editor*** is displayed. Refer to *Sections 4.3.9.2 Creating a New Plate Configuration* or *4.3.9.4 Create a New Slide Configuration* 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).
	Save As	Saves a plate/slide configuration file in a different location or with a different name. Click ***Save As***, browse to the desired location and enter a name under which to save the configuration file.

4.3.9.2 Creating a New Plate Configuration

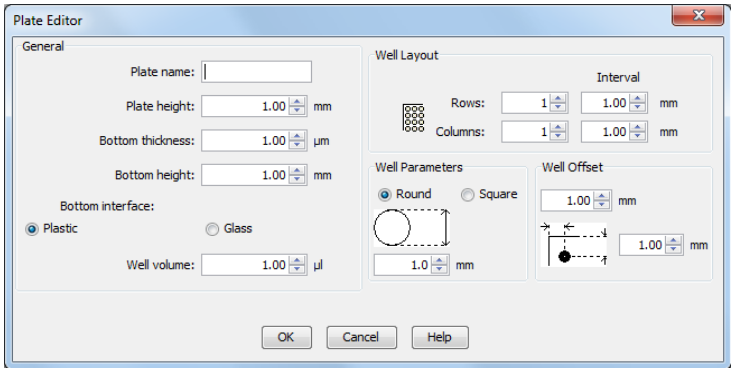
The ***Plate Editor*** is used to specify the spatial parameters of the plate. This information can be found in the specification sheet provided by the plate manufacturer.

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*** icon on the ***Plate/Slide Manager*** toolbar. The ***Plate Editor*** opens.

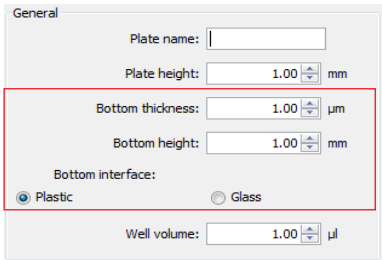
Fig 4-49. Plate Editor Window - New.



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 theplate bottom 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.

- *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. Then, click ***OK*** to exit the ***Plate/Slide Manager***.

4.3.9.3 Microtiter Plate Types Supported on IN Cell Analyzer 6000

Table 4-4. Microtiter plates supported in line-scanning mode

	20×/40×/60× Objective
96-well plate	Nunc 265302 Greiner uClear 655090
384-well plate	Greiner uClear 781097
1536-well plate	Greiner Sensoplate 783892

Table 4-5. Microtiter plates supported in line-confocal mode

	2×/4× Objective	10×/20×/40× Objective
6-well plate	Costar 3506/16	Costar 3506/16
12-well plate	Costar 3512/13	Costar 3512/13
24-well plate	Costar 3526	Costar 3526
48-well plate	Costar 3548	Costar 3548
96-well plate	PE ViewPlate-96 6005182 BD Falcon 353948 Nunc 160376 Nunc 265302 Greiner uClear 655090	PE ViewPlate-96 6005225 BD Falcon 353948 Nunc 160376 Nunc 265302 Greiner uClear 655090
384-well plate	n/a	Corning 3712/3985 Greiner uClear 781097
1536-well plate	n/a	Greiner Sensoplate 783892

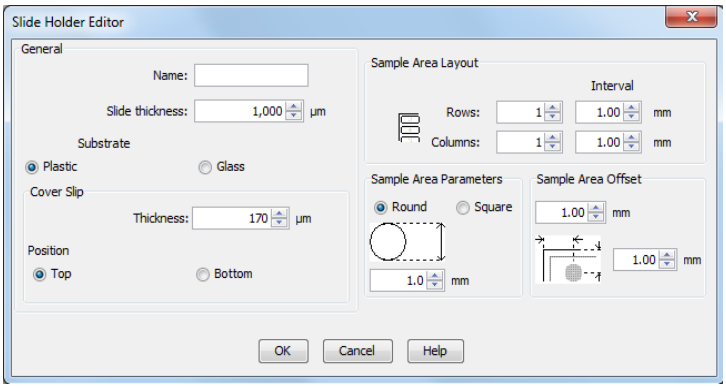
4.3.9.4 Create a New Slide Configuration

The process of defining slide parameters is similar to that for creating a plate definition described in *section 4.3.9.2 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 4-50. 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 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 slide during acquisition.
- *Sample Area Layout* specifies 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* Indicate 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***.

4.3.9.5 Creating New Plates and Slides from Existing Configurations

You may modify existing plate and slide definitions or you may chose to create a new slide definition using an existing file as a template. *Note:* When creating a new configuration from an existing configuration, the fields will contain the parameters of the original file.

- To open an existing plate configuration not already in the list, click the ***Plate/ Slide Manager toolbar|Open*** icon . Then, browse to and select the plate file (**.xplt*) you want to open.

- To edit the configuration settings for a particular plate, click the ***Plate/Slide Manager toolbar|Edit*** icon . The ***Plate Editor*** window is displayed, including all of the settings for the currently selected plate.

4.3.9.6 Saving and Deleting Plate and Slide Configurations

- To save a plate configuration file in a different location or with a different name, click the ***Plate/Slide Manager toolbar|Save As*** icon . 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 ***Plate/Slide Manager toolbar|Delete*** icon . A confirmation window is displayed. Click ***OK*** to delete the file.

Note: *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 **Save As**, **Edit**, or **Delete** the file.*

4.3.10 Liquid Handling

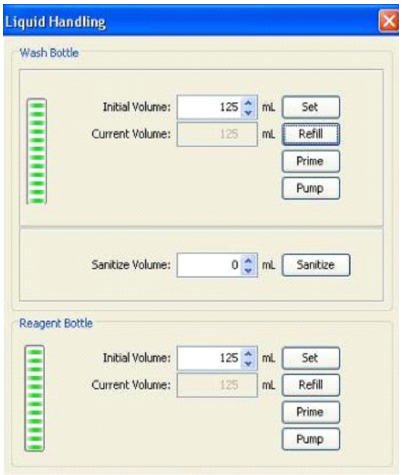
If installed, the IN Cell Analyzer 6000 has a liquid handling system for dispensing or aspirating a reagent from 96- and 384-well plates and for cleaning the system via a wash station. Controls for the ***Liquid Handling*** module can be found on the Main toolbar, and in the ***Protocol Designer***. 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 Section 5.2.1.9 Liquid Handling card.*

To start the ***Liquid Handling*** system:

1. Click the ***Main toolbar|Liquid Handling*** icon . The ***Liquid Handling*** dialog opens.

Fig 4-51. Liquid Handling dialog



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 reagent.

d. When it becomes necessary to refill the wash bottle, to the standard 250 mL, click the ***Refill*** button. The system will clear the connecting tubing of any fluid so the Wash bottle can be removed.

3 In the *Reagent 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.

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 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 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 so the Wash bottle can be removed.

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 *Section 5.2.1.9 Liquid Handling card* for detail on completing the ***Protocol Designer|Liquid Handling*** card.

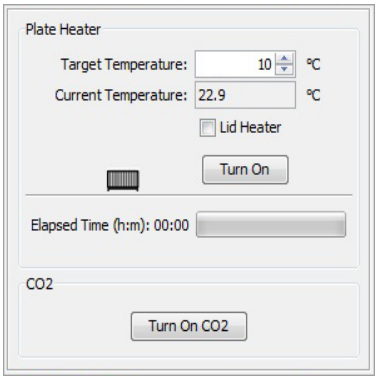
4.3.11 Environmental Control

The ***Environmental Control*** dialog is used to control plate heating and the delivery of humidified CO_2 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  icon. The **Environmental Control** window is displayed.

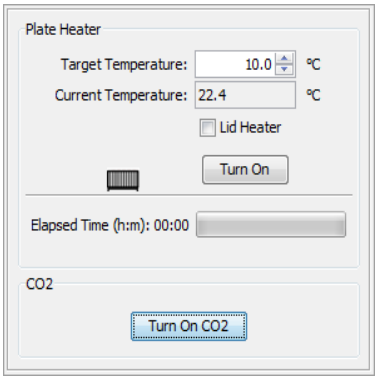
Fig 4-52. Environmental Control dialog.



- *Plate Heater* - To enable the plate heater, use the arrow keys to specify the *Target Temperature* (ºC). Check the ***Lid Heater*** checkbox to enable the lid heater to the same target temperature as the plate heater.

  Click the ***Turn On*** button, and allow the plate and/or 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.

- *CO_2 chamber* - Click the ***Turn On CO2*** button to enable the CO_2 humidifier. Allow the humidifier to run for a minimum of 60 minutes before beginning any experiments. Verify CO_2 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 *Chapter 10.2* before turning on the CO_2 .

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.

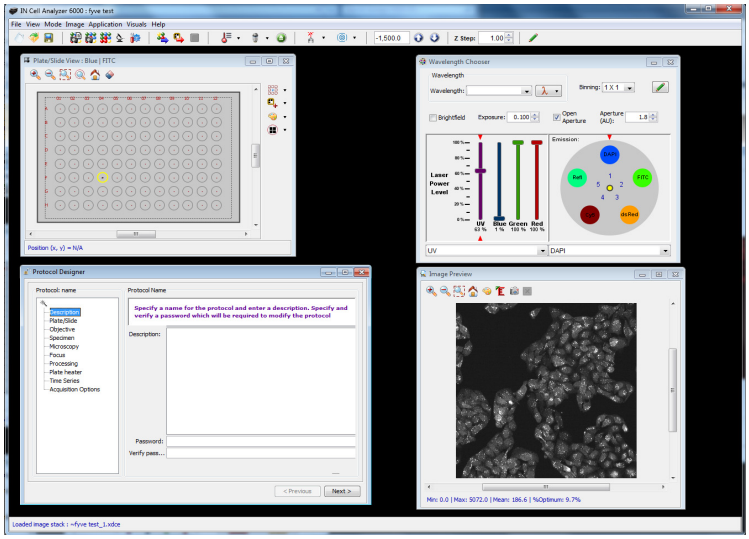
When the predefined protocol is launched, the system works without user input to complete the experiment, which can last from seconds to days.

To begin designing an experiment, open ***Assay Development*** by either:

- Selecting ***Mode|Assay Development*** from the Main menu bar.
- Clicking the ***Assay Development*** icon on the ***Main toolbar***.

The ***Plate/Slide View***, the ***Protocol Designer***, the ***Wavelength Chooser***, and the ***Image Preview*** all open as the Assay Development Workspace.

Fig 5-1. Assay Development Mode.



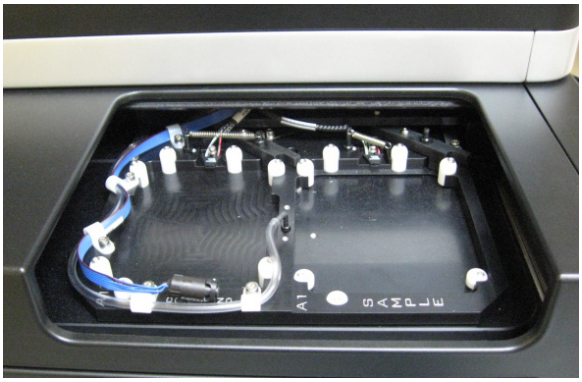
5.1 Loading a Sample

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 6000:

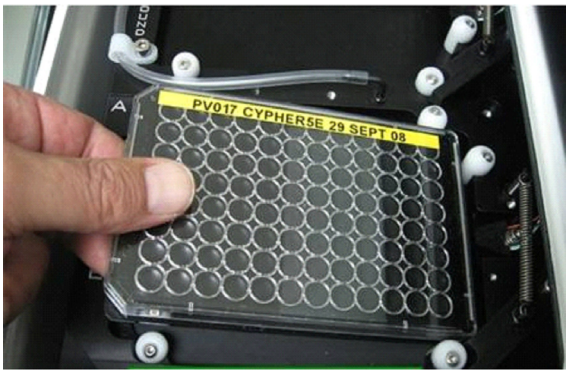
1 Click the ***Main Toolbar|Eject*** icon. The access door on the top left of the instrument slides to the rear of the instrument to open.

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/Slide View*** window. The plate is automatically positioned for imaging.

