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CellInsight™ CX7 Pro High Content Screening (HCS) Platform

USER GUIDE

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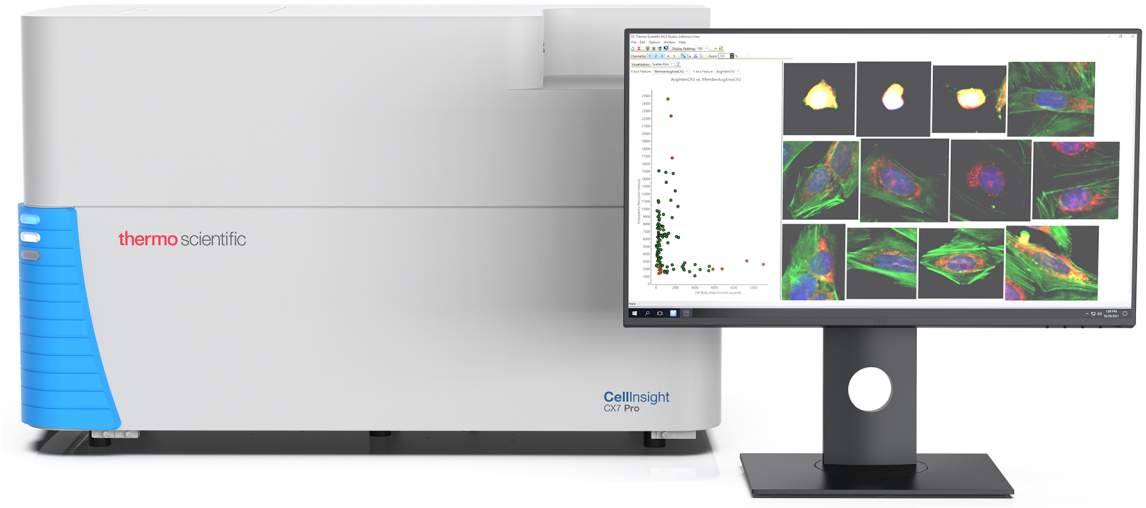

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0026044

Revision	Date	Description
B.0	26 May 2022	Update user guide to include the new light engine and laser specifications, update instrument specifications, consolidate safety precautions in the Safety appendix, add information on the AbCam FirePlex assay and cell painting, and instructions to change the camera mode from 15-bits to 16-bits, update screenshots and instrument images where relevant, expand Replacement parts and accessories appendix.
A.0	12 January 2022	New user guide.

Contents

Introduction

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

This guide is a useful reference tool and contains important safety instructions. The chapters that follow this introduction are described below.

- **Chapter 1, Overview**—provides an overview of the system hardware (see page 9).
- **Chapter 2, CellInsight™ CX7 Pro HCS Reader operation**—provides instructions on system operation (see page 16).
- **Chapter 3, Using the illumination correction tool**—describes how to use the Illumination Correction Image Acquisition Wizard (see page 52).
- **Chapter 4, CellInsight™ CX7 Pro HCS Reader maintenance**—outlines system maintenance procedures (see page 69).
- **Appendix A, Technical specification**—lists the technical specifications of the system (see page 92)
- **Appendix B, Automated spectral selection for fluorescence imaging**—describes the key spectral elements of the 7-color CellInsight™ CX7 Pro HCS Platform that enable its fluorescence imaging capabilities (see page 0)
- **Appendix C, Automated spectral selection for transmitted light imaging**—describes the key spectral elements of the colored brightfield transmitted light imaging capability of the CellInsight™ CX7 Pro HCS Platform (see page 112)
- **Appendix D, How to change the camera setting from 15-bit to 16-bit mode**—provides instructions on how to switch the Pro camera mode from 15-bits to 16-bits (see page 118)
- **Appendix E, Replacement parts and accessories**—provides a list of system replacement parts and accessories (see page 119)
- **Appendix F, Safety**—describes safety precautions and requirements related to the operation and maintenance of the system (see page 120)

Terminology, conventions, and symbols

This guide assumes that you have a basic knowledge of computers using the Microsoft™ Windows™ operating system and that you have experience working with windows, menus, commands, buttons, tabs, dialog boxes, and other Microsoft™ Windows™ elements. If you are unfamiliar with these terms, refer to Microsoft™ Windows™ documentation.

Throughout this guide certain terminology, conventions, and symbols are used consistently. These conventions are described below.

Terminology

Term	Definition
Click	This term means to place the mouse pointer over the item, then depress and release the primary mouse button (usually the left button) in one quick motion
Right-click	This term means to place the mouse pointer over the item, then depress and release the secondary mouse button (usually the right button) in one quick motion
Double-click	This term means to place the mouse pointer over the item, then depress and release the primary mouse button twice in quick succession.
Drag	This term means to place the mouse pointer over the item, depress and hold down the left mouse button, move the pointer (and the object) to some target location, then release the mouse button.
Press	This term means to push and release a key on the keyboard. For example, press the **Tab** key.
Shift Ctrl Alt	When any of these terms appear before any of the above terms, it means to hold down the specified keyboard key while taking the hyphenated action. For example, **Shift-click** means to hold down the **Shift** key while clicking an item.
Shortcut menu	This is a menu that appears when you right-click an item.

Conventions

- Menu names, menu items, buttons, and options appear in bold type. For example: From the **File** menu, select **Save Protocol.**
- Window titles and dialog box names begin with uppercase letters. For example: “The Save As dialog box displays.”

Symbols

Several symbols appear throughout the documentation in order to draw your attention to important information such as operating tips and suggestions, as well as the presence of hazards. Refer to the "Safety" appendix of this guide for a description of each symbol. When these symbols appear on the equipment, consult product documentation to identify the nature of any potential hazard and to determine the actions to perform.

1 Overview

The Thermo Scientific™ CellInsight™ CX7 Pro High Content Screening (HCS) Platform consists of the CellInsight™ CX7 Pro HCS Reader and the CellInsight™ computer.

In combination with the BioApplication and scanning software, the platform images fluorescently labeled cells and other biological samples within the wells of standard microplates and automatically measures the multiparametric effects of cell treatments.

The CellInsight™ CX7 Pro HCS Platform provides the following:

- Complete automation of image acquisition and analysis processes with automated 3 position objective changer and automated filter changes
- Ultra-stable solid state illumination source for Hoechst™, FITC, TRITC, CFP, YFP, Cy5™, and Texas Red™-like dyes, and laser illumination for fluorescent near-IR dyes.
- Wide fluorescence spectral range from the blue to near infra-red, enabling up to 5-color simultaneous multiplexing
- Spectral flexibility through independently controlled multi-position dichroic mirror and emission filter wheels enabling advanced fluorescence imaging modes
- Ability to acquire transmitted light images of chromophore labeled samples through the color brightfield capability
- Ability to image multi-dimensional structures in three dimensions with an integrated confocal unit
- Integrated laser autofocus
- Simple to use software that fits your workflow and gives results in minutes
- An intuitive data viewer that allows you to review plate features immediately after scanning
- An integrated 10 GB Thermo Scientific™ Store Express Image and Database Management Software solution

The CellInsight™ CX7 Pro HCS Reader is pictured in Figure 1.

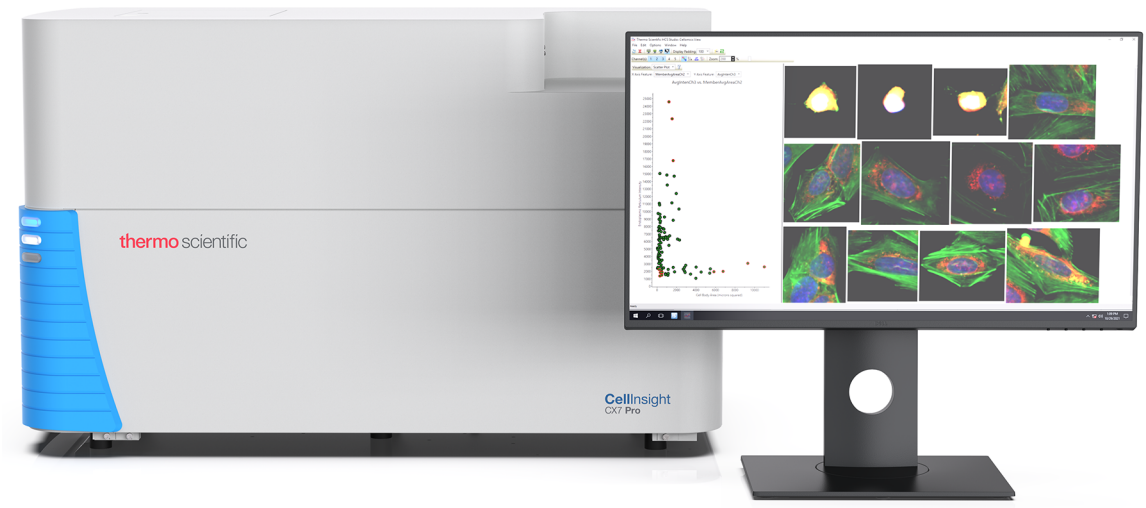


Figure 1 CellInsight™ CX7 Pro HCS Reader

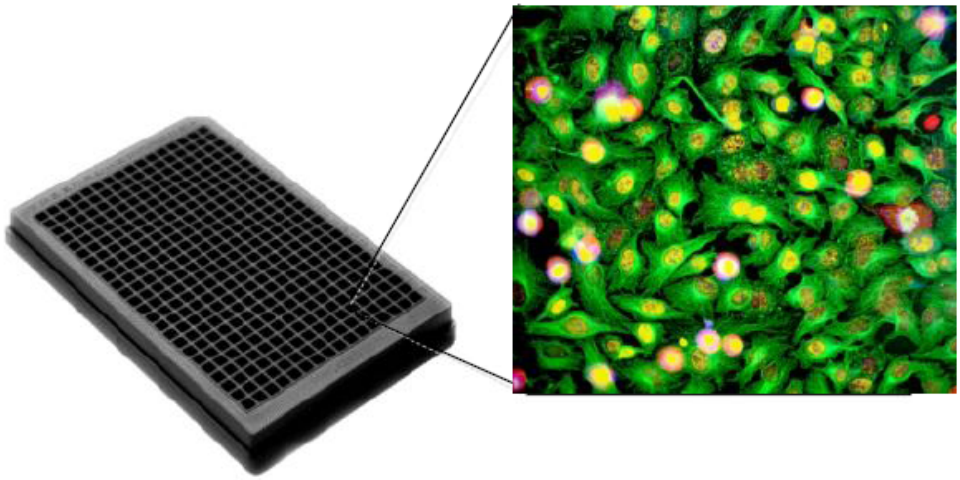


Figure 2 Imaging cells within wells of microplates

The unique optical path of the CellInsight™ CX7 Pro HCS Reader is optimized for performing rapid automated imaging scans in clear-bottom microplates. The reader automatically focuses on a field of cells and acquires images at each selected channel. The installed HCS Studio™ Cell Analysis Software scanning application, Thermo Scientific™ Scan Application, identifies and measures individual features and structures within each cell in a field of cells, so that hundreds of cells are analyzed in parallel. The software then tabulates and presents the results automatically. All of the raw data, including images of individual cells, are available for inspection and analysis.

The software provides for multicolor imaging, automated cell-based image analysis, and data management for archiving, analysis, and creation of reports. An intuitive, graphical user interface guides you through image acquisition and image analysis, as well as data review and reporting.

CellInsight™ CX7 Pro HCS Reader

The CellInsight™ CX7 Pro HCS Reader contains the opto-mechanical hardware, light sources, camera, plate scanning stage, and barcode reader with the following specifications and features.

- Plate Scanning Stage

  Compatible with slides and multi-well plates with up to 1536 wells
- Barcode Reader

  Identifies microplates according to the attached barcode label and automates the tracking and identification of microplates
- Laser Autofocus

  Allows for focusing of cells by defining a reference point where cells are in focus and seeking that position during scanning, thus alleviating the need for well-defined cells and allowing focusing on sparse fields
- Fluorescence Light Source

  High intensity 7-color mixed LED and laser light source providing long term stability for high quality fluorescence imaging
- Transmitted Light Source

  High intensity 4-color LED source providing long term stability for high quality brightfield imaging
- Confocal Imaging

  Integrated confocal spinning disk unit
- Camera

  High-resolution and high sensitivity sCMOS camera
- Objectives

  Multiple objectives for an automated 3-position objective turret providing magnified, detailed images of intact cells and cell components
- Independently controlled 5-position dichroic mirror wheel and 6-position emission filter wheel
- (*Optional*) Robotic Plate Handlers

  Enables barcode tracking and “walk-away” operation.

CellInsight™ computer

The CellInsight™ computer components include a PC connected to a high definition monitor, keyboard, and mouse. The CellInsight™ software, HCS Studio™ Cell Analysis Software, runs on the computer, which controls the essential functions of the CellInsight™ CX7 Pro HCS Reader.

Confocal imaging

The CellInsight™ CX7 Pro HCS Reader offers the ability to enable the latest advancements in multi-dimensional high-content imaging and analysis via Confocal microscopy based imaging. Multi-dimensional structures can be imaged in three dimensions leading to more information and deeper understanding about biological change. Confocal imaging uses the same light source, light path, and filters as the Widefield detection mode.

Confocal imaging provides the following capabilities:

- Spinning disk confocal system with two pinhole sizes
- Robust, long life, seven-color illumination using solid state LEDs and laser light sources
- User defined Z-range with 0.1 micrometer resolution step size for confocal Z-planes
- Multiple options for projections (including images acquired in conventional epi-fluorescence mode, referred to as Widefield illumination mode).

For more information, see “Confocal imaging software setup procedures” on page 35.

Brightfield

The CellInsight™ CX7 Pro HCS Reader also offers the ability to acquire transmitted light images through the Brightfield capabilities.

Highlights include:

- Ability for multiplexed transmitted light illumination with different colors - blue, green, amber (yellow), red, and white - enabling the detection of a wide range of chromophores with high contrast
- Integrated software control for acquisition and focusing of transmitted light images
- Transmitted light images of a field can be added as another channel in addition to the fluorescence images, or used on its own
- Enables chromophore-labeled tissue analysis.

For more information, refer to “Brightfield software setup procedures” on page 29.

Laser autofocus

Laser autofocus defines a reference point where the cells are in focus. The laser then seeks this position when plates are being scanned, which alleviates the need for well-defined cells and allows the instrument to focus on sparse fields.

For more information, see “Laser autofocus software setup” on page 27.

CellInsight™ Onstage Incubator

The CellInsight™ Onstage Incubator is an optional module, which allows you to incubate live cells on the CellInsight™ automated stage. This allows for the capture of images from live cells over time in a controlled environment.

The CellInsight™ Onstage Incubator consists of an environmental chamber located inside the CellInsight™ instrument and a separate exterior control unit that supplies the power and gas (air or air-CO_2 premix, CO_2, and N_2 for O_2 displacement in hypoxia experiments), and controls the humidity and temperature.

For more information about the Onstage Incubator module, refer to the *HCA On-Stage Incubator User Guide* (Part. No. LC07730101).

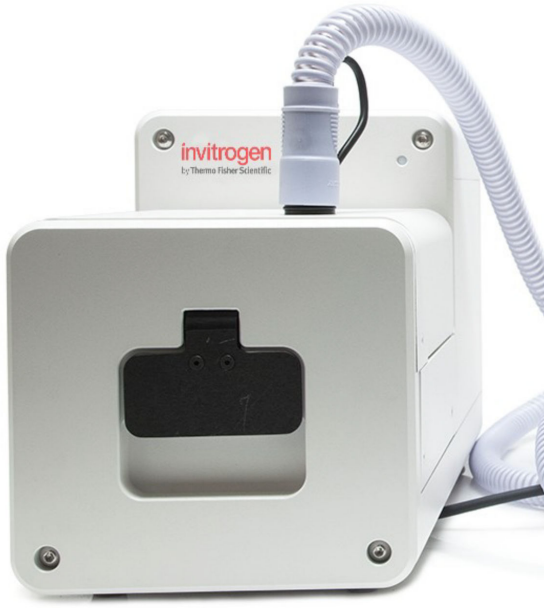


Figure 3 CellInsight™ Onstage Incubator Control Unit

Robotic plate handler

When using an optional robotic plate handler with the CellInsight™ CX7 Pro HCS Reader, you can automatically load plates onto the Reader stage for unattended continuous operation.

Contact Technical Support for a list of integrated robots and for more information on integrating additional robots. For instructions on using the integrated robots with the CellInsight™ CX7 Pro HCS Reader, refer to your robotic plate handler user documentation.

If you are using an Orbitor™ RS Microplate Mover, refer to the *Thermo Scientific™ Orbitor™ RS User Guide* (Pub. Part. No. LC07500100).

HCS Studio™ Cell Analysis Software – data analysis and exploration

The HCS Studio™ Cell Analysis Software includes the Scan Application, BioApplication Software, View Application, Illumination Correction Tool, and optional Robotic Plate Handler software. Each application is described in the following sections.

Scan application

The Scan Application within the HCS Studio™ Cell Analysis Software runs the essential functions of the CellInsight™ CX7 Pro HCS Reader. Refer to online help for more information. To access online help, click on the question mark icon in the right upper corner of the scan application screen. 

BioApplication software

The BioApplication software allows you to perform measurements of fluorescence intensities, variations, and morphometry that may be relevant in determining cellular responses. The applications are multi-parametric; they measure the fluorescence from different fluorophores (and chromophores in some cases) simultaneously. Some of the biological situations that can be evaluated are as follows:

- Membrane permeability
- Presence of several proteins in the nucleus (e.g., transcription factors)
- Expression of GFP in cells
- Presence of molecules in the cell that identify it as being in a particular physiological state
- Identification of specific cells in a mixed cell culture
- Simultaneous measurements in the same cell of the above properties
- Rare event analysis
- Identification of chromophore-labeled antigens in tissue samples.

View application

The Thermo Scientific™ HCS Studio™ View Application software provides the following features:

- Rich visualization of data down to the cell level
- Interactive visual analysis of multi-feature, cell-based data down to the image
- Multi-plate, multi-well, and multi-cell feature comparisons.

Illumination correction tool

The Illumination Correction Tool allows you to monitor the health of your optical system by measuring fluorescence intensity differences within a field, which may change over time. The analysis of a fluorescent standard plate made available as a Reagent kit will produce a report that may describe common optical system changes to include filter degradation and mechanical interferences. If necessary, you may enhance the precision with which cellular measurements are made by correcting the images for the measured spatial heterogeneity, when necessary, before data extraction. The enhancement consists of routine flat field correction. The Illumination Correction Tool is designed to monitor the CellInsight™ CX7 Pro HCS Platform for spatial heterogeneity and correct for abnormalities, when necessary.

Optional robotic plate handler software

If you are using an optional robotic plate handler with the CellInsight™ CX7 Pro HCS Platform, use the robot software to setup and run the essential functions of the robot. For instructions on using the software with the CellInsight™ CX7 Pro HCS Platform, refer to your robotic plate handler user documentation.

2 CellInsight™ CX7 Pro HCS Reader operation

WARNING! This instrument is only to be operated by trained laboratory personnel only. Use this product only in the manner described in this guide. When used other than as specified, the safety protections may be impaired. Refer to the "Safety" appendix in this document to ensure safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, read the instructions, warnings, and precautionary measures supplied in this guide and with accessories. Failure to adhere to safety precautions and/or procedures outlined in this document can result in system failure, personal injury, or death. Thermo Fisher Scientific Inc. shall not be held liable under any circumstances. Thermo Fisher Scientific Inc. does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories. The manufacturer cannot assume any liability for any other applications, possibly also involving individual modules or single parts. This also applies to all service or repair work which is not carried out by authorized service personnel. All claims against warranty will be forfeited in these cases.

CellInsight™ CX7 Pro HCS Reader startup

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

IMPORTANT! If you are using a robotic plate handler with the CellInsight™ CX7 Pro HCS Reader, refer to your robotic plate handler user documentation for system startup instructions.

Start the CellInsight™ CX7 Pro HCS Reader without a robotic plate handler

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

1. Turn on the CellInsight™ CX7 Pro HCS Reader power switch located on the back of the instrument.

   Note: Some instruments can require up to 45 seconds for hardware intialization after turning on the instrument power switch before opening the scan software.

2. Turn on the CellInsight™ computer and the monitor.

The instrument status lights are shown in the following image. A description of each status light color is provided in the following table.

Light Color	Meaning
Blue	The instrument is powered on.
White	The instrument has undergone stage and focus drive calibration and is ready for use.
Green	A microplate scan is occurring.

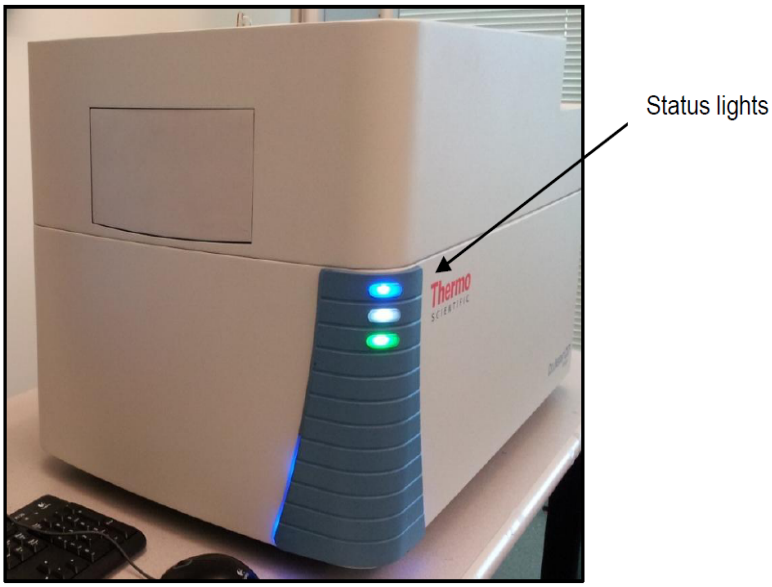
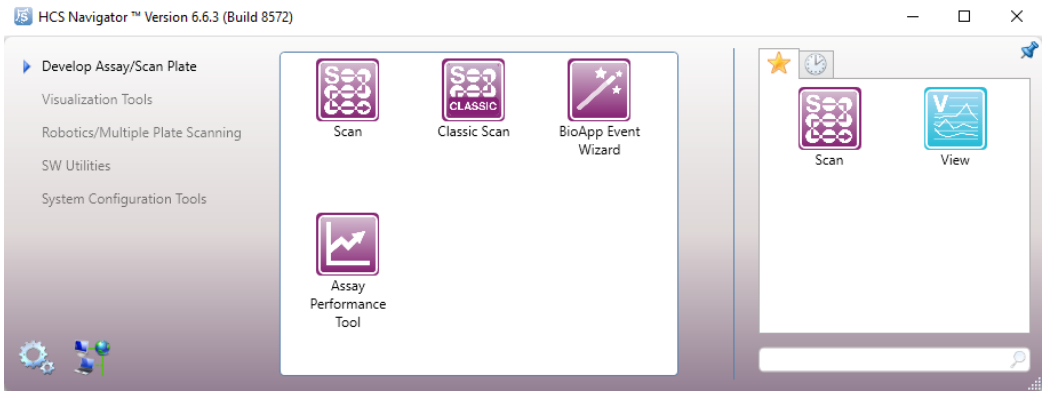


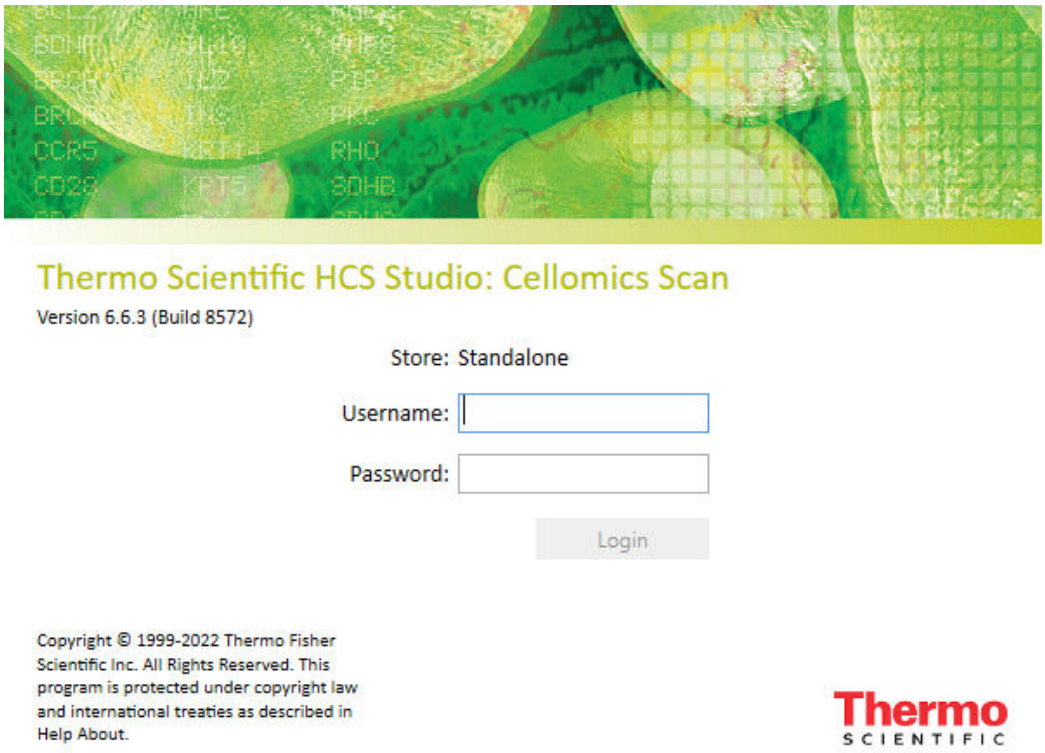
Figure 4 Instrument status lights

Start the scan application

1. Double-click the **HCS Studio™** icon on the desktop.
   The HCS Studio™ Navigator opens.



2. Double-click the **Scan** icon. The Scan Login screen opens.



Log on to the CellInsight™ CX7 Pro HCS Reader

1. Enter your **user name** and **password**. Refer to the *System Configuration Tool Administrator's Guide* (Part. No. LC06310702) for information on how to establish passwords.

2. Click **Login**. Refer to the online help for more information about the software features.

CellInsight™ CX7 Pro HCS Reader startup troubleshooting

If there is a problem during the startup, do the following:

- Verify that all system components are plugged into their power sources. Refer to the Chapter 4, "CellInsight™ CX7 Pro HCS Reader maintenance" in this guide for diagrams showing component connections.
- Check the CellInsight™ CX7 Pro HCS Reader instrument fuse and change it if necessary. Refer to the Chapter 4, "CellInsight™ CX7 Pro HCS Reader maintenance" in this guide for fuse replacement instructions.

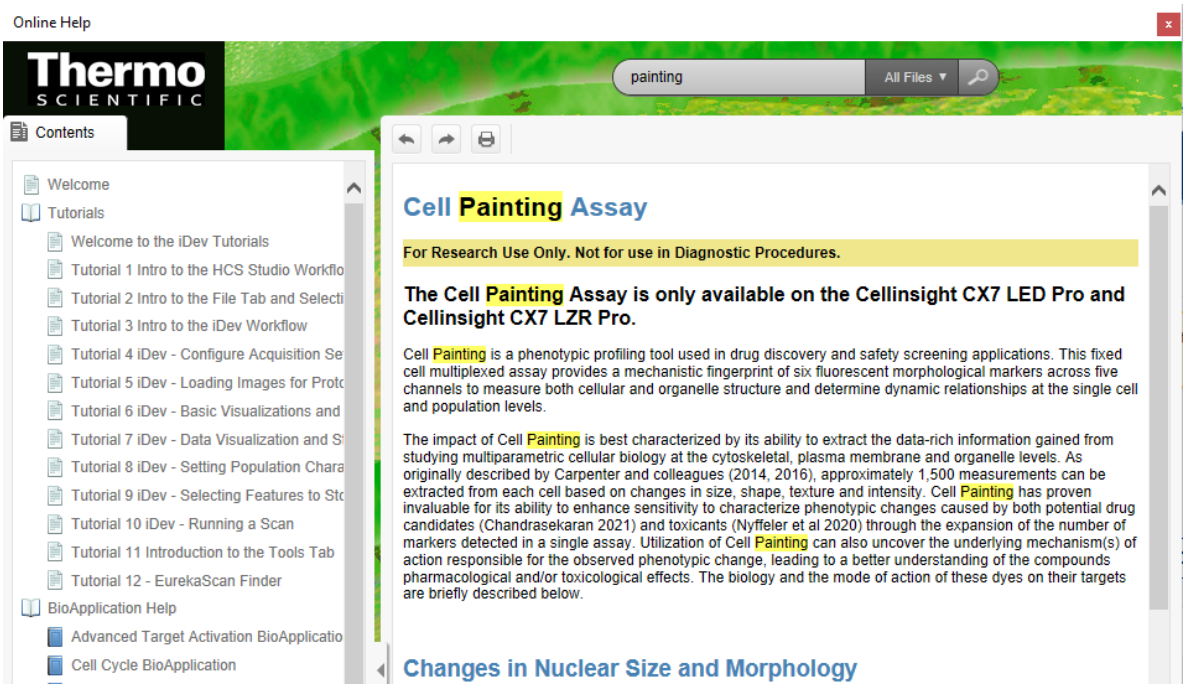
Platform setup procedures

Before scanning a microplate on the CellInsight™ CX7 Pro HCS Platform, you must first perform a series of setup procedures to ensure that the platform is properly configured to scan the microplates. The following checklist provides a summary of the setup tasks you must perform before scanning.

Each checklist item refers you to a specific page within this guide or to an HCS Studio™ online help tutorial topic to obtain more information. If you are referred to an online help topic, follow the instructions below to access the online help.

To access online help from the HCS Studio™ software,

1. Click the **Help** button located in the upper right corner of the HCS Studio™ software.
2. The HCS Studio™ online help appears with the table of contents displayed along the left side of the help screen. Click on the appropriate help topic within the table of contents to view it.



Robotic Plate Handler Setup Procedures

- If using a robot, see your robotic plate handler user documentation.

Setup Tasks for Platform Use

- Label microplates for barcode reader, if desired (see “Label microplates” on page 22)
- Insert microplates in the CellInsight™ instrument (see “Insert a microplate in the CellInsight™ CX7 Pro HCS Reader stage” on page 23)
- Specify barcode settings (see “Specify barcode settings” on page 25)
- Verify that desired objectives are installed (see “Inspect and clean the objective” on page 72)
- Install the desired objective and adjust correction collar, if needed (see “Change the objective” on page 74)

- Verify that the Brightfield unit is in place and specify settings (see “Brightfield software setup procedures” on page 29)
- Create a protocol via Assay or BioApplication template (see Online Help Tutorial 2)
- Use EurekaScan Finder, if desired (see Online Help Tutorial 12)
- Perform system calibration using the Calibration Verification Wizard
- Calibrate the specific form factor using the Calibration Verification Wizard
- Select a Form Factor (see Online Help Tutorial 10)
- Perform Illumination Correction (see “Overview of the illumination correction feature” on page 54)

Setup Tasks for Data Acquisition

- Choose objective (see Online Help Tutorial 4)
- Choose Camera Acquisitions Mode, Binning (see Online Help Tutorial 4)
- Choose Software Autofocus or Laser Autofocus (see “Laser autofocus software setup” on page 27 and Help Tutorial 4)
- Choose number of channels to acquire (see Online Help Tutorial 4)
- Choose Imaging Mode: Widefield, Brightfield, Confocal (see “Confocal imaging software setup procedures” on page 35 and Help Tutorial 4)
- Set exposure and focus settings (see “Laser autofocus software setup” on page 27 and Help Tutorial 4)
- Contrast Stretching (see Online Help Tutorial 6)
- Use Image Projection, if desired (see “Create an assay protocol using the confocal options” on page 35 and Help Tutorial 4)

Setup Tasks for Plate Scanning

- Change reference wells (see Online Help Tutorials 7 and 8)
- Save images and Z-stacks (see Online Help Tutorial 10)
- Select the scan area (see Online Help Tutorial 10)

Robotic plate handler setup procedures

If you are using a robotic plate handler with the CellInsight™ CX7 Pro HCS Reader, refer to your robotic plate handler user documentation for system setup procedures.

Label microplates

If you are using the barcode reader, it is necessary to place a barcode label on the side of each microplate that you want to scan. Refer to the "Additional system specifications" on page 97 in this guide for a list of supported barcode symbologies.

To label the microplate, place the barcode label as shown in the following example for a 96-well microplate. Note the location of well A1.

Figure 5 Barcode label placed on a 96-well microplate

Insert a microplate in the CellInsight™ CX7 Pro HCS Reader stage

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

IMPORTANT! If you are using a robotic plate handler with the CellInsight™ CX7 Pro HCS Reader, refer to your robotic plate handler user documentation for instructions on how to insert microplates and other system setup procedures.

DANGER! **RISK OF EYE INJURY!**

Wear safety glasses designed to filter infrared wavelengths and high intensity visible light. **Wearing laser protective glasses with an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm** is required anytime the instrument is opened for servicing and there is risk of exposure. These glasses will filter infrared wavelengths.

CAUTION! **MOVING MECHANICAL PARTS!**

The CellInsight™ CX7 Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. DO NOT MANUALLY OPEN THE STAGE DOOR WHEN THE READER IS PERFORMING A SCAN!

To load a microplate in the Reader stage,

1. From the Scan Application, choose the **Tools** tab, then select **Load/Unload Plate**. This places the microplate stage in a position that provides easy access to the microplate holder. When the stage is in position, the **Plate Load/Unload** dialog is displayed.

2. If a plate is present, remove the plate by grasping the sides of the microplate and lifting the microplate straight up from the stage insert. To insert a new plate, locate the label marked **A1** on the Reader stage, then rotate the microplate so that the location of well A1 matches the corner with the sticker.

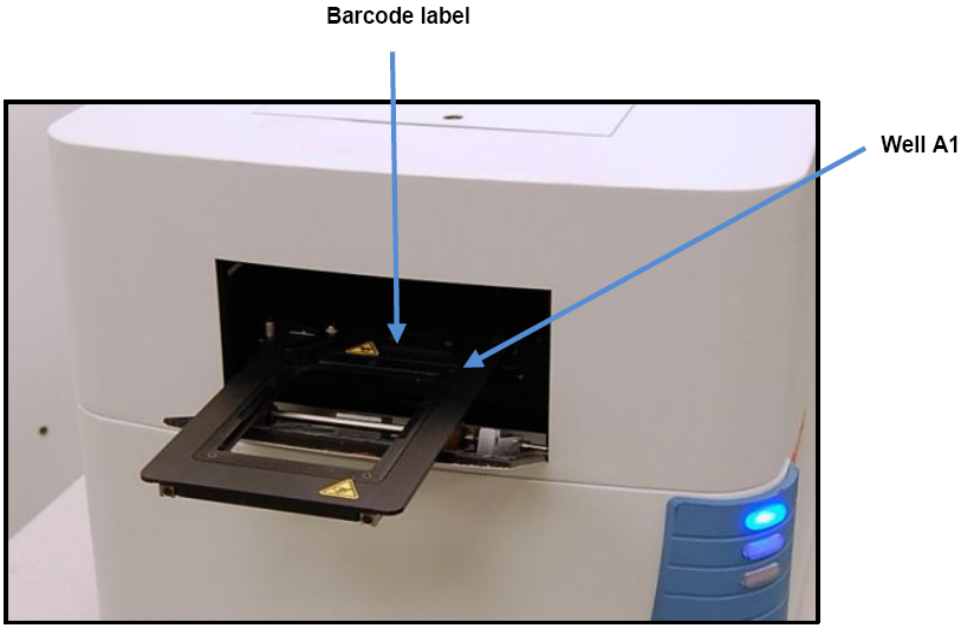


Figure 6 Microplate placed in CellInsight™ CX7 Pro HCS Reader stage

3. Insert the microplate into the stage.
4. Gently press down on the microplate to ensure that it rests flat against the stage.
5. After the plate is in place, press and hold the **Ctrl** key, then click **Ctrl-OK** in the **Plate Load/Unload** dialog.

