

CellInsight™ CX7 LZR Pro High Content Screening (HCS) Platform USER GUIDE

Catalog Number HCSDCX7LZRPRO

Document Part Number 710427

Publication Number MAN0026043

Revision A.0



Life Technologies Corporation | 22025 20th Ave SE Ste. 100 | Bothell, Washington 98021 USA

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Revision history: Pub. No. MAN0026043

Revision	Date	Description
A.0	13 January 2022	New user guide.

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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 instrumentation hardware (see Chapter 1, “Overview”).
- **Chapter 2, Safety precautions**—describes safety precautions relating to the operation of the instrumentation (see Chapter 2, “Safety precautions”).
- **Chapter 3, CellInsight™ CX7 LZR Pro HCS Reader operation**—provides instructions on instrumentation operation (see Chapter 3, “CellInsight™ CX7 LZR Pro HCS Reader operation”).
- **Chapter 4, Using the illumination correction tool**—describes how to use the Illumination Correction Image Acquisition Wizard (see Chapter 4, “Using the illumination correction tool”).
- **Chapter 5, CellInsight™ CX7 LZR Pro HCS Reader maintenance**—outlines instrumentation maintenance procedures (see Chapter 5, “CellInsight™ CX7 LZR Pro HCS Reader maintenance”).

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, please 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. Thus, 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. Please refer to the Chapter 2, “Safety precautions” 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.

Contacting Technical Support

If you have a technical question that you are unable to answer after consulting the documentation, please contact Technical Support.

Service Location: U.S., Pittsburgh, PA (Eastern Time)

Phone: 800-955-6288 Choose option #3, then choose option #1

E-mail: instrumentservices@lifetech.com

Before contacting Technical Support for service or support, it is helpful if you are prepared to answer the following questions:

- What were you doing when the problem occurred?
- Can you reproduce the problem?
- Did you try to solve the problem? If so, what steps did you take and what did you observe?
- Which error messages, if any, appeared?
- What is the serial number of your instrument?

Having these answers will help us provide you with a solution as quickly as possible.



Overview

The Thermo Scientific™ CellInsight™ CX7 LZR Pro High Content Screening (HCS) Platform consists of the CellInsight™ CX7 LZR 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 LZR Pro HCS Platform provides the following:

- Complete automation of image acquisition and analysis processes with automated 3 position objective changer and automated filter changes
- Continuous-wave solid-state laser illumination for Hoechst™, FITC, TRITC, CFP, YFP, Cy5™, Texas Red™, and near-IR like dyes
- Wide fluorescence spectral range from the blue to near infra-red, enabling up to 6-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 module
- Simple to use software that fits your workflow and gives results in minutes
- Intuitive data viewer that allows you to review plate features immediately after scanning
- Integrated 10 GB Thermo Scientific™ Store Express Image and Database Management Software