5.2 Establishing Protocol Settings

The steps to designing a successful experiment include:

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

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

5.2.1 Designing the Experiment - The Protocol Designer

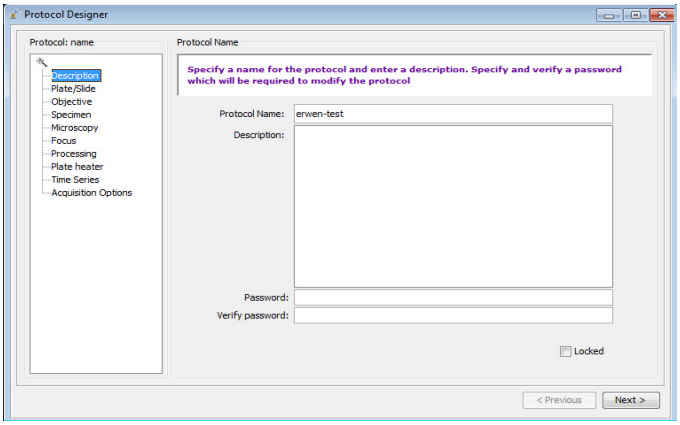
The ***Protocol Designer*** is used to define each of the parameters of an imaging experiment, and is located at the lower-left of the Assay Development Workspace.

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

5.2.1.1 Description card

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

Fig 5-4. Protocol Name Window.



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.

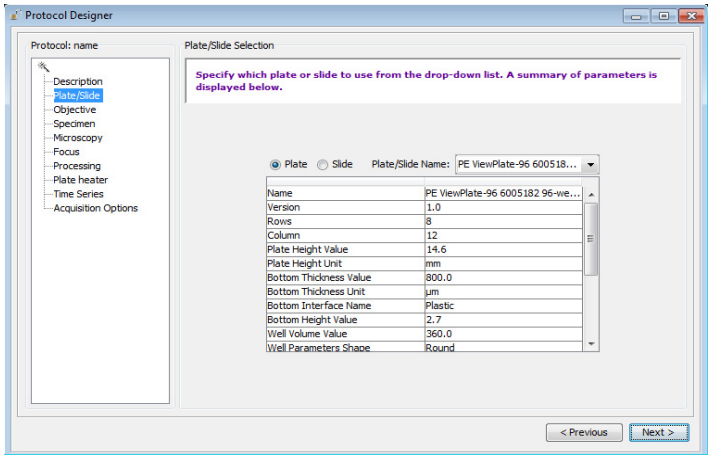
3 *Optional* : Use the ***Password*** field to enter a password for this protocol. Verify the password in the ***Verify Password*** field.

4 Click ***Next*** to proceed, or select a card from the list.

5.2.1.2 Plate/slide card

Clicking ***Next*** from the ***Description*** card, or clicking the ***Plate/Slide*** card option opens the ***Plate/Slide Selection*** window.

Fig 5-5. Plate/Slide Selection Window.



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.

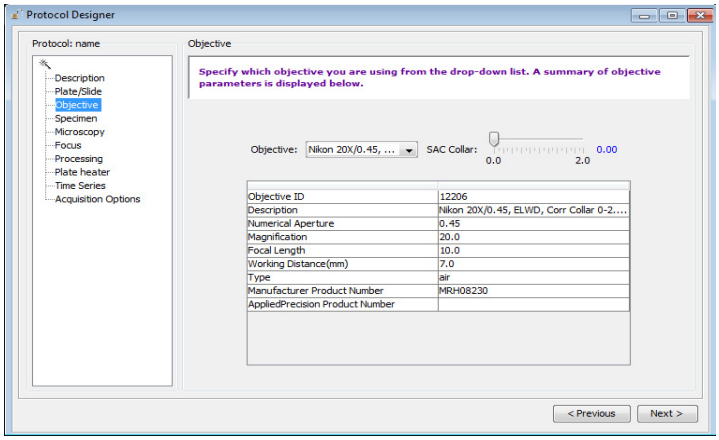
Note: *To define a new plate/slide holder type, if you cannot locate the type you need, use the* ***Plate/Slide Manager****, accessed from the* ***Main Menu|Applications|Plate/Slide Manager****. See 4.3.9 Plate/Slide Manager.*

2 Click ***Next*** to proceed, or select a card from the list.

5.2.1.3 Objective card

Clicking ***Next*** from the ***Plate/Slide*** card, or clicking the ***Objective*** card option opens the ***Objective*** window.

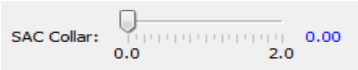
Fig 5-6. Objective Window.



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.

Note: *The objective selected in the **Protocol Designer** represents the default objective that will be used when the protocol is run. The objective listed as active in the **Objective Changer** is the objective currently set within the instrument.*

2 If the selected objective is equipped with an *SAC collar* (Spherical Aberration Correction), the ***SAC Collar*** slider is enabled. Adjust the *SAC collar* by moving the slider left or right, to the value of the plate bottom thickness, and minimize any observed effects of spherical aberration.



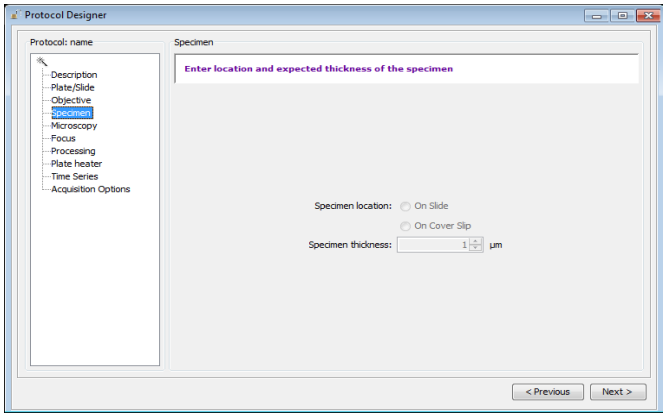
3 Click ***Next*** to proceed, or select a card from the list.

5.2.1.4 Specimen card

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

Clicking ***Next*** from the ***Objective*** card, or clicking the ***Specimen*** card option opens the ***Specimen*** window.

Fig 5-7. Specimen Window.



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

1 In the ***Specimen location*** field, mark either the ***On Slide*** or ***On Cover Slip*** radio button to indicate the location your specimen.

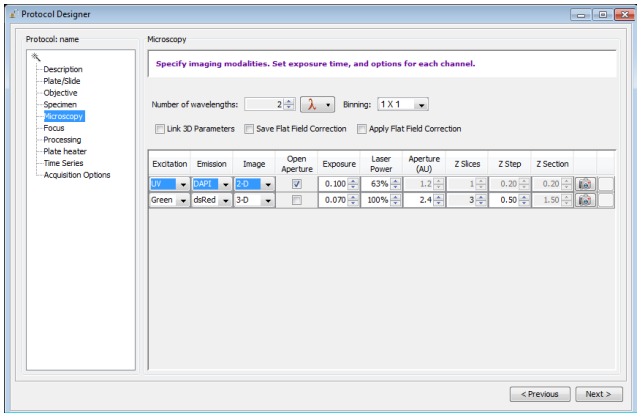
2 Enter the thickness of the specimen into the ***Specimen thickness*** field. You may enter a value or use the arrow keys.

3 Click ***Next*** to proceed, or select a card from the list.

5.2.1.5 Microscopy card

Clicking ***Next*** from the ***Specimen*** card, or clicking the ***Microscopy*** card option opens the ***Microscopy*** window.

Fig 5-8. Microscopy Window.



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.

To view or define a new wavelength pairs (shortcuts), click the ***Wavelength*** icon λ to on the ***Wavelength Definition*** window. Refer to section *4.3.7 Wavelength Chooser*.

2 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 Slices and Z Step columns of the first ***3D Image*** selection (1) are replicated in all subsequent ***3D Image*** selections (2).

The Z-slice and Z-step columns for all (subsequent) ***3D Image*** selections (3) automatically adjust to the settings you choose for the first 3D image selection.

Fig 5-9. Link 3D paramenters.

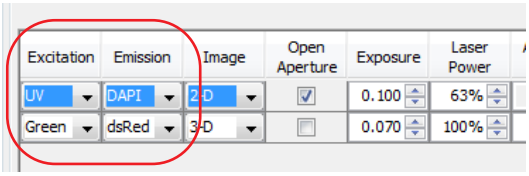
3 Mark the ***Save Flat Field Correction*** checkbox to save the flat field correction files as part of the experimental data.

4 Mark the ***Apply Flat Field Correction*** checkbox to apply the flat field correction to the current experiment.

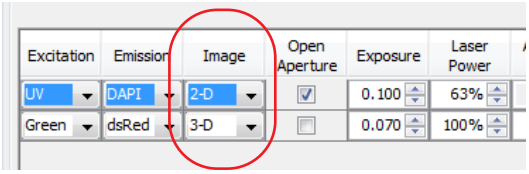
Define the wavelengths to be used in the experiment in the grid.

1 Select the ***Excitation*** laser and ***Emission*** filter for each of the wavelengths from the drop-down menus. Any defined wavelength shortcuts will also included in the dropdown list. When you select a defined wavelength

shortcut in the ***Exitation*** drop-down list, the wavelength in the ***Emission*** field is automatically selected.



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



The available modalities are:

Type	Description
2-D	The system acquires a standard two-dimensional image. 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 Mark the ***Open Aperature*** checkbox to disable confocality, and open the aperture to it's widest setting (wide-field imaging).

4 In the ***Laser Power*** field, use the arrow keys to set the percent age of the laser power that is applied to the sample being acquired.

5 In the ***Aperture (AU)*** field, use the arrow keys to select the size of the aperture.

6 Using either the arrow keys or typing a value into the field, and select ***Return*** or ***Enter*** after each value:

- Set the exposure time for each wavelengths in the experiment in the ***Exposure*** field.
- Set the number of Z Slices for each wavelength in the ***Z Slices*** field.
- Set the number of Z Steps for each wavelength in the ***Z Steps*** field.
- Set the Z Section size for each wavelength in the ***Z Section*** field.

Note: *The Z section field will be disabled, as the value is automatically set.*

7 Click ***Next*** to proceed, or select a card from the list.

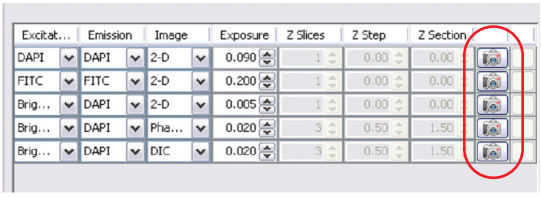
5.2.1.5.1 Previewing the Settings - Digitize

Use the ***Digitize*** button on the ***Microscopy*** 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 ***Image Preview*** window. The ***Digitize*** feature will take an image at the current Z-position shown in the ***Main toolbar|Z position*** box. 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.

At any point from the ***Microscopy*** card, you can sample for the selected wavelength, and display the resulting image is displayed in the ***Image Preview*** 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 ***Image Preview*** window.

Fig 5-10. Using the Digitize button.



Note: *Clicking the **Digitize** button in the **Image Preview** window will not generate an image based on the current protocol. Rather, this button generates an image based on the current settings in the **Plate/Slide View**, the **Wavelength Chooser**, and the **Objective Changer**.*

Note: *When you select the **Digitize** button from the **Microscopy** card, the binning and exposure time are automatically updated in the **Wavelength Chooser**.*

Note: *The digitize button performs no focusing operations.*

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

The statuses are:

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.

5.2.1.6 Focus card

Multiple focus methods are available on the IN Cell Analyzer 6000. 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.

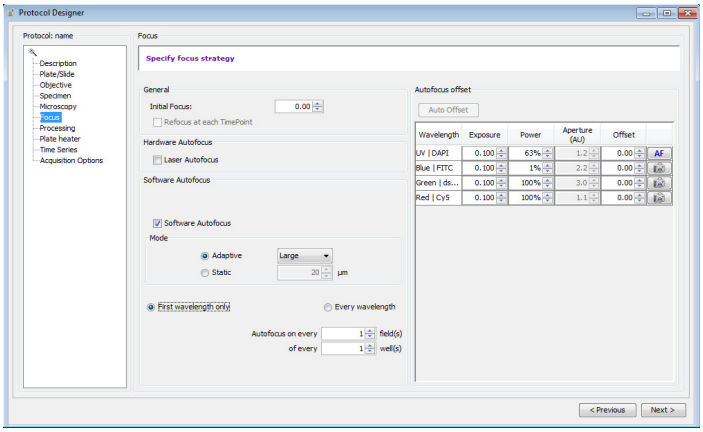
- Perform Laser Autofocus only – automatically finds interface between the air and the glass surface underneath the slide or cover slip or the liquid, substrate interface at the bottom of a well within a multi-titre plate. The software can then calculate the position of the sample. However, this may not be the optimal plane of focus for imaging the samples. The HWAF 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).