Specify barcode settings

Note: If you have purchased the optional CellInsight™ Onstage Incubator, and you are using the Robotic Plate Handler, the barcode label is read from a barcode reader mounted to the robot. The procedures described in this section are not applicable to this type of barcode implementation.

WARNING! If the barcode reader is mounted to the Robotic Plate Handler, note the following:

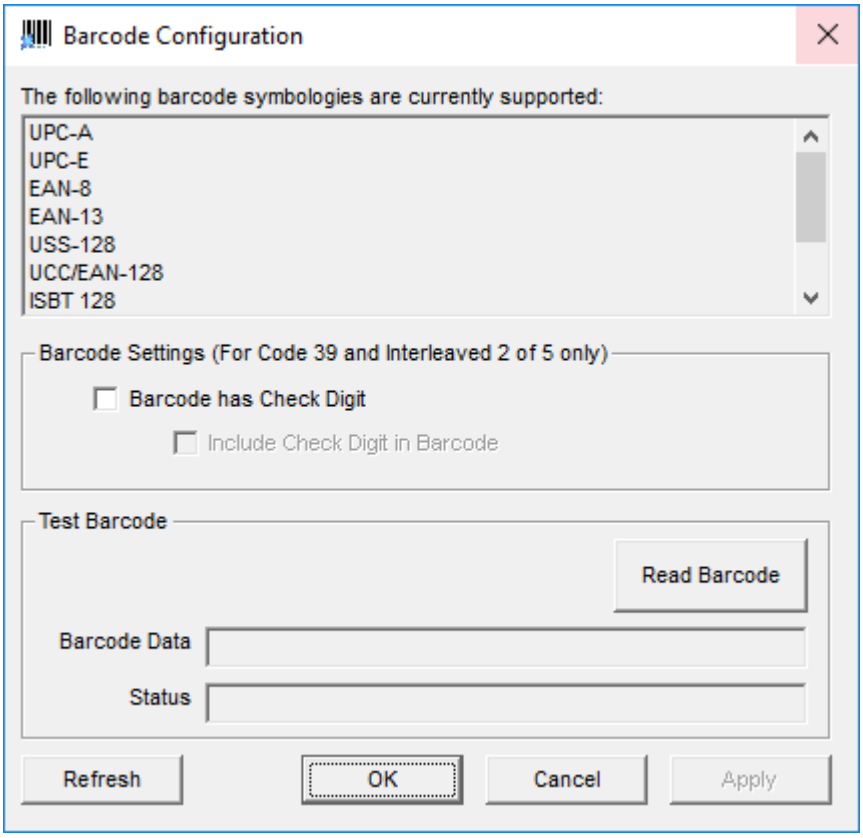
The instrument is equipped with a dual LED barcode scanner. This scanner incorporates an LED device of laser safety class 2. The output power is 1 mW maximum (continuous wave). During operation, laser radiation outside the instrument will occur. Intentional deflection of the beam by means of tools or mirrors may be possible but constitutes an improper use of the instrument.

If you have placed a barcode label on the plate for identification, you can specify barcode settings and test the barcode scan using the **Barcode Configuration** dialog.

To specify barcode settings,

- From the **Tools** tab, select **Configure Barcode**.
  The Barcode Configuration dialog appears.

Note: A list of all supported barcode symbologies appears at the top of the dialog.



If you are using Code 39 or Interleaved 2 of 5 symbologies, you have the option of using and displaying check digits.

If the barcode label includes a check digit,

1. Check **Barcode has Check Digit**.
2. If you want to include the check digit as part of the barcode, check **Include Check Digit in Barcode**.
3. Click **OK** to apply the settings and close the dialog. Click **Apply** to apply the settings without closing the dialog.

To reset the barcode settings to the default settings,

- Click **Refresh**. All settings that are not saved by clicking **Apply** will be lost.

Barcode testing

Note: If you have purchased the optional CellInsight™ Onstage Incubator, and you are using the Robotic Plate Handler, the barcode label is read from a barcode reader mounted to the robot. The procedures described in this section are not applicable to this type of barcode implementation.

The Barcode Reader Testing option allows you to test the barcode label on an individual plate.

To test the barcode reader,

1. Load a labeled microplate in the stage (see instructions and safety precautions that appear earlier in this chapter).
2. From the **Tools** tab, select **Configure Barcode.**
3. Specify the check digit options, if applicable.
4. Click **Read Barcode**.

   The stage moves to the barcode read position and scans the barcode label on the plate, after which the barcode data from the scan is displayed in the Barcode Data field. (This is the Plate ID displayed in the Scan Application.) The barcode symbology type is displayed in the Status box if the barcode is found.
5. Verify that the barcode reader is reading the label correctly. If the barcode label cannot be read, the Status box will display **NO BARCODE**. Verify that the plate is in the stage and the label is placed in the proper location.
6. Click **OK** to apply the settings and close the dialog. Click **Apply** to apply the settings without closing the dialog.

   Note: Contact Technical Support if you wish to discuss barcode options not provided in the Barcode Configuration dialog.

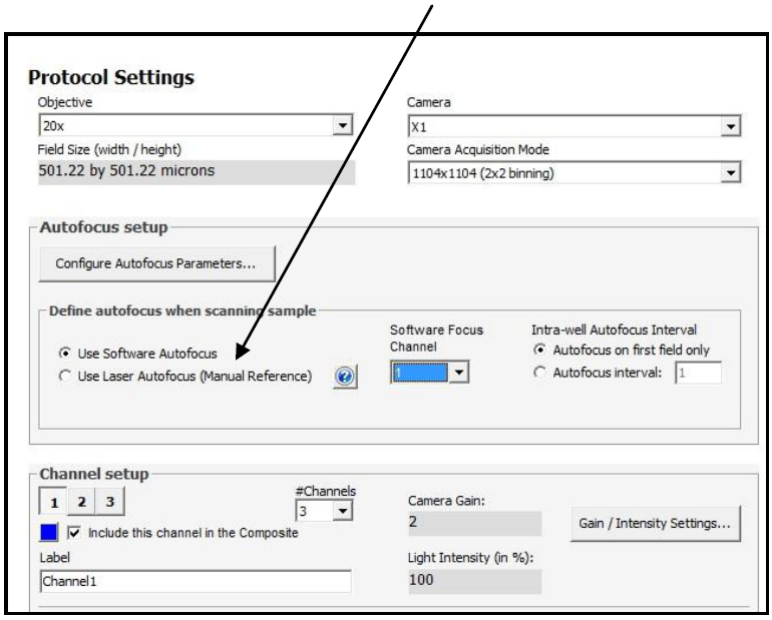
Laser autofocus software setup

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

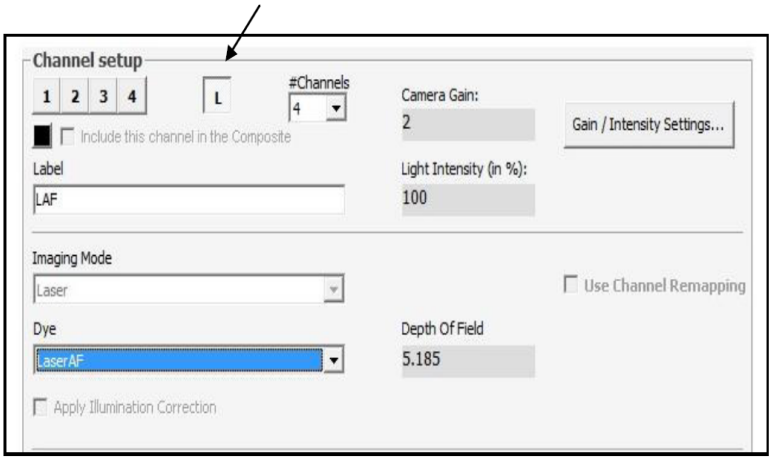
Laser autofocus allows for the focusing of cells by defining a reference point where the cells are in focus. The laser then seeks for this position during scanning of plates alleviating the need for well-defined cells, and allows for focusing on sparse fields.

To enable laser autofocus,

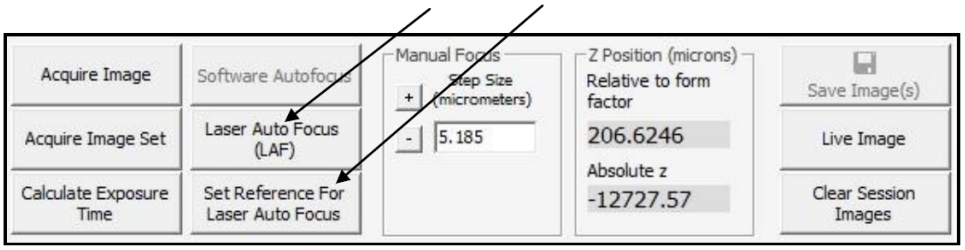
1. Select the **Use Laser Autofocus (Manual Reference)** option in Autofocus setup.



2. A channel labeled **L** is now added to Channel setup, showing you that you have selected to use **Laser Autofocus (LAF)** for focusing during a plate scan. Note that some of the channel setup options are not available for the **L** channel.

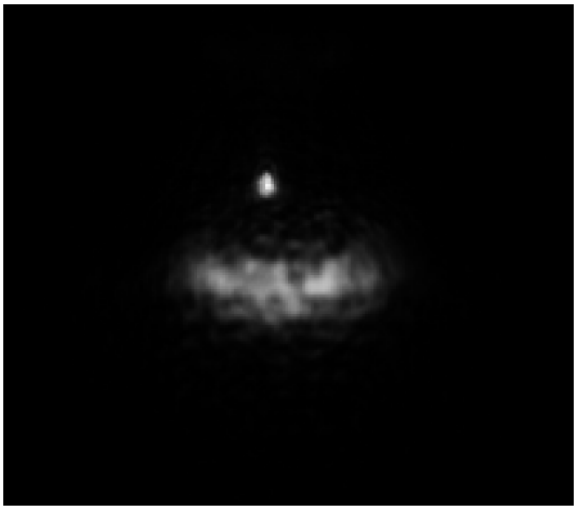


Note: When the Laser Imaging Mode is enabled, two additional controls are available under the Software Autofocus : **Laser Autofocus (LAF)** and **Set Reference for Laser Autofocus**.



To train the laser autofocus,

1. Ensure that the cells are in focus in the software autofocus channel (often channel one).
2. Click **Set Reference For Laser Auto Focus**.
3. Select the **L** channel, then click **Acquire Image**. In most cases, you will see an intense bright spot and a more diffuse semi-circle in the **L** channel image. The following figure shows an example using the 10X objective.



4. Click **Calculate Exposure Time** to set an appropriate exposure time for the LAF.

To verify that laser autofocus position is correct,

1. Move to another well or field in the plate.
2. Click **Laser Autofocus (LAF)**. The software moves to the laser channel during interrogation, then moves back to the channel(s) where the cells are imaged. The sample image must be in clear crisp focus.

   Settings for the laser autofocus are stored in the protocol and will be used for scanning with laser autofocus.

Brightfield software setup procedures

In the **Configure Acquisition** tab of the Scan Application, the Imaging Mode includes a Brightfield option. When mode is set to Brightfield, the available colors for transmitted light Brightfield illumination are displayed in the **Dye** drop-down list. The Brightfield feature may be used to acquire images in any channel, including the focus channel.

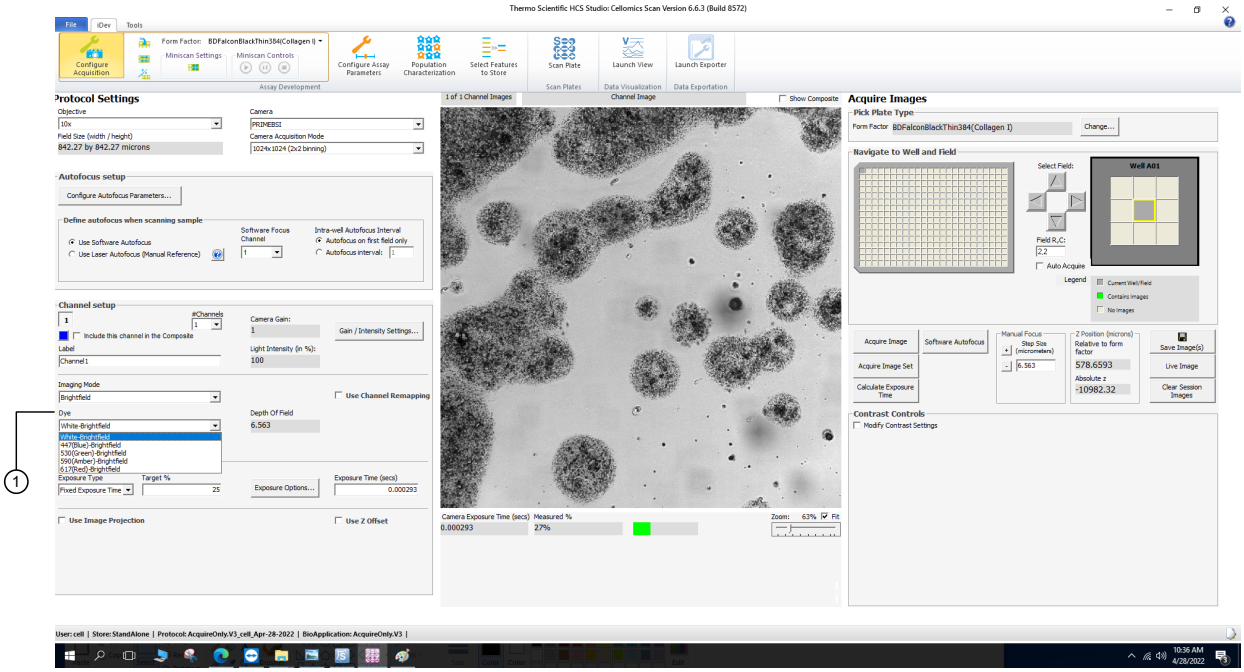


Figure 7 Configure Acquisition tab

① Dye dropdown

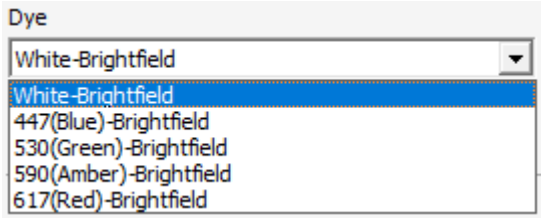


Figure 8 Dye dropdown menu

In addition to performing standard white light brightfield illumination, the Brightfield feature can use different colors of light to illuminate the sample for transmitted light imaging. This takes advantage of the ability of chromophores to differentially absorb light of different wavelengths. In this manifestation, instead of taking a picture of the sample with a color camera, the image is acquired by combining images of the sample illuminated with the appropriate absorbance wavelength of the dye being imaged. For example, a haematoxylin and eosin (H&E) stained slide differentially absorbs blue, green, and red light; it would thus be imaged with Blue, Green, and Red LED wavelengths, and when combined in the Scan or iView Applications would encode to the typical blue nuclei and pink tissue of the stains (see Appendix C, “Automated spectral selection for transmitted light imaging” for more information).

Visualizing brightfield images

To visualize the chromophores similar to what is seen by a color camera, subtractive light mixing is required, because chromophores absorb versus emit the detected light color. This is opposite to what is done for fluorescence, since fluorophores emit photons of the detected color, additive color mixing is used for visualization. In other words, for a multiplexed fluorescence sample with three fluorophores emitting blue, green, and red photons, the color image should visualize the blue fluorescence with a blue pseudo-color visualization, the green fluorescence with a green pseudo-color, and red fluorescence with a red pseudo-color. However, for proper visualization of an H&E stained sample illuminated with blue, green, and red transmitted light, the blue illuminated image should be visualized with a red pseudo-color, the red illuminated image with a blue pseudo-color, and the green illuminated image will remain with a green pseudo-color, as was done in Figure 51 on page 116.

Brightfield imaging and optical density calculations

The user interface provides the ability to work with transmitted light microscopy. For instance, chromological stains are generally measured in ‘optical density’ (OD) rather than ‘intensity’. Optical density measurements are calculated on a per channel basis, and are based on the equation:

$$OD = -\log_{10}\left(\frac{Intensity_c}{Intensity_m}\right)$$

where $Intensity_c$ is the current intensity of the pixel, and $Intensity_m$ is the maximum intensity that is available to the sample.

When a BioApplication, such as the Histology BioApplication, uses OD, there is an additional image setting for visualization within the **Configure Assay Parameters** screen. This image setting is called **OpticalDensityChN**. This image is a negative version of the ‘dark on light’ object type.

Focusing with the Brightfield feature depends on multiple factors including, but not limited to, cell/specimen type, image contrast, per-field object density, objective lens, exposure time, debris, plate type, plate lid, plate seals, condensation on inner surfaces of a seal, and meniscus effects. Typically, the more contrast that is obtained in the image, the more accurate the focusing will be. Additionally, you may have to change the focus parameters from plate to plate and sample to sample when attempting to focus using the Brightfield channel because of the multiple factors involved in determining contrast. Plate scan times may be different when using the Brightfield channels for focusing and should be considered before starting a run. We recommend that you focus on channels that have sufficient fluorescence for object detection when possible.

For more information on using transmitted light brightfield imaging, see Appendix C, “Automated spectral selection for transmitted light imaging”.

Engage the Brightfield unit

Before using the Brightfield unit, you must first verify that the Brightfield arm is in the engaged position.

To verify that the Brightfield arm is in the engaged position,

1. Open the service door as shown in Figure 9.

Note: A key is required to open this door.

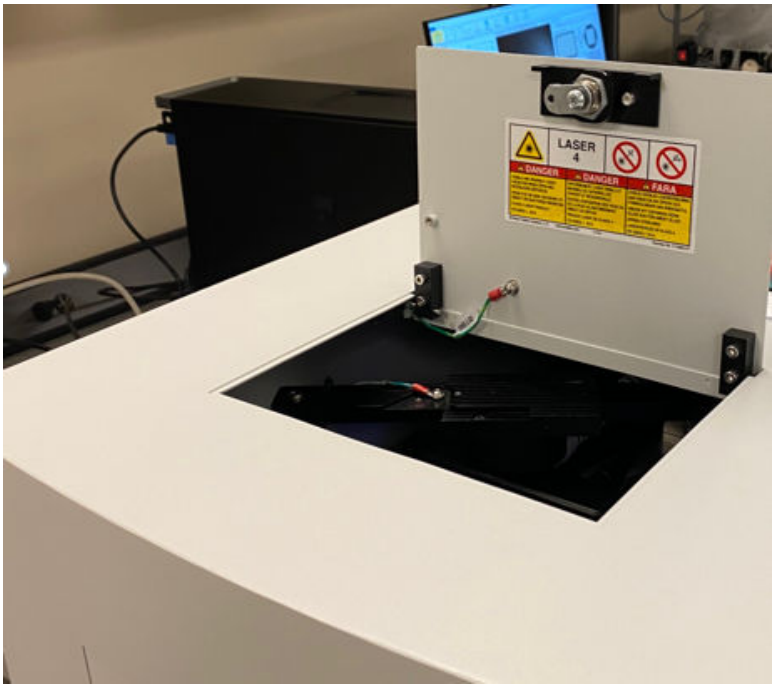


Figure 9 CellInsight™ CX7 Pro HCS Reader service door

2. If the Brightfeld arm is in place (engaged), it will be in the position as shown in Figure 10. If the arm is not in this position, follow the next step to engage the arm.

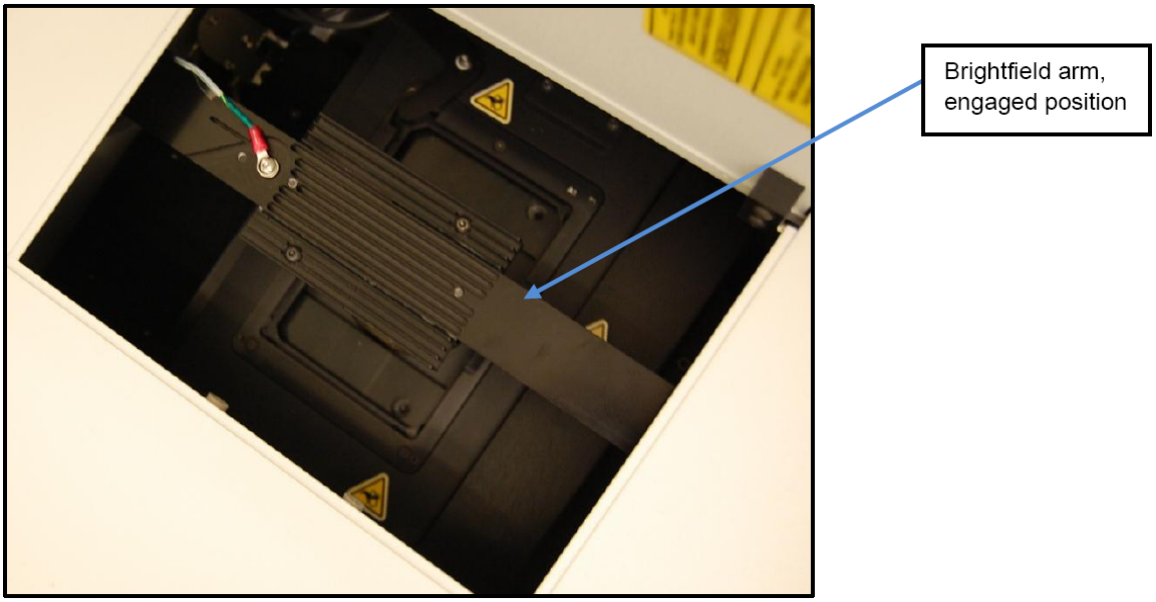


Figure 10 CellInsight™ CX7 Pro HCS Reader open service door with engaged Brightfield arm.

3. If the Brightfeld arm is not in the engaged position (Figure 11), you must physically move the arm to the engaged position. To move the arm, grasp the arm as indicated in the following image, then move the arm until it is in the engaged position (as shown in Figure 10).

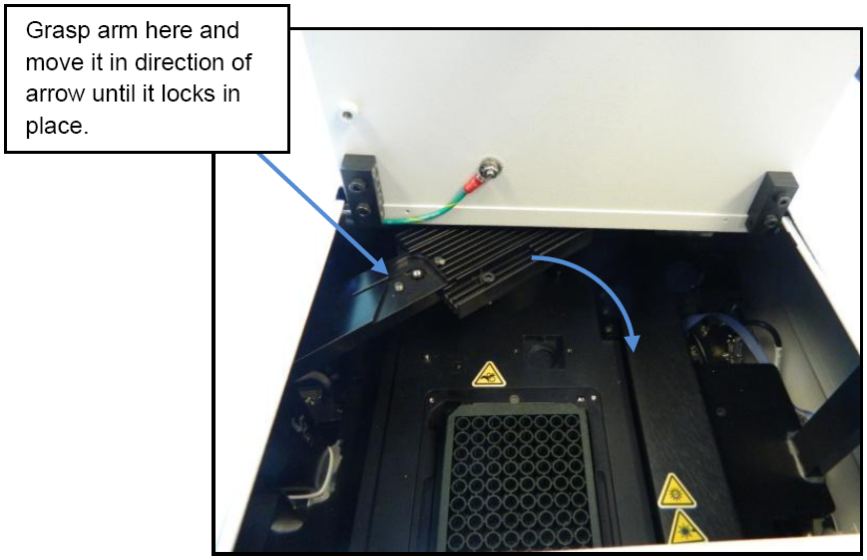


Figure 11 CellInsight™ CX7 Pro HCS Reader open service door with disengaged Brightfield arm.

4. Close and lock the service door.

Note: The service door must be locked or the laser safety interlock will prevent the Laser Autofocus or the near IR laser from turning on.

Specify the Brightfield settings

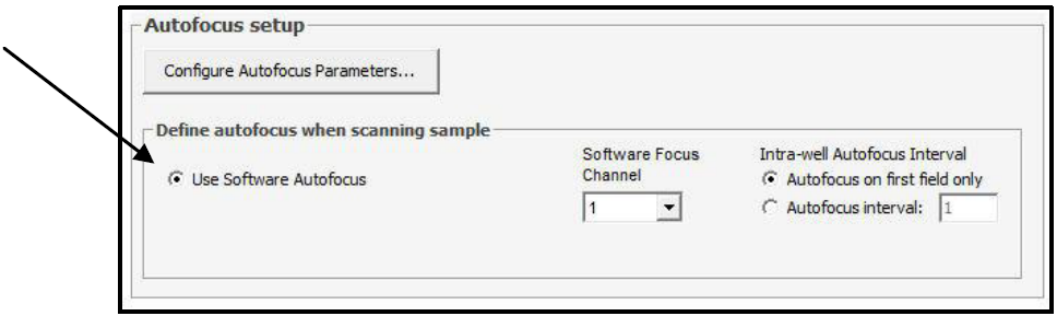
To specify Brightfield channels,

1. From the **Configure Acquisition** icon in the iDev tab of the Scan Application, click on the channel number(s) for the desired Brightfield.
2. From **Imaging Mode,** choose the **Brightfield** option.
3. From the **Dye** drop-down menu, select the desired **Brightfield illumination color**. The Brightfield illumination colors are specified by their wavelength (except for white) followed by the color. The available options are:
   - White-Brightfield
   - 447 (Blue)-Brightfield
   - 530 (Green)-Brightfield
   - 590 (Amber)-Brightfield
   - 617 (Red)-Brightfield.

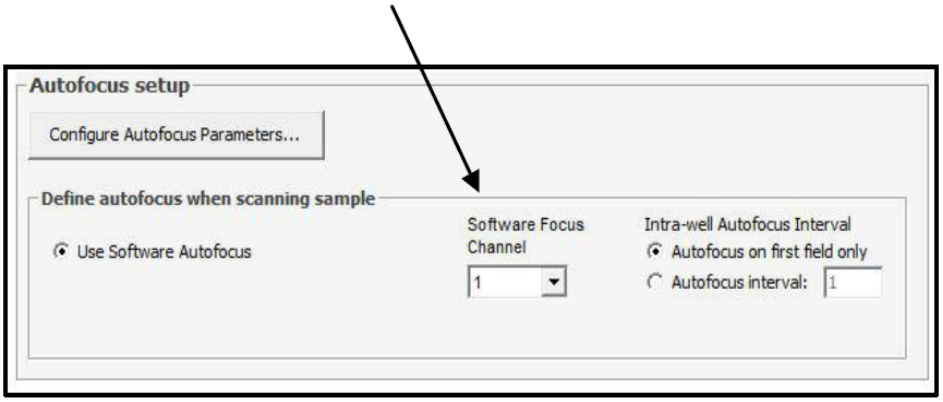
Note: To use software autofocus, continue with the following procedure. Otherwise, refer to "Laser autofocus software setup" on page 27 for instructions on using the laser autofocus option.

To specify a Brightfield focus channel,

1. From **Configure Acquisition**, choose **Use Software Autofocus**.

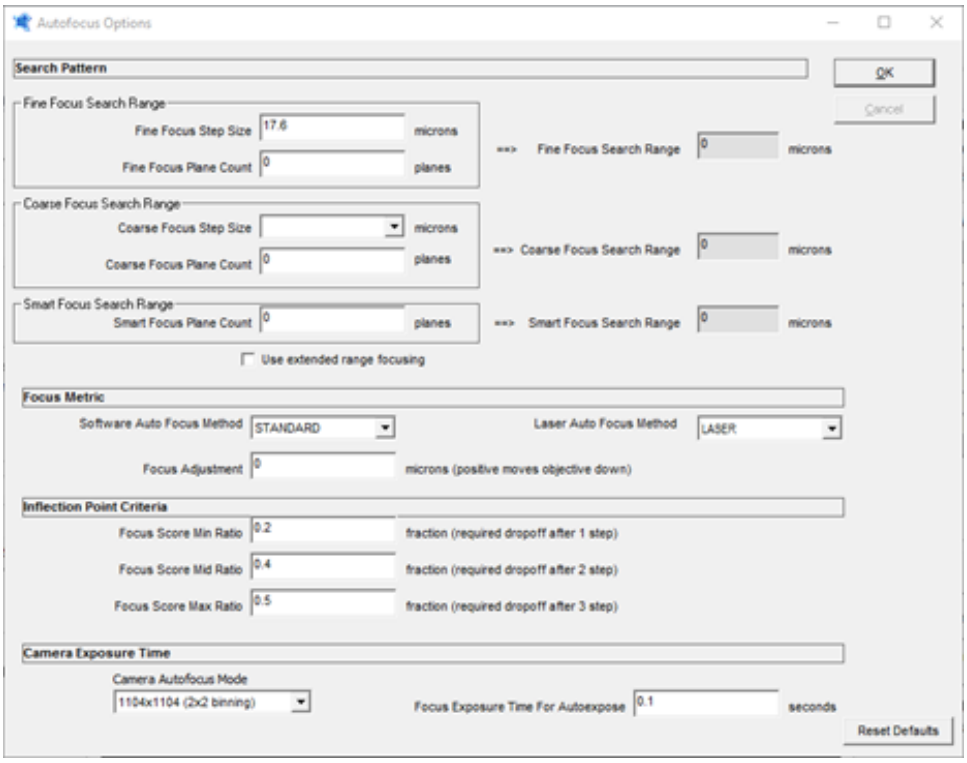


2. From the **Software Focus Channel** drop-down list, select the **Brightfield** channel to use for the Focus channel.



To autofocus using a Brightfield channel image,

1. Select **Configure Acquisition**.
2. Click **Acquire Image**. The image appears in the Image Display area.
3. Click **Configure Autofocus Parameters**. The Autofocus Options dialog appears.



4. For best results, under Focus Metric, select **BRIGHTFIELD** from the **Software Auto Focus Method** drop-down menu.
5. Click **OK**.

Confocal imaging software setup procedures

This section describes how to use Confocal imaging with the Thermo Scientific™ HCS Studio™ Cell Analysis Software on the CellInsight™ CX7 Pro HCS Reader. The following topics are described in detail:

- Creating an Assay Protocol Using the Confocal options
- Scanning a Plate Using Assay Protocols with the Confocal Detection Mode
- Running a Disk Scan

Create an assay protocol using the confocal options

When you want to apply optical sectioning using the Confocal imaging unit, you must specify these unit options in the Assay Protocol. The settings are accessible within the **Configure Acquisition** screen of the HCS Studio™ Cell Analysis Software. Instructions are provided below.

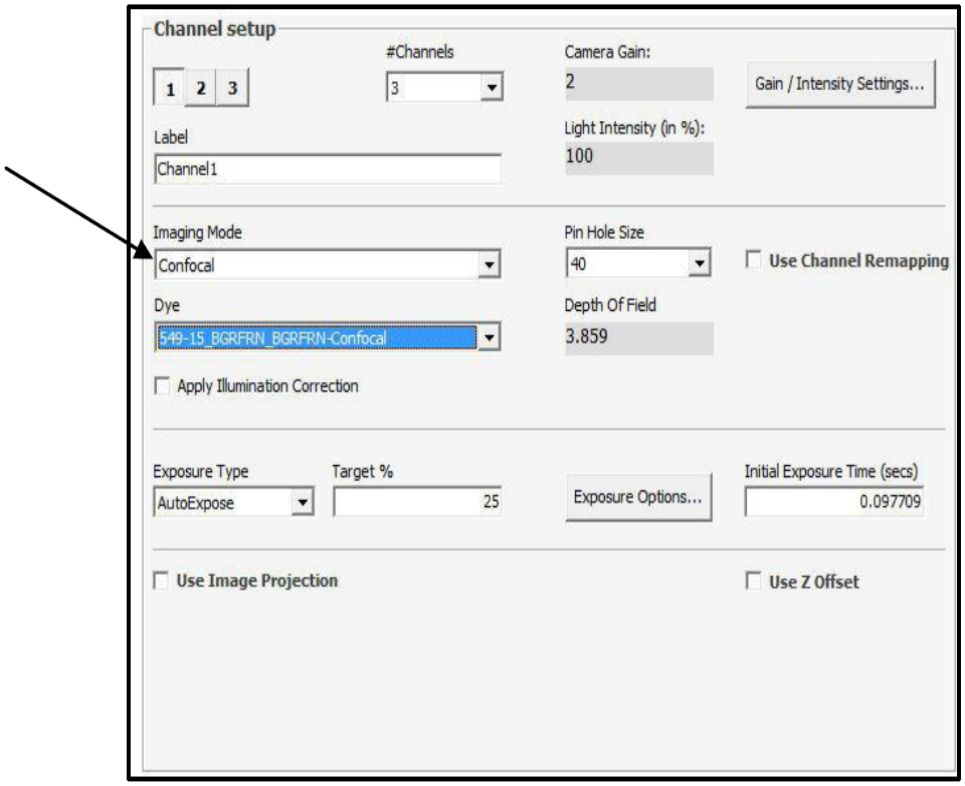
Note: You cannot create an Assay Protocol that requires the Confocal options when working on an instrument that does not have the Confocal unit installed, or the Confocal unit is not enabled via the ArrayScan™ Configuration Tool. By default, the Confocal unit is enabled. If for some reason the Confocal mode does not display under Imaging Mode, ensure that the Confocal unit is enabled and the appropriate filters are available using the ArrayScan™ Configuration Tool.

Specify the Confocal options in HCS Studio™ Cell Analysis Software

This section describes how to implement the Confocal options when working within the HCS Studio™ Cell Analysis Software.

1. Open the **Configure Acquisition** screen. Refer to the software tutorials within the software online help for instructions.

2. Under Channel Specific Parameters, select the desired channel number, then select **Confocal** from the **Imaging Mode** options.



3. The filter options are dependent on the **Imaging Mode** selection. Select the desired Confocal filter set from the **Dye** drop down menu.

   Note the naming convention of the filters. For example: **386-23_BGRFRN _BGRFRN-Confocal** refers to exciting the sample with 386 nm light with a 23 nm range, using a dichroic filter that passes blue (B), green (G), red (R), far red (FR), and near infra-red (N) light, and using an emitter filter that passes BGRFRN light.

4. The 70 micrometer pinhole is set as default when selecting the confocal mode. However, you can choose between the 70 or 40 micrometer pinholes for confocal image acquisition.

Note: Using the smaller 40 micrometer pinhole may significantly increase exposure time to achieve the desired camera saturation. We strongly recommend that you use the high NA objectives for optimal performance.

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5. The Depth of Field is displayed in micrometers. The default depth of field for each objective is listed under **Depth of Field**. This is the depth of field for a single acquired image.

6. Specify the desired Exposure settings.

Note: For shorter exposure times, consider using the 70 micrometer pinhole and 4×4 camera binning, because the image resolution and detail under these settings may be sufficient for your needs. For brighter signal, consider using the higher NA objectives (i.e., the 40X/0.95 or 20X/0.7 objectives).

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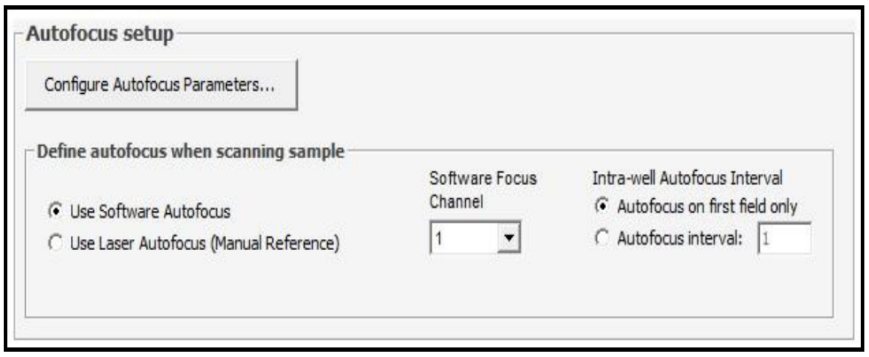
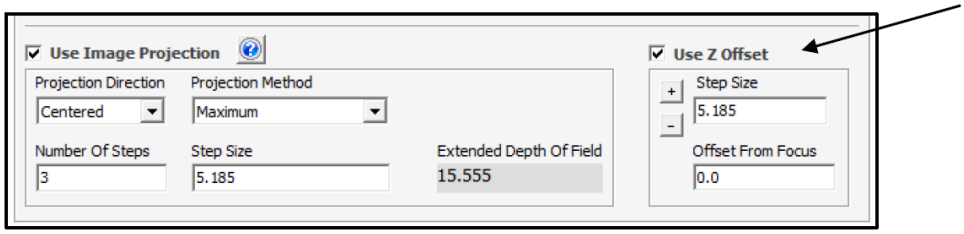
7. From the **Software Focus Channel** drop down, select the channel to be used for the Software Autofocus or Laser Autofocus channel. Specify the Autofocus settings.