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

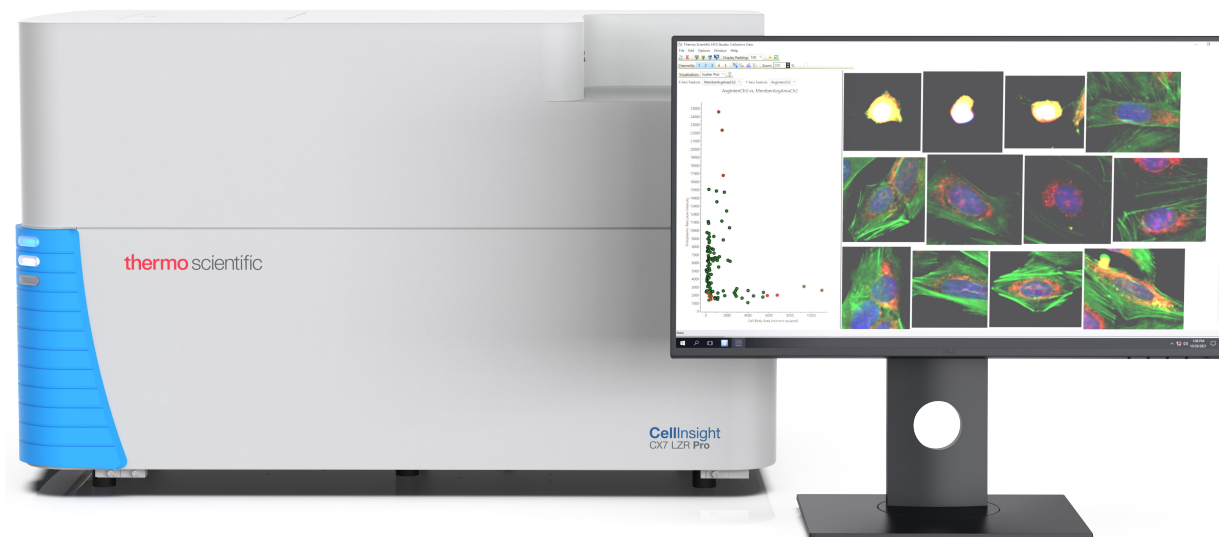


Figure 1 CellInsight™ CX7 LZR Pro HCS Reader

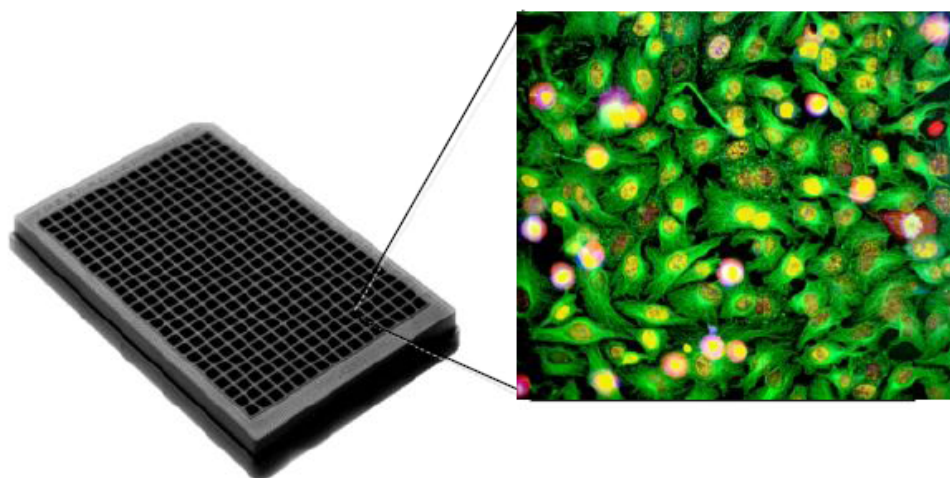


Figure 2 Imaging cells within wells of microplates

The unique optical path of the CellInsight™ CX7 LZR Pro HCS Reader is optimized for performing rapid automated imaging scans in clear-bottom microplates. The CellInsight™ CX7 LZR Pro HCS 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 LZR Pro HCS Reader

The CellInsight™ CX7 LZR 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**
Seven high intensity continuous-wave solid-state lasers 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 LZR Pro HCS Reader.

Confocal imaging

The CellInsight™ CX7 LZR 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 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).

Refer to “Confocal imaging software setup procedures” on page 58 for more information.

Brightfield

The CellInsight™ CX7 LZR 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.

The following image shows the user accessible portion of the Brightfield feature. Refer to “Laser autofocus software setup” on page 49 for more information

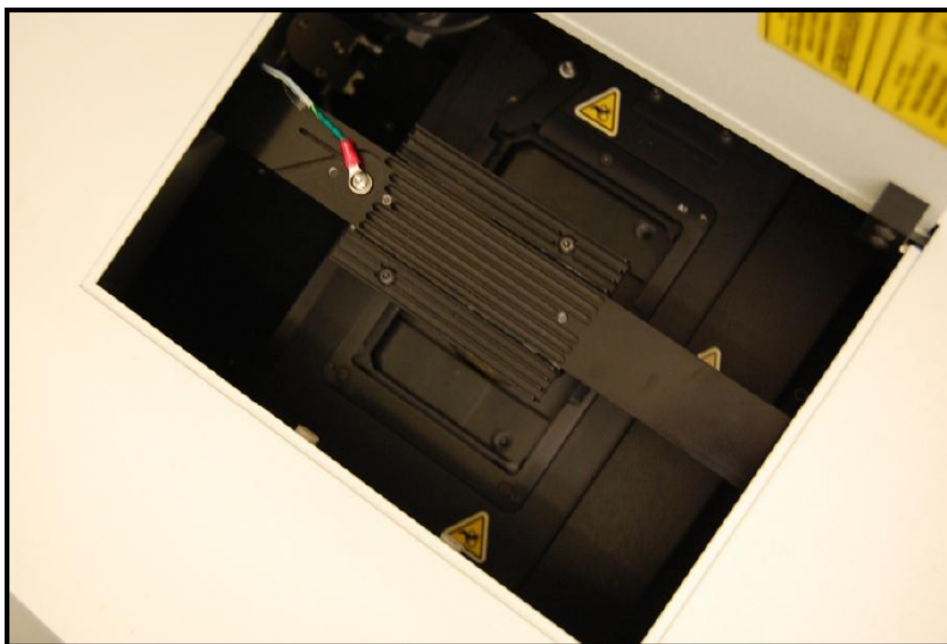


Figure 3 Brightfield components

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-CO₂ premix, CO₂, and N₂ for O₂ displacement in hypoxia experiments), and controls the humidity and temperature.