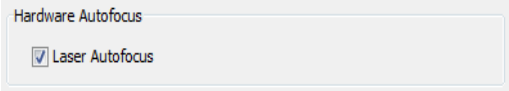
Clicking ***Next*** from the ***Microscopy*** card, or clicking the ***Focus*** card option opens the ***Focus*** window.

Fig 5-11. Focus Window.



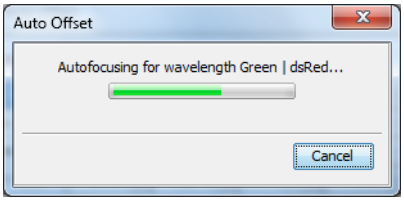
5.2.1.6.1 Hardware Autofocus Search

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.

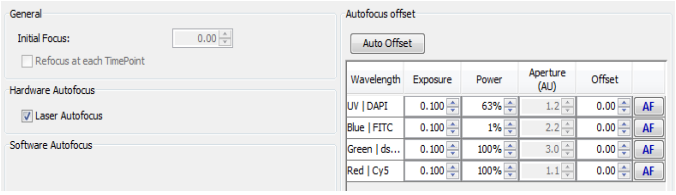


2 In the *Focus Offset* section, the ***Auto Offset*** button is only available if you selected to use the ***Laser Autofocus*** without using the ***Software Autofocus***. Use this button to have the system automatically set the autofocus offsets for this experiment. When you click the ***Auto Offset***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

Fig 5-12. Auto Offset dialog.



The table shows each selected wavelength pair, its exposure time, and its offset.



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

When ***Hardware Autofocus*** is selected, clicking AF 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 for that wavelength will display an in-focus image with the new exposure settings applied in the ***Image Preview*** window.

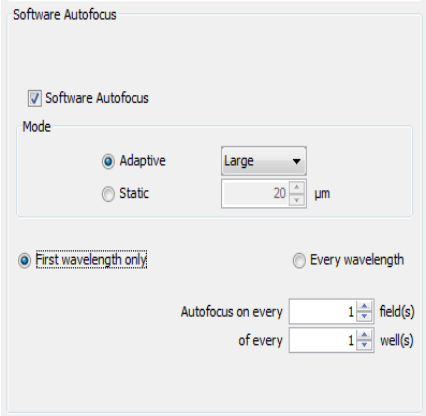
The returned offset value can manually be edited by inserting a new offset into the box, and pressing ***Return*** or ***Enter*** or by using the up/down arrows. A new image using these settings can be visualized in the ***Image Preview*** window by pressing the AF button.

5.2.1.6.2 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 6000 searches between adjacent focal planes until the specified accuracy level is reached.

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

Fig 5-13. Software Autofocus.



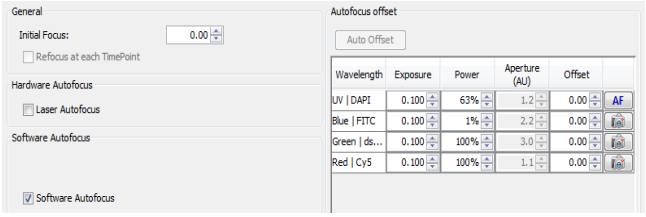
2 Choose the search range to use for this experiment. Note that using higher increments will increase the experiment's duration. In the *Mode* section, select either the ***Adaptive*** or ***Static*** mode by marking the radio button.

Adaptive Mode	For each Z-step, autofocus starts from the previous in-focus point. The drop-down list next to the radio button indicates the search range from the previously discovered in-focus point. ***Large*** specifies a 400 µm search range, ***Medium*** specifies a 200 µm search range, and ***Small*** 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 drop-down as a search range. This mode is the faster 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 AF in the far-right column only in the first row of the

Autofocus Offset table. The button is displayed for the remaining rows.



- If you have selected the ***Every wavelength*** radio button, the *Focus Offset* table displays AF 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.

Use the icon from the ***Main*** toolbar to find focus in the first field and the first well of the experiment, and then click the icon.

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 -1500µm, click the ***Laser Autofocus*** icon to provide coarse focus, and then the ***Software Autofocus*** icon to provide 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 AF , buttons to preview the result of the offset for the wavelength using all 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 ***Image Preview*** window. Based on this image, you can manually adjust the exposure time and offset for the wavelength as necessary.

5.2.1.6.3 Determining the Optimal Focus Settings

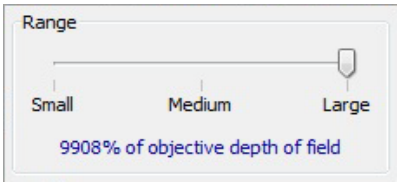
To generate the highest quality images from the IN Cell Analyzer 6000 system, it is important that you use all of the information available to attain the best focus possible. This section outlines one method for determining optimal focus. For

additional information on setting up focus strategies, refer to the beginning of this section (*Section 5.2.1.6 Focus card*).

To determine optimal focus:

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

Fig 5-14. Software Autofocus Range slider.



For the ***Software Autofocus*** to be effective, the scale is defined as follows

Small	The sample should be within 100 µm.
Medium	The sample should be within 200 µm.
Large	The sample should be within 400 µm.

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 the focus range (small, medium, or large). Or select ***Static*** and indicate the size of the search range in the value field.

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 ***Every wavelength*** in the experiment. *Note:* Selecting ***First wavelength only*** will decrease the experiment's duration.

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

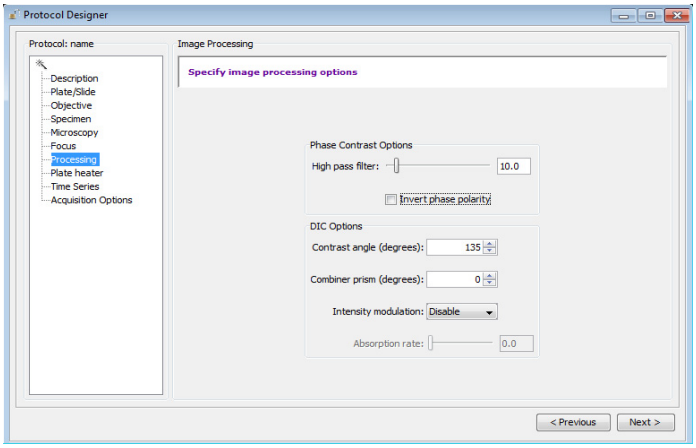
6 Click the ***Main toolbar|Save Protocol*** icon to save the settings in the currently active protocol file.

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

5.2.1.7 Processing card

Clicking ***Next*** from the ***Focus*** card, or clicking the ***Processing*** card option opens the ***Image Processing*** window.

Fig 5-15. Image Processing window.



This window is divided into two sections:

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.

5.2.1.7.1 Phase Contrast Options section

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.

5.2.1.7.2 DIC Options section

1. Use the up/down arrows to indicate the ***Contrast Angle***.
2. In the ***Combiner prism*** field, use the up/down arrows to select the correct angle of the combiner prism.

3 In the ***Intensity modulation*** field, select from the drop-down list:

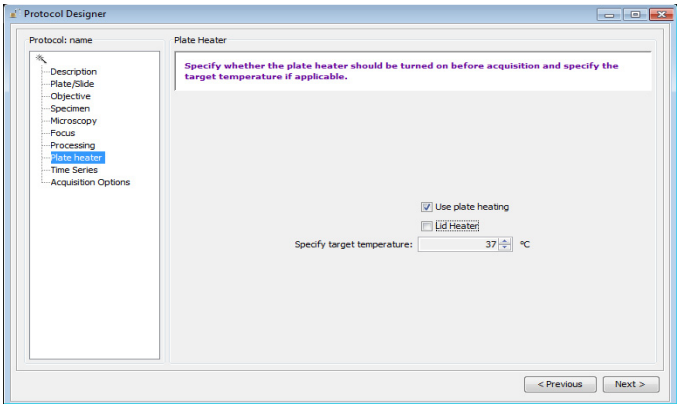
Modulation Type	Description
Disable	Amplitude data is not included in the processed image.
Standard	Amplitude data is included in the processed image.
Advanced	Absorption effects are included in the image.

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

5.2.1.8 Plate heater card

Clicking ***Next*** from the ***Processing*** card, or clicking the ***Plate Heater*** card option opens the ***Plate Heater*** window.

Fig 5-16. Plate Heater Window.



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

2 Check the ***Lid Heater*** checkbox to enable the lid heating unit.

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

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

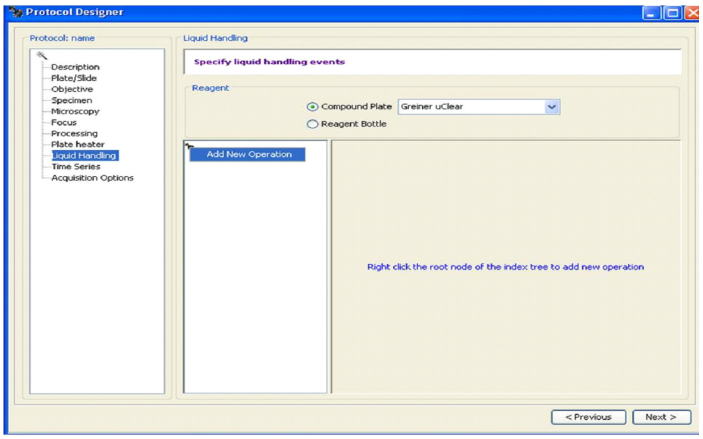
4 Click ***Next*** to proceed, or select a card from the list.

5.2.1.9 Liquid Handling card

Note: *If the **Liquid Handling** card is unavailable, this optional feature is not installed on your system. See Section 2.3 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.



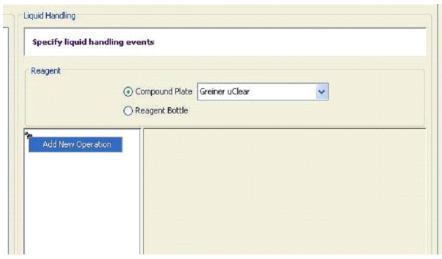
2 In the *Reagent* section, mark the radio button for either:

- *Compound Plate* - select the type of compound plate you will be using for this experiment from the dropdown list.
- *Reagent Bottle* - the reagent/compaound is supplied from a bottle rather than a compound plate.

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

3 Place the mouse cursor on the root node icon in the panel below the *Reagent* section.

4 Click the right mouse button, and then click ***Add New Operation***.

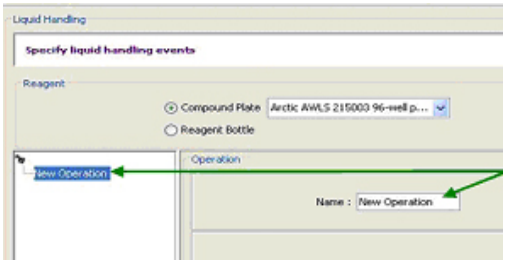


Note: *Each operation you define will be available when you set up time points on the* ***Time Series*** *card.*

5 An operation node is added to the tree, by default 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.

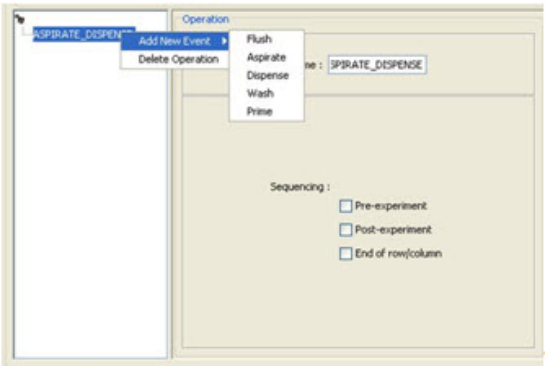
Note: *To remove any operation/task from the tree, right-click on the operation name, and select* ***Delete Operation*** *from the context menu.*



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 or bottle. (see Step 10).
Dispense	Dispenses the specified volume (10 - 100 µl) of solution into the specified well of the sample plate. (see Step 10).
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 10).
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.

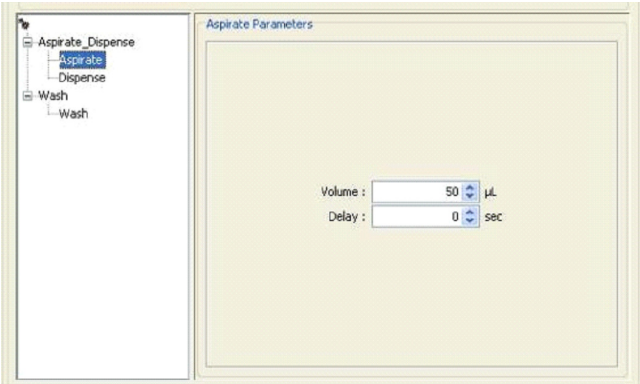
9 To add more operations, repeat from Step 3.