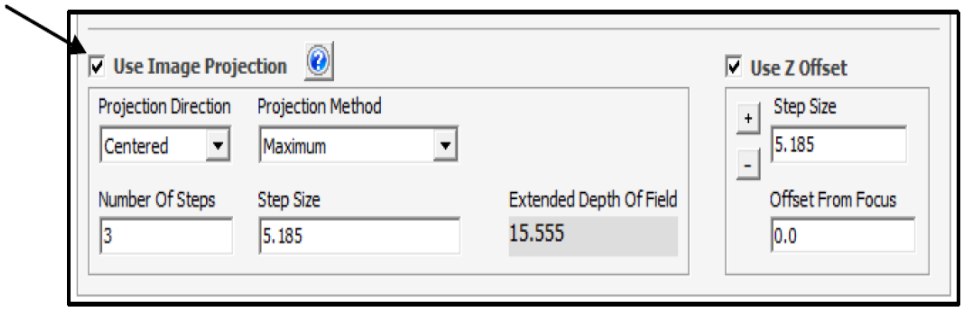


8. Check the **Use Z Offset** checkbox. This is the offset, in micrometers, relative to the Focus position. Negative values indicate a position higher into the well, and positive values indicate a position closer to the bottom of the well. To acquire an image at an offset from the focus plane, enter a step size in the field provided and click **Positive** or **Negative Step Size** to adjust the setting.

Note: You may have to set an offset from focus to obtain sharp confocal images.



9. Check **Use Image Projection** if you want to use a projected image for analysis.



10. The following Projection settings are available. Based on descriptions below and the following Z-stack Projections schematic, adjust the settings as desired.

Setting	Description
Projection Direction	Location of the Z-stack relative to the *Offset From Focus* position, where the possible values are as follows. **Note:** A Z-stack is two or more two-dimensional images of the same field and channel, acquired at different Z-positions. None: No projection Above: Z-stack starts at *Offset From Focus* position and extends higher into the well (negative Z direction) Center: Z-stack is centered about the *Offset From Focus* position Below: Z-stack starts at *Offset From Focus* position and extends toward the bottom of the well (positive Z direction)
Number of Steps	Number of images in the Z-stack, where the distance between each image is defined by the *Step Size.* The value must be greater than 1.
Projection Method	Defines the algorithm used to combine the images from the Z-stack into a single image. The possible methods are as follows: Maximum: On a per-pixel basis, selects the pixel with the largest pixel intensity across the Z-stack. It is the most commonly used image Z-stack visualization method in fluorescence microscopy (bright objects on a dark background Nearest Neighbor Deblurring (NND): Based on optical sectioning followed by software deconvolution, this method improves image quality by eliminating out-of-focus light from image planes adjacent to the focus plane in the z-direction (along the thickness of the sample). Three consecutive images, acquired along the z-axis, are used to create a deblurred middle image. The image on the top and bottom of the triplet can be thought of as out-of-focus haze that was added to the middle image. The deblurred images are then used for analysis and saved to the database. You can select the direction for optical sectioning to be either centered (recommended) around the focus plane, or above or below the focus plane. You can also define the number of images (steps in the z-direction) to be acquired and used in the deblurring process. The minimum required for nearest neighbor deblurring is 3 steps in the z-direction. **Note:** The greater the number of steps, the longer it will take to process the images and hence can lead to slower plate scans. Although the Step Size can be changed, it is recommended that you do not change the Step Size, as this is determined by the system based on the objective magnification and NA. As with other projection methods or channel specific parameter settings, the nearest neighbor deblurring step size and number of steps are saved as part of the protocol. Nearest neighbor deblurring is typically used when you are imaging thick samples such as tissue samples, stem cell colonies, neuronal cells with extending neurites, etc. It can also be used in imaging monolayer of cells, when it is necessary or desirable to improve image quality by removing out-of-focus light, as when imaging rare events such as fluorescent proteins expressed in low levels.

(continued)

Setting	Description
Projection Method *(continued)*	**Note:** The NND method is implemented for the Maximum Projection only. Minimum: On a per-pixel basis, selects the pixel with the smallest pixel intensity across the Z-stack. This projection method is most useful where the object-of-interest comprises dark pixels on a bright background, or when only the background is of interest. Max Min Difference: On a per-pixel basis, calculates the intensity range (maximum minus the minimum) across the Z-stack. This will perform better that the Maximum Projection Method when there is background noise that remains constant across the Z-stack images. Mean: On a per-pixel basis, calculates the average pixel intensity across the Z-stack. This is most useful when the volume of information is across the Z-stack, rather than just the value of pixel maximum (or minimum) intensity, has to be considered. Standard Deviation: On a per-pixel basis, calculates the standard deviation in pixel intensity across the Z-stack. This is most useful for object-of-interest detection in Brightfield (BF) microscopy where the background of Z-stack images doesn't change across the Z-stack while the object-of-interest intensity varies from being above to being below the background.
Step Size	Distance, in micrometers, between each image in the Z-stack. The default value is the *Depth of Field.*

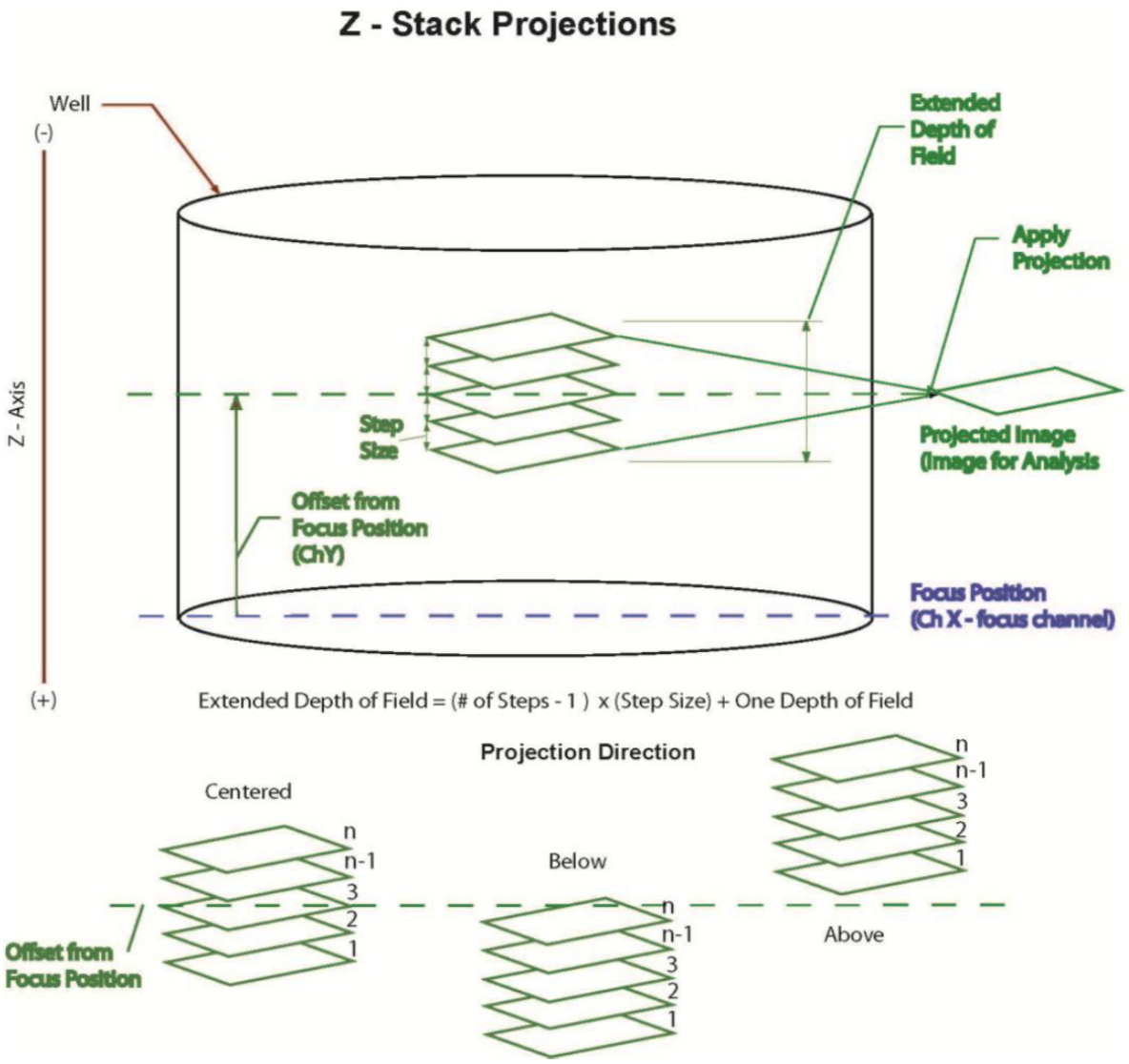


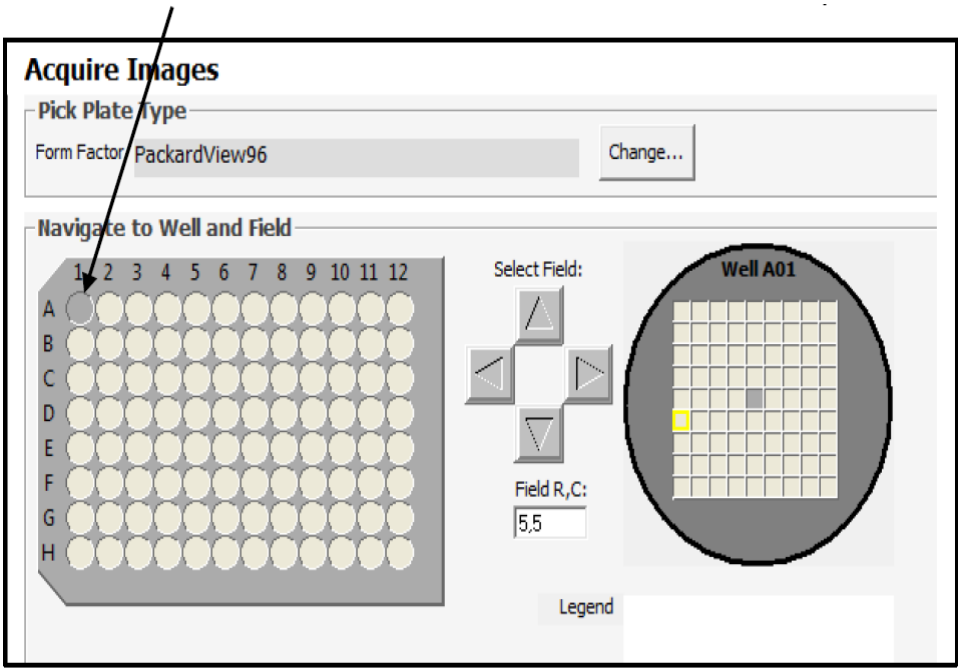
Figure 12 Z-Stack Projections

Acquire images

Clicking **Acquire Image** in the **Configure Acquisition** screen displays a single image. If a projection direction and projection method is selected for a particular channel, then the projected image from the acquired Z-stack images is displayed by default. Refer to the following procedures for details on how to display individual Z-stack images.

To evaluate the confocal options,

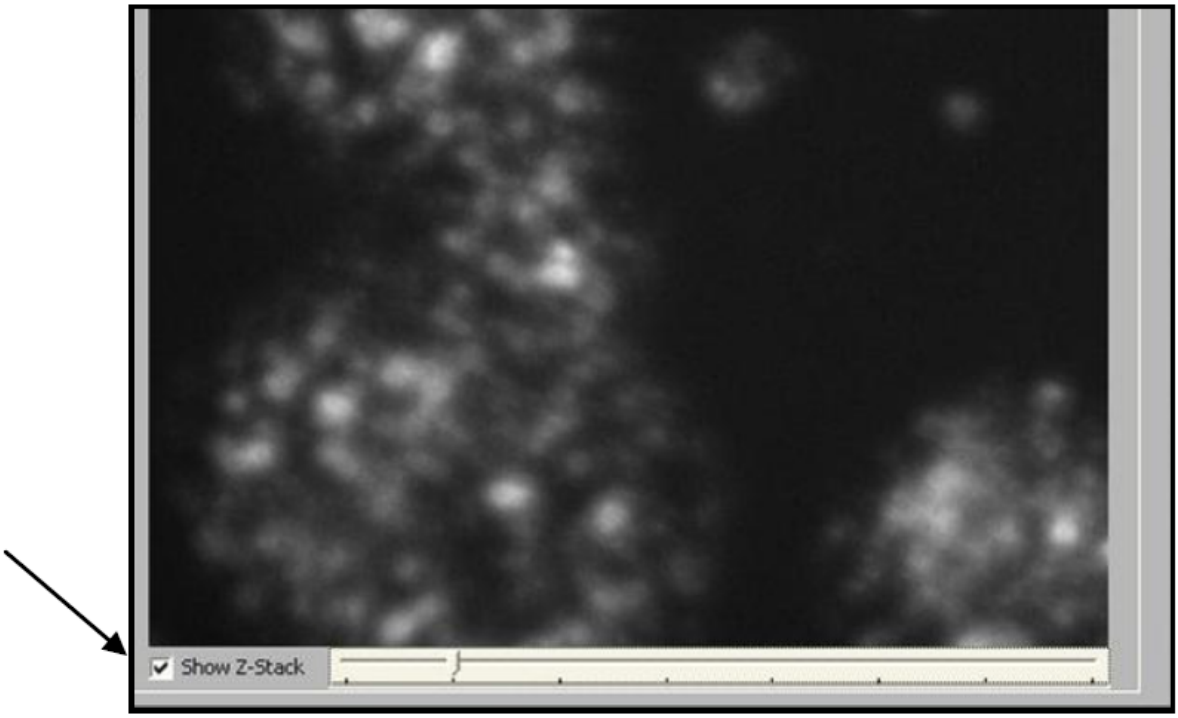
1. Open the **Configure Acquisition** screen. Refer to the software tutorials in the HCS Studio™ Cell Analysis Software online help for instructions.
2. Select the **Form Factor**.
3. Specify the **Confocal Detection Mode** options as described before.
4. From the plate representation, click on the desired well. Well A1 is selected by default.



5. Focus on the sample using the Widefield detection mode.
6. Choose the channel for confocal detection mode. Specify Offset from Focus settings, if needed.
7. Click **Acquire Image**. The image is acquired with the settings defined in the Projection tab.

To display the individual Z-stack images,

1. Select **Show Z-Stack** as shown in the following image.

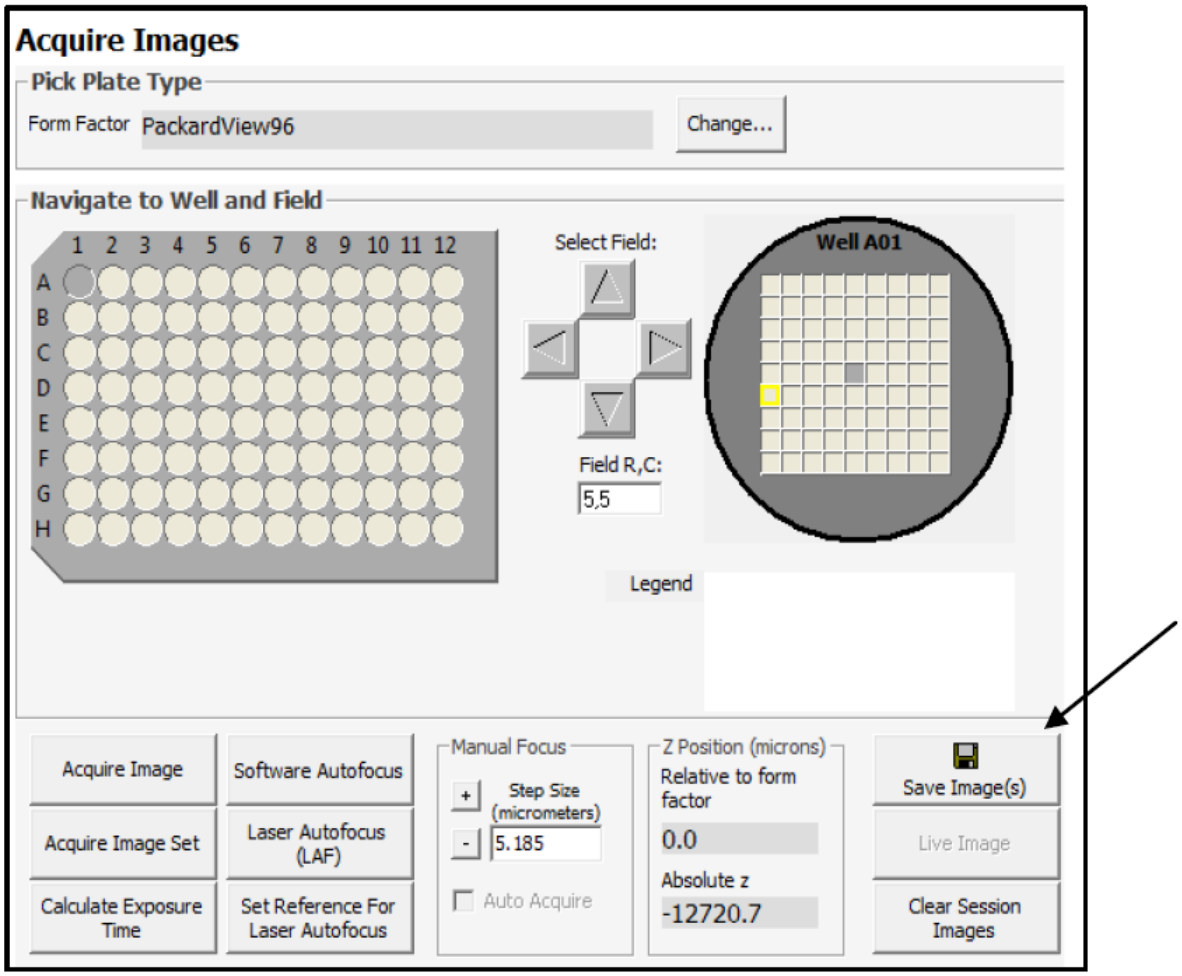


2. When the Z-stack option is selected, you can view each image of the Z-stack by clicking on the slider bar or using the up/down arrow keys on your keyboard.

When you click **Acquire Image Set**, the instrument acquires confocal images for channels that were specified to use the Confocal Detection Mode and non-confocal images (Widefield or other detection mode) for channels that were not specified to use the Confocal Detection Mode.

To save the Z-stack images to a local disk,

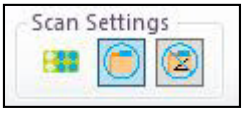
1. Display the desired image in the **Image Display** window.
2. Click **Save Image(s)**.



3. Choose to save the current channel or all available channel images, desired file format, location of the images to be saved, and image filenames. Click **Save**.

Note: You can also save Z-stack images from channels that were specified to use the Widefield detection mode or other detection modes such as Brightfield. The term "Widefield" is used for conventional Epi-fluorescence.

Note: You can also save Z-stack images locally when running a scan by clicking on the **Save Z Stack** icon in the **Scan Settings** area of the Scan toolbar. Note that a significant amount of storage space may be needed when this option is enabled. The z-stack images are saved only on the local hard drive; the images are not spooled to the Store database.



Using the Full-Width-Half-Max (FWHM) tool

This tool is available within the HCS Studio Configure Acquisition task, when acquiring an image stack on the CellInsight™ instrument. It is available for all detection modes.

This tool provides the ability to measure the total intensity of a rectangular region in the image over a range of Z-positions, on a per channel basis. From these total intensity values, the software produces a graph of the Z-position versus Total Intensity, and reports statistics about these values, including the FWHM value, percentage of area in tail, peak total intensity, and floor total intensity. The terms are described below. In addition, you can export the data to be used in other analysis tools, such as the Microsoft™ Excel™ software application.

The tool can be used for the following two purposes:

- To evaluate the amount of out-of-focus light that is blocked by the system. This can be applied on any sample and can be useful for comparing the effects of the different detection modes.
- To measure the optical Z-resolution of the system. For best accuracy, perform this measurement with fluorescent beads that are smaller than the resolving power of the optics.

Table 1 Definition of terms

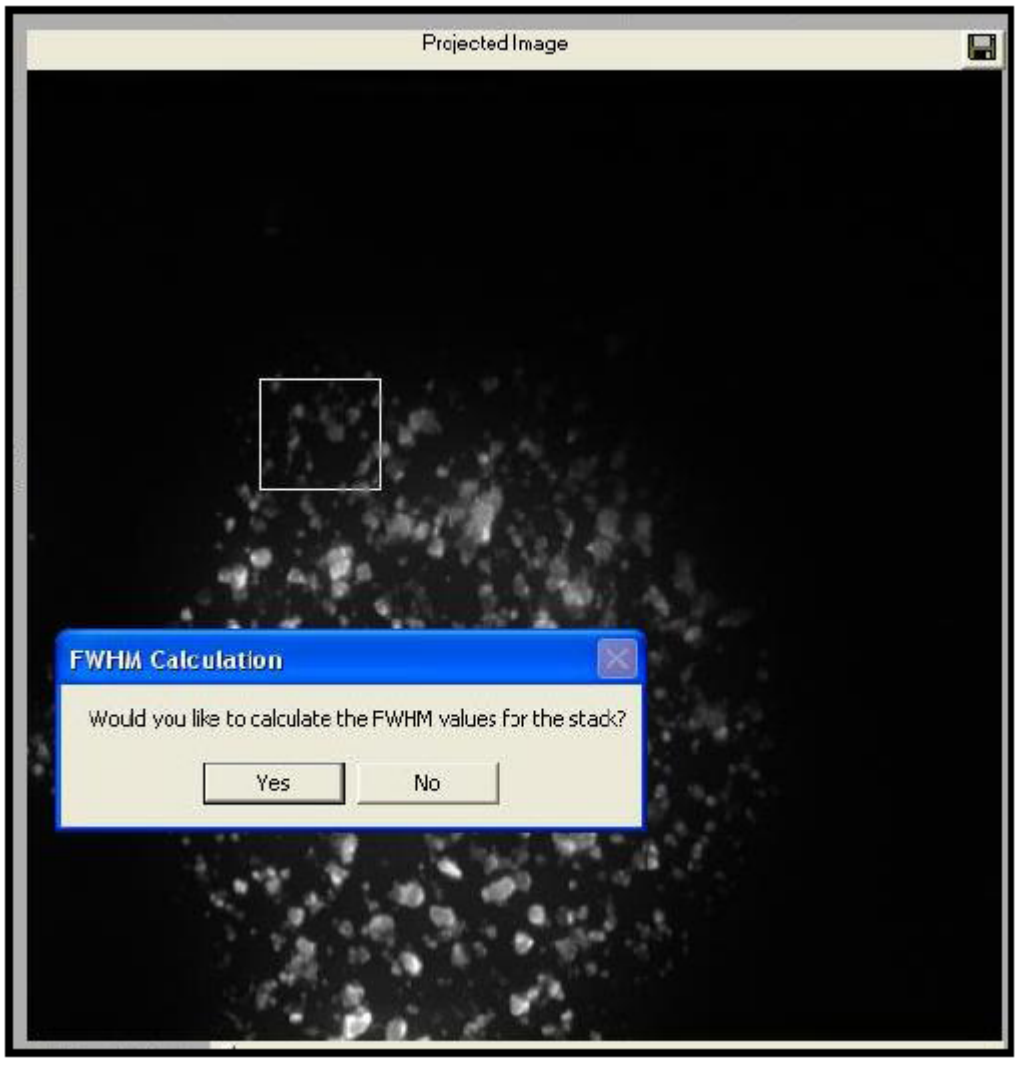
Setting	Description
Z-position	Z-position, in micrometers, of the image within the Z-stack, relative to the focus position
Total Intensity	Sum of the pixel intensity values within the selected rectangular region of the image, for a specific Z-position
Peak Total Intensity	Largest Total Intensity value of all of the images in the Z-stack
Floor Total Intensity	Estimated Total Intensity of the floor. This is estimated by finding the smallest pixel intensity value across all images in the Z-stack, and using the equation: Floor Total Intensity = MinPixelIntensity × Width × Height, where the Width and Height are the dimensions of the selected rectangular region
FWHM	The width, in micrometers, of the main lobe at the Half Total Intensity value; where the Half Total Intensity value is the midpoint between the Peak Total Intensity and Floor Total Intensity. *This value is useful for defining the Z-resolution of the optics.*
Percent Area In Tails	The area of the curve outside the “full width” relative to the total area of the curve. *This value is useful for defining the sharpness of the cutoff of out-of-focus light.*

Use the Full-Width-Half-Max (FWHM) tool

1. Acquire an image stack in the HCS Studio Configure Acquisition task. To acquire a Z-stack, the **Projection Direction** and **Projection method** must be set for that channel by clicking on the **Projection** tab and setting the parameters.

   Note: FWHM calculations cannot be done on single images, which do not have Z-stacks.

2. While holding the left mouse button, select the rectangular region in the image to measure. Refer to the following image; this can be done when viewing the Projection image or the Z-stack images. The location and size of the rectangular region is reported in the task bar. You can also click on an individual pixel in the field and calculate the FWHM for that pixel.



3. The following message is displayed: “Would you like to calculate the FWHM values for the stack?” Click **Yes**.

4. As a result, a window plotting the Z-position versus Total Intensity is displayed. Along with the plot, this window reports the calculated statistics.

5. If desired, click **Expand** to expand the plot to full screen.

6. To export the data, double-click on the plot. On the Export dialog that appears, click **Export** to export the plot.

Scan a plate using confocal assay protocols

Note: Review the following information when scanning one or more plates using Assay Protocols that require confocal imaging.

Autofocus and autoexpose

- For Assay Protocols that require confocal imaging, perform autofocus on a conventional Widefield image on the channel set for autofocusing rather than on a confocal image. Typically this is Channel 1 using the Widefield Detection Mode.
- For channels configured for Confocal, perform autoexpose on the confocal image.

Illumination correction

- For Assay Protocols requiring Confocal imaging and using Illumination Correction, illumination correction is performed on the confocal slice or projected image.

Run a disk scan

When using the Scan Application on a desktop computer, you can create and load Assay Protocols that require Confocal imaging if the Scan Application is configured to use confocal imaging. When working in the Scan Client software, you can turn the Confocal option on and off and you can configure projection settings; however, you cannot interact with the images.

If you cannot create or load confocal enabled Assay Protocols, contact Technical Support for assistance.

Remove the microplate from the CellInsight™ CX7 Pro HCS Reader stage

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

IMPORTANT! If you are using a robotic plate handler with the CellInsight™ CX7 Pro HCS Reader, refer to your robotic plate handler user documentation for instructions on how to insert microplates and other system setup procedures.

WARNING! BIOHAZARD!

Use proper laboratory practices when handling hazardous materials.
Make sure that biohazards do not contaminate CellInsight™ CX7 Pro HCS Reader components.
In the event of system contamination, contact Technical Support for applicable cleanup procedures.
The CellInsight™ CX7 Pro HCS Reader is not equipped with any special devices for protection from substances which are corrosive, toxic, radioactive, or otherwise hazardous to health. All legal regulations for accident prevention, particularly those in the respective countries, must be observed when handling such substances.

DANGER! **RISK OF EYE INJURY!**

Wear safety glasses designed to filter infrared wavelengths and high intensity visible light. **Wearing laser protective glasses with an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm** is required anytime the instrument is opened for servicing and there is risk of exposure. These glasses will filter infrared wavelengths.

CAUTION! **MOVING MECHANICAL PARTS!**

The CellInsight™ CX7 Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. DO NOT MANUALLY OPEN THE STAGE DOOR WHEN THE READER IS PERFORMING A SCAN!

To remove a microplate from the CellInsight™ CX7 Pro HCS Reader stage,

1. From the **Scan Application**, choose the **Tools** tab, then select **Load/Unload Plate.** This places the microplate stage in a position that provides easy access to the microplate.
2. When the plate is in the unload position, remove the plate from the stage.
3. After the plate has been removed, click **Ctrl-OK** in the **Plate Load/Unload** dialog. This command places the stage inside the CellInsight™ CX7 Pro HCS Reader.

Manually remove a microplate from the stage

Under normal operating conditions, refer to "Remove the microplate from the CellInsight™ CX7 Pro HCS Reader stage" on page 47 for instructions on how to remove a single microplate from the CellInsight™ CX7 Pro HCS Reader stage.

In the event of a power failure, you may need to manually remove a plate from the CellInsight™ CX7 Pro HCS Reader stage.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

WARNING! BIOHAZARD!

Use proper laboratory practices when handling hazardous materials.
Make sure that biohazards do not contaminate CellInsight™ CX7 Pro HCS Reader components.
In the event of system contamination, contact Technical Support for applicable cleanup procedures.
The CellInsight™ CX7 Pro HCS Reader is not equipped with any special devices for protection from substances which are corrosive, toxic, radioactive, or otherwise hazardous to health. All legal regulations for accident prevention, particularly those in the respective countries, must be observed when handling such substances.

DANGER! RISK OF EYE INJURY!

Wear safety glasses designed to filter infrared wavelengths and high intensity visible light. **Wearing laser protective glasses with an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm** is required anytime the instrument is opened for servicing and there is risk of exposure. These glasses will filter infrared wavelengths.

CAUTION! MOVING MECHANICAL PARTS!

The CellInsight™ CX7 Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. DO NOT MANUALLY OPEN THE STAGE DOOR WHEN THE READER IS PERFORMING A SCAN!

To manually remove a plate without the use of the Scan Application,

1. In the event of a power failure, turn off the CellInsight™ CX7 Pro HCS Reader main power switch.
2. Turn the power off to terminal strip, then remove terminal strip power cord from outlet. If terminal strip is not used, remove the CellInsight™ CX7 Pro HCS Reader power cord from the outlet.
3. Open the service door as shown in the following image.

   Note: A key is required to open this door.

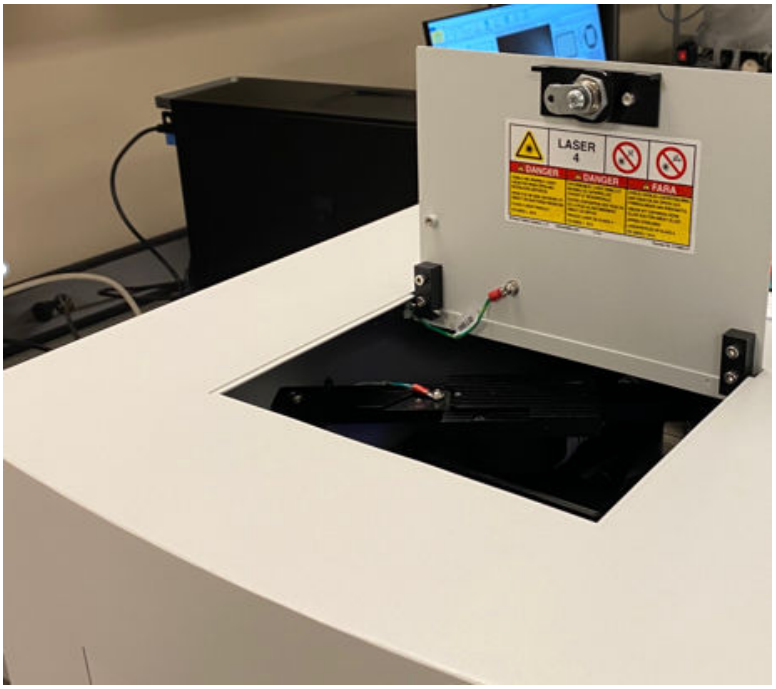


Figure 13 CellInsight™ CX7 Pro HCS Reader service door

4. If the Brightfeld arm is in place (engaged), move the arm to the side by grasping the arm as indicated in the following image and moving the arm to the disengaged position (see the following two figures). When the arm is in the disengaged position, you will feel a magnet holding the arm in place.

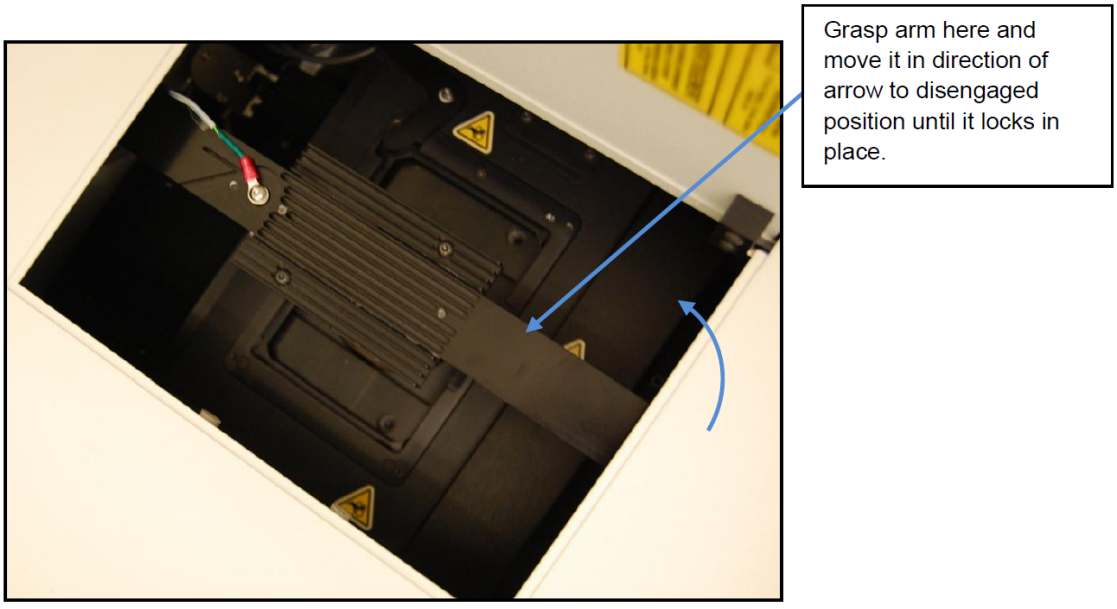


Figure 14 CellInsight™ CX7 Pro HCS Reader open service door with engaged Brightfield arm.

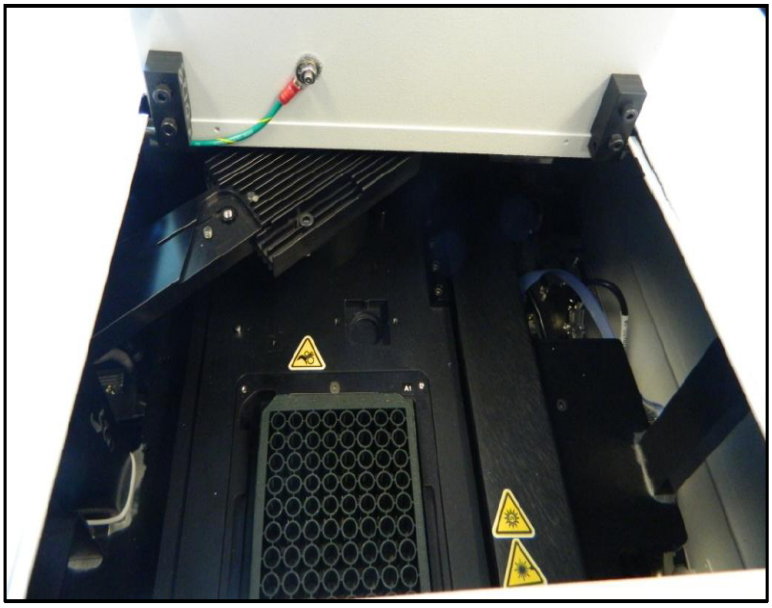


Figure 15 CellInsight™ CX7 Pro HCS Reader open service door with disengaged Brightfield arm.

5. Grasp the sides of the microplate and lift the microplate straight up from the stage insert.
6. Close and lock the service door.

Note: The service door must be locked or the laser safety interlock will prevent the Laser Autofocus or the near IR laser from turning on.

CellInsight™ CX7 Pro HCS Reader shutdown

Note: If you are using a robotic plate handler with the CellInsight™ CX7 Pro HCS Reader, refer to your robotic plate handler user documentation for system shutdown instructions.

Scan application shutdown

From the **File** menu, select **Exit** to close the Scan Application.

CellInsight™ CX7 Pro HCS Reader shutdown

1. Follow the instructions above for closing the software application.
2. From the Windows™ **Start** menu, select **Shut down.**
3. Choose the **Shut down** option, then click **OK.**
4. Turn off the computer and the monitor.
5. Turn off the CellInsight™ CX7 Pro HCS Reader power switch located on the back of the instrument.

3 Using the illumination correction tool

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Illumination Correction on the CellInsight™ CX7 Pro High Content Screening (HCS) Platform provides the following:

- Diagnostic indicators of optical conditions
- Flat field illumination correction
- Dark current subtraction

Industrial-scale automation is subject to continuous operation by a range of users and therefore is under continuous stress. Such stresses can subtly effect optical performance and include lamp age, optical filter deterioration, and mechanical perturbations. These stresses affect the performance of all optical systems and are detectable through sCMOS imaging as variations in the uniformity of field fluorescence. When severe, the lack of uniformity can impact the extraction of spatially resolved data.

The illumination correction wizard on the CellInsight™ CX7 Pro HCS Platform serves as an optical performance monitoring tool and will facilitate awareness of performance changes along with corrective measures to include automated image correction if desired by the operator. The use of a fluorescent standard reference plate enables the analysis and reporting on the extent to which fluorescence intensities vary across an image of a stable homogenous fluorescent cocktail (Thermo Scientific™ OptiTracker™ II Performance Monitoring Kit). After an automated scan of the fluorescent standard, the operator is presented with an opportunity to print a performance report.

When correction is desired, a dark current subtraction for background camera noise is performed. This subtraction is executed on all images prior to data extraction and prior to flat field illumination correction (see below). This dark current subtraction effectively improves the contrast in the image.

The CellInsight™ CX7 Pro HCS Platform, when properly configured, produces illumination with variation significantly lower than the biological heterogeneity in a cellular sample. However, results of single cell analyses can be affected by uneven illumination. Illumination correction is designed to correct images for the unevenness in the illumination across a field of view due to the physical limitations inherent in any optical system.

Illumination correction involves a flat field correction by using images acquired from the Illumination Correction Plate. Illumination correction images are collected using the Illumination Correction Image Acquisition Wizard (described below) to represent the illumination pattern for each filter set and objective installed in the CellInsight™ CX7 Pro HCS Platform. The Wizard also provides diagnostic indicators of significant optical issues resulting in uneven illumination in specific fluorescence channels or in the general optical pathway.

This chapter discusses the following Illumination Correction topics:

- General Process of Using Illumination Correction
- Overview of the Illumination Correction Feature
- Preparing the Illumination Correction Plate
- Running the Illumination Correction Image Acquisition Wizard
- Determining the Status of the Current Illumination Correction Images Stored on the CellInsight™ CX7 Pro HCS Platform
- Using the Illumination Correction Feature in your Scans
- Status Messages with Using the Illumination Correction Wizard
- Diagnostics to Restore Optimal Illumination
- Guidelines on the Use of the Illumination Correction Plate
- Frequently Asked Questions

Overview of the illumination correction feature

The general process for Illumination Correction on the CellInsight™ CX7 Pro High Content Screening (HCS) Platform is:

1. Prepare the Illumination Correction Plate according to the package directions, or use a previously prepared and stored Illumination Correction Plate.
2. Run the automated Illumination Correction Image Acquisition Wizard.
3. The CellInsight™ CX7 Pro HCS Platform automatically performs Illumination Correction during a plate scan as well as when acquiring images in the Configure Acquisition task, provided that the **Apply Illumination Correction** checkbox is checked as shown in the following image.

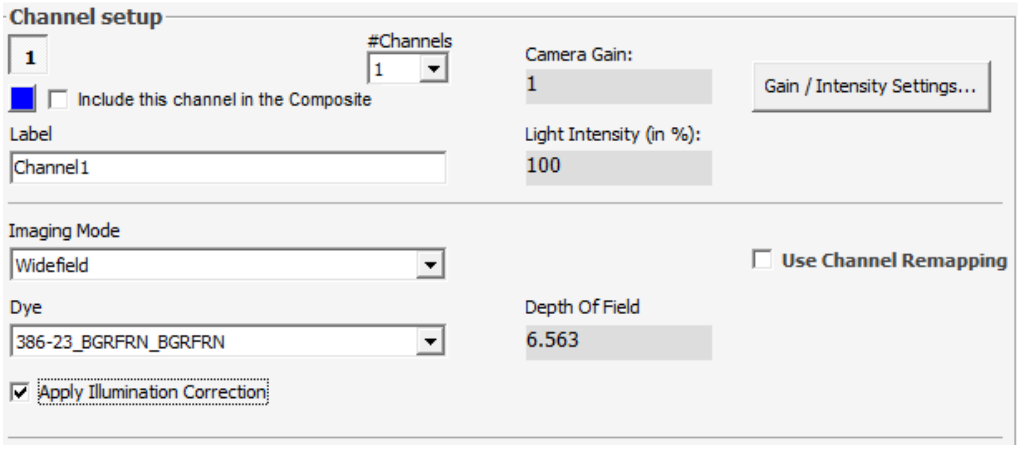


Figure 16 Apply Illumination Correction checked

We recommend that you use the Illumination Correction feature for all channels of all BioApplications when intensity variations (COV) are high (see the status message table on page 65). You can select this option for one or more channels (when optical performance is passed).

The **Image Display Mode** is set to **Image Stretch** by default. **Stretch Max** exaggerates pixel differences for visualization purposes. You can see details within cells or at the cell periphery that may not be seen without contrast stretching. This Image Display Mode also shows gradients of unevenness even though the range of pixel intensity is minimal. We recommend that you maintain **Image Stretch Max** to enhance visualization even though subtle changes in intensity may appear more pronounced. All data is extracted from the raw, non-contrast stretched image.

For examples that illustrate these guidelines, see Figure 17 and Figure 18.

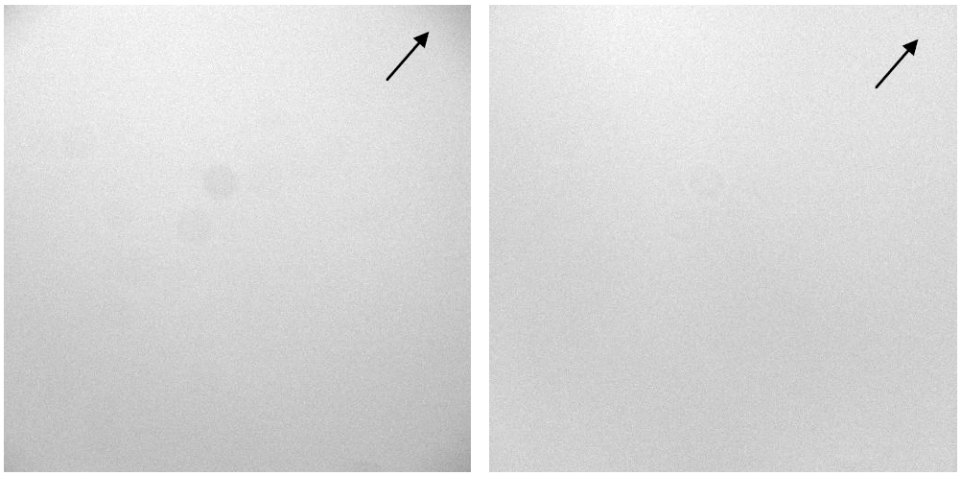


Figure 17 The Illumination Correction image in the left panel shows the subtle shading on a Contrast Stretch Max displayed image that is uncorrected with a COV = 7.2%. The right panel has been corrected and now appears more uniform with a COV = 5.2%.

In a qualitative demonstration of the Illumination Correction Image Acquisition Wizard, images were obtained from a cytoplasm to nucleus translocation experiment using a custom protocol. The protocol was set up such that one illumination-corrected and one non-corrected image were obtained for each field within a well (Figure 18). It is visually apparent that in this particular case, using a 4X objective, the Illumination Correction Tool had a noticeable effect in reducing spatial heterogeneity (correction removed the darker corners of the uncorrected initial image).

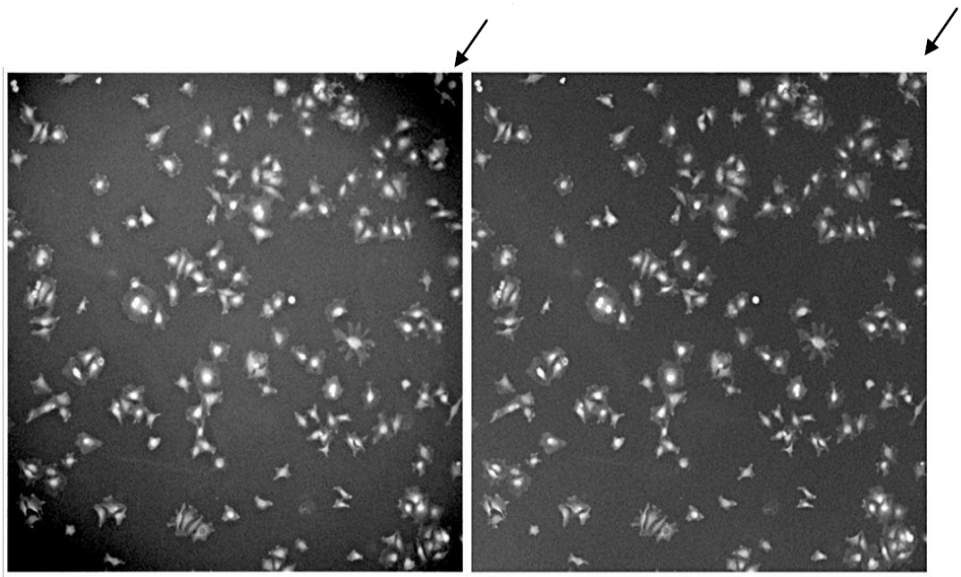


Figure 18 The image on the left shows an uncorrected 4X, TRITC image. The image on the right shows an illumination corrected 4X, TRITC image.

A quantitative demonstration of the Illumination Correction Tool was performed using the same plate and well as above. In this study, multiple field images were obtained using the 4X, 10X, and 20X objectives. Approximately 5000 cells were measured. One of the cellular features extracted, the average nuclear intensity of NF-κB, was calculated for all the cells at each magnification. The *percentage change between the uncorrected intensity and the corrected intensity* for each cell was calculated

and plotted relative to the position of each cell in the imaging field. The results obtained using the 4X objective showed that only 9% of the cells required a correction of greater than 13%. The spatial heterogeneity within the same well when imaged with either a 10X or 20X objective did not require any cellular image values to be corrected more than 13%.

Prepare the illumination correction plate

WARNING! The Illumination Correction Solution contains fluorescent reagents in a DMSO solution. Follow all safety procedures given in the MSDS included with the OptiTracker™ solution kit.

Prepare the Illumination Correction Plate according to the directions that accompany the OptiTracker™ II product. These directions guide you in placing the correct volume and concentration of the solution into the applicable plate type.

After you have prepared the OptiTracker™ II plate, you are ready to use the Illumination Correction Image Acquisition Wizard.

Run the Illumination Correction Image Acquisition Wizard

The Illumination Correction Image Acquisition Wizard images the OptiTracker™ plate to capture the illumination pattern for each filter-dichroic set and each objective for which correction is desired.

To run the Wizard,

1. From the **HCS Studio™ Navigator**, select **System Configuration Tools**. Double-click the **Illumination Correction** icon.

2. The **Illumination Correction Image Acquisition Wizard** screen appears and provides a brief description of the correction method. Select **Next** to continue.

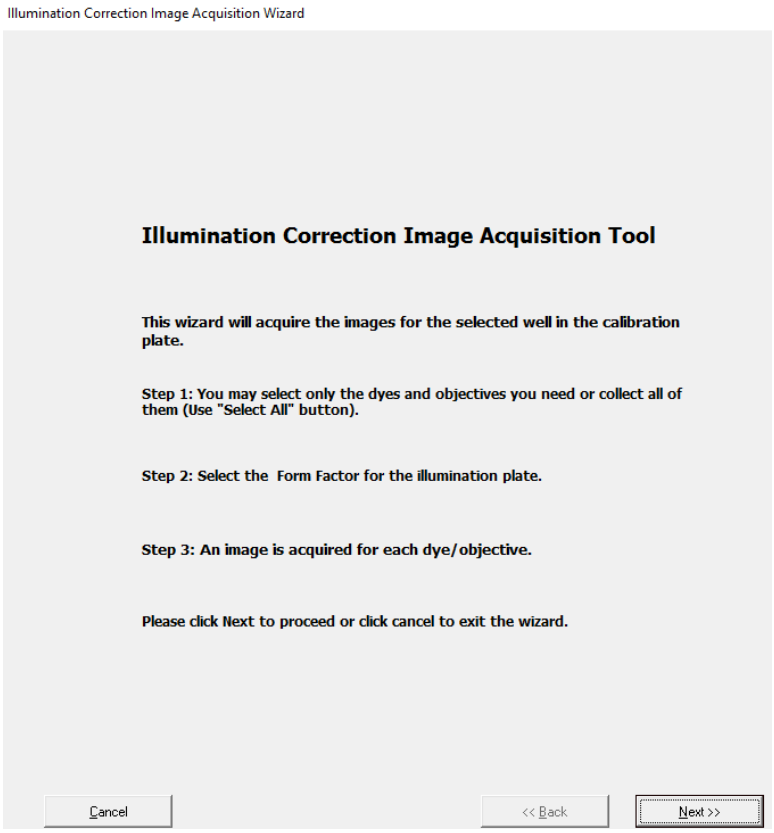


Figure 19 Illumination Correction Image Acquisition Wizard

3. You are then prompted to insert the Illumination Correction Plate into the instrument. Select **OK** to continue.

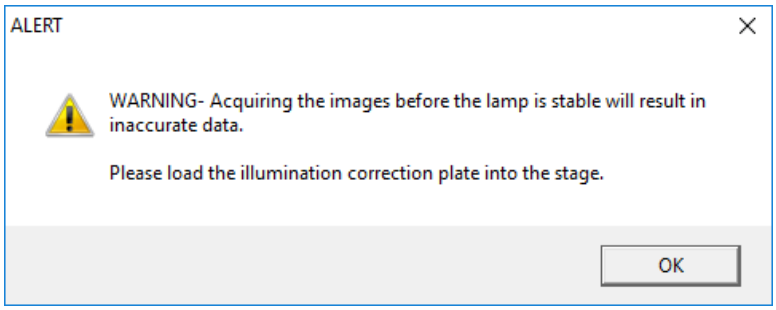


Figure 20 Warning message: Load the Illumination Correction Plate into the instrument.

4. The **Dye and Objective Selection** screen appears, which allows you to specify the filter-dichroic combinations and the objective(s) to be included in the correction procedure.

5. Choose the objective(s) and dyes that are needed for your scan. If you want to select all dyes and objectives, click **Select All**. Click **Next** to continue.

Note: We do not recommend selecting any of the dyes that start with 438-24, such as the 438-24_C_C or the 438-24_C_Y, or the LaserAF dye. The dye solution that is used to obtain illumination correction images does not have a dye that can be excited with the 438-24 wavelength light source.

You should have a total of 78 dyes if you have selected all dyes/objectives, then unselected the dyes mentioned above.

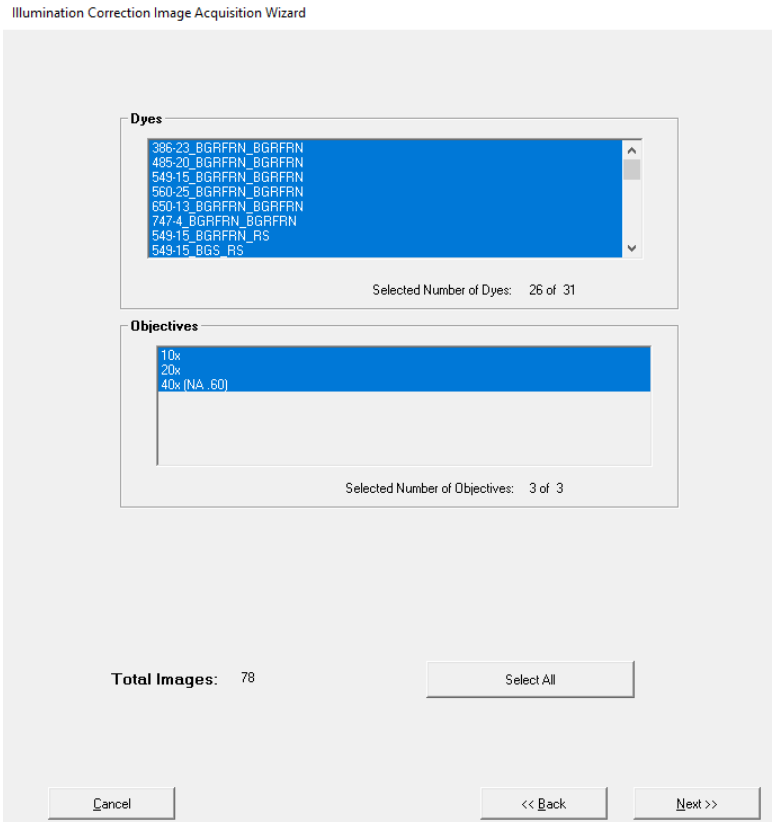


Figure 21 Illumination Correction Image Acquisition Wizard: Select dyes and objectives. In this example, the user has selected all objectives and dyes, then unselected all 438 and LaserAF dyes.

6. The **Plate and Well Selection** screen appears, which allows you to specify the **Plate Manufacturer** and the **Plate Type of the Illumination Correction Plate**. In this screen, you also indicate which well contains the reference dye solution. Click on the desired well in the plate representation. Select **Next** to continue.

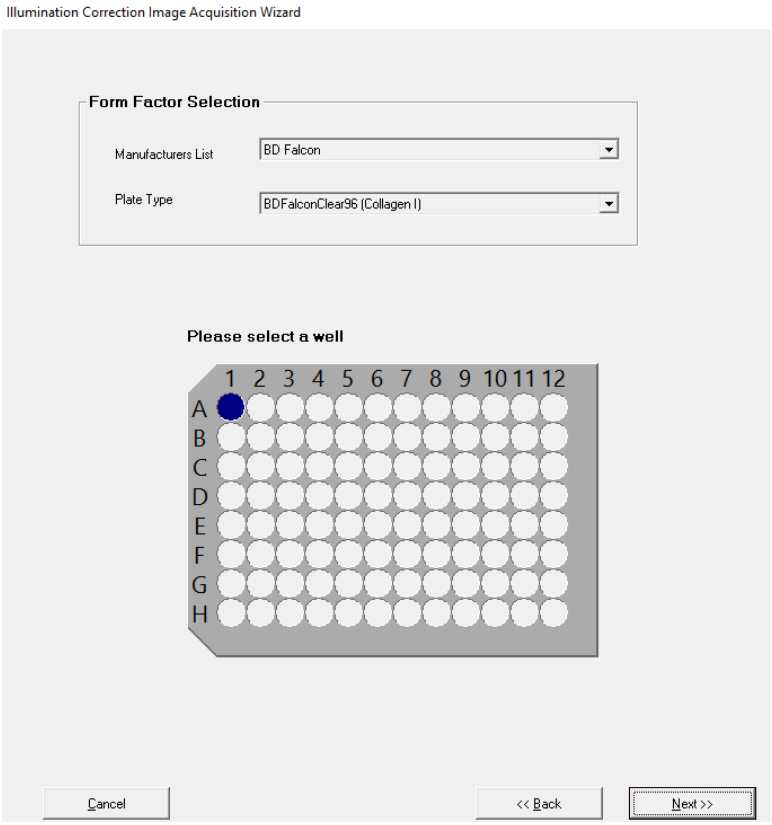


Figure 22 Illumination Correction Image Acquisition Wizard: Select plate manufacturer, plate type, and well.

7. The **Selections Summary** screen appears. This screen provides a summary of the options selected. Review the option settings. To make changes to your selections, click **Back**. If the option settings are correct, click **Start Acquisition Process**. The screen is updated during the image acquisition process.

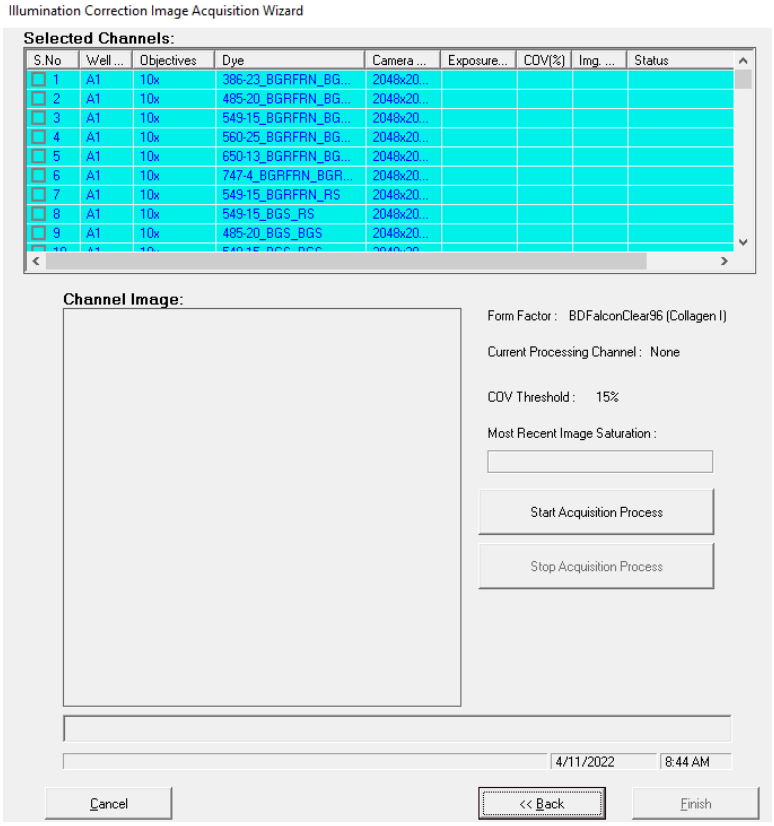


Figure 23 Illumination Correction Image Acquisition Wizard: Option setting summary.

8. Ignore all messages that prompt you to change the objective on the objective turret and click **OK** to proceed.

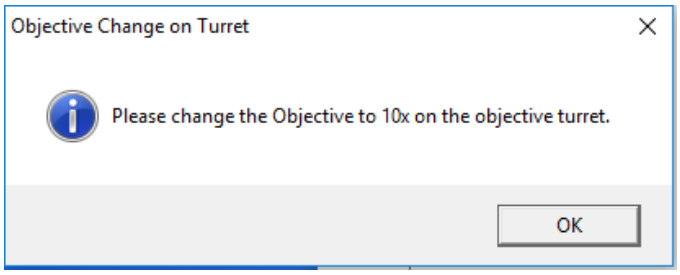


Figure 24 Warning message: Objective Change on Turret.

9. After the correction process has completed, the **Correction Done** screen appears as below.

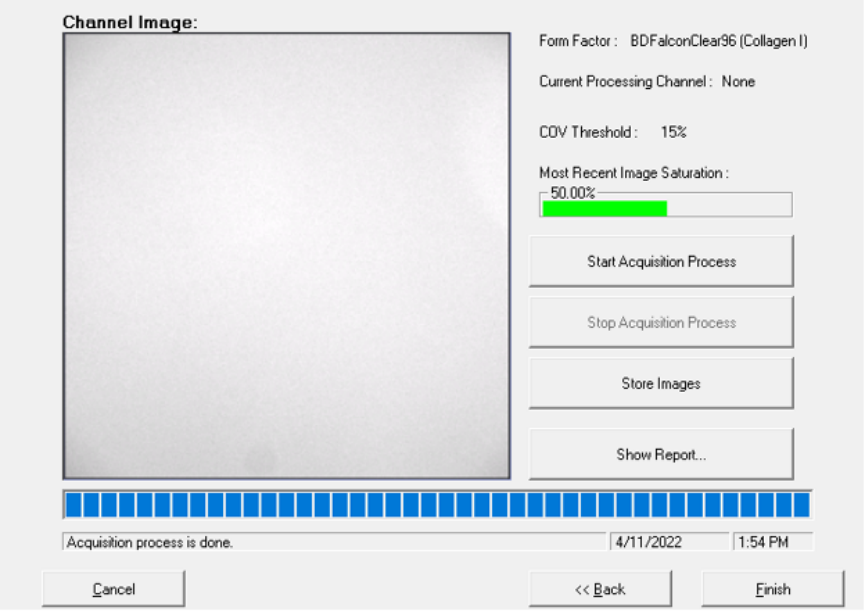
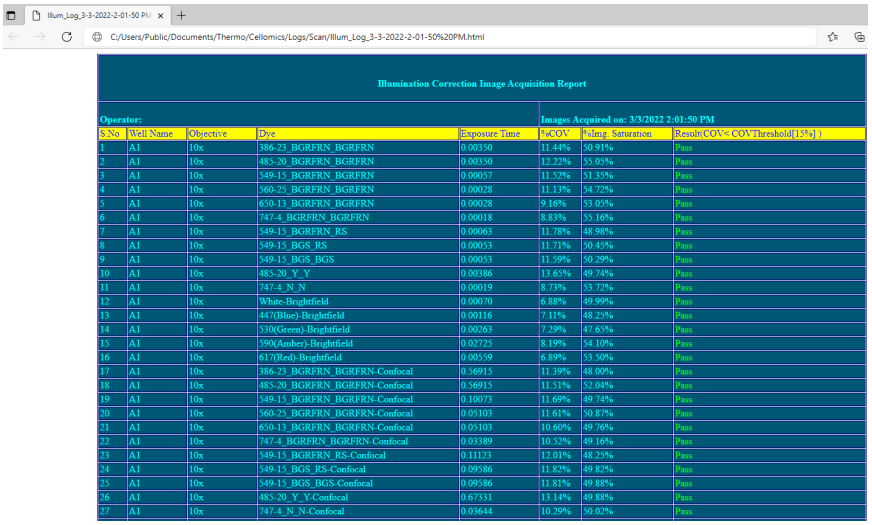


Figure 25 Illumination Correction Image Acquisition Wizard: Processing complete.

10. To view the entire report, click **Show Report**.

11. A web browser page showing the report appears. To print a copy, select **Print** from the **File** menu. You can then close the window, if desired.



Illumination Correction Image Acquisition Report

Operator: **Images Acquired on: 3/3/2022 2:01:50 PM**

S.No	Well Name	Objective	Dye	Exposure Time	%COV	%Img. Saturation	Result(COV< COVThreshold[15%])
1	A1	10x	386-23_BGRFRN_BGRFRN	0.00350	11.44%	50.91%	Pass
2	A1	10x	485-20_BGRFRN_BGRFRN	0.00350	12.22%	55.05%	Pass
3	A1	10x	549-15_BGRFRN_BGRFRN	0.00057	11.52%	51.35%	Pass
4	A1	10x	560-25_BGRFRN_BGRFRN	0.00028	11.13%	54.72%	Pass
5	A1	10x	650-13_BGRFRN_BGRFRN	0.00028	9.16%	53.05%	Pass
6	A1	10x	747-4_BGRFRN_BGRFRN	0.00018	8.83%	55.16%	Pass
7	A1	10x	549-15_BGRFRN_RS	0.00063	11.78%	48.98%	Pass
8	A1	10x	549-15_BGS_RS	0.00053	11.71%	50.45%	Pass
9	A1	10x	549-15_BGS_BGS	0.00053	11.59%	50.29%	Pass
10	A1	10x	485-20_Y_Y	0.00386	13.65%	49.74%	Pass
11	A1	10x	747-4_N_N	0.00019	8.73%	53.72%	Pass
12	A1	10x	White-Brightfield	0.00070	6.88%	49.99%	Pass
13	A1	10x	447(Blue)-Brightfield	0.00116	7.11%	48.25%	Pass
14	A1	10x	530(Green)-Brightfield	0.00263	7.29%	47.65%	Pass
15	A1	10x	590(Amber)-Brightfield	0.02725	8.19%	54.10%	Pass
16	A1	10x	617(Red)-Brightfield	0.00559	6.89%	53.50%	Pass
17	A1	10x	386-23_BGRFRN_BGRFRN-Confocal	0.56915	11.39%	48.00%	Pass
18	A1	10x	485-20_BGRFRN_BGRFRN-Confocal	0.56915	11.51%	52.04%	Pass
19	A1	10x	549-15_BGRFRN_BGRFRN-Confocal	0.10073	11.69%	49.74%	Pass
20	A1	10x	560-25_BGRFRN_BGRFRN-Confocal	0.05103	11.61%	50.87%	Pass
21	A1	10x	650-13_BGRFRN_BGRFRN-Confocal	0.05103	10.60%	49.76%	Pass
22	A1	10x	747-4_BGRFRN_BGRFRN-Confocal	0.03389	10.52%	49.16%	Pass
23	A1	10x	549-15_BGRFRN_RS-Confocal	0.11123	12.01%	48.25%	Pass
24	A1	10x	549-15_BGS_RS-Confocal	0.09586	11.82%	49.82%	Pass
25	A1	10x	549-15_BGS_BGS-Confocal	0.09586	11.81%	49.88%	Pass
26	A1	10x	485-20_Y_Y-Confocal	0.67331	13.14%	49.88%	Pass
27	A1	10x	747-4_N_N-Confocal	0.03644	10.29%	50.02%	Pass

Figure 26 Illumination Correction Image Acquisition Report. In this example, the Status indicator, Pass, specifies that the spatial heterogeneity of each filter-dichroic and objective combination lies within a range where application of Illumination Correction is appropriate. Refer to the Status Message table that appears later in this chapter for a description of all other status messages.

12. Click **Store Images** using the button on the previous page, if you wish to save the corrected images. When saved, you can apply these corrected images to future scans.

Note: Failing images are not stored, so that they cannot be used for image correction.

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13. The **Images Stored** message appears. Click **OK** to continue.

Figure 27 Message box: Images Stored.

14. Remove the Illumination Correction Plate from the stage. Store the plate at room temperature protected from light (place upright in closed drawer, wrap in foil). Dispose of the plate properly if there is any indication of solution leakage.

Use the illumination correction feature in your scans

WARNING! Before proceeding, read and understand the information in the “Safety” appendix in this document.

1. Insert an assay cell plate into the stage.
2. In the **Configure Acquisition** task of the HCS Studio™ Scan software, verify that the **Apply Illumination Correction** checkbox is checked for each channel.

   Note: If you want to use Illumination Correction in the Configure Acquisition task, ensure that you have valid Illumination Correction images.

3. Scan the plate as usual. All images processed through the Store Image and Database Management Software from this scan will have the background subtracted and the Illumination Correction applied. These corrections are applied before the algorithm extracts cellular data.

   Note: If images have expired (>4320 hours), a warning will ask if you would like to proceed. If you proceed, the warning and expiration time of the images appears in the **View Comments** box associated with that plate.

Status messages when using illumination correction

The Illumination Correction Image Acquisition Wizard Report provides status conditions for operation and diagnostics. The status indicates if you should continue to operate your CellInsight™ CX7 Pro HCS Platform, or if you should contact Technical Support for assistance. You can operate your CellInsight™ CX7 Pro HCS Platform when you receive either a **Pass** or **Pass/Illumination Correctable** condition. All possible status messages and recommended actions are outlined in the following table. Perform the recommended actions before contacting Technical Support.

Status Message	COV[1]	Correction Images Saved	Recommended Action
Pass	0–13%	Yes	None
Pass/ Illumination Correctable	>13–25%	Yes	None needed, but issue should be brought up at next available Service visit or Preventative Maintenance visit. You can continue to operate your CellInsight™ CX7 Pro HCS Platform. We recommend that you review the **Diagnostic Checklist** to see if the COV can become an unconditional Pass.
Fail/ Illumination Issue	>25%	No	Review the Diagnostic Checklist. If this does not reduce the COV to above Pass conditions, contact Technical Support. **We recommend that you do not use your instrument with any filter/objective combinations that fail due to this issue.**
Fail/ Exposure Too Short	—	No	Determine if you are imaging the correct well and the correct dilution in the plate. Review the Diagnostic Checklist. If this does not restore images to Pass conditions, contact Technical Support.
Fail/ Exposure. Too Long	—	No	Determine if you are imaging the correct well and the correct dilution in the plate. Review the Diagnostic Checklist. If this does not restore images to Pass conditions, contact Technical Support.

[1] Coefficient of Variation within the OptiTracker™ solution image used for diagnostic pass/fail criteria and illumination image correction for data extraction during scanning.

Diagnostics to restore optimal illumination

When the Wizard displays a message that indicates a Pass/Illumination Correctable (COV >13%–25%) condition or a Fail/Illumination Issue (COV >25%), refer to the "Diagnostic checklist" below to assist you in troubleshooting or restoring the CellInsight™ CX7 Pro HCS Platform to optimal functioning.

Diagnostic checklist

- The Illumination Correction Plate is properly inserted in the stage and the door is closed.
- The selected plate manufacturer and plate type matches the Illumination Correction Plate.
- The objective type being used is compatible with the plate type being used.
- The Illumination Correction Solution is present, ~100–200 µL remains in the well, the **correct well is selected,** the plate is sealed properly, and it is not past expiration.
- The well plate used for Illumination Correction has been properly calibrated using the Form Factor Calibration wizard with a cellular sample.

After correcting any of the above, **restart the Wizard and repeat collection** of Illumination Correction images.

If these actions fail to lower the COV to <25%, we recommend that you contact Technical Support for possible corrective action. Generate and print a report, along with a screen shot, for reference when emailing or speaking with Technical Support.

Guidelines on OptiTracker™ plate use

- The OptiTracker™ II solution and the sealed OptiTracker™ plate (see OptiTracker™ II Insert) are to be stored at room temperature, away from light.
- The seal on the plate should be intact to prevent evaporation or leaks. Approximately 100–200 µL should remain in each Illumination Correction Solution-containing well.
- The sealed plate can be used for scanning all filters and objectives for at least 1 month.
- The same well can be used for illumination correction imaging up to 5 times.
- A label on the plate can record the number of times a well has been used.

Note: Do not place the label over any of the wells containing solution.

- We recommend that you include all channels in your assay when running the Illumination Correction Image Acquisition Wizard.
- When running the Illumination Correction Image Acquisition Wizard, select the channels and objectives that you will use during your single or multi-plate scan.
- Follow proper procedures and safety precautions when preparing and using the Illumination Correction plate.