10 Refer to *Section 5.2.1.9.1 Event Parameters* for instruction on completing the *Aspirate, Dispense,* and *Wash* parameter options.

11 Press ***Next*** to move to the ***Time Series*** card.

5.2.1.9.1 Event Parameters

- **Aspirate Parameters**

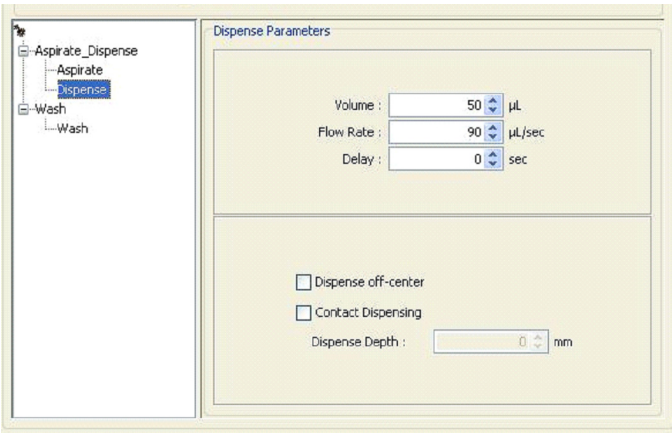


1 In the Volume field, enter a volume (10 - 100 µL in quantities of 10 µL) to be aspirated from the reagent bottle or compound plate.

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.

Note: *When using a compound plate, the Aspirate and Dispense volume should be equal.*

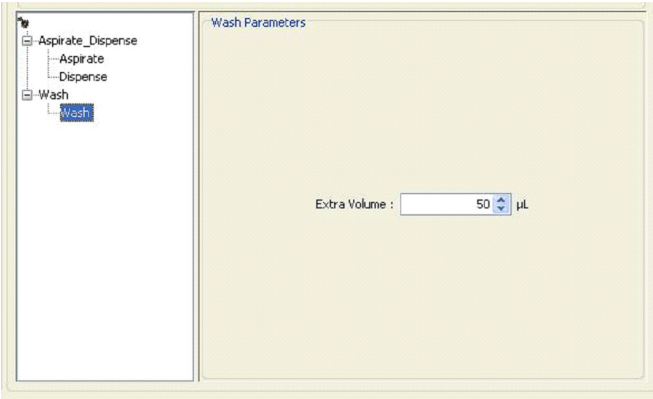
2 In the ***Flow Rate*** field, enter the amount of liquid (in µL) to be dispensed per second.

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.

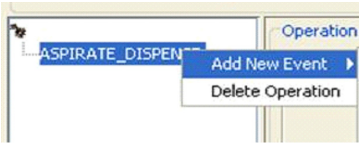
- **Wash Parameters.**



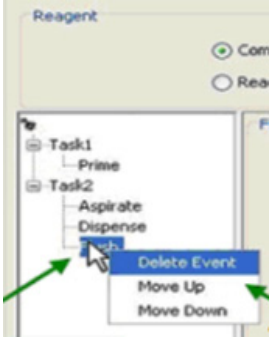
1 In the ***Volume*** field, enter the volume of wash fluid (in µL) to use for each wash event.

5.2.1.9.2 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.



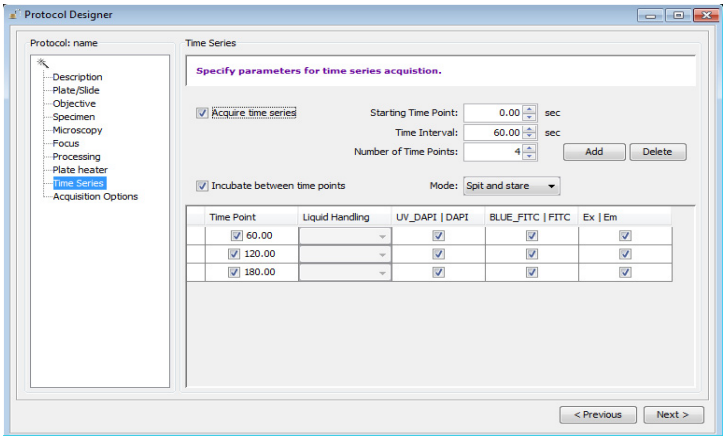
5.2.1.10 Time Series card

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. The amount of time needed before the next time point can be determined by hovering over the time pointto display the duration for that time point's sequence of events.

Note: *When adding liquid handling procedures, the liquid handling operations MUST BE defined before the time series can be specified (see Section 5.2.1.9 Liquid Handling card).*

Clicking ***Next*** from the ***Plate Heater*** card, or clicking the ***Time Series*** card option opens the ***Time Series*** window. If the *Liquid Handling* option is installed, click ***Next*** from the ***Liquid Handling*** card, or click the ***Time Series*** card.

Fig 5-17. Time Series Window



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.

2 Check the ***Re-focus at each time point*** checkbox on the ***Focus*** card.

Note: *It is recommended that you enable this feature to allow the system to re-focus after incubation since the stage returns to the neutral position during the incubation cycle.*

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

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

Note: *The first time point is usually 0.00, but may also be set to any time point within the range of the experiment*.

4 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*.

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

Enable the ***Incubate between time points*** checkbox to allow the sample plate to return to the ***Load*** position, where it will incubate between time points. This is useful if the time intervals are somewhat long (as for a live-cell experiment).

Note: *Do not use if running fast kinetic imaging*.

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

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

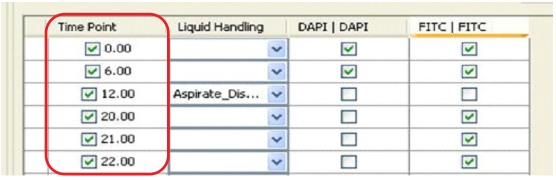
7 In the ***Mode*** field, select the type of time series tp be executed from the dropdown list. There are two modes available:

- *Spit and Stare* - used for short experimental time periods. In this mode, the *IN Cell Analyzer 6000* captures the complete time series from one well before moving to the next well.
- *Look Walk Look* - useful when considering assays over a longer time period. In this mode, the *IN Cell Analyzer 6000* 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.

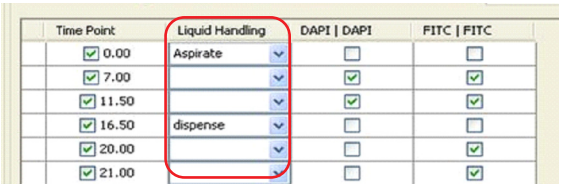
8 In the time series table:

a. Under the ***Time Point*** column, unmark the checkbox, to the left of each

time point to deselect and exclude them from your experiment.



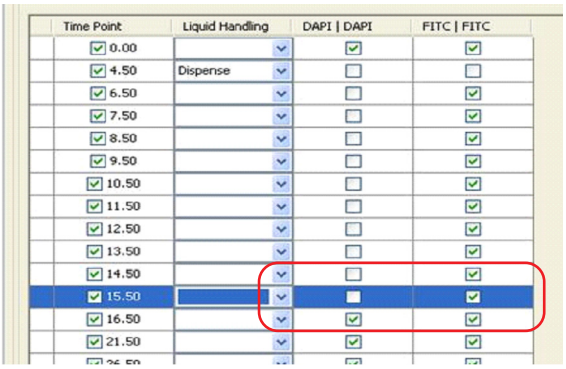
b. In the ***Liquid Handling*** column, select a Liquid Handling instruction for the time point you require the operation to occur from the dropdown list. If the *Liquid Handling* option is not installed on the instrument, the fields in this column will appear disabled.



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.



9 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.

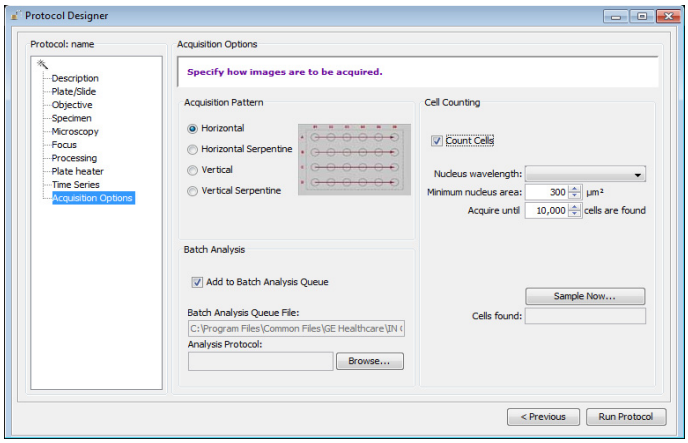
10 Click ***Next*** to proceed, or select a card from the list.

5.2.1.11 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 ***Next*** from the ***Plate Heater*** card, or clicking the ***Acquisition Options*** card option opens the ***Acquisition Options*** window.

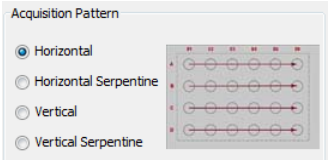
Fig 5-18. Acquisition Options window.



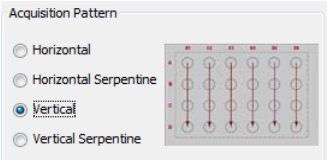
1 In the *Acquisition Pattern* section, mark the radio button of the acquisition pattern to use when traversing the plate wells. Each option is graphical depicted to help you choose as shown below:

Table 5-1. Acquisition Pattern Options.

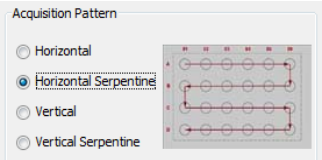
Horizontal:



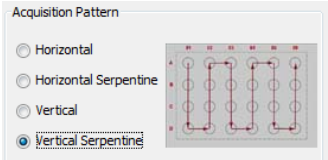
Vertical:



Horizontal Serpentine:

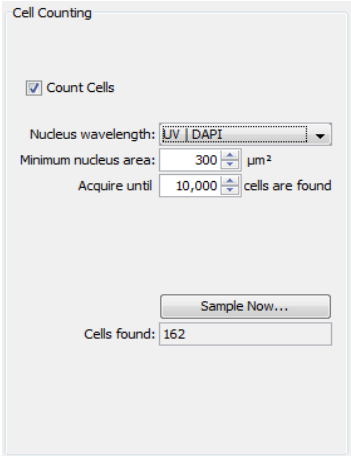


Vertical Serpentine:



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 (i.e., *.xanp* or *.xeap*).

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

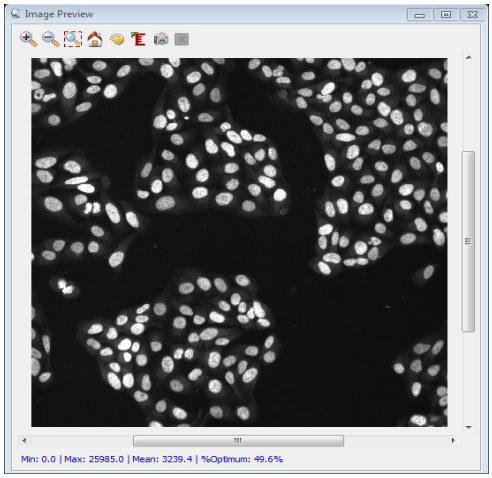


- In the ***Nucleus Wavelength*** field, select a wavelength from the dropdown list.
- Select the Minimum Nucleus Area from the dropdown list, or enter a value in the ***Minimum Nucleus Area*** field.
- In the ***Acquire Until*** field, enter a value, or use the arrow keys, to set the upper limit for the number of cells acquire.

Note: *There must be enough Fields to Acquire enabled to cover the number of cells that you want to acquire. Refer to section 6.3.2 Fields to Acquire for detail.*

4 Click ***Sample Now*** to sample the cell counting parameters and preview the results in the ***Image Preview*** window. The ***Cells found*** field will update with the number of cells found in the sampled image.

Fig 5-19. Cell Counting results in Image Preview Window.

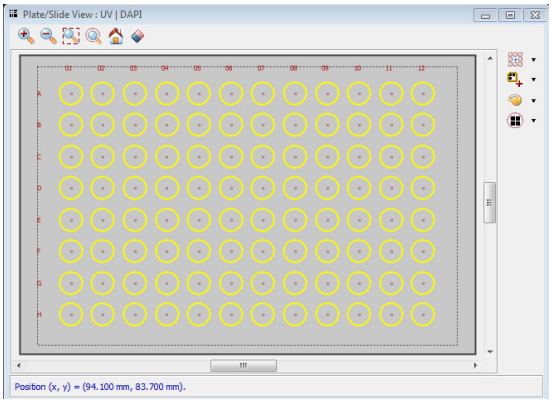


5 Click ***Run Protocol*** to run the current protocol from the Protocol Designer. The ***Acquisition Session*** dialog will open. Refer to *Chapter 6 Acquisition Mode - Conducting Experiments* on detail on completing this dialog.