Frequently asked questions

Question	Answer
Can I use the illumination images that I acquired two weeks ago for use with the scan that I want do today?	Yes, the CellInsight™ CX7 Pro HCS Platform prompts you to collect a new set of images before scanning a plate only if >6 months (4320 hours) has passed since the last set of illumination correction images was collected.
How long does it take to acquire the illumination correction images?	• If you choose to take images for only a single objective and filter set, the Wizard completes the task in less than 1 minute. • If you choose to take images for all filters with all objectives, the Wizard completes the task in about 5 minutes. If needed, you can walk away and return later after clicking **Start Acquisition Process**.
When viewing images in the Scan Application, am I looking at raw images or illumination corrected images?	• If you did not check **Apply Illumination Correction** for that channel, the images that you see will be contrast stretched only. • If you check **Apply Illumination Correction** for that channel, the images that you see will be background subtracted and illumination corrected.
Should I always use Illumination Correction on all channels when I scan plates on the CellInsight™ CX7 Pro HCS Platform?	We recommend using Illumination correction only on channels with altered optical performance.
Under what circumstances can using illumination correction be detrimental to my data?	If the images have expired, they may not provide optimal correction. Always check the startup window reporting the illumination status and note the age of your images. **Note:** When using a robotic microplate handler with a protocol that uses illumination correction, the correction will be done even if the images are expired.
If I can visibly detect by eye some degree of illumination non-uniformity, should I stop using my instrument and contact Technical Support?	• The Illumination Correction feature can correct significant visible illumination gradients. Therefore, if the COVs for all the filters and objectives you need to use are below 25%, you can still use your instrument. • If your Illumination Correction COVs are over 13%, review the Diagnostic List to determine if you can remedy any of the common sources of uneven illumination and to restore it to an unconditional pass. For some channels, a COV between 13% and 25% is normal. • If the COVs are above 25% for filters and objectives in use, do not use your instrument. Contact Technical Support for assistance.

4 CellInsight™ CX7 Pro HCS Reader maintenance

This chapter describes routine maintenance and cleaning procedures.

CAUTION! Before performing the following procedures, read and understand the information in the "Safety" appendix in this document.

Note: All maintenance procedures described in this guide can be safely performed by QUALIFIED SERVICE PERSONNEL. Maintenance not covered in this guide must be performed only by Thermo Fisher Scientific service personnel.

Routine care and maintenance

To ensure proper operation of the CellInsight™ CX7 Pro HCS Reader:

- Periodically check all power cords and data cables for secure connection and proper condition. Refer to "System schematics" on page 81.
- Keep the CellInsight™ CX7 Pro HCS Reader dust free. Check microscope objective monthly for dust and dirt accumulation and clean as necessary. Refer to Reader Cleaning and Objective Cleaning instructions later in this chapter.
- Avoid exposure of the instrument to extreme climatic influences such as temperature shocks or high humidity, and to mechanical shocks.
- Avoid jarring the unit, especially during its operation.

CellInsight™ CX7 Pro HCS Reader maintenance

CellInsight™ CX7 Pro HCS Reader cleaning

WARNING! Ensure that the CellInsight™ CX7 Pro HCS Reader is turned off and AC power cords are disconnected from the unit before cleaning.

Never spray or pour any liquid directly onto the CellInsight™ CX7 Pro HCS Reader.
Dust and dirt can impair the performance of optics and moving parts. When performing any maintenance procedure, do not leave the CellInsight™ CX7 Pro HCS Reader doors open for an extended period.

WARNING! BIOHAZARD!

Use proper laboratory practices when handling hazardous materials.
Ensure that biohazards do not contaminate CellInsight™ CX7 Pro HCS Reader components.
In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.
The CellInsight™ CX7 Pro HCS Reader is not equipped with any special devices for protection from substances which are corrosive, toxic, radioactive, or otherwise hazardous to health. All legal regulations for accident prevention, particularly those in the respective countries, must be observed when handling such substances.

To ensure proper operation of the CellInsight™ CX7 Pro HCS Reader, perform the following cleaning procedures:

- Clean the outer surfaces of the CellInsight™ CX7 Pro HCS Reader with a clean cloth dampened with a mixture of water and a mild detergent. Use another lint-free cloth to wipe the instrument dry. Do not use any solvent.
- Always protect the instrument from dust and moisture. Remove dust particles with a clean brush or rubber blower.
- If any spills occur, turn OFF the instrument, disconnect the AC power cords from the unit, and clean up any spills immediately.
- Clean the sample compartment every six months or when it has become soiled with sample substance.

CellInsight™ CX7 Pro HCS Reader fuse replacement

If the CellInsight™ CX7 Pro HCS Reader does not activate when you turn on the power switch, verify the following before replacing the fuse:

- The terminal strip is plugged firmly into the wall outlet and the switch on the terminal strip (if present) is on.
- The CellInsight™ CX7 Pro HCS Reader power cord is plugged firmly into the terminal strip and firmly attached to the back of the unit.

IMPORTANT! Perform fuse replacement only if it is known that a building over current or a short circuit caused the instrument fuse failure. In all other fuse fault scenarios, contact Technical Support.

WARNING! RISK OF ELECTRIC SHOCK!

Disconnect the mains power cord before changing the fuses. To reduce the risk of fire or shock, replace fuses only with fuses of the same type and rating. The use of makeshift fuses and the short-circuiting of fuse holders are not permitted.

To replace the CellInsight™ CX7 Pro HCS Reader fuse,

1. Turn off the main power switch.
2. Remove the AC power cord from the unit.
3. The fuse holder is located on the power entry module under the AC power connection on the back of the instrument (see following figure). The fuse holder contains two fuse locations.

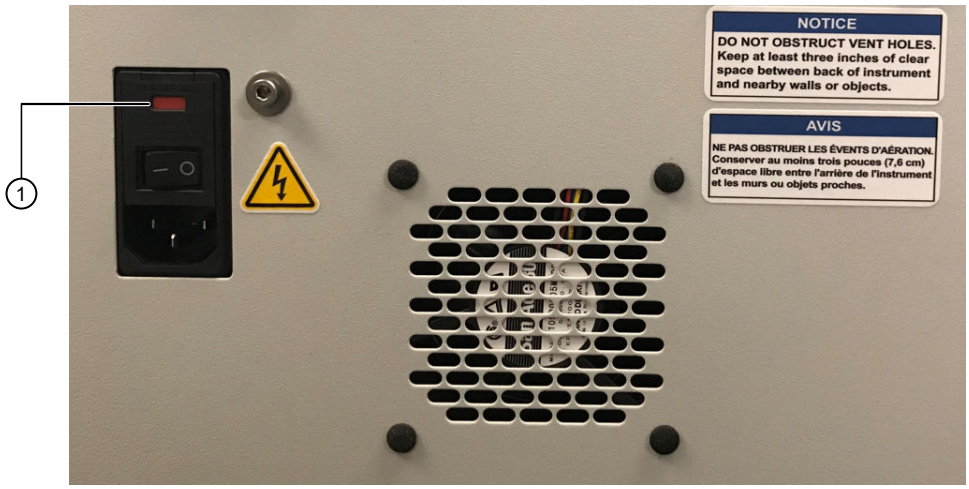


Figure 28 Fuse holder on the back of the instrument

① Fuse holder

4. Using a flat-head screwdriver, pry the fuse holder from the instrument panel.

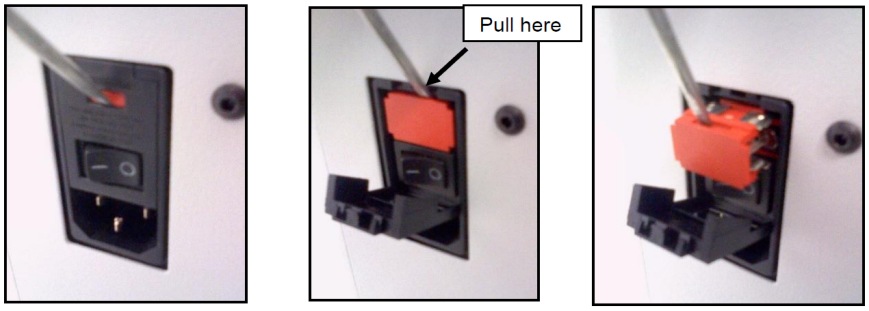


Figure 29 Removing the fuse holder from back of the instrument

5. Remove one or both damaged fuses. Visually inspect and check for continuity. If necessary, replace fuse with a fuse of the same type and rating:

   110–120V: 6A Medium Acting Fuse

   220–240V: 6A Medium Acting Fuse

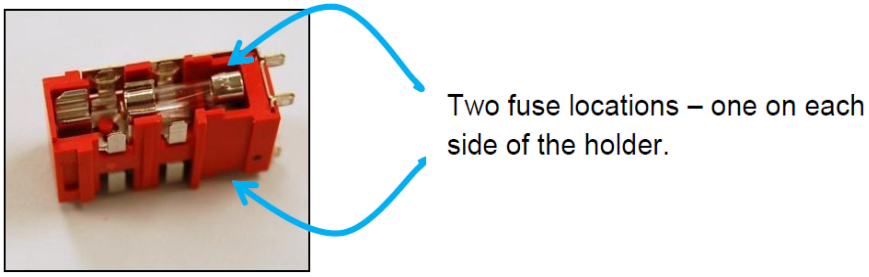


Figure 30 Fuse locations

6. The power entry module on the instrument requires dual fuses for both the hot and neutral AC lines. It requires two identical fuses in both locations. Therefore, the orientation of the fuse holder while replacing it back into the instrument panel is not critical.

   Note: The fuse holder requires two identical fuses in both locations.

7. Plug the power cord into the power input module. Verify the following: (a) all power connections are correct, and (b) the terminal strip is turned on.

8. Turn on the power to the CellInsight™ CX7 Pro HCS Reader.

9. If the CellInsight™ CX7 Pro HCS Reader still does not power up, or if the fuse fails again, contact Technical Support for assistance.

Inspect and clean the objective

Inspect the objective monthly or when there is any indication of reduced performance.

WARNING! RISK OF ELECTRIC SHOCK!

Disconnect the AC power cord before inspecting the objective.

CAUTION! MOVING MECHANICAL PARTS!

The CellInsight™ CX7 Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. DO NOT MANUALLY OPEN THE STAGE DOOR WHEN THE READER IS PERFORMING A SCAN!

WARNING! RISK OF EYE INJURY!

Wear safety glasses designed to filter ultraviolet wavelengths and high intensity light. **Wearing laser protective glasses with an optical density greater than 6 (OD > 6) for wavelengths less than 400 nm, and an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm** is required anytime the instrument is opened for servicing and there is risk of exposure. These glasses will filter ultraviolet wavelengths and high intensity light.

WARNING! CLASS 4 and 3R LASERS – INVISIBLE LASER RADIATION – AVOID EXPOSURE TO BEAM! WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

To inspect the objective,

1. If a microplate is inserted in the stage, follow the procedures on “Remove the microplate from the CellInsight™ CX7 Pro HCS Reader stage” on page 47 to remove the microplate.
2. Turn off the main power switch.
3. Remove the AC power cord from the unit.
4. Open the CellInsight™ CX7 Pro HCS Reader service door.

   Note: A key is needed to unlock the door.

5. Use a magnifying glass to look for contamination (e.g., dust or fingerprints) on the optical surface. If contamination is seen on the surface of the lens, continue with the following cleaning instructions.

   The objective should be cleaned when contamination is seen on the surface of the lens or if there is any indication of reduced performance in the appearance of images.

   Note: All optical elements are delicate and should be handled as carefully as possible. The glass and antireflective (AR) coated surfaces will be damaged by any contact, especially if abrasive particles have come into contact with the surface. In most cases, it is best to leave minor debris on the surface.

To clean the objective,

6. Place a clean lens paper on the front surface of the objective. Do not use other tissues which contain abrasive additives. Place a drop of a 1:1 solution of glass cleaner and distilled water on the lens paper. Holding the lens paper at one corner, gently draw it over the objective.

7. If this does not remove the dust, twist the edge of the lens paper into a wick, wet it with the glass cleaner/ water solution, and drag it over the surface of the lens, moving in a single direction.

   If additional cleaning is required, use a new sheet of lens paper for each swipe. Do not scrub or scour the optical surface of the objective with the lens paper. Drawing the fluid over the lens in this way safely cleans it; scrubbing the lens will apply pressure that will damage the optics.

8. Close and lock the CellInsight™ CX7 Pro HCS Reader service door.

9. If you are unable to clean the objective in this way, contact Technical Support for assistance.

Change the objective

You may need to periodically change the objective due to magnification requirements of your Assay Protocols. After you have inspected and cleaned the desired objective, follow the procedures below to change the objective.

Changing objectives is accomplished using the **Objective Change Wizard**. The Objective Change Wizard is an automated wizard that walks you through the required steps of removing an objective from the optional objective turret position, known as the Load/Unload Position, and installing a new objective in its place.

Note: You must run the Objective Change Wizard each time you wish to install a new objective. Only one optional objective can be installed at any given time.

CAUTION! Do not attempt to place the new objective in any position other than the position specified (**Load/Unload Position**). Doing so could cause damage to the objective and instrument.

CAUTION! MOVING MECHANICAL PARTS!

The CellInsight™ CX7 Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. DO NOT MANUALLY OPEN THE STAGE DOOR WHEN THE READER IS PERFORMING A SCAN!

WARNING! RISK OF EYE INJURY!

Wear safety glasses designed to filter ultraviolet wavelengths and high intensity light. **Wearing laser protective glasses with an optical density greater than 6 (OD > 6) for wavelengths less than 400 nm, and an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm** is required anytime the instrument is opened for servicing and there is risk of exposure. These glasses will filter ultraviolet wavelengths and high intensity light.

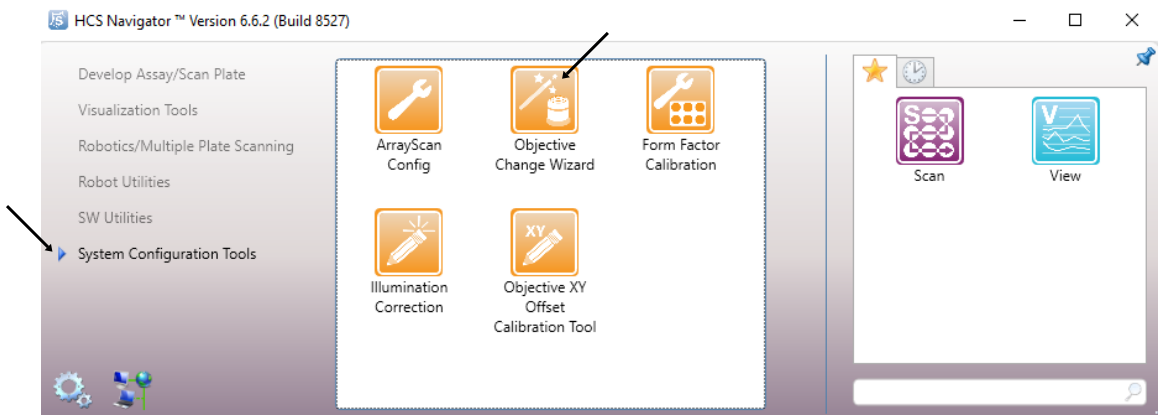
WARNING! CLASS 4 and 3R LASERS – INVISIBLE LASER RADIATION – AVOID EXPOSURE TO BEAM! WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

To change the objective using the Objective Change Wizard,

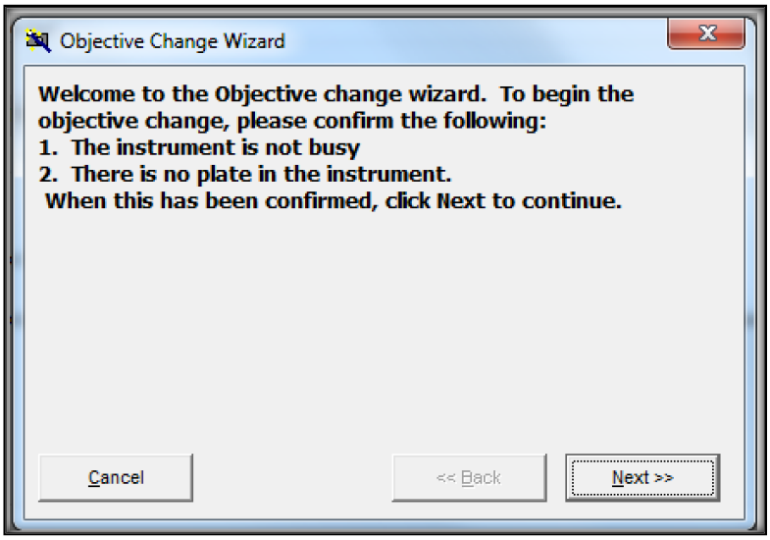
1. If a plate is inserted in the Reader stage, follow plate removal procedures on “Confocal imaging software setup procedures” on page 35 to remove the plate from the Reader stage.

2. Verify that the HCS Studio™ Scan software is closed.

3. From the HCS Studio™ Navigator, select the **System Configuration Tools** option. Then double-click on the **Objective Change Wizard** icon.



4. The Objective Change Wizard welcome screen appears.



5. Confirm the items listed on the welcome screen, then click **Next** to begin instrument initialization and calibration.

6. When the instrument is initialized and calibrated, click **Next** to continue.

7. Wait for system motion to stop. Open the service door as shown in the following image.

Note: A key is required to open this door.

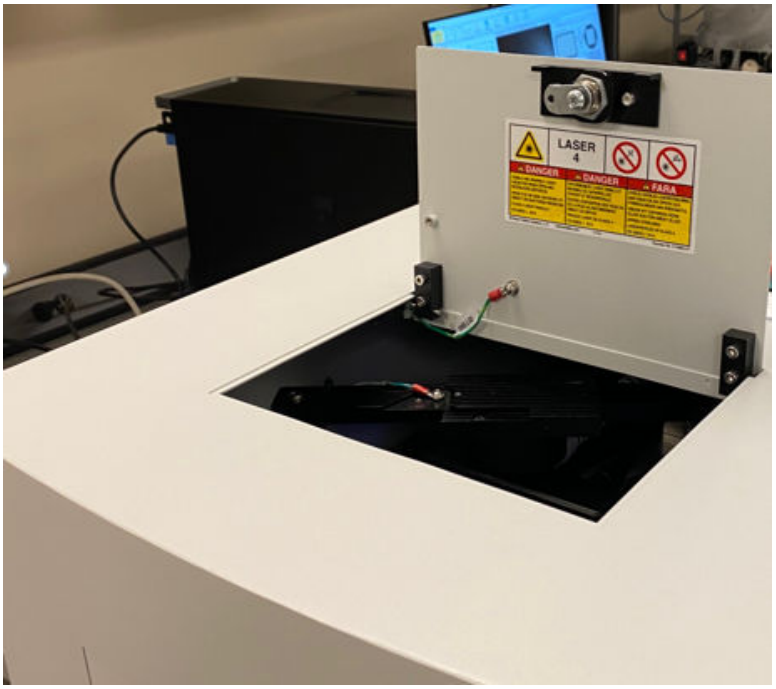


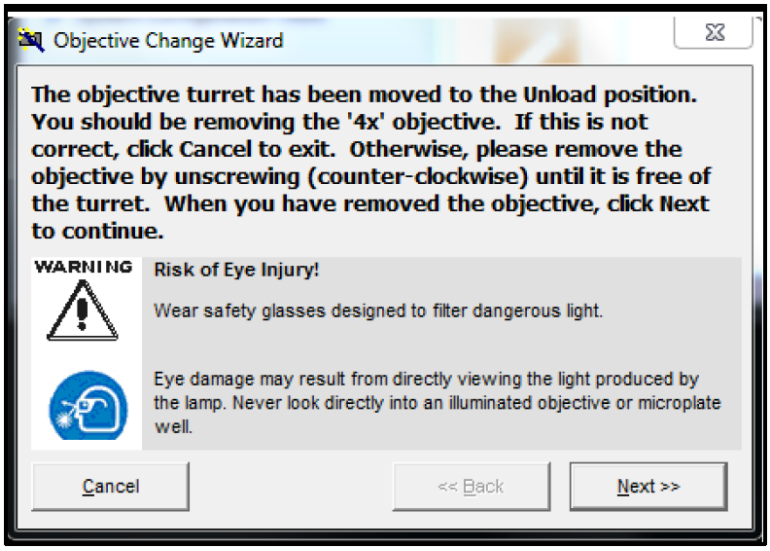
Figure 31 CellInsight™ CX7 Pro HCS Reader service door

8. Do one of the following:

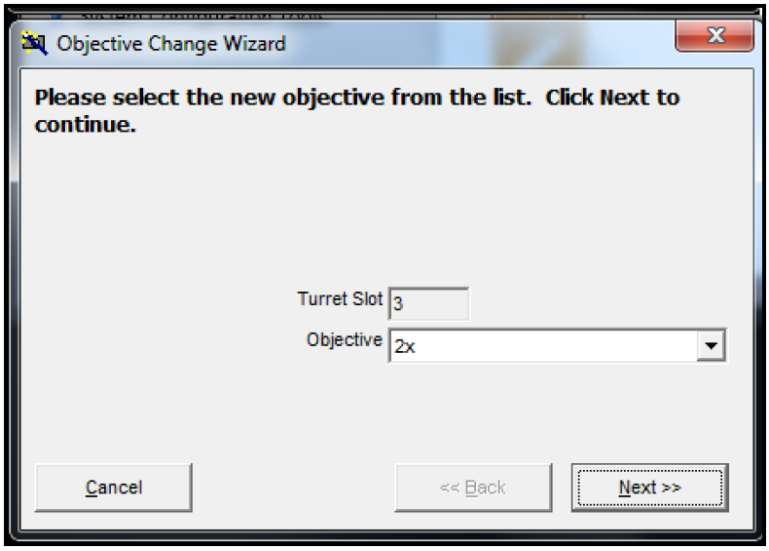
   - **If the system does not detect an objective in the Load/Unload Position,** skip ahead to the next step.
   - **If the system detects that an objective has been installed in the Load/Unload Position**, the turret will be moved such that the objective in the Load/Unload Position is most accessible and the following screen will appear. Follow the instructions on the screen. After removing the objective located in the Load/Unload Position, click **Next**.

CAUTION! To avoid system damage, uninstall and install objectives only in the **Load/Unload Position**.

Note: All optical elements are delicate and should be handled as carefully as possible. The glass and antireflective (AR) coated surfaces will be damaged by any contact, especially if abrasive particles have come into contact with the surface. In most cases, it is best to leave minor debris on the surface.

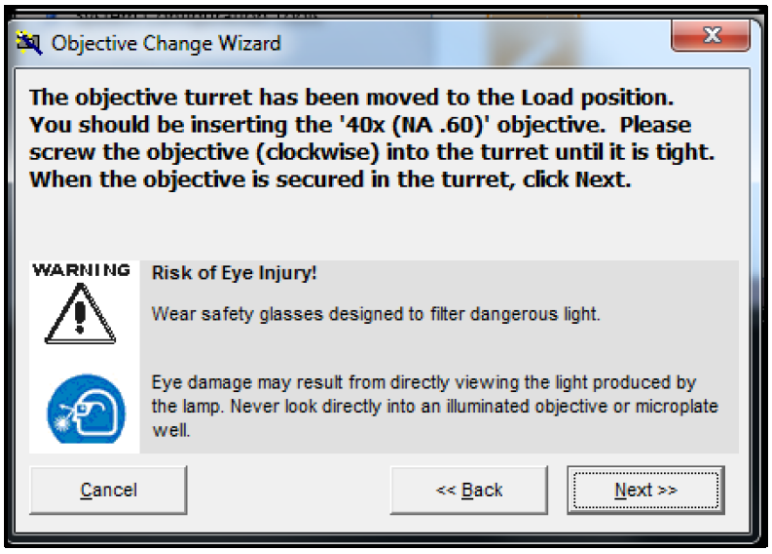


9. From the Objective list, select the objective that you are installing, then click **Next**.



10. The following screen appears. Insert the new objective in the Load/Unload Position by following the instructions on screen. Close and lock the service door. Click **Next** to continue.

Note: All optical elements are delicate and should be handled as carefully as possible. The glass and antireflective (AR) coated surfaces will be damaged by any contact, especially if abrasive particles have come into contact with the surface. In most cases, it is best to leave minor debris on the surface.



11. The following screen appears and indicates that the objective change is complete. Click **Close** to close the Objective Change Wizard.

12. When using the newly installed objective, be sure to use a compatible microplate type.

Note: Thin clear-bottom plastic or glass plates are required when using the high NA objectives.

Note: You must run the Objective Change Wizard each time you wish to install a new objective. Only one optional objective can be installed at any given time in order to avoid interference between the objectives and the stage.

IMPORTANT! After changing an objective, you must verify that the objective chosen in the **Configure Acquisition** tab of the Scan Application matches the actual objective installed on the CellInsight™ CX7 Pro HCS Reader.

Adjust the correction collar on the Olympus™ 20X and 40X objectives

The correction collar, when rotated, separates or brings together some of the internal glass elements of the objective. If the correction collar is not in its optimal position for the plate type, the images can appear soft and slightly out of focus. Rotating the collar can correct for thick culture vessels by eliminating spherical aberration and provide the highest quality image.

CAUTION! MOVING MECHANICAL PARTS!

The CellInsight™ CX7 Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. DO NOT MANUALLY OPEN THE STAGE DOOR WHEN THE READER IS PERFORMING A SCAN!

WARNING! RISK OF EYE INJURY!

Wear safety glasses designed to filter ultraviolet wavelengths and high intensity light. **Wearing laser protective glasses with an optical density greater than 6 (OD > 6) for wavelengths less than 400 nm, and an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm** is required anytime the instrument is opened for servicing and there is risk of exposure. These glasses will filter ultraviolet wavelengths and high intensity light.

WARNING! CLASS 4 and 3R LASERS – INVISIBLE LASER RADIATION – AVOID EXPOSURE TO BEAM! WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

To adjust the correction collar,

1. From the Scan Application, select the **Tools** tab, then select **Move to Objective Change Position.** The stage begins to move to the objective change position. A message informs you when the stage is in the objective change position. In this message, click **OK**. Do NOT remove the objective.

2. To access the objective, wait for system motion to stop. Open the service door as shown in the following image.

   Note: A key is required to open this door.

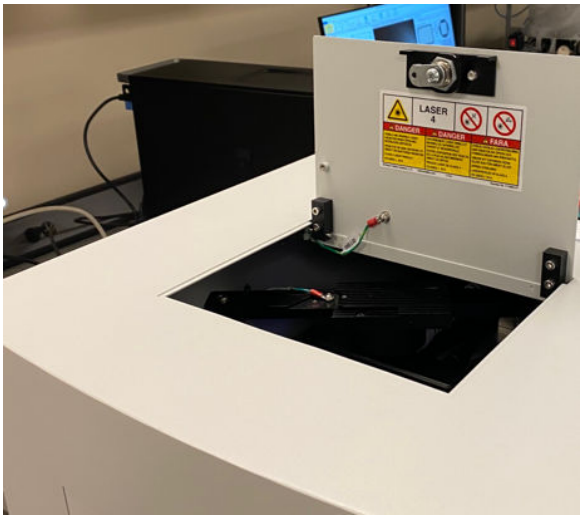


Figure 32 CellInsight™ CX7 Pro HCS Reader service door

3. Based on the plate thickness and the guidelines in the following table, adjust the collar to an appropriate starting position.

Plate Manufacturer and Type	Plate Thickness	Recommended Collar Position
Greiner µClear coated plates	Thin (0.19 mM)	0.17
Corning™ BioCoat™ plates (aka Falcon™)	Thick (1.11 mM)	1.1

4. Place your plate on the stage, click **OK** to close the stage door, then **Autofocus** the instrument. If autofocus fails, try to manually focus using 100 micron steps. If you can manually focus, set proper exposure time and verify that autofocus succeeds and images are in focus. If they are not in focus, proceed to step 5.

5. Rotate the correction collar very slightly to the left or right and re-focus the objective to determine if the image has improved or degraded.

6. Repeat step 1–step 4 to determine if the image is improving or degrading as the correction collar is turned in a single direction (left or right). If the image has degraded, follow the same steps and rotate the correction collar in the opposite direction to find the position offering optimum resolution and contrast.

7. When the collar position has been determined, record the collar position and use this whenever the same type of plate is used.

8. Close and lock the service door.

System schematics

Internal AC system diagram – power A/C

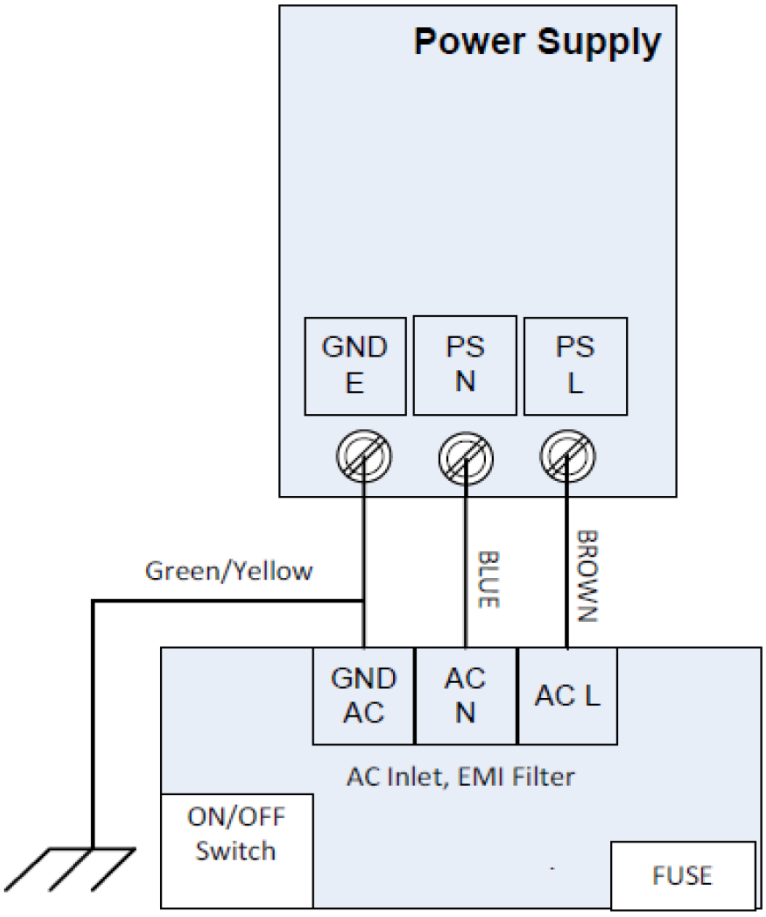


Figure 33 CellInsight™ CX7 Pro HCS Reader System Connection Diagram – Power A/C and D/C

System connection diagram – communication layout

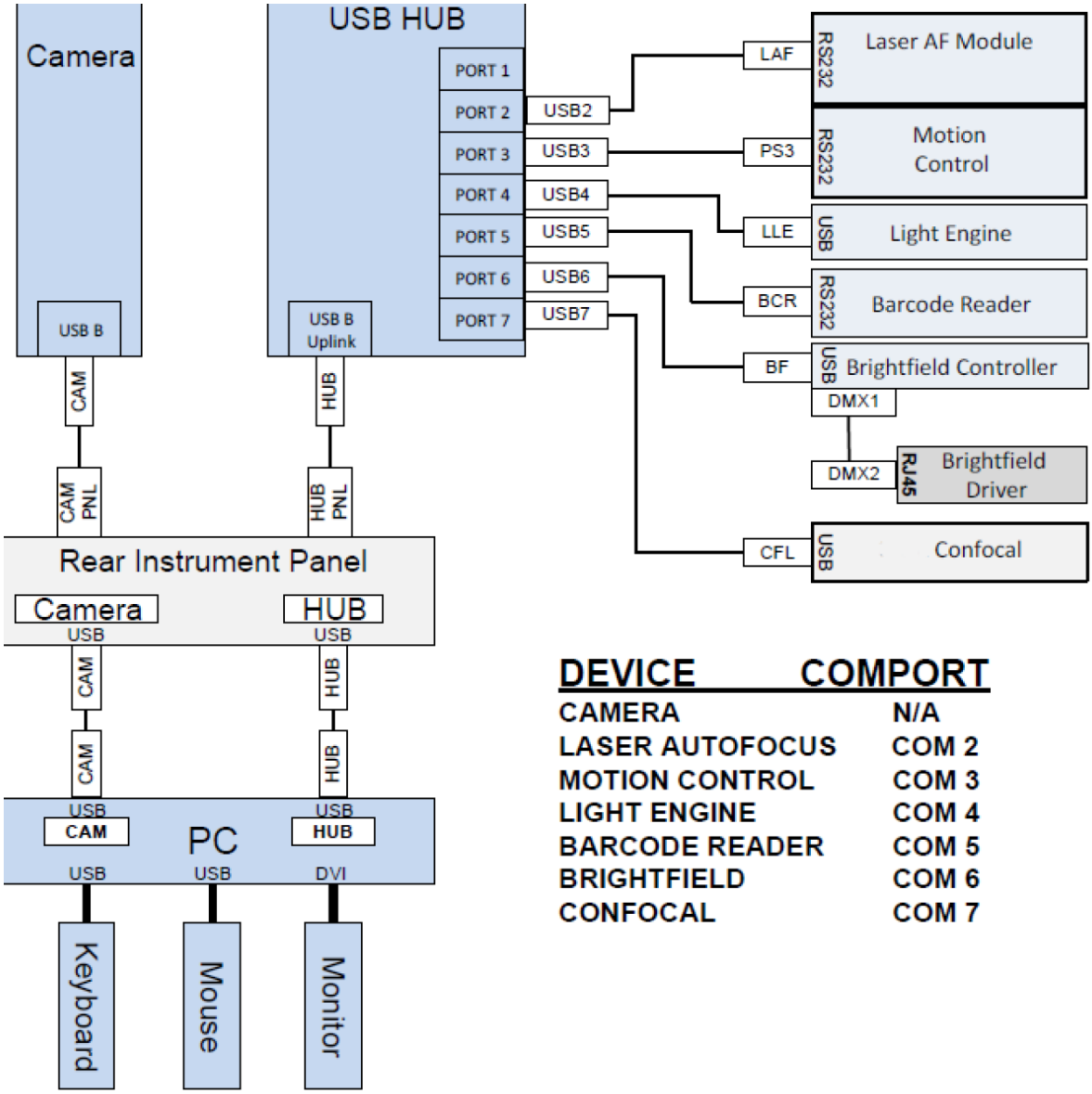


Figure 34 CellInsight™ CX7 Pro HCS Reader Connection Diagram – Communication Layout

Power connection diagram – 110–120 VAC

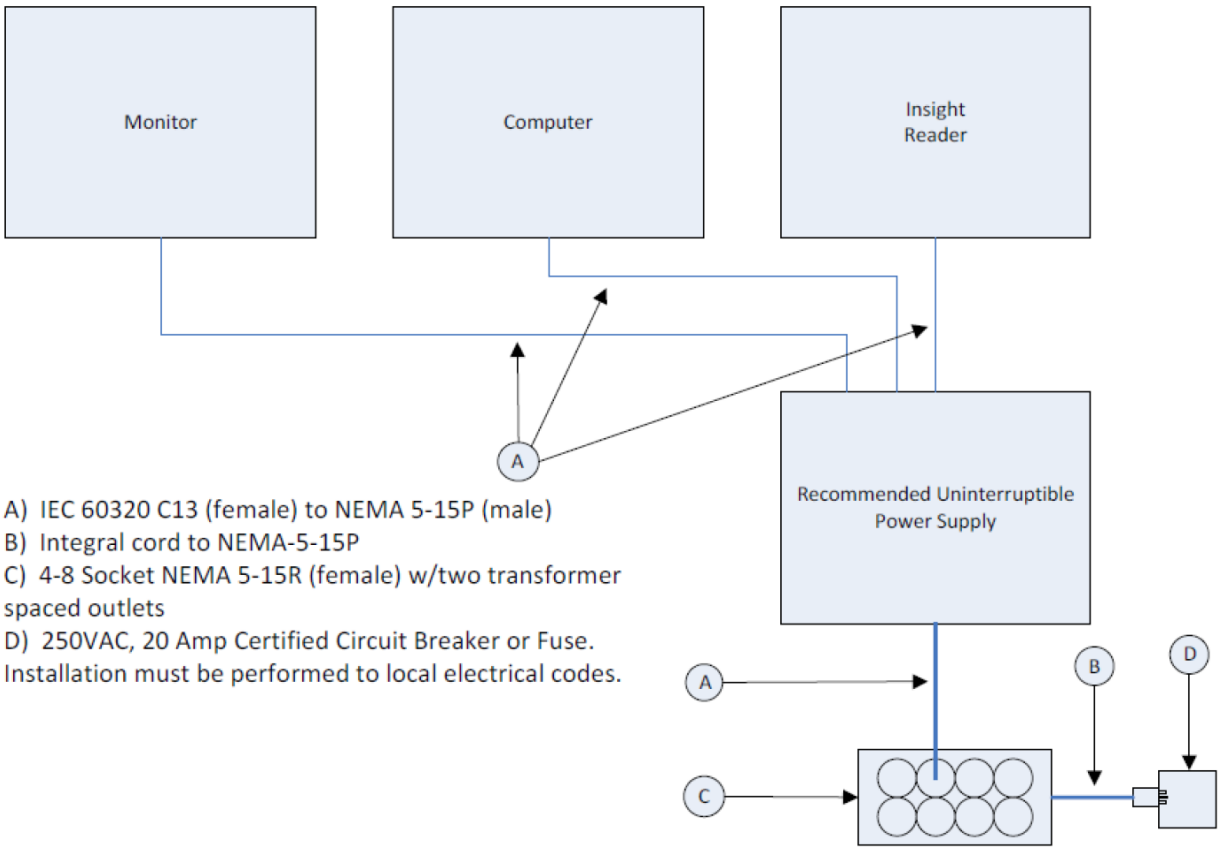


Figure 35 CellInsight™ CX7 Pro HCS Reader System Connection Diagram – Power – 110–120 V

Power connection diagram – 220–240 VAC

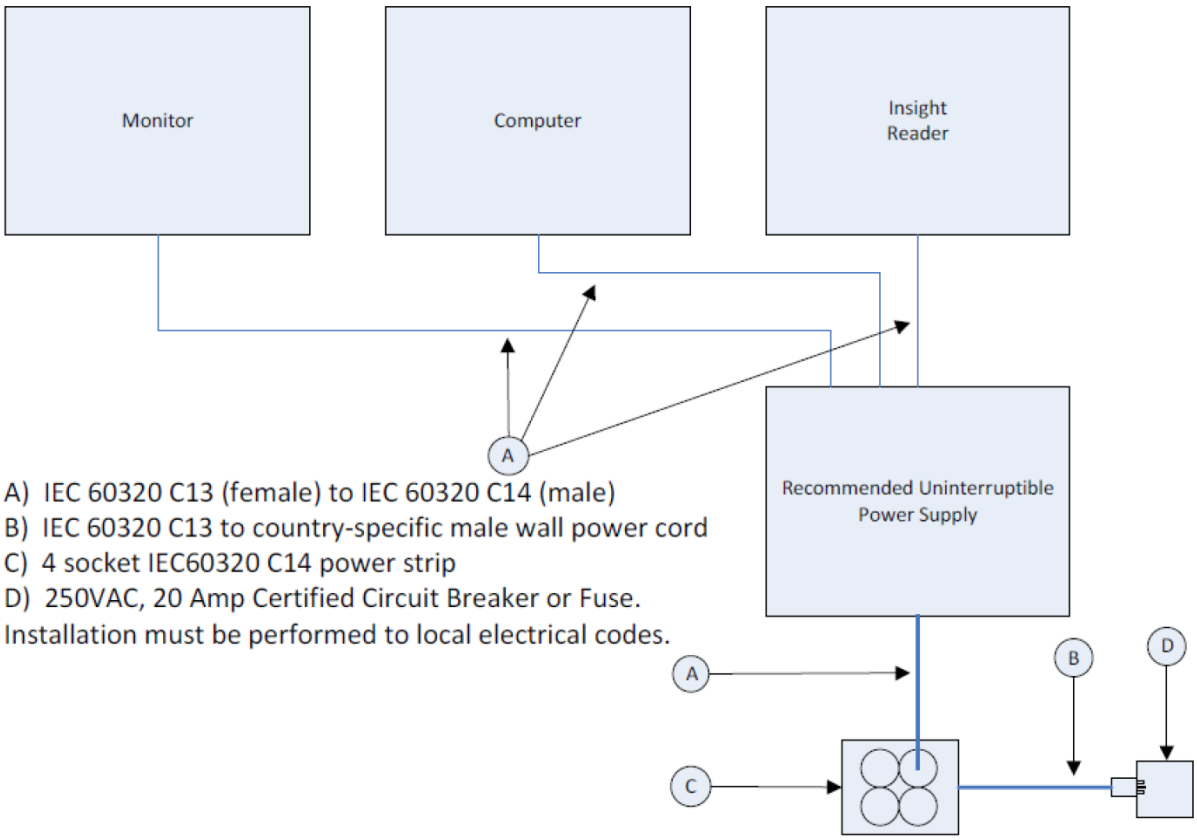


Figure 36 CellInsight™ CX7 Pro HCS ReaderSystem Connection Diagram – Power – 220–240 V

Connection diagram – optional onstage incubator

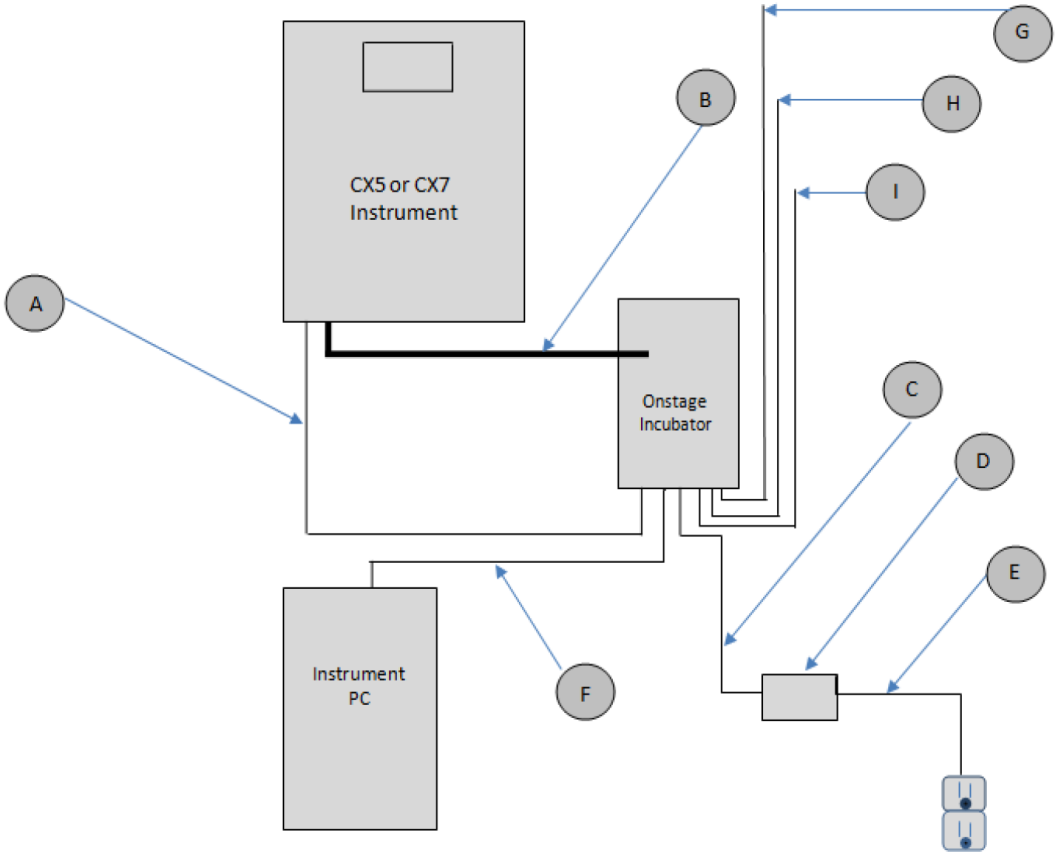


Figure 37 CellInsight™ CX7 Pro HCS Reader System Connection Diagram – CellInsight™ Onstage Incubator

A: 6-Pin Sensor Data Cable

B: Chamber Gas Supply Hose

C: Onstage Incubator Power Supply Cable

D: AC/DC Power Switching Adapter

E: 3-Pin Line Power Cable

F: USB 2.0 A to B Cable

G: CO_2 Inlet Line

H: N_2 Inlet Line

I: Compressed Air Inlet Line

Computer and monitor

WARNING! RISK OF ELECTRIC SHOCK!

Ensure that the computer and monitor are turned off and AC power cords are disconnected from the computer and monitor before cleaning.
Never spray or pour any liquid directly onto the screen or case.

Clean computer monitor

To clean the monitor screen,

1. Ensure that the computer and monitor are turned off and AC power cords are disconnected from the computer and monitor before cleaning. Wipe the screen with a clean, soft, lint-free cloth. This removes dust and other particles.

2. If still not clean, apply a small amount of *non-ammonia, non-alcohol* based glass cleaner onto a clean, soft, lint-free cloth, and wipe the screen. Always use an anti-static screen cleaner.

To clean the monitor case,

1. Ensure that the computer and monitor are turned off and AC power cords are disconnected from the computer and monitor before cleaning. Wipe with a soft, dry cloth.
2. If still not clean, apply a small amount of *non-ammonia, non-alcohol* based mild non-abrasive detergent onto a clean, soft, lint-free cloth, and wipe the case.

Clean computer case exterior

Ensure that the computer and monitor are turned off and AC power cords are disconnected from the computer and monitor before cleaning. Periodically use a clean, dry, soft cloth to wipe the exterior surfaces. Use a mild, non-abrasive detergent and clean, soft, lint-free cloth if further case exterior cleaning is needed.

Disassembly for a short distance move

The CellInsight™ CX7 Pro HCS Reader requires careful handling. The following safety precautions and instructions pertain to moving the instrument a short distance, for example from room to room within a building.

Note: If you are using a robotic plate handler with the CellInsight™ CX7 Pro HCS Reader, refer to your robotic plate handler user documentation for disassembly instructions.

WARNING! RISK OF ELECTRIC SHOCK!

Switch OFF the power and disconnect the power cord from the power supply before performing any procedure that requires moving the instrument components.

WARNING! Always handle the equipment with care when moving. The CellInsight™ CX7 Pro HCS Reader weighs approximately 82 kg (180 lbs). At least two people are required for transport. Take the proper lifting precautions to avoid injury and always lift the CellInsight™ CX7 Pro HCS Reader by grasping the underside of the instrument frame. Do not attempt to lift the instrument by grasping the enclosure panels.

Before moving the CellInsight™ CX7 Pro HCS Reader,

- Contact Technical Support to assist in planning a move.
- Ensure that the system will be protected from jarring, shock and vibration, liquids and moisture, and temperature extremes during the move. The system should remain within approximately 15 degrees of level throughout the move.
- Prepare the new location by clearing an appropriately sized bench area. Refer to the requirements for bench size, supporting weight, environment, and clearances that are specified in the Safety Precautions chapter of this guide.
- Position the instrument so that you can reach the AC inlet and avoid a trip hazard created by any of the cords, such as the AC cord.
- Ensure that the robotic plate handler arm, if present, is not obstructed in its motion.
- Ensure that there is a power outlet, network, and phone line connection at the new location. Refer to the Appendix A, “Technical specification” for system power requirements.
- Use a cart or other suitable transport that can safely support the system weight. Transport over smooth surface; avoid rough tile flooring, etc.
- If moving from floor-to-floor within a building, we recommend using an elevator.

Note: If the instrument has experienced temperature differences during transport, allow 2–3 hours before powering on to avoid any possible damage due to condensation.

Prepare the CellInsight™ CX7 Pro HCS Reader for a short move

1. Stop any current plate scan and, if present, remove the microplate from the CellInsight™ CX7 Pro HCS Reader stage. Ensure that the stage door is closed.
2. Shut down the application software, the computer and monitor, and the CellInsight™ CX7 Pro HCS Reader. See “CellInsight™ CX7 Pro HCS Reader shutdown” on page 51 for instructions.
3. Disconnect all cables between the instrument and the computer, as well as the robotic microplate handler, if present. Mark all cables so you can easily connect them at the new location. Coil and tie the cords. Unplug the terminal strip from the wall socket. If you are using a robotic plate handler, refer to the robotic plate handler manufacturer documentation for instructions on preparing the robot for a move.

   Note: The weight of the CellInsight™ CX7 Pro HCS Reader is approximately 82 kg (180 lbs). At least two persons are required for transport. Take the proper lifting precautions to avoid injury. Lift the CellInsight™ CX7 Pro HCS Reader by grasping the underside of the instrument frame. Do not attempt to lift the instrument by grasping the enclosure panels.
4. At the new location, place the instrument on the workbench. Position the instrument so that you can reach the AC inlet and avoid a trip hazard created by any of the cords, such as the AC cord.
5. Reconnect all cables between the instrument and computer and robotic plate handler, if present.
6. Plug all power cords into the terminal strip. Plug the terminal strip into the wall socket.

Disassembly and packing for shipping

The CellInsight™ CX7 Pro HCS Reader requires careful handling. Do not attempt to pack and ship the system without contacting Technical Support.

Long-term shutdown

If you will not be using the CellInsight™ CX7 Pro HCS Reader for an extended period of time (30 days or more), perform the following steps to protect the system.

Note: If you are using a robotic plate handler with the CellInsight™ CX7 Pro HCS Reader, refer to your robotic plate handler user documentation for system shutdown instructions.

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1. Remove the plate, if present, from the CellInsight™ CX7 Pro HCS Reader.
2. Follow the shutdown procedures described in “CellInsight™ CX7 Pro HCS Reader shutdown” on page 51.
3. Turn off the power switch on the terminal strip.
4. Unplug the terminal strip power cord from its power outlet.
5. Cover the components with a plastic or cloth cover.

Sterilization procedure

In the event that a hazardous material is spilled onto or into the components of the CellInsight™ CX7 Pro HCS Reader, follow the sterilization procedure as described below. **However, if the hazardous material is a biohazard, refer to "Biological hazard safety"** **on page 142** **and contact Technical Support for applicable cleanup procedures.**

CAUTION! IMPORTANT!

Do **not** subject the CellInsight™ CX7 Pro HCS Reader to UV sterilization. UV degrades many materials, including plastic. Damage from UV exposure is not covered under the manufacturer's warranty.

CAUTION! CLEANING AND DECONTAMINATION

Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

WARNING! BIOHAZARD!

Use proper laboratory practices when handling hazardous materials. Refer to "Biological hazard safety" on page 142.

- Ensure that biohazards do not contaminate system components.
- In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.
- The CellInsight™ CX7 Pro HCS Platform is not equipped with any special devices for protection from substances which are corrosive, toxic, radioactive, or otherwise hazardous to health. All legal regulations for accident prevention, particularly those in the respective countries, must be observed when handling such substances.

Sterilize components in the event of a non-biohazard material spill

1. Turn OFF the CellInsight™ CX7 Pro HCS Platform and all optional components/modules and accessories, then disconnect the AC power cords from the units.
2. Lightly wipe working surfaces of the CellInsight™ CX7 Pro HCS Reader with paper towels or Kimwipes™ tissues dampened with 70% ethanol or 4,000 ppm hydrogen peroxide (H_2O_2).

CAUTION! IMPORTANT!

Do **not** soak any surface in sterilization solution. NEVER spray liquid anywhere on the instrument. Always wipe surfaces with dampened paper towels instead.

Dismantling and recycling the CellInsight™ CX7 Pro HCS Reader equipment

IMPORTANT! Do not dispose of the CellInsight™ CX7 Pro HCS Reader equipment with other unsorted municipal or household waste. ontact Technical Support for more information.

The CellInsight™ CX7 Pro HCS Reader contains hazardous substances. Follow all national and local regulations with regard to dismantling, recycling, and disposal. Contact Technical Support for detailed information.

Contact the manufacturer of the CellInsight™ CX7 Pro HCS Reader computer for detailed information regarding the dismantling and recycling of the computer equipment.

A Technical specification

Note: Technical specifications are subject to change without notice.

CellInsight™ CX7 Pro HCS Reader instrument

Maximum power consumption	720 W
Frequency range	50–60 Hz
AC voltage	100–240 V
Current	6 A maximum
Supported microplates	• Compatible with clear bottom 6-, 12-, 24-, 48-, 96-, 384- and 1536-well microplates • Slideport™ Slide Inserts for up to 4 glass microscope slides
Stage travel	327 mm × 78 mm
XY resolution	0.04 µm
Z resolution	0.006 µm
Minimum Z-stack step size	0.1 µm
Wavelength range	Specific wavelengths between 386 nm–747 nm
Objectives	Olympus™ UPLXAPO 10X/0.4NA (3.1 mm WD); Olympus™ LUCPLFLN 20X/0.45NA w/CC (6.60–7.80 mm WD); Olympus™ UCPLFLN 20X/0.7NA w/CC (0.80–1.80 mm WD); Olympus™ LUCPLFLN 40X/0.6NA w/CC (2.70-4.00 mm WD) The following optional objectives are available: Olympus™ PLAPON 1.25X/0.04NA (5 mm WD); Olympus™ PLAPO 2X/0.08NA (6.2 mm WD); Olympus™ UPLXAPO 4X/0.16NA (13 mm WD); Olympus™ UPLXAPO 20X/0.8NA (0.6 mm WD); Olympus™ UPLXAPO 40X/0.95NA (0.18 mm WD); Olympus™ UPLFLN 60X/0.9NA (0.2 mm WD)
Max number of channels	6

Camera	Photometrics Prime BSI: High-resolution, thermo-electrically cooled to –20°C; up to 16-bit dynamic range (15-bit range for HCS Studio™ Cell Analysis Software, 16-bit for acquire only); FOV 2048 × 2048 pixel array; 6.5 × 6.5 micron pixel size; >95% peak Quantum Efficiency (QE); USB 3.0 (no additional CameraLink or Firewire Cards needed) See Figure 38 for the camera quantum efficiency vs. wavelength graph.
Optics	Infinity corrected system
Standard objective field size	10X = 870.1 µm 20X = 435 µm 40X = 217.5 µm
Image compatibility	JPG, BMP, GIF, PNG, TIF
Instrument dimensions	80 (D) × 48.5 (W) × 48.5 (H) cm 31.5 (D) × 19.1 (W) × 19.1 (H) in. Height includes the feet which the instrument sits on. The feet should not be removed.
Instrument weight	82 kg (180 lbs)
Instrument shipping dimensions	104.1 (D) × 101.6 (W) × 86.4 (H) cm 41 (D) × 40 (W) × 34 (H) in.
Instrument shipping weight	140 kg (308 lbs) (includes all items and packaging)
Optical filters	Refer to Appendix B, “Automated spectral selection for fluorescence imaging” for detailed information.
NIR laser radiation mode	Continuous wave
NIR laser maximum power	4 W
NIR laser average power range	250–400 mW

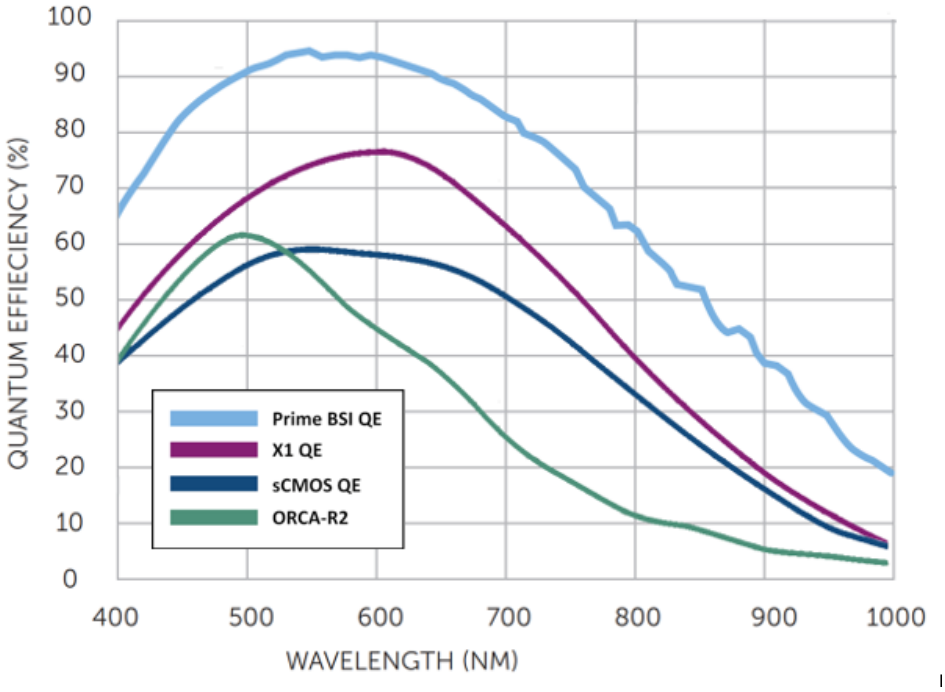


Figure 38 Camera quantum efficiency vs. wavelength

Dimensions and clearances

The recommended bench size is as follows:

60 in. wide by 25 in. deep if the CellInsight™ computer can be placed under the bench

-or-

72 in. wide by 25 in. deep if the CellInsight™ computer can be placed on the same bench top

Note the required clearances.

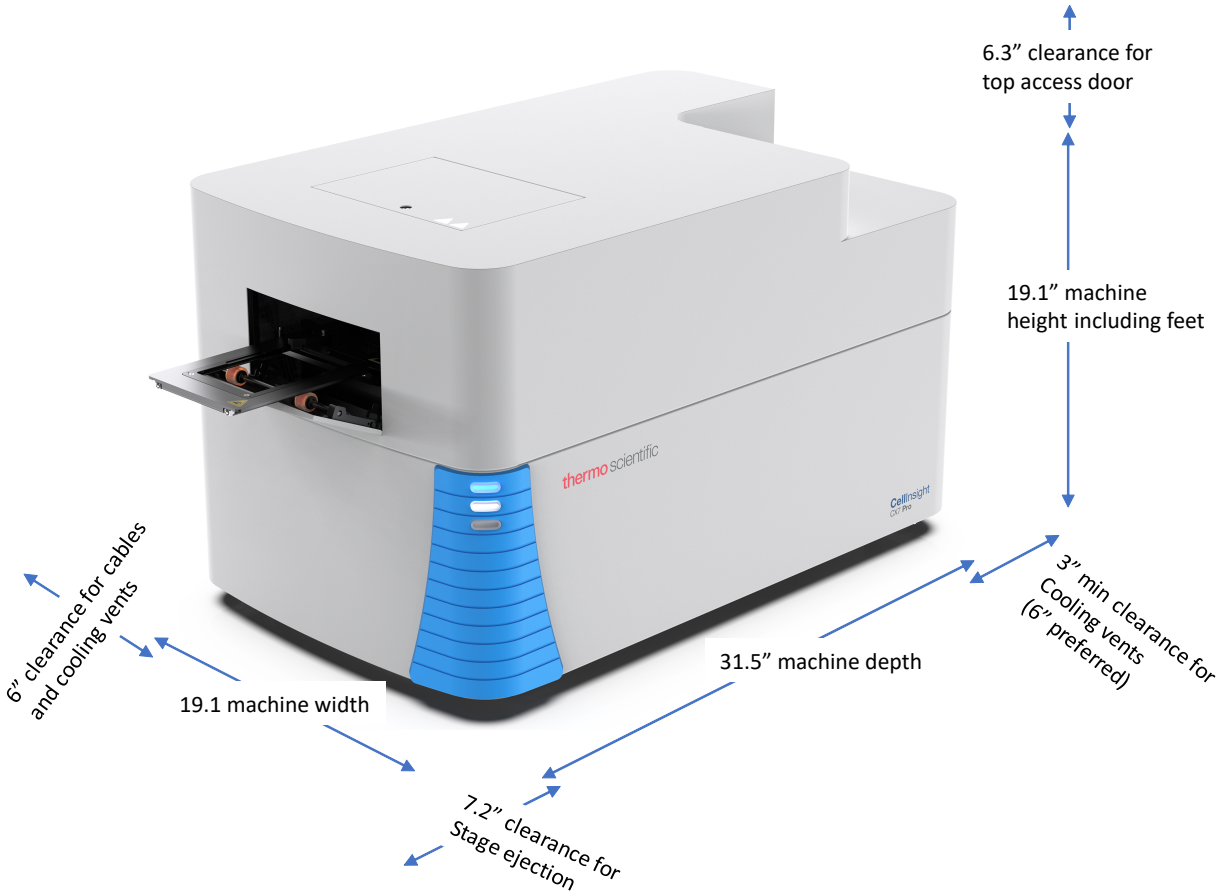


Figure 39 Dimensions and clearances

Robotic plate handler

Refer to the appropriate robot manufacturer documentation for detailed specifications.

If you are using an Orbitor™ RS Microplate Mover, refer to the *Thermo Scientific™ Orbitor™ RS User Guide* (Pub. Part. No. LC07500100).

Light source

The system is equipped with a 7 color light source consisting of 6 LEDs and a near-infra red laser, all contained within the solid-state light engine for fluorescence excitation.

Barcode reader

The following barcode symbologies are supported:

UPC-A

UPC-E

EAN-8

EAN-13

USS-128

UCC/EAN-128

ISBT 128

Code 39

Code 39 Full ASCII Conversion

Interleaved 2 of 5

CellInsight™ computer

Refer to the computer manufacturer documentation for specifications.

Additional system specifications

Earthquake protection	None
Electrical safety	Conforms to IEC 61010-1:2010
Enclosure	IP 20
Intended site	Closed room
Interference suppression	Conforms to EN 61326-1:2013
Line frequency	50/60 Hz
Line voltage	110–120 VAC ± 10% (220–240 VAC ± 10%)
Maximum elevation of site above sea level	2000 m
Noise immunity	Conforms to EN 61326-1:2013
Sound pressure Lp	< 70 dB (A)
Overall space required for entire system	637.5 mm (D), 645.2 mm (H), 1059.2 mm (W)
Overvoltage category	II
Permissible ambient temperature range during operation	+ 18°C to +25°C
Permissible ambient temperature range during transport	+ 5°C to +50°C
Permissible atmospheric pressure range	800 hPa to 1.060 hPa
Permissible relative humidity during operation (non-condensing)	< 75% at 30°C
Phytosanitary measures	Transport crates conform to IPPC Standard No. 15
Pollution degree	2
Power line fuses	110–120V: 6A Medium Acting Fuse 220–240V: 6A Medium Acting Fuse
Protection class	I

B Automated spectral selection for fluorescence imaging

This appendix describes the key spectral elements of the 7-color CellInsight™ CX7 Pro High Content Screening (HCS) Platform that enable its fluorescence imaging capabilities. Fluorescence imaging is enabled by an epi-illumination optical geometry where the fluorescence emission travels back along the same optical path as the illumination (Figure 40). Two options are available for fluorescence imaging: widefield and confocal fluorescence imaging. The CellInsight™ CX7 Pro HCS Platform is also capable of brightfield microscopy in five different colors in a transmitted light optical geometry, which can be multiplexed with the different fluorescence microscopy options; the transmitted light imaging capabilities are described in a different section.

In this appendix, the spectral output of the 7-color excitation source, and the transmission spectra for the independently controlled dichroic mirror and emission filter options are described. Also described are the key components enabling widefield and confocal fluorescence imaging. Recommendations of common fluorophores, fluorescent proteins, and other fluorescent probes that can be used with the filters in this instrument are also given, as well as their peak wavelengths for excitation (absorbance) and emission (fluorescence at fixed excitation wavelength).

The instrument comes with a range of multi-band and single band dichroic mirrors and emission filters. These mirrors and filters are available in a basic configuration that enables a wide range of fluorescent probes to be used, as well as in an advanced configuration that enables non-standard imaging modes such as ratiometric imaging, FRET imaging (fluorescence resonance energy transfer), and the imaging of quantum dots. Note that in this appendix, all spectra are normalized, therefore, quantitative inferences based on the figures are not necessarily valid. The micro-environment of a fluorophore in a specimen can be quite different from samples used to obtain these spectra (e.g., pH and other components of buffers, natural auto-fluorescence of the bio-specimen or substrate, quenching, and FRET can all affect the spectra). Moreover, the relative intensities in a multi-labeled specimen are unlikely to be equivalent, and imaging exposure times (sCMOS integration) will likely differ. Additionally, there is potential for cross-talk/bleed-through with multi-labeled samples.

The following topics are discussed in this chapter:

Instrument configurations

Widefield – confocal configuration

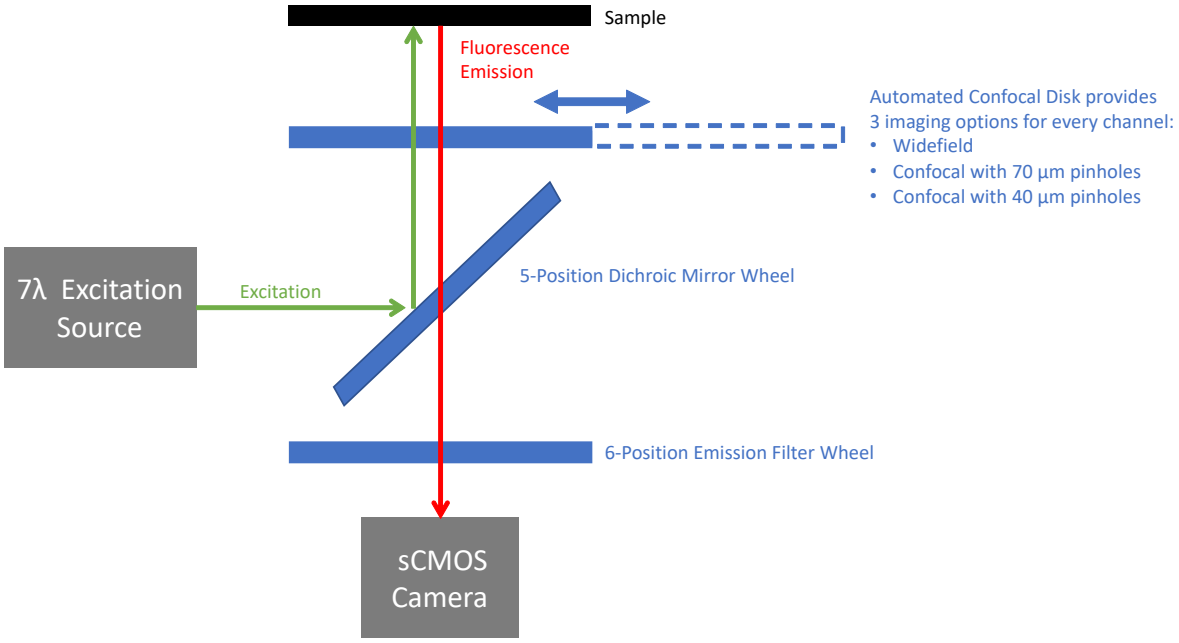


Figure 40 The Widefield – Confocal configuration for the 7-color CellInsight™ CX7 Pro HCS Platform

The Widefield – Confocal configuration has the following elements:

- The confocal and widefield imaging modes follow the same optical path; depending on the imaging mode selected, the confocal disk slides out of the optical path for widefield imaging or it is in place with two different pinhole options (40 µm or 70 µm) for confocal imaging.
- The same high intensity 7-color light source is used for fluorescence excitation for the widefield and confocal imaging modes
- The same automated five-position dichroic mirror filter wheel and six-position emission filter wheel provide different filter options for the widefield and the confocal imaging modes

Light source for fluorescence excitation

The instrument has a 7-color light source for fluorescence excitation; the 6 lower excitation wavelengths are generated from LEDs, and the 747 nm excitation is from a laser diode. Each color generated from the LEDs is further conditioned by an internal emission filter before being emitted from the light source. The use of LEDs with internal excitation filters eliminates the need for additional excitation filter wheels in the light path. The excitation light source has been purposely built for these seven colors, which range from the ultra violet (UV) to the near infra-red (NIR). Figure 41 shows the spectral power output of the seven emitted colors from the light source – each color is described by the center wavelength, and the FWHM (full width of the half maximum) of the emitted spectral band. For example, 386_23 refers to exciting the sample with 386 nm light with a 23 nm band. The spectral power output from the 6 lower wavelength LED lines are normalized. Note that the excitation filters cannot be changed to broaden the range of excitation using the LED light source; nonetheless these seven colors can excite a broad range of fluorescent probes, giving you a lot of options and flexibility in choosing fluorescent dyes (Figure 42).

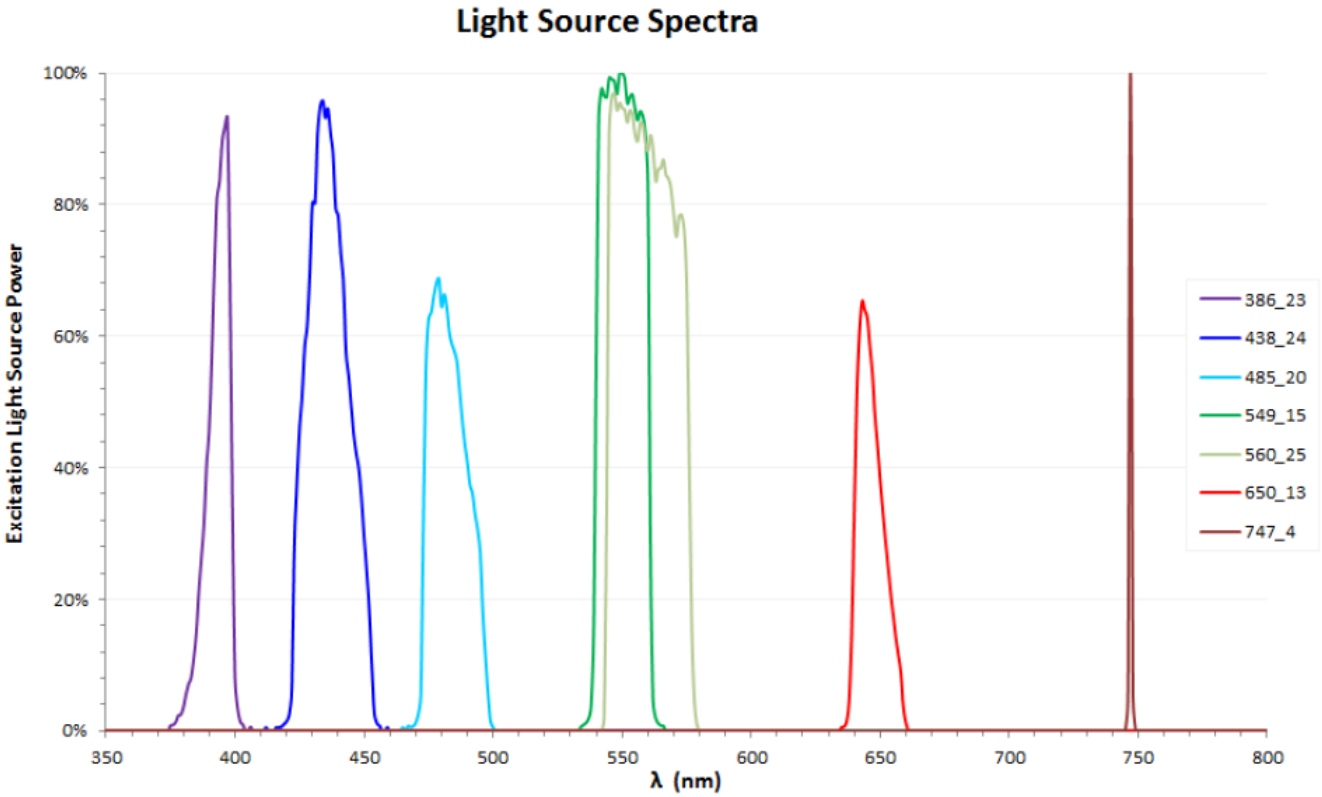


Figure 41 Spectral power output from the 7-color excitation light source

Note: "386_23 nm" means center-wavelength of 386 nm and full-width-at-half-maximum band of 23 nm.