5.2.2 Previewing Plate/Slide Regions

In the upper-left portion of the Assay Development Workspace, the ***Plate/Slide View*** window displays. Use this window to define preview scans and set up the fields of view containing particular regions of interest within the wells. Also see *Section 4.3.1 Plate/Slide View*.

Fig 5-20. Plate / Slide View window.



5.2.2.1 Set up preview scan:

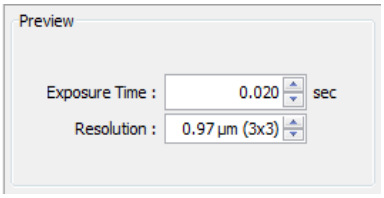
The ***Preview Scan*** feature allows a low resolution view of a user-defined region of the plate/slide, providing the ability to pre-scan for sample position and refine the position of the fields-of-view to acquire the best possible images.

Note: *Preview Scan is independent of the protocol and uses the settings from the Objective Changer, and Wavelength Chooser windows.*

To perform a preview scan:

1. Click the arrow to the right of the ***Preview Scan*** icon to open the ***Preview Scan*** window.
2. Use the arrow keys toset the ***Exposure Time*** and ***Resolution*** for the preview.

Fig 5-21. Preview Scan window.



3. Close ***Preview Scan*** window. Holding down the left mouse button, drag the mouse across the ***Plate/Slide View***. A brown box outline defines the preview region.
4. Click on the ***Preview Scan*** icon in the main toolbar to start. Images will populate the box.

Note: *Higher binning levels combine pixels in the image, so exposure time*

should be lowered as binning is increased.

Note: *Do not use binning if preview scanning with Brightfield*

5 If the resulting images are out of focus, use the ***Laser Autofocus*** or ***Software Autofocus*** position icons and to improve the clarity from the Main toolbar.

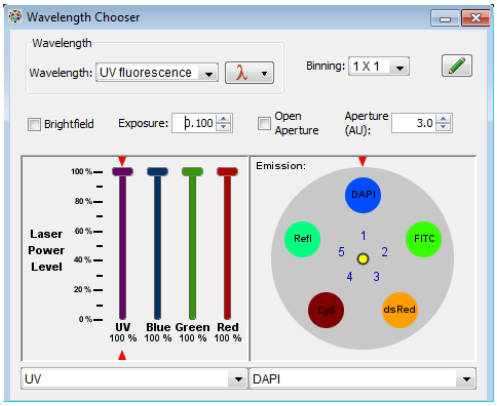
Note: *If you are using Phase Contrast or DIC imaging, however, you'll need to set the exposure times in the* ***Microscopy*** *card and digitize each wavelength to preview your images.*

5.2.3 Choosing the Wavelengths

The ***Wavelength Chooser*** window displays in the upper-right portion of the Activity Workspace. Use this window to help define the wavelength settings or monitor the wavelength settings during image acquisition in the Acquisition Workspace.

During the protocol design process, the ***Wavelength Chooser*** can be used to help determine the wavelength filters to use, define new wavelength pairings, and explore possible wavelength settings. Changes to the wavelength settings can be included in the protocol by clicking the ***Save to Protocol*** icon . The settings will display on the ***Protocol Designer|Microscopy*** card. See the *Wavelength Chooser Section 4.3.7* for detail.

Fig 5-22. Wavelength Chooser.

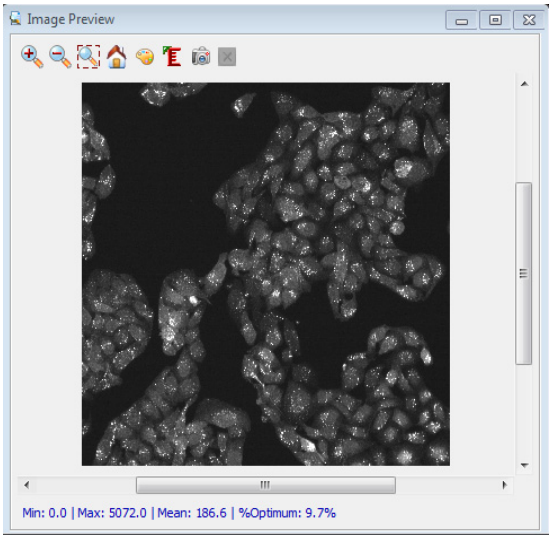


Note: *Higher binning levels combine pixels in the image, so exposure time may need to be lowered as binning is increased.*

5.2.4 Previewing the Image

In the lower-right portion of the Assay Development Workspace, the ***Image Preview*** window is displayed. Images acquired using ***Preview Scan*** or ***Protocol Designer*** will display in this window. Click on a well in the ***Plate/Slide View*** window, and then click the ***Digitize*** button to acquire the image based on the current protocol settings. Refer to the *Section 4.3.5 Image Data Review* for detail.

Fig 5-23. Image Preview Status Bar.



5.3 Saving the Protocol

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

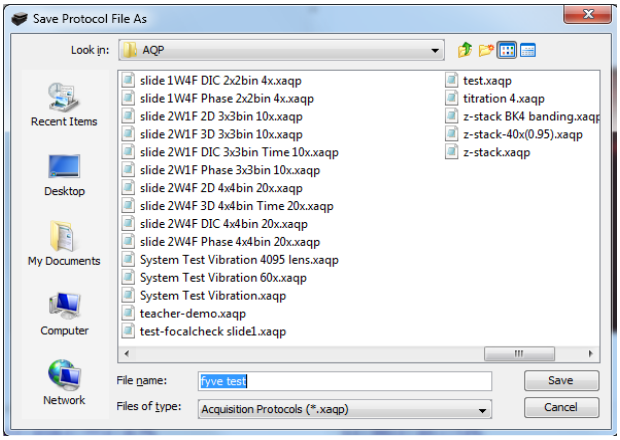
- Save the protocol for future use.
- Save a protocol with a different name (as when modifying any protocol settings).

5.3.1 Save a Protocol Without Running the Acquisition

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

5.3.2 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***.

5.4 Editing Protocols

The *Protocol Designer* allows previously created protocols to be modified. From the Main menu, click ***Open***, or  from the Main toolbar, and select a protocol from the ***Open Protocol File*** dialog to display the ***Protocol Designer*** with the selected protocol settings. Go to ***Mode|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. You can also use the ***Previous*** and ***Next*** buttons at the bottom of the window to navigate to your choice.

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.

6 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 6000 system works without user input to complete the experiment which can last from seconds to days.

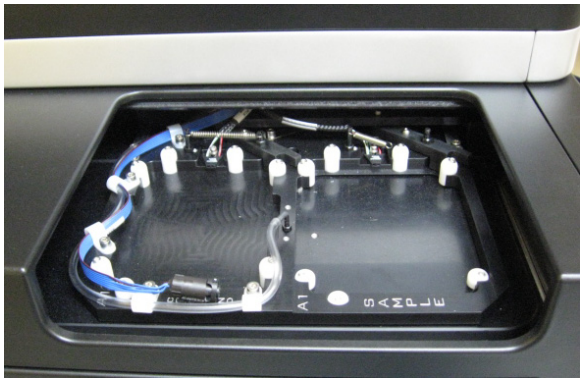
6.1 Loading a Sample

To begin acquiring images, a microplate should be loaded. 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 6000:

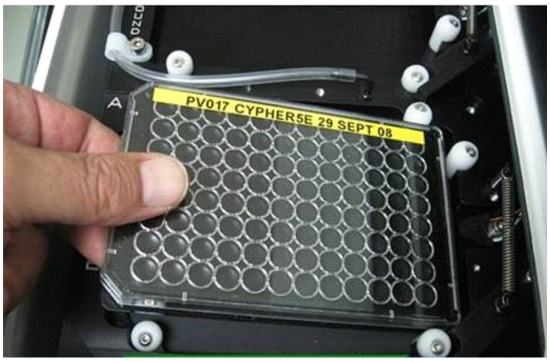
1. Click the ***Eject*** icon on the Main toolbar. The access door on the top of the system slides open toward the back of the instrument.

Fig 6-1. Plate Access Door.



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

Fig 6-2. Plate carrier.



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

6.2 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 *Section 5.2.1 Designing the Experiment - The Protocol Designer* for instruction on creating a protocol.

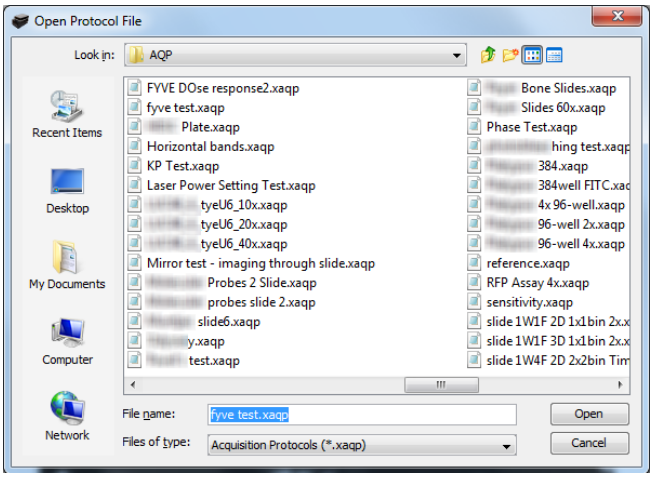
1 Open the Acquisition protocol file from either:

- ***Main menu|File|Open***.
- Clicking the ***Open File*** 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 6-3. Open protocol File dialog.



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 6000 main window.

Fig 6-4. Acquisition 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 ***Main menu|File|Save As.***

6.3 Run the Acquisition

Acquiring images takes place only while the IN Cell Analyzer 6000 system software is in Acquisition Mode. An acquisition protocol can be run from:

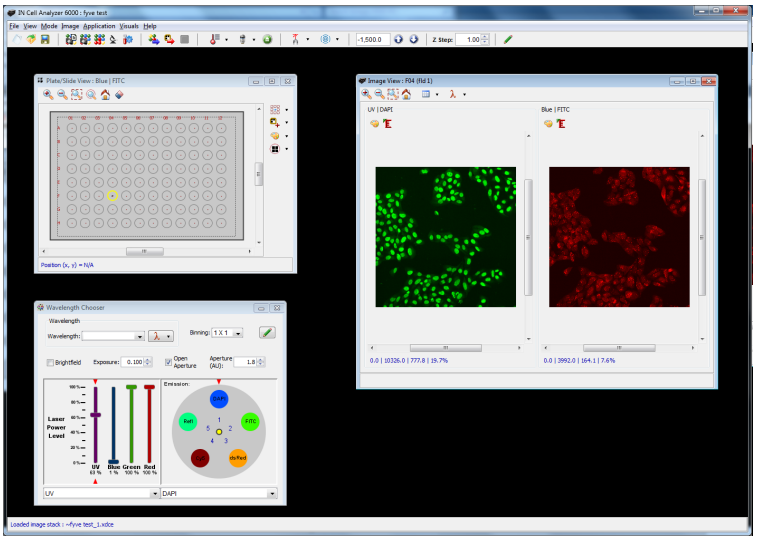
- Select ***Run Protocol*** from the ***Protocol Designer|Acquisition*** options card for the current protocol.
- ***File|Open*** to open a saved protocol in the ***Protocol Designer***.

Note: *If you start the imaging process while the system software is in any other mode, it will switch automatically to Acquisition Mode.*

To open the Acquisition Mode and display the Acquisition Workspace, select either:

- Select ***Main menu|Mode|Acquisition***.
- Click  on the Main toolbar.

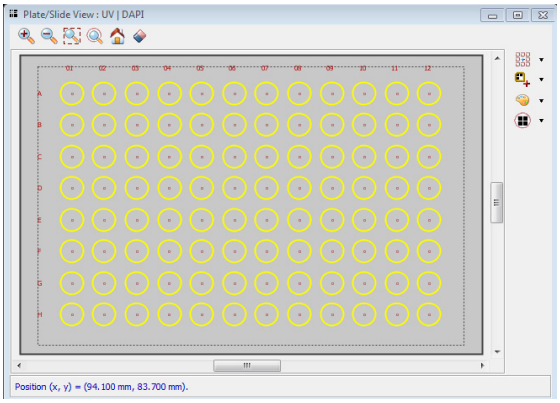
Fig 6-5. Acquisition Mode.



6.3.1 Select the Region of Interest

In the upper-left portion of the Acquisition Workspace, the ***Plate/Slide View*** window displays. Use this window to define preview scans and set up the fields of view containing particular regions of interest within the wells. Refer to *Section 4.3.1 Plate/Slide View* and *Section 5.2.1 Designing the Experiment - The Protocol Designer* for detail on uses of the ***Plate/Slide View*** window.

Fig 6-6. Plate/Slide View window.



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:

1. Select a well on the plate to be imaged.
2. From the Main Toolbar, click the icon. Select a low magnification objective to view a larger area of the sample. Refer to *Section 4.3.8 Objective Changer* for detail on the ***Objective Changer***.
3. In the Plate/Slide View window, click the well in which you are interested to take a snapshot. The image will display in the Image preview window. Perform HWAF or SWAF autofocus to achieve best image quality.Use this image to determine the best possible targets for imaging.

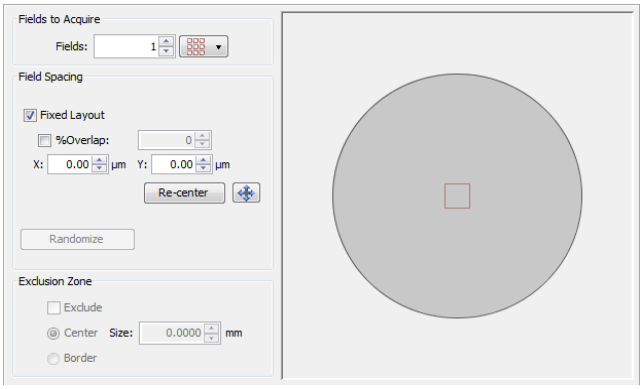
6.3.2 Setup Fields for Acquisition

You can acquire multiple fields of view within each well of a plate. The software gives you flexible control over positioning, so that fields of view can be grouped together or distributed throughout the well. Use the ***Fields to Acquire*** window to position the defined fields over your region of interest.

To define the fields for acquisition:

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

Fig 6-7. Set up Fields to Acquire window.



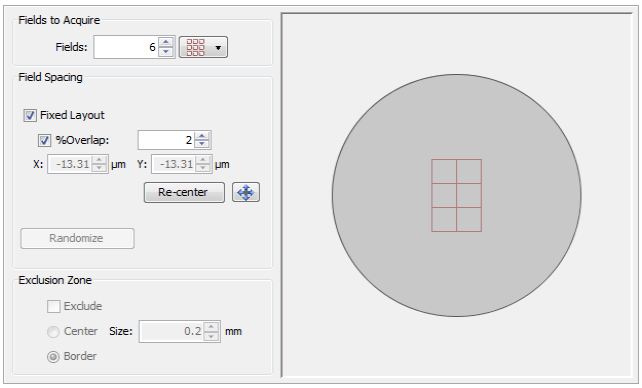
2 In the ***Fields to Acquire*** section, use the arrow keys to select the number of fields to acquire, or click the icon to set up fields of view in a specific number of rows and columns.

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.

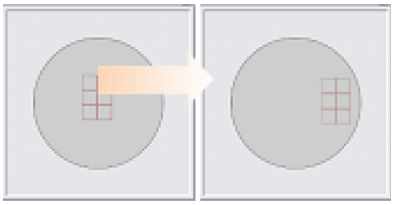
Fig 6-8. Set up field spacing.



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 button and using click-and-drag to move the group of fields to the desired location.

Fig 6-9. Moving the position of the fields.



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

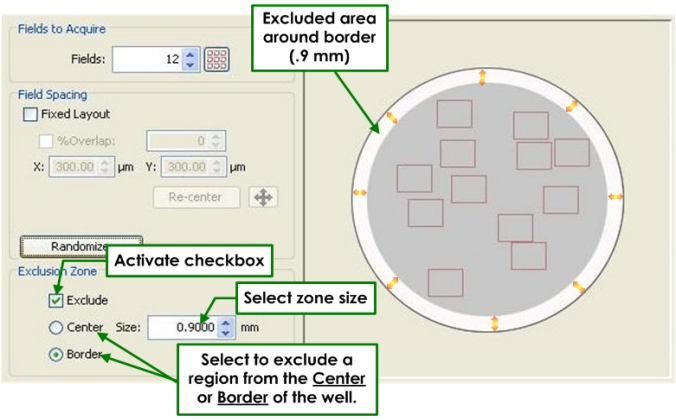
Note: *You can view the fields as they move over the preview scan in the Plate/ Slide View window.*

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 6-10. Excluding specific regions of the well.

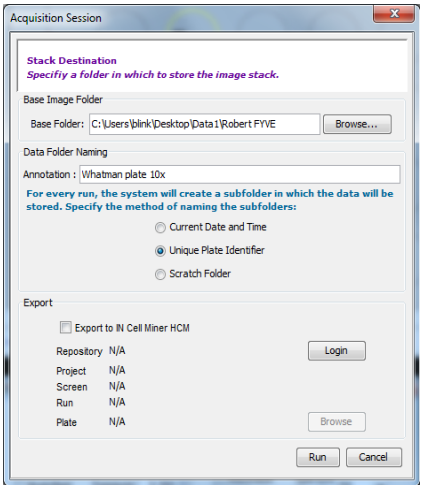


6 When you are satisfied with the settings, close the ***Fields to Acquire*** window.

6.3.3 Start The Acquisition

From ***Main menu |Application Settings***, click ***Start Acquisition Session*** to start an image scan using the currently defined protocol. The ***Acquisition Session*** window is displayed.

Fig 6-11. Acquisition Session window.



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 6000* system.

2 Select the folder containing the image stack to analyze.

3 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.

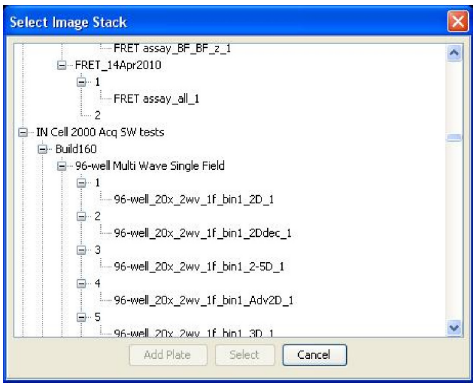
4 In the *Export* section, mark the ***Export to IN Cell Miner HCM*** checkbox to export the acquisition data (*.xdce* file) to ***IN Cell Miner HCM***. You may store the data in the database for future analysis with ***IN Cell Investigator*** or data mining using ***IN Cell Miner HCM***.

5 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 .

6 Click ***Login*** to log in to the ***IN Cell Miner HCM*** application. Return to the ***Acquisition Session*** dialog.

7 Click on ***Browse*** button on the ***Acquisition Session*** dialog to open up the ***IN Cell Miner HCM*** project browser window.

8 Select the target Project, Screen, Run and Plate. You will see a dialog similar to one below.

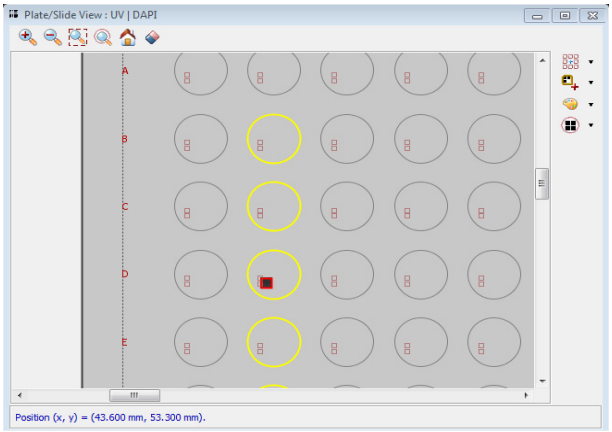


9 Click the ***Run*** button to start the acquisition session and import data to ***IN Cell Miner HCM***.

6.3.4 Monitor the Acquisition Session

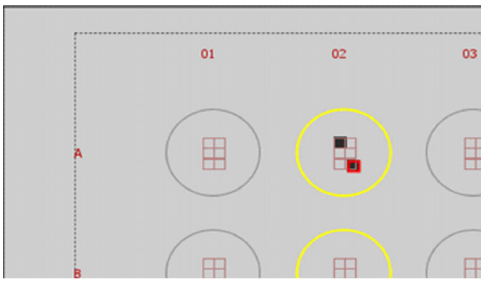
In the upper-left portion of the Activity Workspace use the ***Plate/Slide View*** window to monitor the acquisition process.

Fig 6-12. Plate / Slide View window.



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 6-13. Enlarged view of the Plate/Slide View window.



Use the toolbar options to manipulate the wells.

Button	Function	Description
	Zoom in	Enlarges the displayed image. See *Section 4.3.1.1.3 Image magnification.*
	Zoom out	Reduces the size of the display. See *Section 4.3.1.1.3 Image magnification.*
	Zoom for selection	Zooms to a desired portion of the plate. See *Section 4.3.1.1.3 Image magnification.*
	Zoom to Well	Zooms to a selected well. See *Section 4.3.1.1.3 Image magnification.*
	Reset zoom	Resets the display to its original size. See *Section 4.3.1.1.3 Image magnification.*

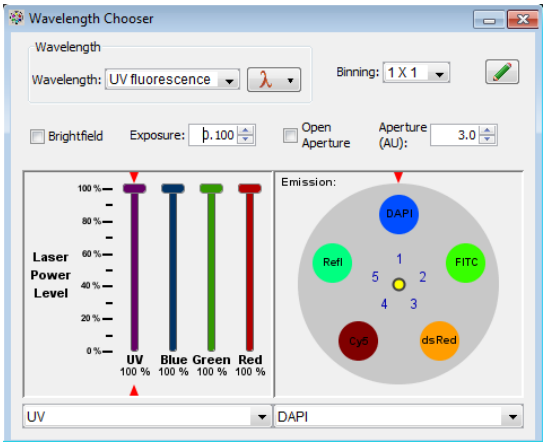
Button	Function	Description
	Clear Plate View	Clears any image preview data (thumbnails) from the Plate/Slide View window.

Button	Function	Description
	Setup Preview	Previews a selected region of the plate or slide. See *Section 4.3.1.1.1 Set up preview scan area*. Click the arrow to open the ***Preview Scan*** from which to set exposure time and resolution/binning options.
	Fields to Acquire	Opens ***Fields to Acquire*** window to define the system's image acquisition on specific regions of interest.
	Visuals	Displays the ***Visuals*** window showing a line graph of the current image. See *Section 4.3.1.1.3 Image magnification*.
	Show Current Well	Displays the ***Current Well*** window. Click the arrow to open the ***Set Current Well*** window from which to select a location.

6.3.5 Monitor the Wavelength Settings

The ***Wavelength Chooser*** window is displayed to monitor the system's wavelength settings during an acquisition. 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. Additionally, the ***Wavelength Chooser*** can be used to explore other choices once the acquisition has completed. Refer to *Section 4.3.7 Wavelength Chooser* for more detail.

Fig 6-14. Wavelength Chooser.

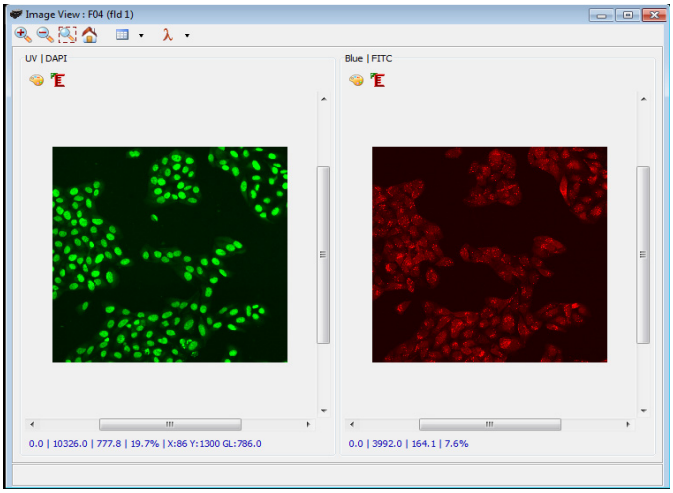


6.3.6 Viewing Acquired Images

The ***Image 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 ***Image 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 *Section 4.3.4 Image View* for more detail.

Fig 6-15. Image View window set to two wavelengths.



Use the toolbar options to manipulate the wells.