Basic configurations for excitation light source, dichroic mirrors, and emission filters

The instrument has two matched multi-band dichroic mirror and emission filter sets and several single band dichroic mirrors and emission filters. The individual excitation colors from the excitation light source are matched with various dichroic-emitter combinations to enable a wide range of fluorophores, fluorescent proteins and other fluorescent probes. The excitation sources and filters for imaging are available in two modes: Basic and Advanced.

- **Basic mode:** Enables fluorescence measurement from a wide range of fluorophores, fluorescent proteins, and other fluorescent probes across the visible spectra, from UV to NIR.
- **Advanced mode:** Enables non-standard fluorescence imaging such as: FRET (fluorescence resonance energy transfer) imaging, ratiometric imaging, and imaging quantum dots.

The nomenclature convention that is used to describe the different excitation-dichroic mirror-emission filter combinations is based on the fluorophore emission colors that this system supports – i.e., blue (B), cyan (C), green (G), Yellow (Y), red (R), scarlet (S), far red (FR), and near-infra-red (N). The wavelength ranges of these colors are shown in Figure 42. The naming convention specifies the LED, dichroic mirror, and emission filter combination. For example, 386-23_ BGRFRN_BGRFRN refers to exciting the sample with 386 nm light with a 23 nm band, and using the penta-band dichroic mirror along with a penta-band emission filter that transmits blue (B), green (G), red (R), far red (FR), and near-infra-red (N) light. Likewise, 438-24_C_Y refers to exciting the sample with 438 nm light with a 24 nm band, and using the single-band dichroic mirror that transmits cyan (C) light and emission filter that transmits yellow (Y) light. All filter combinations that work (i.e., do not cause bleed through of excitation light) are available, in either the basic or advanced spectral configurations.

Figure 42 summarizes this instrument's basic spectral capability; where for each of the 7 colors, it shows the excitation wavelength and range from the excitation light source, the emission filter transmission band for that color, and common fluorescent probes for that color channel. Note that the fluorescent probes, proteins and fluorophores are a representative set used as an example. There are many other fluorescent probes that could be used. The specific excitation light source, dichroic mirror, and emission filter combinations that are available in the basic configuration, and that enable the imaging of the range of fluorophores shown in Figure 42, is laid out in Figure 43. Figure 44-Figure 46 show the normalized excitation powers and the transmission traces of all the basic filter combinations.

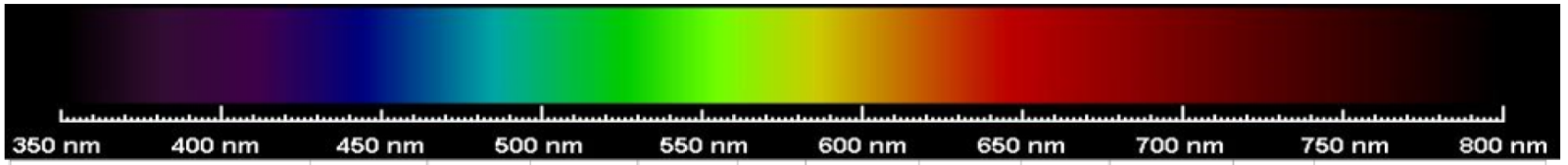
Specifically, the system comes with two multiband dichroic mirrors that have matched multiband emission filters:

- **BGRFRN:** The penta-band (i.e., 5-color) BGRFRN dichroic mirror and emission filter enable the multiplexed imaging of up to five different colors (Figure 43-Figure 44).
- **BGS:** The tri-band (3-color) BGS dichroic mirror and emission filter enable the multiplexed imaging of up to three different colors. This combination has broader transmission and thus brighter detection for green, red, and scarlet dyes than the BGRFRN penta-band set (Figure 43-Figure 45).
- **RS:** Additionally, there is a broad RS (red scarlet) single band emission filter which can be used with either the BGRFRN or BGS dichroic mirrors. With 549_15 or 560_25 nm excitation, the RS emitter enables brighter detection of red and scarlet dyes than with the BGRFRN emitter. The 485_20 nm exciter with the RS emitter also enables doing FRET between green (donor) and red or scarlet (acceptor) dyes.

The basic configuration also has three pairs of matched single band dichroic mirrors and emission filters that expand the spectral capability of the instrument (transmission traces in Figure 46):

- **C:** The matched C (cyan) dichroic mirror and emission filter, which with 438_24 nm excitation, enables excitation of versions of CFP (cyan fluorescent protein).
- **Y:** The matched Y (yellow) dichroic mirror and emission filter, which with 485_20 nm excitation, enables excitation of versions of YFP (yellow fluorescent protein).
- **N:** The matched N (near infra-red) dichroic mirror and emission filter, which with 747_4 nm laser excitation, enables excitation of versions of fluorescent probes that fluoresce in the near infra-red range.

CellInsight™ CX7 Fluorescence Spectral Capability



Nomenclature *(based on dye emission)*	Blue (B)	Cyan (C)	Green (G)	Yellow (Y)	Red (R)	Scarlet (S)	Far Red (FR)	LASER AUTOFOCUS λ (730 nm)	Near IR (N)
Excitation: 7λ LED & laser (center λ/FWHM) (nm)	*386/23*	*438/24*	*485/20*		*549/15*	*560/25*	*650/13*		*747/4*
BGRFRN Emission Filter (center λ/FWHM) (nm)	*438/47*		*521/22*		*604/30*		*704/54*		*810/85*
BGS Emission Filter (center λ/FWHM) (nm)	*432/44*		*517/31*		*615/71*				
4 Single Band Emission Filters (center λ/FWHM) (nm)		*C 482/25*		*Y 542/27*	*RS 612/69*				*N 809/81*
Typical Fluorophores & Fluorescent Proteins (Ex λ max / Em λ max)	DyLight 405 (400/420) Alexa Fluor® 405 (401/421) DyLight 350 (353/432) Whole Cell Blue (350/440) Alexa Fluor® 350 (346/442) AMCA (350/450) DAPI (345/455) Pacific Blue (403/455) BFP (402/457) Hoechst (350/461)	ECFP (433/475) TagCFP (458/480)	EGFP (488/507) Cy2™ (492/510) DyLight 488 (493/518) Whole Cell Green (493/518) Alexa Fluor® 488 (499/519) FITC (495/520)	EYFP (514/527) TagYFP (508/524)	mOrange (549/565) Alexa Fluor® 555 (555/565) Whole Cell Orange (550/568) TRITC (550/570) Cy3™ (550/570) Alexa Fluor® 546 (556/573) Mitotracker® Orange (551/576) Dylight 550 (562/576) PE (496/578)	DsRed (557/582) mCherry (587/610) Texas Red (596/620) Alexa Fluor® 594 (591/618) DyLight 594 (593/618) Rhodamine Red-X (570/590) Alexa Fluor® 568 (578/603)	DyLight 633 (638-658) Alexa Fluor® 647 (650/665) Cy5™ (650/670) DyLight 650 (652/672) Whole Cell Red (654/673) Alexa Fluor® 660 (663/690) Cy5.5™ (675/694) DRAQ5 (646/696)		Cy7™ (742-767) Alexa Fluor® 750 (749/775) DyLight 755 (754/776) DyLight 800 (777/794) Alexa Fluor® 790 (784/814)

Independent Control of:
- 7λ excitation source
- 5 position dichroic mirror wheel
- 6 position emission filter wheel

Figure 42 7-color basic spectral capability of the CellInsight™ CX7 Pro HCS Platform

7 Color LED Light Engine Exciter	5 Position Dichroic Mirror Wheel	6 Position Emission Filter Wheel	Use
386_23, 485_20, 549_15, 560_25, 650_13, 747_4	BGRFRN	BGRFRN	• 5λ multiband dichroic & emitter covering visible spectrum to simultaneously detect 5 colors (Blue, Green, Red/Scarlet, Far Red & Near-IR), for speed • Uses 6 of the 7λ LLE excitations • 560_25 exciter not recommended with BGRFRN dichroic & RS emitter
		RS	• Brighter detection of Red & Scarlet dyes with RS emitter • Green/Red-Scarlet FRET with RS emitter
386_23, 485_20, 549_15	BGS	BGS	• Optimized 3λ multiband for Blue, Green, & Red/Scarlet dyes • Brighter detection of Green, Red & Scarlet dyes than with BGRFRN set
438_24	C (Cyan)	C (Cyan)	Enables detecting CFP
485_20	Y (Yellow)	Y (Yellow)	Enables detecting YFP
747_4	N (Near IR)	N (Near IR)	• Dedicated Near IR/Near IR channel for optimized detection of near IR dyes • Can be used with BGS set, to avoid near-IR dye excitation by UV LED

Figure 43 Basic configuration for excitation light source, dichroic mirrors, and emission filters

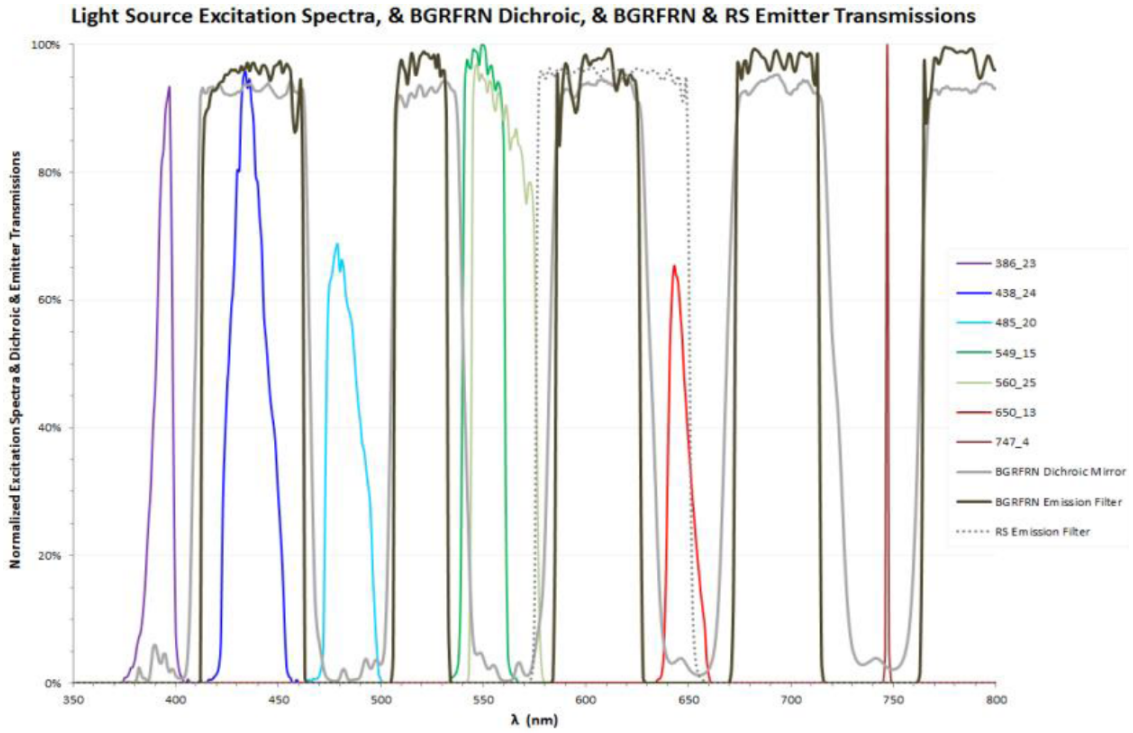


Figure 44 Penta-band BGRFRN dichroic mirror and emission filter transmission spectra, overlaid with the normalized spectral power distribution from the excitation 7-color light engine

Also shown is the single band RS emission filter's transmission. Only the excitation sources that fall between transmission bands should be used to excite dyes whose emission falls within a transition band to the right of the excitation. Excitation with a wavelength that falls inside a transition band (e.g., 438_24 for this set) will cause bleed through, and thus should be avoided.

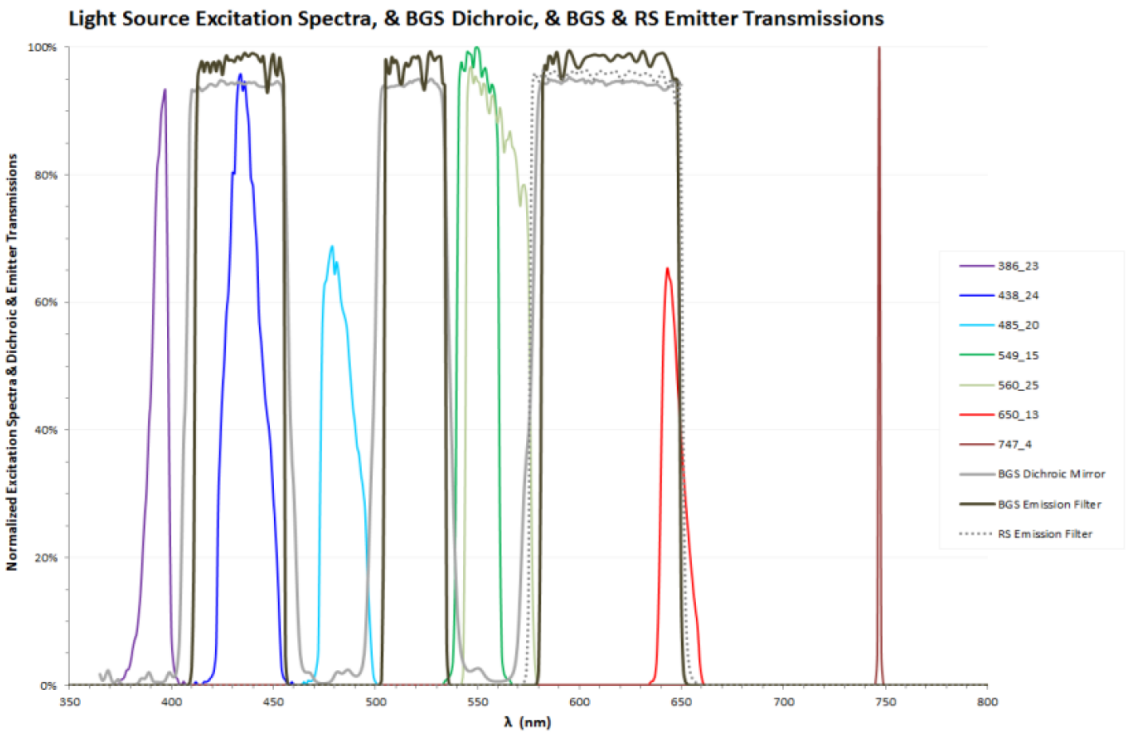


Figure 45 Tri-band BGS dichroic mirror and emission filter transmission spectra, overlaid with the normalized spectral power distribution from the excitation 7-color light engine

Also shown is the single band RS emission filter's transmission. Only excitation sources that fall between transmission bands should be used to excite dyes whose emission falls within a transition band to the right of the excitation. Excitation with a wavelength that falls inside a transition band (e.g., 438_24 and 650_13 for this set) will cause bleed through, and thus should be avoided.

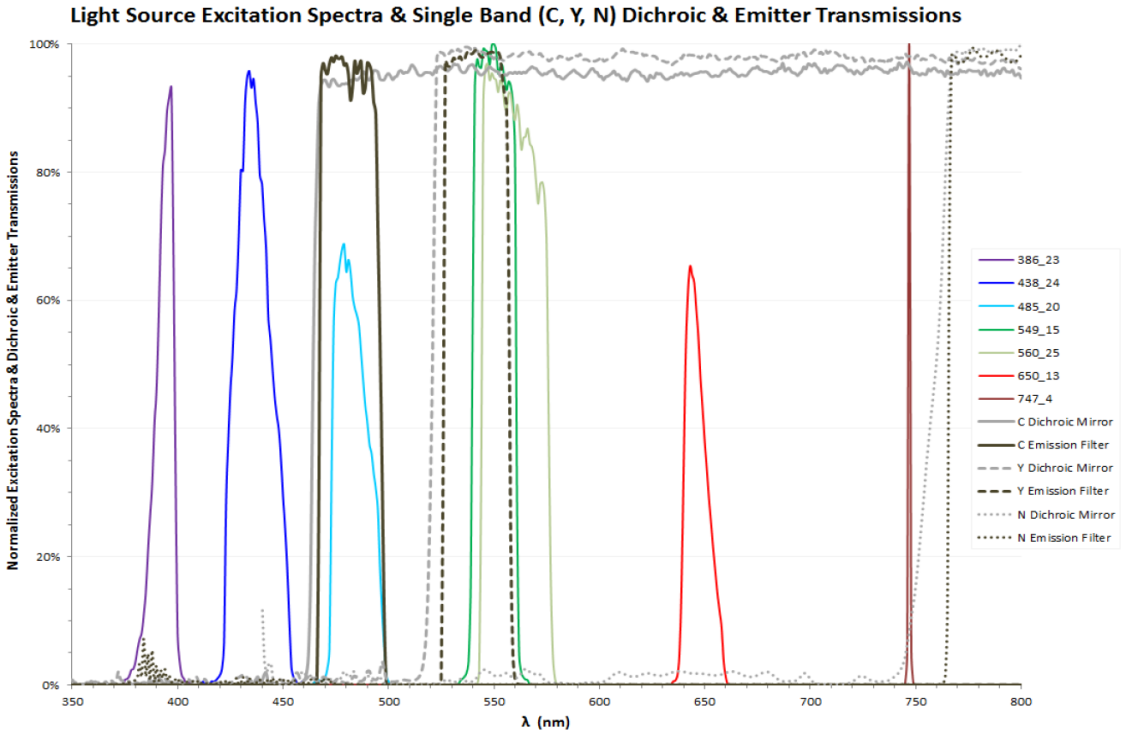


Figure 46 Single band matched C, Y, and N dichroic mirrors and emission filters transmission spectra, overlaid with the normalized spectral power distribution from the excitation 7-color light engine

Advanced configurations for excitation light source, dichroic mirrors, and emission filters

The instrument's exciters, dichroic mirrors, and emission filters are all independently controlled by software. This enables pairing up of non-matched dichroic mirrors and emission filters to enable non-standard fluorescence imaging. Figure 48 shows the different combinations that are available in the advanced mode, as well as the types of fluorescence assays that can be run with them. The system is enabled to do three types of advanced fluorescence imaging assays:

- **FRET (fluorescence resonance energy transfer):** A "donor" dye is excited, and instead of emitting a fluorescent photon, its excited state energy is non-radiatively transferred to an "acceptor" dye, which is then excited and emits a fluorescent photon. Since FRET only occurs when the donor and acceptor fluorophores are within a specific short distance (the Förster radius), it is usually used as a proximity indicator between molecules. Common ways of doing FRET is to excite the donor dye, then monitor the (lack of) fluorescence from the donor or the (gain of) fluorescence from the acceptor. Figure 48 lays out exciter, dichroic mirror, and emitter combinations that enable FRET of different donor-acceptor pairs.
- **Quantum Dots:** Quantum Dots (QDot) are excited in the blue or UV wavelengths, and can emit over a wide spectral range depending on the specific Qdot™ probe. Figure 48 shows the exciter, dichroic mirror, and emitter combinations that enable imaging of different quantum dots.
- **Ratiometric Imaging:** Fluorescent probes have long been used to monitor the environment inside cells. Many environmental sensors can give an accurate measurement of the cell's environment when used in a calibrated ratiometric mode. Both excitation and emission ratio imaging is done. In excitation ratio imaging, as done with the calcium indicator Fura-2, the fluorescent probe is excited by two different excitation wavelengths, and monitored by the same emission filter. In emission ratio imaging, a single excitation is used, and the emission at two different wavelengths is monitored and ratioed. The independent control of the excitation wavelengths, dichroic mirrors, and emission filters enable a variety of different ratiometric imaging to be performed on this platform.
- **FirePlex® Particle Imaging:** The CellInsight™ CX7 Pro and CellInsight™ CX7 LZR Pro platforms are validated to run the AbCam FirePlex® multiplex immunoassays, including the new high-throughput, no-wash format. The Pro camera has been validated to run FirePlex® assays using both 15- and 16-bit imaging formats. See the following representative scatterplot that was obtained using the CellInsight™ CX7 Pro HCS Platform against FirePlex® standard control samples, demonstrating the high reproducibility and specificity of the assay (Figure 47).

  To perform the FirePlex® multiplex immunoassays on CellInsight™ CX7 Pro or CellInsight™ CX7 LZR Pro platforms, select 4X objective for image acquisition, use laser autofocus or software autofocus on channel 2, and use the following filter sets:

Channel	3 Channel Assay	2 Channel Assay
Channel 1	485-20 C_C	485-20 C_C
Channel 2	560-25 BGS_RS	560-25 BGS_RS
Channel 3	560-25 BGRFRN_BGRFRN	—

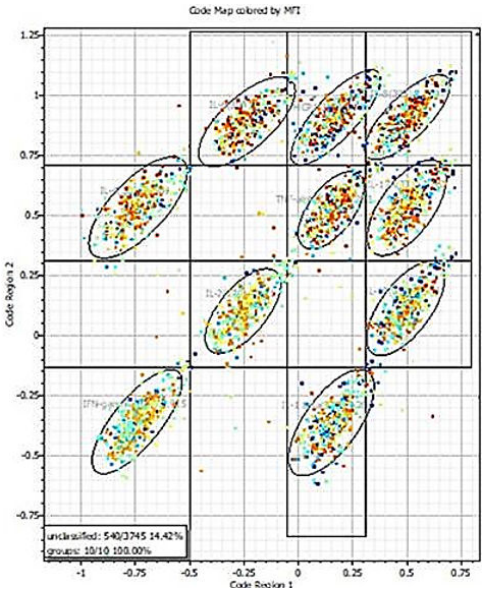


Figure 47 Representative scatterplot demonstrating the high reproducibility and specificity of the AbCam FirePlex® multiplex immunoassay run on the CellInsight™ CX7 Pro HCS Platform.

- **Cell Painting:** Cell Painting is a mechanistic profiling tool used in drug discovery and safety applications. The assay provides a fingerprint of six fluorescent markers across five multiplexed channels to measure both cellular and organelle processes at the single cell level. You can perform cell painting on the CellInsight™ CX7 Pro and CellInsight™ CX7 LZR Pro platforms using the following filter sets:
  - Nuclei: 386-23_BGRFRN
  - Endoplasmic Reticulum + Nucleoli: 485-20_BGRFRN_BGRFRN
  - Golgi + Cell Membrane: 560-25_BGRFRN_BGRFRN
  - Mitochondria: 650-13_BGRFRN_BGRFRN
  - F-Actin: 549-15_BGS_BGS

7 Color LED Light Engine Exciter	5 Position Dichroic Mirror Wheel	6 Position Emission Filter Wheel	Use
386_23	BGRFRN or BGS	Y (Yellow)	• Qdots 525, 565 • BFP/GFP FRET
		RS	• Qdots 565, 585, 605, 625, or 655
438_24		Y (Yellow)	• Qdots 525, 565 • Single band green detection
		RS	• Qdots 565, 585, 605, 625, or 655
485_20		Y(Yellow)	• GFP/YFP FRET • Single band green detection
		RS	• Green/Red-Scarlet (e.g. GFP/RFP) FRET
386_23	C (Cyan)	C (Cyan)	• Detection of cyan dyes
438_24		Y(Yellow)	• Qdots 525, 565
			• CFP/YFP FRET & BRET
485_20			• GFP/YFP FRET & BRET
485_20	Y(Yellow)	C (Cyan)	• Detection of FirePlex® particle barcode region
560_25	BGS	RS	• Detection of FirePlex® particle analyte region
386_23 438_24	Y(Yellow)	Y(Yellow)	• Qdots 525, 565 • BFP/GFP FRET • GFP/YFP FRET
386_23	N (Near IR)	N (Near IR)	• Qdots 705, 800

Figure 48 Advanced configuration for excitation light source, dichroic mirrors, and emission filters

General guidelines to optimize scan speeds

The excitation light source is configured to have high transmittance in the seven supported wavelengths. The excitation filter wheel moves are relatively rapid, whereas turret moves require more time (~0.1 and 1.0 seconds for the excitation filter wheel and turret moves respectively). The system performs fastest when no motion is involved, and just the multi-band matched dichroic mirror – emission filter combinations are used: i.e., for an assay for up to four colors, use the BGRFR dichroic-emitter filter combination, or for an assay up to three colors, use the BGS combination. For example, use BGRFR for a 4-color assay that uses Hoechst™, Alexa Fluor™ 488, Alexa Fluor™ 555, and Alexa Fluor™ 647; use BGS for a 3-color assay that uses Hoechst™, Alexa Fluor™ 488, and Texas Red™.

In general, for the fastest, optimized imaging, we recommend the following:

1. Perform no filter wheel (dichroic and/or emission) moves, if possible.
2. If filter wheel moves are necessary, then minimize their number.
3. If filter wheel moves are necessary, then consider the acquisition sequence. Do not create a protocol that moves the filter wheel back and forth. Faster scan times are achieved when acquisition sequences take all images using one filter wheel position before moving to another position (stage moves and other events occur after an image set is collected for a field).

   Note: Some BioApplications require a specific acquisition sequence for image processing reasons.

4. Perform system and form factor calibrations routinely.
5. Ensure that field service personnel have optimized stage insert flatness during installation or preventive maintenance visits.

Automated spectral selection for transmitted light imaging

The CellInsight™ CX7 Pro HCS Platform is also capable of brightfield microscopy in five different colors in a transmitted light optical geometry, which can be multiplexed with the different fluorescence microscopy options. This appendix describes the key spectral elements of the colored brightfield transmitted light imaging capability of the CellInsight™ CX7 Pro HCS Platform. Brightfield illumination is enabled by an illumination source above the sample; the illumination light passes through the sample with some of it possibly absorbed, and then the remaining transmitted light is detected by the sCMOS camera on the other side of the sample.

This appendix describes the 4-color LED illumination source and the resulting 5-color transmitted light options, and its transmission compatibility with the penta-band fluorescence dichroic mirror and emission filters. Also described are recommendations of absorption wavelengths and illumination strategies for common chromophores, and suggestions for effective multicolor chromophore visualization.

Transmitted light microscopy configuration

Transmitted light configuration

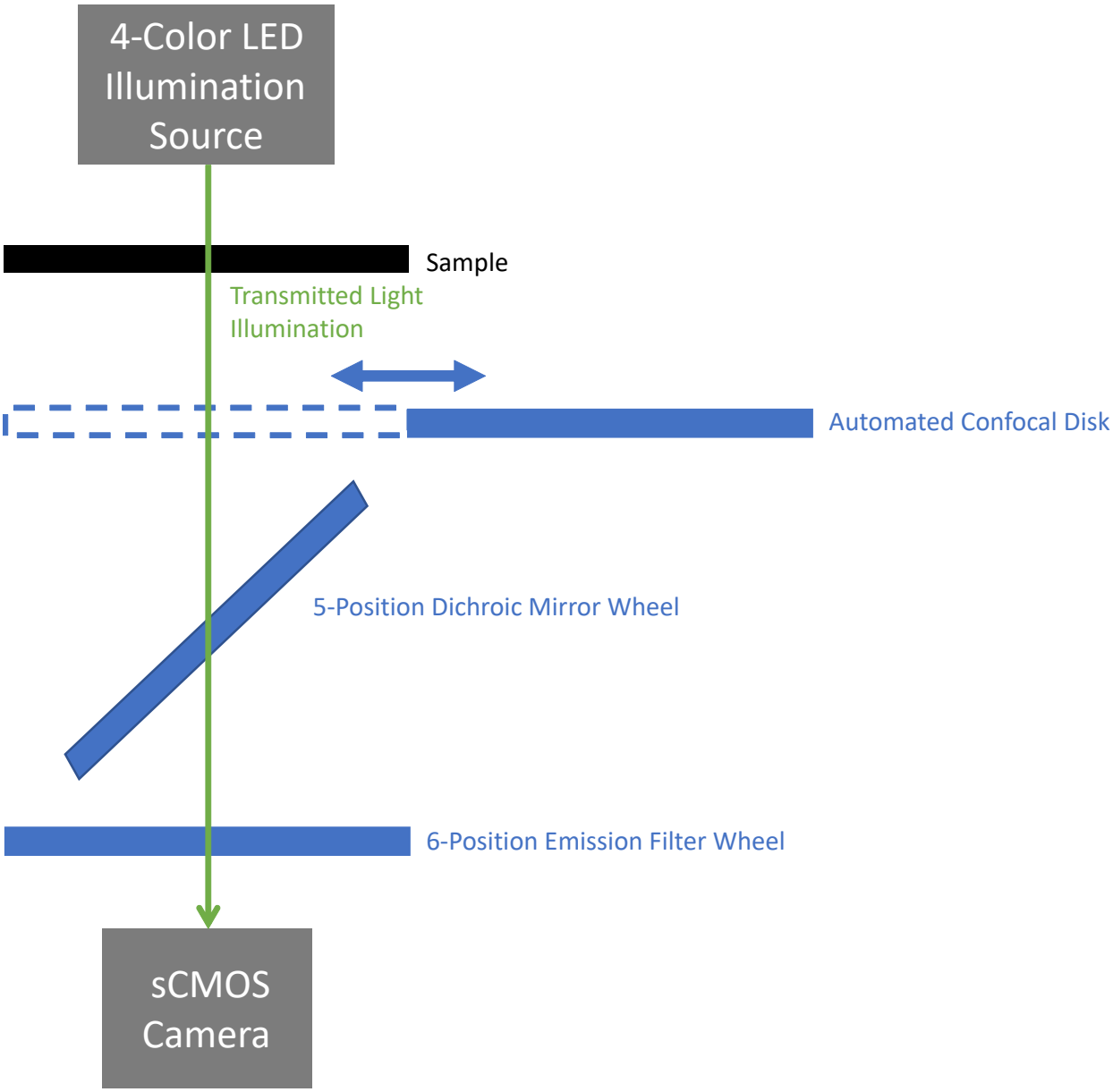


Figure 49 The Transmitted light configuration for the CellInsight™ CX7 Pro HCS Platform

The instrument comes with a range of multi-band and single band dichroic mirrors and emission filters for fluorescence microscopy. For transmitted light imaging, the penta-band BGRFRN dichroic mirror and emission filter are automatically placed in the optical path, because the different transmitted light colors can pass through them and onto be detected by the sCMOS camera (Figure 52). This enables a wide range of chromophores to be detected.

Illumination in the transmitted light configuration is from a 4-color LED light source above the sample. The colors and wavelengths of the LEDs are:

- Blue: 447.5 nm
- Green: 550 nm
- Amber: 590 nm
- Red: 617 nm

A fifth color option, White, is also available, and when selected, is automatically achieved by the simultaneous illumination by the red, green, and blue LEDs. In addition, the power output of each colored LED can be individually changed to alter the tint of the illumination beam.

The illumination passes through the sample. Depending on the chromophores present in the sample, some of the illumination may be absorbed by the sample. Different chromophores absorb different wavelength lights differently. Figure 50 shows the visible absorption band for 35 common biological stains, overlaid by the four wavelengths of the transmitted light LEDs. This figure shows that every stain absorbs at least one, if not more, of the illumination wavelengths. Thus with the differential absorption of the four illuminating colors, sufficient contrast can be achieved to differentiate biological structures labeled by these chromophores. To image a chromophore with optimal contrast, choose illumination colors in the different channels which are absorbed differently by referring to Figure 50 – i.e., choose illumination wavelengths that fall both within and outside the absorption band of the stain.

As an example, Figure 51 shows an image of human breast cancer tissue immuno-histochemically stained with the proliferation marker KI67-DAB and counterstained with H&E (Haemotoxylin and Eosin). The monochrome images shows the differential absorption of the samples when trans-illuminated by the blue, green, and red lights. Combining the three monochrome images in software resulted in the color image (see below for optimal color visualization tips).

After transmitting through the sample, the light follows the same optical path as fluorescence emission, in that it passes through the confocal unit, dichroic mirror, and emission filter before being detected by the sCMOS camera. For brightfield transmitted light imaging, the confocal disk is automatically removed from the optical path, and the penta band BGRFRN dichroic mirror, and emission filter are automatically used. As shown in Figure 52, the four wavelengths from the brightfield LED are not blocked but instead transmitted through the penta-band dichroic mirror and emission filter.

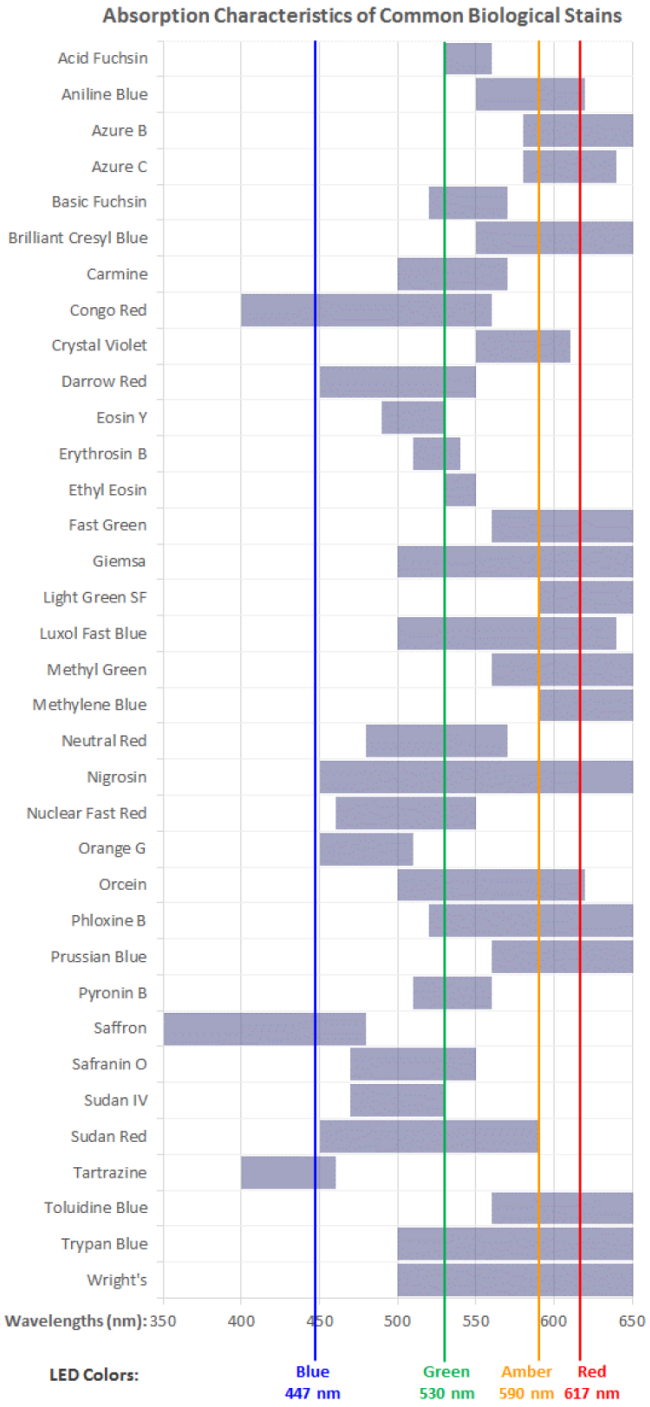


Figure 50 Absorption ranges of common biological stains, overlaid by the four LED wavelengths from the transmitted light source on the CellInsight™ CX7 Pro HCS Platform

For optimal contrast, illuminate the stained sample with wavelengths that are both within and outside the absorption band of the stain.