Button	Function	Description
	Zoom in	Enlarges the displayed image. Refer to *Section 4.3.1.1.3 Image magnification.*
	Zoom out	Reduces the size of the display. Refer to *Section 4.3.1.1.3 Image magnification.*
	Zoom to selection	Displays a desired portion of the plate. Refer to *Section 4.3.1.1.3 Image magnification.*
	Reset zoom	Resets the display to its original size. Refer to *Section 4.3.1.1.3 Image magnification.*
	Image View Layout	Sets up the display in the Image Viewer. See *Section 4.3.4.1.1 Image View Layout.*
	Wavelength	Displays the wavelengths window. See *Section 4.3.4.1.2 Wavelength Selection.*
Wavelength toolbar		
	Visuals	Opens the ***Visuals*** window. Refer to *Section 4.3.1.1.4 Adjusting image appearance.*
	Ruler	Enables the ***Ruler*** feature. Refer to *Section 4.3.3.1.1 Ruler feature.*
	Digitize	Enables re-imaging upon making modifications to an acquired image.

7 Data Review Mode - Reviewing Collected Data

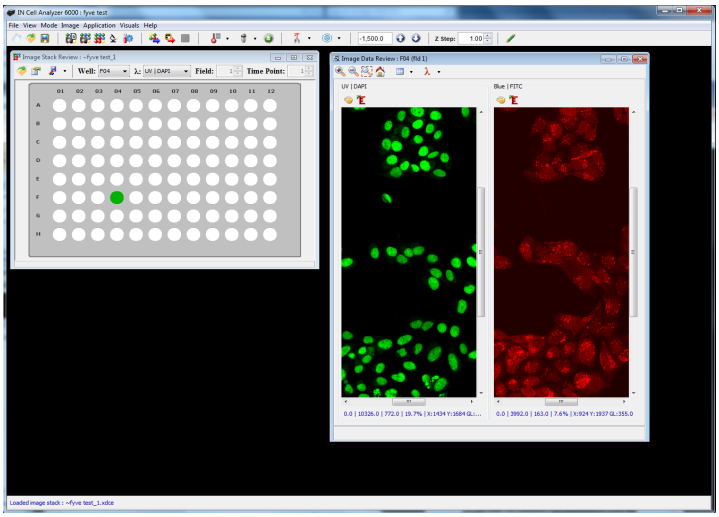
The Data Review Mode allows you to review acquired image stacks and their associated data.

7.1 Viewing Acquired Images

To view the acquired data, open to *Data Review Mode* by either:

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

Fig 7-1. Data Review Mode.

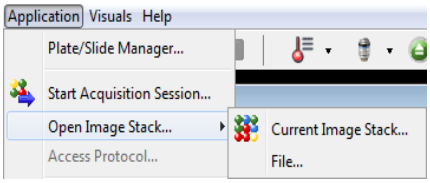


7.1.1 Open the Image Stack to View

From ***Application |Open Image Stack***:, select:

- ***Current Image Stack*** to open the stack currently active in the IN Cell Analyzer 6000 system.
- ***File...*** to browse to and select a different image stack.

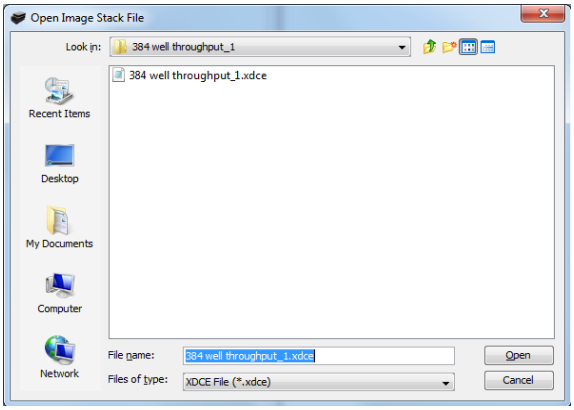
Fig 7-2. Main Menu|Application Menu.



7.1.1.1 Opening an Image Stack (.xdce)

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

Fig 7-3. Open Image Stack File Window.

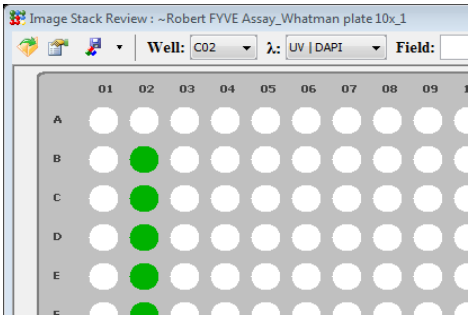


2 Browse to the location of the image stack file *(.xdce)* and select ***Open***. When the image stack file is finished loading, a status message is displayed at the status bar of the IN Cell Analyzer 6000 application window.

7.1.2 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.

Fig 7-4. Image Stack Review window.



The ***Image Stack Review*** window contains a toolbar for using the Image Stack Review features.

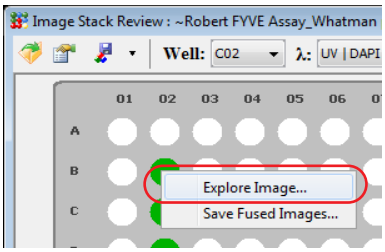
Item	Function	Description
	Open Image Stack file	Displays the ***Open Image Stack File*** window. Browse to the location of the image stack file (.xdce).
	Properties	Opens the ***Image Properties*** window, listing the acquisition protocol attributes.
	Save Fused image	Saves fused images for the selected wells, at the selected field and time point. See *section 4.3.2.1.2 Fused Images*. Clicking the arow opens a dropdown list to select the images to fuse.
Well: C02	**Well Number**	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 ***Image Data Review*** window.
λ: UV | DAPI	**Wavelength**	Use the drop-down list in the ***Wavelength*** field to select the wavelength pair you want to examine.
Field: 1	**Field Number**	Use the arrow keys in the ***Field*** portion of the toolbar to select the specific field you want to examine.
Time Point: 1	**Time Point**	Use the arrow keys in the ***Time Point*** portion of the toolbar to select the specific time point you want to examine
Z Section: 1	**Z Section Number**	Use the arrow keys in the ***Z Section*** field to select the specific Z Section you want to examine.

7.1.3 Image Explorer / Image Data Review window

The ***Image Data Review*** window is designed to show you the acquired image data from each of the defined wavelengths.

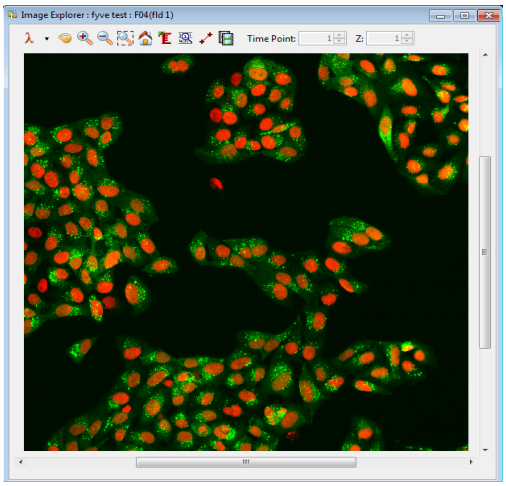
7.1.3.1 To Open Image Explorer:

From the ***Image Stack Review*** window, right-click on any of the wells and select ***Explore Image*** from the pop-up menu. More details regarding the ***Image Explorer*** window are provided in *Section 4.3.6 Image Explorer (Data Review Mode)*.



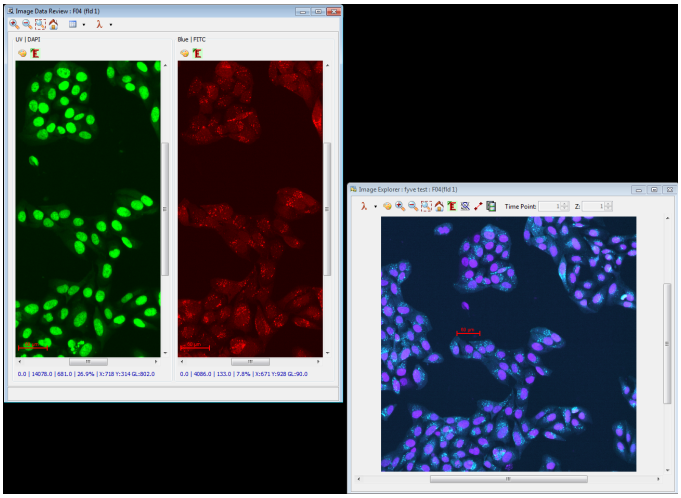
The ***Image Explorer*** window is displayed.

Fig 7-5. Image Explorer Window.



You can use the ***Image Explorer*** window to display specific information about the specified field or well.

Fig 7-6. Image Explorer Window.



Using the ***Image Explorer*** toolbar at the top of the window, you can modify the displayed image, measure distances between points, and generate movies from Z stacks.

The ***Image Explorer*** window contains a toolbar for using the ***Data Review*** features. When you have multiple views set up in the **Image Data Review** window, each of the views will also contain individual ***Visuals*** and ***Ruler*** tools.

Button	Function	Description
	Wavelength	Displays the ***Wavelengths*** window. Refer to *Section 4.3.4.1.2 Wavelength Selection.*
	Visuals	Opens the ***Visuals*** window. Refer to *Section 4.3.1.1.4 Adjusting image appearance.*
	Zoom in	Enlarges the displayed image. Refer to *Section 4.3.1.1.3 Image magnification.*
	Zoom out	Reduces the size of the display. Refer to *Section 4.3.1.1.3 Image magnification.*
	Zoom to Selection	Displays a desired portion of the plate. Refer to *Section 4.3.1.1.3 Image magnification.*
	Reset zoom	Resets the display to its original size. Refer to *Section 4.3.1.1.3 Image magnification.*
	Display Ruler	Enables the ***Ruler*** feature. Refer to *Section 4.3.3.1.1 Ruler feature.*

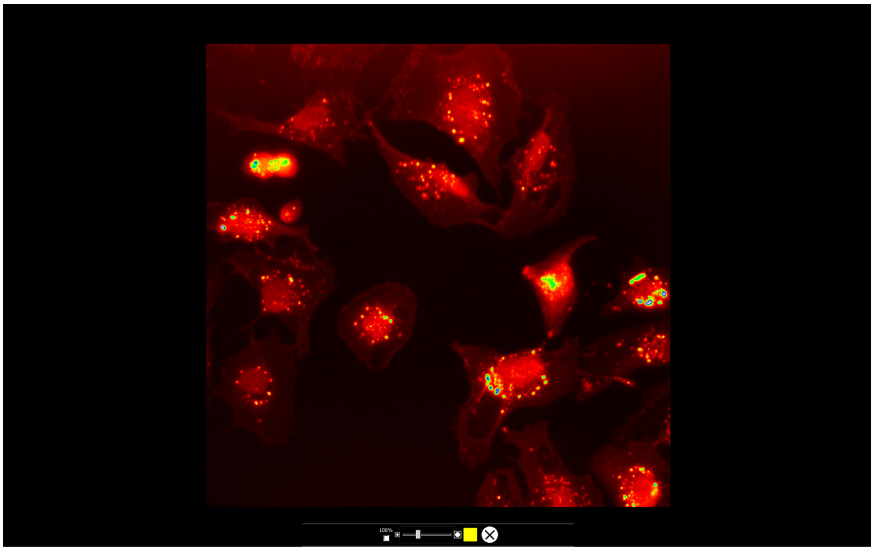
Button	Function	Description
	Point Values	Used to determine wavelength intensity values at points within an image. Refer to *section 4.3.6.1.1 Point Values: Capturing Wavelength Intensities of Points within an Image.*
	Measure	Used to calculate distances between various points within the image data to determine exact locations for regions of interest. See section *4.3.6.1.2 Measure: Calculating Distances Between Points in an Image.*
	Movies	Generates movies from multiple images, such as time series and Z-stack acquisitions. See *Chapter 9 Creating an Image Slideshow.*
Time Point: 1	**Time Point Selection**	Selects a time point to view the corresponding images.
Z: 1	**Z Section Selection**	Selects a Z-section plane to view the corresponding images.

8 Microscope Mode - Using IN Cell Analyzer 6000 Full-screen

When you switch the IN Cell Analyzer 6000 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  icon. The ***Microscope Mode*** screen is displayed using your entire display area.

Fig 8-1. Microscope Mode



8.1 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.

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.
	Pans the zoomed image.
	Exit Full-screen mode (Esc).

8.2 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.

The following features are available from the ***Acquisition*** toolbar:

Button	Function
	Perform Laser Autofocus.
	Perform Software AutoFocus.
	Z Position (in µm).
	Increase Z Position by Z Step.
	Decrease Z Position by Z Step.
	Z Step.
	Toggle to enable the 'joy stick' allowing the mouse to manipulate the display.
	Pin button - toggle to pin and unpin the Acquisition toolbar.

8.2.1 Accessing Hidden Tools

Pressing the ***spacebar*** in Microscope Mode, when viewing an image, converts the standard mouse pointer to a red *reticle* cursor 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 image 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).

8.2.2 Image Acquisition 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.

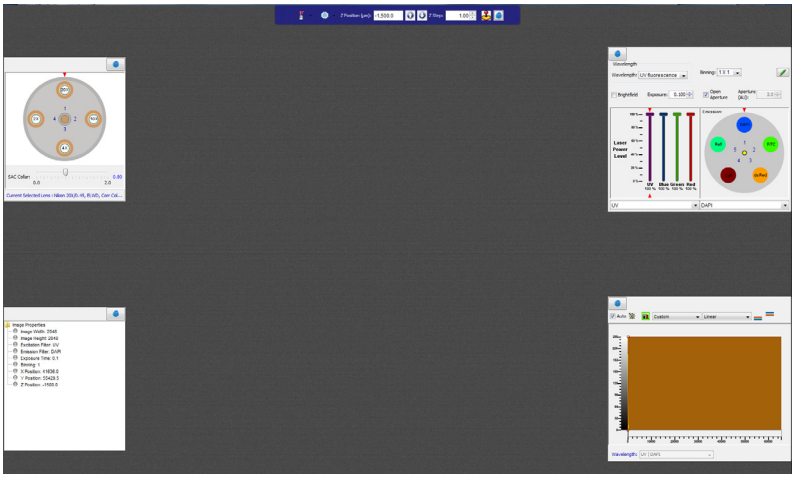
1 From the ***Microscope Mode*** screen, move the mouse to the right or left edge of the screen. The following acquisition tools will pop-up:

Tool	Reference
Objective Changer	Move the mouse to the upper-left edge of the screen; the active objective is denoted by red arrow at the top of the ***Objective Changer*** window . *Section 4.3.8 Objective Changer*
Image Properties	Move the mouse to the lower- left edge of the screen; ***Image Properties*** window provides data for the selected image file. *Section 4.1.1.4.1 Properties*

Tool	Reference
Configure Wavelength	Move the mouse to the upper-right edge of the screen; the ***Wavelength Chooser*** displays the current wavelength settings. *Section 4.3.7 Wavelength Chooser.*
Visuals	Move the mouse to the lower-right edge of the screen; the ***Visuals*** window represents the various intensities of the selected wavelength; manipulate the line graph from either end point to scale the image contrast intensity. *Section 4.3.1.1.4 Adjusting image appearance.*

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 8-2. Microscope Tools windows.



8.2.3 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.

Numeric Keypad:	
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).

9 Creating an Image Slideshow

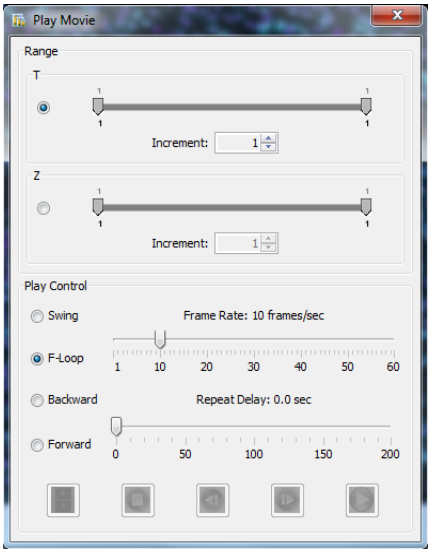
9.1 Generating Movies - Data Review Mode only

From the ***Image Explorer*** window, you can generate movies from multiple images. This feature is most useful for viewing time series, and serial Z-sections.

To use the ***Movie*** tool in the ***Image Explorer*** window, right click on a thumbnail image and choose ***Explore Images***. The ***Image Explorer*** window appears:

1 From the ***Image Explorer*** toolbar, click the icon. The ***Play Movie*** window opens.

Fig 9-1. 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, select one of the control options by marking its radio button:

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

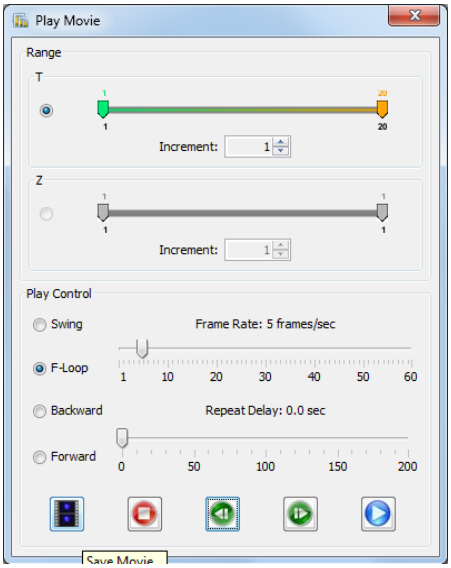
5 Adjust the ***Repeat Delay*** slider to adjust the amount of delay between replays.

6 The control buttons at the bottom of the ***Play Movie*** window function as follows:

Button	Description
	Play- play the currently active Z stack as a movie.
	Stop - 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 as shown: Adjust the **Z Range, Z Increment, Compression Quality**, and **Frame Rate** as desired. Then click [Save] to save the movie.

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

Fig 9-2. Save Movie Window when Save Movie icon is clicked.



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

10 Environmental Control

The *Environmental Control Module* delivers humidified CO_2 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 CO_2 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% CO_2. Regulated CO_2 to the gas flow controller should be at 20 psi. The controller is rated for a maximum inlet pressure of 1.4 bar (~20 psi or 138 kPa). The water bottle is rated for a maximum of 2.1 bar (~30 psi).

10.1 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% CO_2 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 6000 system should be allowed 60 minutes to come to thermal equilibrium. Insert the microplate directly from an incubator into the IN Cell Analyzer system after it has come to thermal equilibrium.

To use the Environmental Control Module:

1. Turn on the plate heater. Allow the system to come to thermal equilibrium.
2. Supply 25% CO_2 at 20 psi to the instrument.
3. Turn on the CO_2 in the user interface. **Allow 60 minutes for CO2 to equilibrate through the system.**

4. Dock the stage by selecting ***Main menu>Applications>Eject***. The plate access door opens.
5. Insert a pre-incubated microplate containing live cells.
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.
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 CO_2 delivery to the Environmental Control Module gas control unit at 20 psi from a 25% CO_2 premix cylinder (25% CO_2 premixed in air).
5. A dual stage regulator (Table 1) is required to set the 20 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 6000.

10.1.1 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% CO_2 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 PSIG outlet pressure,0-4000 PSIG 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

10.2 Removing and Replacing the CO_2 Humidifier

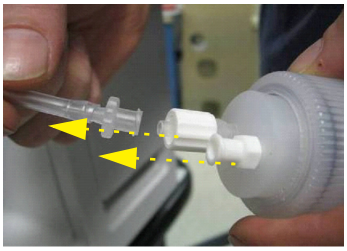
The fluid storage for the CO_2 Humidifier is on the side of the IN Cell Analyzer 6000 system farthest from the access door (outlined in green below).

Fig 10-1. CO_2 compartment access door.

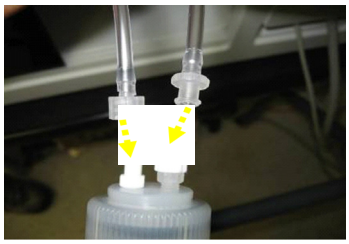
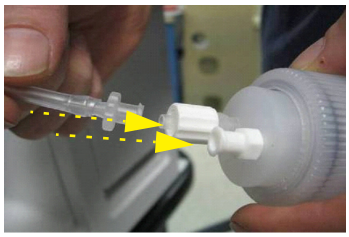
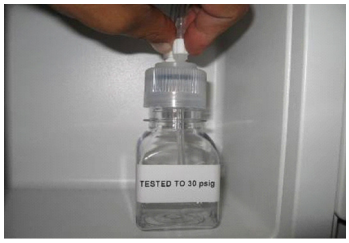
The operator slides a door open to access the fluid storage, removes the tubing from the fluid storage bottle, and refills the bottle.

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

10.2.1 Removing the CO_2 Humidifier Bottle

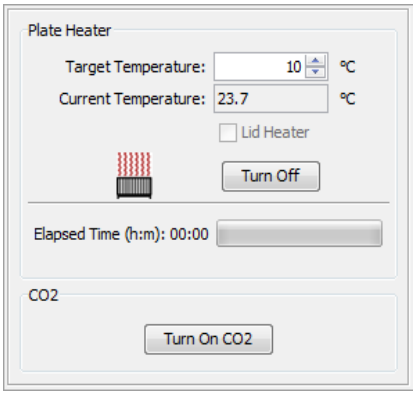
Slide open the plexiglass doors on the fluid storage access area. Gently lift out the CO_2 humidifier bottle.	
Unscrew the tubing from both caps on the humidifier bottle.	
8 Disconnect the two tubes from the bottle. ***Note:*** *The two tubing ends will only fit onto their appropriate connectors on the humidifier bottle. The two tubes are not interchangeable.*	

10.2.2 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 plexiglass doors closed on the fluid storage access area.	

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

Fig 10-2. Environmental Contol dialog



1 Click the ***Turn On CO_2*** button near the bottom of the window.

2 Allow the CO_2 humidifier to run for a minimum of 10 minutes before beginning any experiments.

3 Verify CO_2 humidifier operation by confirming slow bubble production in the humidifier bottle and bubbles moving through the tubing at approximately one bubble every three seconds.

11 Basic System Maintenance

The IN Cell Analyzer 6000 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.

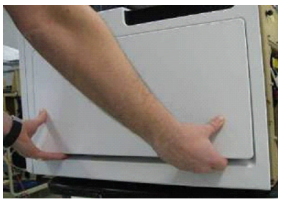
11.1 Removing and Replacing the Air Filter

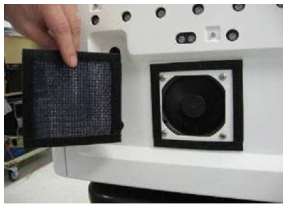
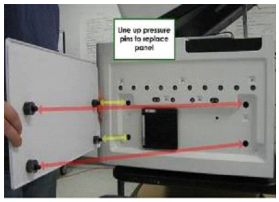
It is necessary to perform this procedure at least every six months (more often if the system is operating in a dirty environment). This will ensure the free movement of air through the system to keep the optics and camera area cool and clean. In this procedure, a portion of the cover is removed and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

11.1.1 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 6000 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 6000 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 IN Cell Analyzer 6000 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 6000 system as normal.

11.2 Daily Checks and Routine Maintenance

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

11.2.1 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.

11.2.2 Environmental Checks

Check that the room where the IN Cell Analyzer 6000 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.

11.3 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.

11.4 IN Cell Analyzer 6000 Instrument

If one of the components in an IN Cell Analyzer 6000 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 cord is connected correctly and that the plug are firmly located in the wall socket.
3. Test the unit again by switching it on.
4. If the unit still does not work, contact your local GE Healthcare service engineer.

11.5 Operating Computer

Please refer to the manual supplied with the operating computer.

Appendix A Site Requirements

A.1 Shipping container

The IN Cell Analyzer 6000 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 6000 weighs 100 kg (221 lbs).

A.2 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

A.3 Moving the IN Cell Analyzer 6000

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.

A.4 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.

A.5 Power requirements

Supply voltage:	100-127/200-240V, 5A/2.5A 50/60 Hz
Maximum power consumption:	500W
Overvoltage category:	Cat II

A.6 Environmental Control Module requirements

May be used at altitudes up to 2000 m

CO2 supply	25% CO_2, externally regulated: 20 psig (1.4 bar). The 25% CO_2 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

A.7 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 6000 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)

A.8 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

A.9 Transport/Storage requirements

Ambient temperature	-40°C to 60°C (-40°F to 140°F)
Relative humidity	10% to 95%, noncondensing

A.10 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 CO_2 supply

A.11 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.

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.

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Notice to purchaser
The IN Cell Analyzer 6000 system is for research purposes only. It is not approved for diagnosis of disease in humans or animals.

GE Healthcare Bio-Sciences AB
Björkgatan 30 751 84, Uppsala, Sweden

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

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/contact

GE Healthcare UK Ltd
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA, UK

www.gelifesciences.com

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