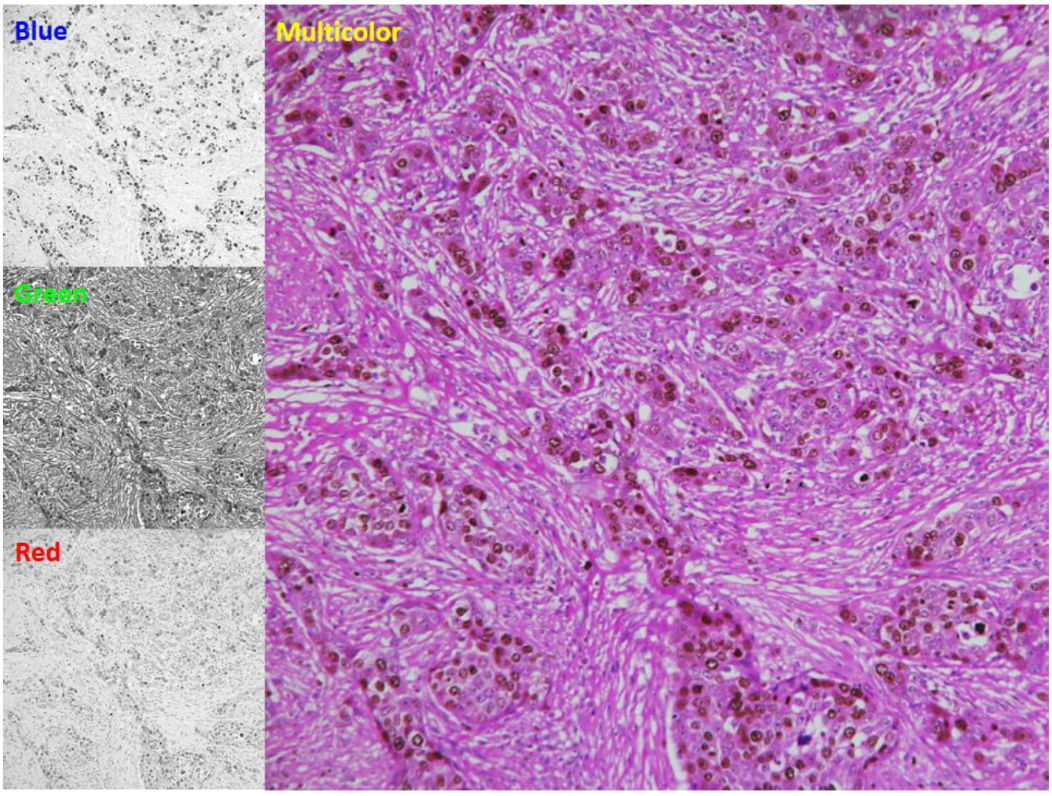


Figure 51 Human breast cancer tissue stained with the proliferation marker KI67-DAB and counterstained with H&E (Haemotoxylin and Eosin)

The monochrome images show the differential absorption of the samples when trans-illuminated by the blue, green, and red lights on the CellInsight™ CX7 Pro HCS Platform. Combining the three monochrome images in software resulted in the color image.

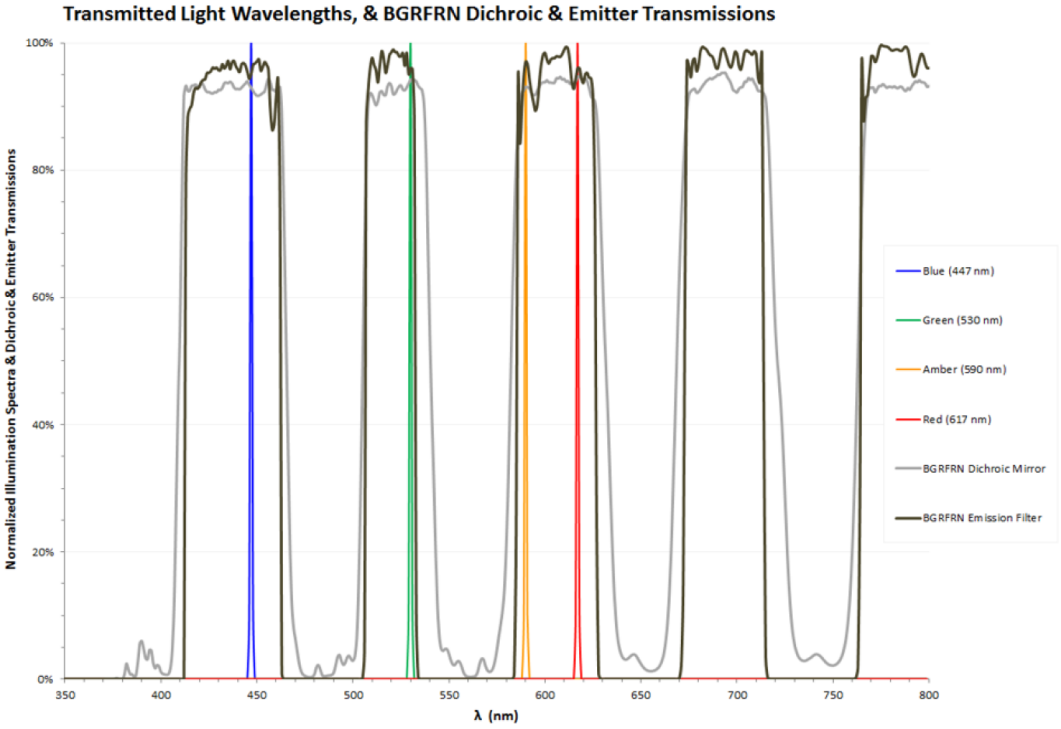


Figure 52 Penta-band BGRFRN dichroic mirror and emission filter transmission spectra, overlaid with the normalized spectral power distribution from the transmitted light source's four illumination colors

The four illumination wavelengths are within the transmission bands and thus will not be blocked by the filters and will reach the detector.

Color visualization of transmitted light images

To visualize the chromophores similar to what is seen by a color camera, subtractive light mixing is required because chromophores absorb versus emit the detected light color. This is opposite to what is done for fluorescence, since fluorophores emit photons of the detected color, additive color mixing is used for visualization. In other words, for a multiplexed fluorescence sample with three fluorophores emitting blue, green, and red photons, the color image should visualize the blue fluorescence with a blue pseudo-color visualization, the green fluorescence with a green pseudo-color, and red fluorescence with a red pseudo-color. However, for proper visualization of an H&E stained sample illuminated with blue, green, and red transmitted light, the blue illuminated image should be visualized with a red pseudo-color, the red illuminated image with a blue pseudo-color, and the green illuminated image will remain with a green pseudo-color, as was done in Figure 51.

D How to change the camera setting from 15-bit to 16-bit mode

Follow the instructions below to switch the Pro camera mode from 15-bits to 16-bits.

1. Open the **HCS Navigator**.
2. Click on the **Gears** icon.
3. Click on the **Config Files** tab.
4. Click on the **Scan** folder.
5. Click on the **cameraif.ini** configuration setting.
6. Scroll down to **[PRIMEBSI]** and find the **BitDepth** setting.
7. Change the **BitDepth** setting from **15** to **16**.

E Replacement parts and accessories

To order replacement parts or accessories that are not customer orderable, contact your local sales representative or contact Technical Support directly.

Part Name	Part Description	Part Number
Power cord[1]	Power cord, United Kingdom, Ireland 230V	AMEP4645
	Power cord, Japan 15A	AMEP4783
	Power cord, Continental Europe, 10A	AMEP4646
	Power cord, China 10A	AMEP4782
	Power cord, North America 115V	AMEP4644
Stage Locking Kit	CellInsight™ CX5/CX7 stage locking kit required for instrument shipment	HCSP0001
Fluorescent calibration plate[1]	Instrument system calibration plate	714140400
Main instrument fuse	Replacement line fuse for instrument mains (2 per instrument)	312485
OptiTracker™ Solution Kit, 1.5 mL[1]	Kit containing OptiTracker™ solution and product insert for use with Illumination Correction tool.	AMEP4773
Objective, 1.25X (0.04 NA) Plan Apo[1]	Olympus™ 1.25X Plan Apochromat dry optional objective	AMEP4736
Objective, 2X (0.08 NA) Plan Apo[1]	Olympus™ 2X Plan Apochromat dry optional objective	NX1002X
Objective, 4X (0.16 NA) U-Plan[1]	Olympus™ 4X X-Line S-Apo dry optional objective	NX1004X
Objective, 20X (0.80 NA) X-Apo[1]	Olympus™ 20X X-Line Apochromat dry optional objective	AMEP4906
Objective, 40X (0.95 NA) X-Apo[1]	Olympus™ 40X X-Line Apochromat dry optional objective	AMEP4907
Objective, 60X (0.90 NA) U-Plan[1]	Olympus™ 60X Fluorite (S-Apo) dry optional objective	AMEP4849

[1] Indicates that the product is customer orderable.

F

Safety

This chapter presents a series of safety precautions related to the operation and maintenance of the CellInsight™ CX7 Pro HCS Platform. The CellInsight™ CX7 Pro HCS Reader is in compliance with the following:

2014/35/EU	Low Voltage Directive
2014/30/EU	Electromagnetic Compatibility Directive
2011/65/EU & Commision Delegated Directive (EU) 2015/863	RoHS Directive

IMPORTANT! This device is classified and conformant to IEC\EN 60825-1:2014, 21 CFR 1040.0, and CFR 1040.11 as an applicable Class 1 laser product.

To help ensure the safe operation of the equipment, observe all the instructions and warnings contained in this guide.

IMPORTANT! Read these instructions carefully before operating or servicing the instrument. In addition, read the instructions, warnings, and precautionary measures supplied with the accessories before commencing use of these items.

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbols

The following symbols may appear adjacent to safety precaution statements in this guide.

Symbol or label	Signal word/description
	**DANGER** – An imminently hazardous situation which, if not avoided, will result in death or serious injury. Consult product documentation to identify the nature of any potential hazard and determine the actions to perform.
	**WARNING** – A potentially hazardous situation which, if not avoided, could result in death or serious injury. Consult product documentation to identify the nature of any potential hazard and determine the actions to perform.
	**CAUTION** – A potentially hazardous situation which, if not avoided, could result in minor or moderate injury. Consult product documentation to identify the nature of any potential hazard and determine the actions to perform.
	Risk of Fire
	Biohazard
	Moving Parts, Pinch Hazard – Risk of body parts, clothing, hair, jewelry, etc. encountering or catching in moving parts. Keep fingers away from moving parts.
	Hazardous Voltage – Risk of shock injury

(continued)

Symbol or label	Signal word/description
	Ultraviolet Light Radiation – Risk of Eye Injury. Wear safety glasses designed to filter dangerous ultraviolet wavelengths and high intensity light.
	Visible and/or Invisible Laser Radiation – Avoid direct eye exposure. Wear laser protective glasses as specified.
	Risk of Eye Injury – Wear safety glasses designed for laser protection and to filter high intensity light and dangerous wavelengths.
	Hot Surface
NOTICE	A cautionary statement which, if not followed, may result in instrument damage or corruption of data. When this symbol appears on equipment, consult product documentation to identify the nature of any potential hazard and determine the actions to perform.
NOTE	A tip, suggestion, or additional information

Location of safety labels

The following images show the locations of the exterior safety labels on the CellInsight™ CX7 Pro HCS Reader. Additional safety precautions are described in the remaining sections of this chapter.

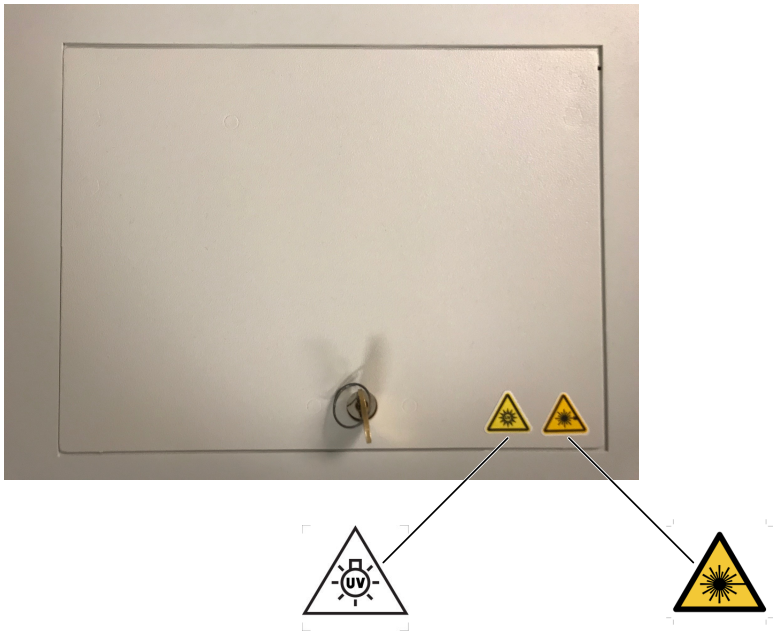


Figure 53 Top view of the CellInsight™ CX7 Pro HCS Reader showing safety precaution label placement (service door closed)

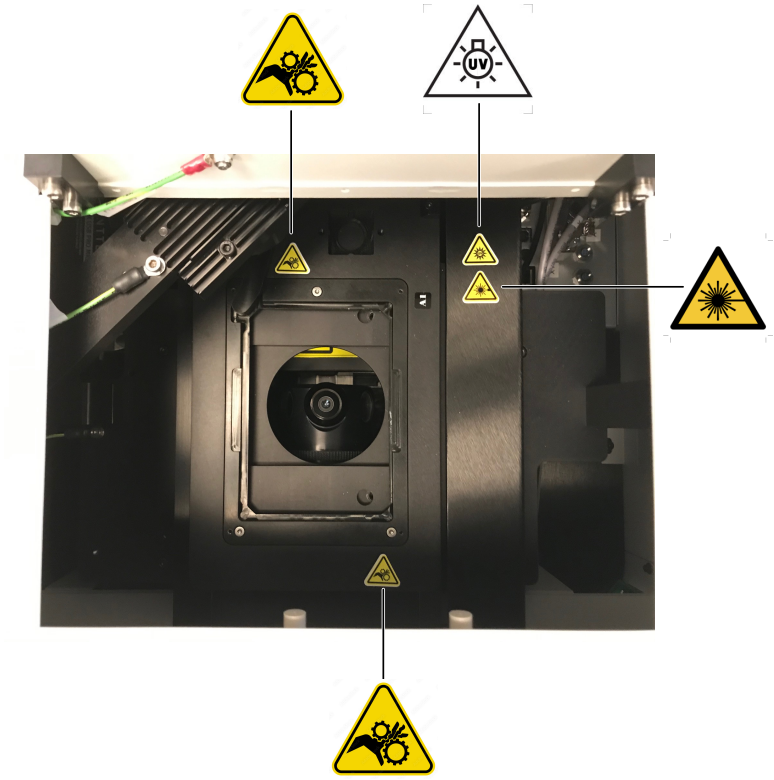


Figure 54 Top view of the CellInsight™ CX7 Pro HCS Reader stage showing safety precaution labels.

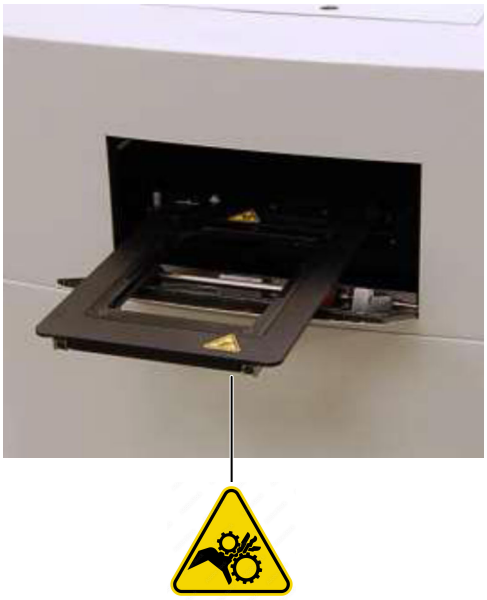


Figure 55 Label placement – CellInsight™ CX7 Pro HCS Reader sample door

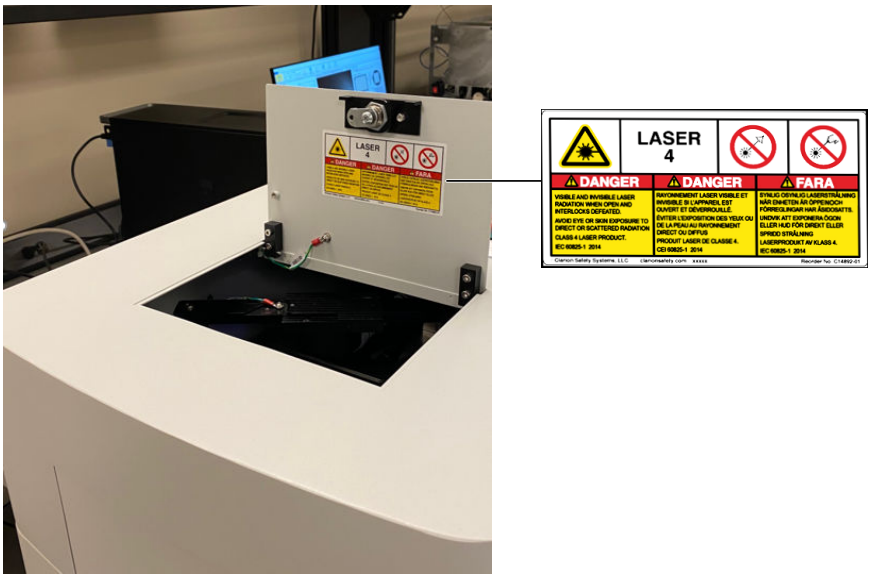


Figure 56 Label placement – CellInsight™ CX7 Pro HCS Reader left side (lid access door open)

Figure 57 Label placement – CellInsight™ CX7 Pro HCS Reader front (front flipper door)

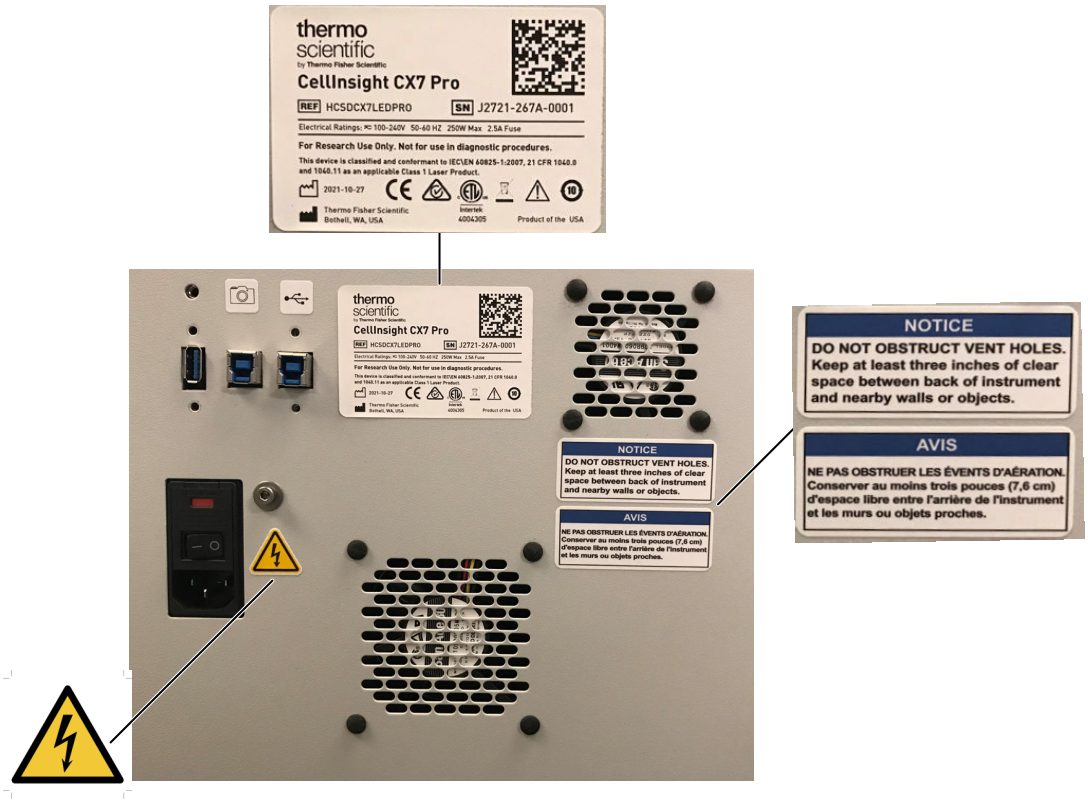


Figure 58 Label placement – CellInsight™ CX7 Pro HCS Reader rear panel

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Protective earthed ground (main ground)

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.
	Indicates conformity with China RoHS requirements.
	Indicates conformity with the WEEE Directive 2012/19/EU. **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options. **European Union Customers:** Call your Customer Service representative for equipment pick-up and recycling. See **thermofisher.com** for a list of customer service offices in the European Union.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General precautions

WARNING! This instrument is only to be operated by trained laboratory personnel only. Use this product only in the manner described in this guide. When used other than as specified, the safety protections can be impaired. Refer to this "Safety" appendix ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, read the instructions, warnings, and precautionary measures supplied in this guide and with accessories. Failure to adhere to safety precautions and/or procedures outlined in this document can result in system failure, personal injury, or death. Thermo Fisher Scientific Inc. shall not be held liable under any circumstances. Thermo Fisher Scientific Inc. does not assume any liability for damages or malfunctions caused by faulty operation, negligence, unauthorized modifications or repairs, or use of unauthorized accessories. The manufacturer cannot assume any liability for any other applications, possibly also involving individual modules or single parts. This also applies to all service or repair work which is not carried out by authorized service personnel. All claims against warranty will be forfeited in these cases.

WARNING! RISK OF ELECTRIC SHOCK!

- The CellInsight™ CX7 Pro HCS Reader contains voltages that are potentially hazardous. To reduce the risk of electric shock or burn, DO NOT REMOVE COVERS. NO USER SERVICEABLE PARTS ARE INSIDE. Periodically inspect power cords and plugs for proper condition and replace as necessary only with Thermo Fisher Scientific provided cords. ONLY THERMO FISHER SCIENTIFIC SERVICE PERSONNEL may perform testing or repairs.
- Do not replace detachable MAINS supply cords by inadequately RATED cords. Only use Thermo Fisher Scientific supplied cords.
- The CellInsight™ CX7 Pro HCS Reader contains voltages that are potentially hazardous. The CellInsight™ CX7 Pro HCS Reader should only be connected to power outlets that are properly grounded and free from electrical or mechanical defects. The grounding effect must not be nullified by an extension cable that does not have a protective ground wire. Ensure that the power voltage in use complies with specification. Using the equipment at any other voltage can cause a fire, electrical shock, and other problems.
- If the protection measures are no longer being met, the instrument must be switched OFF and safeguarded against inadvertent operation.
- Disconnect the mains power cord before changing the fuses. To reduce the risk of fire or shock, replace fuses only with fuses of the same type and rating. The use of makeshift fuses and the short-circuiting of fuse holders are not permitted.

WARNING! CLASS 4 and 3R LASERS – INVISIBLE LASER RADIATION – AVOID EXPOSURE TO BEAM! WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

- The CellInsight™ CX7 Pro HCS Reader contains a Class 4 laser device, conformant to IEC\EN 60825-1:2014. The wavelength of the laser is 742–754 nm CW and the output power is 4 W Max. Avoid direct eye exposure!
- The CellInsight™ CX7 Pro HCS Reader also contains a Class 3R laser device, conformant to IEC\EN 60825-1:2014. The wavelength of the laser is 720–740 nm. The output power is 2.0 mW Max. Avoid direct eye exposure!
- **Wear laser protective glasses with an optical density greater than 6 (OD > 6) for wavelengths less than 400 nm, and an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm.**

WARNING! OVERCURRENT PROTECTIVE DEVICES

The CellInsight™ CX7 Pro HCS Reader and components must be connected to an installation providing an overcurrent protection equal to the fault current at the point of installation (250 VAC, 20 Amps Certified Circuit Breaker or fuse). Installation must be performed according to local codes.

Note: Ensure that the local line voltage and the operating voltage of the CellInsight™ CX7 Pro HCS Reader and all the other electrical components are identical. Incorrect voltage settings that do not meet the specifications can damage the instrument or impair its functions.

Note: The instrument is not intended for outdoor use and can only be used in closed rooms. It can only be used in a laboratory environment that meets the relevant safety standards. The direct ambient temperature and humidity of the system must not exceed the specifications given in the Specifications appendix of this guide.

WARNING! If smoke, abnormal noise, or strange odor is present, immediately shutdown the system and notify Technical Support. It is dangerous to continue using the system.

Note: All maintenance procedures described in this guide can be safely performed by QUALIFIED SERVICE PERSONNEL. Maintenance not covered in this guide must be performed only by Thermo Fisher Scientific service personnel.

CellInsight™ CX7 Pro HCS Reader operation and maintenance precautions

CellInsight™ CX7 Pro HCS Reader

WARNING! RISK OF EYE INJURY!

Wear safety glasses designed to filter ultraviolet wavelengths and high intensity light. **Wearing laser protective glasses with an optical density greater than 6 (OD > 6) for wavelengths less than 400 nm, and an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm** is required anytime the instrument is opened for servicing and there is risk of exposure. These glasses filter ultraviolet wavelengths and high intensity light.

- Eye damage can result from directly viewing the light produced by the light source. The high intensity light and level of UV energy supplied by the light source used in this product can be sufficient to cause damage.
- Never look directly into an illuminated objective, microplate well, or laser beam. The light can damage the cornea and retina of the eye if the light is observed directly.
- Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
- The system must be installed and maintained by a Thermo Fisher Scientific Technical Representative.
- Remove jewelry and other items that can reflect a laser beam into your eyes or those of others.
- Post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing.

Thermo Fisher Scientific Technical Representatives: DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.

WARNING! CLASS 4 and 3R LASERS – INVISIBLE LASER RADIATION – AVOID EXPOSURE TO BEAM! WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

- The CellInsight™ CX7 Pro HCS Reader contains a Class 4 laser device, conformant to IEC\EN 60825-1:2014. The wavelength of the laser is 742–754 nm CW and the output power is 4 W Max. Avoid direct eye exposure!
- The CellInsight™ CX7 Pro HCS Reader also contains a Class 3R laser device, conformant to IEC\EN 60825-1:2014. The wavelength of the laser is 720–740 nm. The output power is 2.0 mW Max. Avoid direct eye exposure!
- **Wear laser protective glasses with an optical density greater than 6 (OD > 6) for wavelengths less than 400 nm, and an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm.**

CAUTION! MOVING MECHANICAL PARTS!

The CellInsight™ CX7 Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. DO NOT MANUALLY OPEN THE STAGE DOOR WHEN THE READER IS PERFORMING A SCAN!

WARNING! BIOHAZARD!

- Use proper laboratory practices when handling hazardous materials.

- Ensure that biohazards do not contaminate CellInsight™ CX7 Pro HCS Reader components.
- In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.
- The CellInsight™ CX7 Pro HCS Reader is not equipped with any special devices for protection from substances which are corrosive, toxic, radioactive, or otherwise hazardous to health. All legal regulations for accident prevention, particularly those in the respective countries, must be observed when handling such substances.

WARNING! The CellInsight™ CX7 Pro HCS Reader is intended for installation in a temperature controlled, low humidity, indoor area free of conductive contaminants.

- Choose a well ventilated area to position the system and provide the specified clearances as shown in the figure on "Dimensions and clearances" on page 95. Do not obstruct the air vents of the CellInsight™ CX7 Pro HCS Reader or components.
- Do not place anything on the equipment that prevents adequate dissipation of heat.
- Avoid exposing the system to direct sunlight or another heat source.
- Ensure that the area around the equipment is clean and free of moisture. Protect equipment from dust and dirt to avoid impairing system performance. Keep the stage door and all covers closed as much as possible. Use a dust cover if the system will not be used for more than a five day period.
- Position the instrument so that you can reach the AC inlet, and be careful to avoid a trip hazard created by any of the cords, such as the AC cord.
- Do not allow liquid or foreign objects on or inside the equipment.
- Do not operate the equipment in an environment where flammable, corrosive, or other damaging liquids or gases are present.
- The minimum bench size for the CellInsight™ CX7 Pro HCS Reader without a robotic microplate handler is 60 in. wide by 25 in. deep if the computer can be placed under the bench, or 72 in. wide by 25 in. deep if the computer can be placed on the same bench top. The supporting surface must be level and must be capable of safely supporting a minimum of 200 lbs. (68 kg). If using a robotic plate handler, increase the bench size and capacity accordingly. Non-level mounting may result in misalignment and equipment malfunction.

CAUTION! Electrostatic Discharge

CellInsight™ CX7 Pro HCS Reader electronics are sensitive to electrostatic discharge (ESD) and transient voltage spikes.
To avoid permanently damaging the system, observe the following precautions:

- Use caution when triggering high-current devices near the system. Electrically noisy devices should be powered from an isolated, conditioned power line or dedicated isolation transformer.
- Never connect or disconnect any power cord or data cable while the system is powered on.

CellInsight™ computer and software

Note: Loading unrelated software on the CellInsight™ computer may cause system damage including but not limited to data corruption and/or software incompatibility. Do not load any software application that has not been provided or approved by Thermo Fisher Scientific. If problems occur as a result of such activity, Thermo Fisher Scientific has no liability and will not be held responsible for damages and repair fees. The customer is responsible for issues that arise from the installation of third party software, including anti-virus software.

Note: It is the responsibility of the customer to ensure that the instrument computer is on a supported operating system. Any negative consequences of running on an unsupported operating system is not covered under any maintenance agreement or plan.

Note: If magnetic field disturbance of the computer components is suspected, move the equipment away from the field source or provide a suitable shielded enclosure. For additional information, contact Technical Support.

Physical injury hazards

WARNING! PHYSICAL INJURY HAZARD!

Use this product only as specified in this document. Using this instrument in a manner not specified by Thermo Fisher Scientific can result in personal injury or damage to the instrument

Moving and lifting the instrument

CAUTION! PHYSICAL INJURY HAZARD!

The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on weight, moving or lifting an instrument may require two or more persons.

WARNING! Always handle the equipment with care when moving. The CellInsight™ CX7 Pro HCS Reader weighs approximately 180 lbs. At least two people are required for transport. Take the proper lifting precautions to avoid injury and always lift the CellInsight™ CX7 Pro HCS Reader by grasping the underside of the instrument frame. Do not attempt to lift the CellInsight™ CX7 Pro HCS Reader by grasping the enclosure panels.

Moving and lifting stand-alone computers and monitors

WARNING! PHYSICAL INJURY HAZARD!

Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See “Documentation and support” on page 143.

Removing covers or parts of the instrument

CAUTION! PHYSICAL INJURY HAZARD!

The instrument is to be serviced only by trained personnel or vendor specified in the user guide. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts. Operators must be trained before being allowed to perform the hazardous operation.

Robotic plate handler

Refer to your robotic plate handler user documentation for additional warnings to ensure safe operation, maintenance, and service of the robotic plate handler.

If you are using an Orbitor™ RS Microplate Mover, refer to the *Thermo Scientific™ Orbitor™ RS User Guide* (Pub. Part. No. LC07500100).

Light source

DANGER! RISK OF EYE INJURY!

Wear safety glasses designed to filter infrared wavelengths and high intensity visible light. **Wearing laser protective glasses with an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm** is required anytime the instrument is opened for servicing and there is risk of exposure. These glasses will filter infrared wavelengths.

- Eye damage can result from directly viewing the light produced by the light source. The high intensity visible light and level of IR energy supplied by the light source used in this product can be sufficient to cause damage.
- Never look directly into an illuminated objective, microplate well, or laser beam. The light could damage the cornea and retina of the eye if the light is observed directly.
- Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
- The system must be installed and maintained by a Thermo Fisher Scientific Technical Representative.
- Remove jewelry and other items that can reflect a laser beam into your eyes or those of others.
- Post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing.

Thermo Fisher Scientific Technical Representatives: DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.

System components

Additional safety precaution statements can be found in the supplied documentation for the following system components:

- Computer
- Monitor

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility. If the line cord is damaged, contact Technical Support.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontaminating the instrument

CAUTION! Using cleaning or decontamination methods other than those recommended by the manufacturer can compromise the safety or quality of the instrument.

CAUTION! CLEANING AND DECONTAMINATION

Only use the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

Note: Clean exterior of the unit with a water dampened cloth and simple detergent only. Refer to "Sterilization procedure" on page 90 for more information.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Laser safety

The CellInsight™ CX7 Pro HCS Platform is classified and conformant to IEC\EN 60825-1:2014, 21 CFR 1040.0, and CFR 1040.11 as an applicable Class 1 laser product.

WARNING! This unit is supplied with an interlock device which protects the end-user from laser radiation in excess of Class 1 limits for Maximum Permissible Exposure under normal operating conditions. However, if the user overrides the interlock device in any manner, the user can be exposed to a Class 4 laser from the Light Engine and a Class 3R from the Laser Autofocus Device.

CAUTION! **Modifications to the instrument outside of normal operation or use may result in hazardous radiation exposure.**

In the event that the user overrides the interlock device (or removes the instrumentation chassis panels or instrument lid), note the following safety precautions regarding the Light Engine and the Laser Autofocus Device.

Lasers can burn the retina, causing permanent blind spots. To ensure safe laser operation:

- Never look directly into the laser beam.
- Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
- The system must be installed and maintained by a Thermo Fisher Scientific Technical Representative.
- Remove jewelry and other items that can reflect a laser beam into your eyes or those of others
- Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing
- DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.
- Use of controls or adjustments or performance of procedures other than those provided in this guide may result in hazardous radiation exposure.

Light engine

The system is equipped with a 7 color LED/laser, solid-state light engine for fluorescence excitation. The Light Engine is a Class 4 Laser Product, 742–754 nm, 4W CW Max.

WARNING! CLASS 4 LASER – VISIBLE AND INVISIBLE LASER RADIATION – AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION! WEAR THE REQUIRED LASER PPE!

The CellInsight™ CX7 Pro HCS Reader contains a Class 4 laser device, conformant to IEC\EN 60825-1:2014. The wavelength of the laser is 750 nm CW and the maximum output power is 4W Max. Avoid direct eye exposure!
Wear laser protective glasses with an optical density greater than 6 (OD > 6) for wavelengths less than 400 nm, and an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm.

Laser autofocus device

The system is equipped with a laser autofocus device. The Laser Autofocus Device is Class 3R Laser Product, 720–740 nm, 2.0 mW Max.

WARNING! CLASS 3R LASER – INVISIBLE LASER RADIATION – AVOID EXPOSURE TO BEAM! WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 Pro HCS Reader contains a Class 3R laser device, conformant to IEC\EN 60825-1:2014. The wavelength of the laser is 720–740 nm. The output power is 2.0 mW Max. Avoid direct eye exposure!
Wear laser protective glasses with an optical density greater than 6 (OD > 6) for wavelengths less than 400 nm, and an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 60825-1 EN 60825-1	*Safety of laser products – Part 1: Equipment classification and requirements*
21 CFR 1040.10 and 1040.11 as applicable	U.S. FDA Health and Human Services (HHS) “Radiological health performance standards for laser products” and “Radiological health performance standards for specific purpose laser products”

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*

(continued)

Reference	Description
ICES-001, Issue 4	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case, you may need to take measures to mitigate the interference. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these can interfere with the proper operation.

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
Directive 2011/65/EU & Commision Delegated Directive (EU) 2015/863	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	“China RoHS” Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at **www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html**.

Chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD!

Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD!

All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL STORAGE HAZARD!

Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

General safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical waste safety

Chemical waste hazard

CAUTION! HAZARDOUS WASTE!

Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposal.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical safety

DANGER! ELECTRICAL SHOCK HAZARD!

Severe electrical shock can result from operating the CellInsight™ CX7 Pro HCS Platform without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD!

For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

DANGER! ELECTRICAL HAZARD!

Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER! ELECTRICAL HAZARD!

Use properly configured and approved line cords for the voltage supply in your facility.

DANGER! ELECTRICAL HAZARD!

Plug the system into a properly grounded receptacle with adequate current capacity.

Physical hazard safety

Moving parts

WARNING! PHYSICAL INJURY HAZARD!

Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Biological hazard safety

WARNING! BIOHAZARD!

Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state / provincial, and / or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications.

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**)
- Your company's / institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

- Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and support

Customer and technical support

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.