Figure 4 CellInsight™ Onstage Incubator Control Unit

Robotic plate handler

When using an optional robotic plate handler with the CellInsight™ CX7 LZR 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 LZR Pro HCS Reader, refer to your robotic plate handler user documentation.

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 LZR Pro HCS Reader. Refer to the online help for more information. To access help, click on the question mark icon. 

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-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 LZR 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 LZR 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 LZR Pro HCS Platform, refer to your robotic plate handler user documentation.

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

2014/35/EU	Low Voltage Directive
2014/30/EU	Electromagnetic Compatibility Directive
2011/65/EU	RoHS Directive

IMPORTANT! This device is classified and conformant to IEC\EN 60825-1:2014 Ed.3, 21 CFR 1040.10, 1040.11, 1010.2, 1010.3, and 1002.10 as an applicable Class 1 laser product.

To help ensure the safe operation of the equipment, the instructions and warnings contained in this guide must be observed.

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.

Equipment safety labels

The product's safety labels use ISO standard icons to indicate hazards.



HAZARD SYMBOL

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. When this symbol appears on equipment, consult product documentation to identify the nature of any potential hazard and determine the actions to perform.

(continued)

Symbol or label	Signal word/description
	WARNING – A potentially hazardous situation which, if not avoided, could result in death or serious injury. When this symbol appears on equipment, 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. When this symbol appears on equipment, 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
	Ultraviolet Light Radiation – Risk of Eye Injury. Wear safety glasses designed to filter dangerous ultraviolet wavelengths and high intensity light.
	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 ultraviolet wavelengths.
	Hot Surface
	Protective Conductor Terminal

(continued)

Symbol or label	Signal word/description
	The CE Mark symbolizes that this product complies with the essential requirements of the relevant European health, safety and environmental protection legislation
	The ETL Mark is proof that this product complies with North American safety standards. Authorities Having Jurisdiction (AHJs) and code officials across the US and Canada accept the ETL Listed Mark as proof of product compliance to published industry standards.
	The C-Tick Mark indicates conformity with Australian and New Zealand standards for electromagnetic compatibility.
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

Environmental symbols

The following symbol applies to all Thermo Fisher Scientific electrical and electronic products placed on the European market after August 13, 20015.

Symbol	Signal word/description
	Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). European Union Customers: Call your Customer Service representative for equipment pick-up and recycling. See www.thermofisher.com for a list of customer service offices in the European Union.

Laser safety notice

The CellInsight™ CX7 LZR 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 3B laser from the Light Engine and a Class 3R from the Laser Autofocus Device.



WARNING!  **Use of controls of adjustments of performance of procedures other than those specified herein 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.

Laser light engine

The system is equipped with a 7-color laser light source for fluorescence excitation. The Light Engine houses seven class 3B lasers (405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW; 647 nm/120 mW; 785 nm/100 mW).



DANGER!  **CLASS 3B – LASER VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!**

 **WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!**

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:

405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

Wear laser protective glasses with 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.



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



The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.

Safety labels

Refer to your CellInsight™ CX7 LZR Pro HCS Reader for the location of safety labels. Additional safety precautions are described in the remaining sections of this chapter.

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

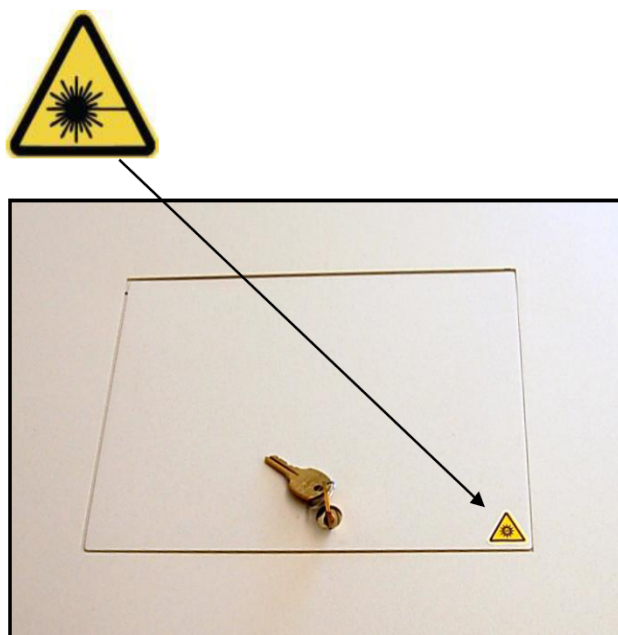


Figure 5 Top view of the CellInsight™ CX7 LZR Pro HCS Reader showing safety precaution label placement (service door closed).

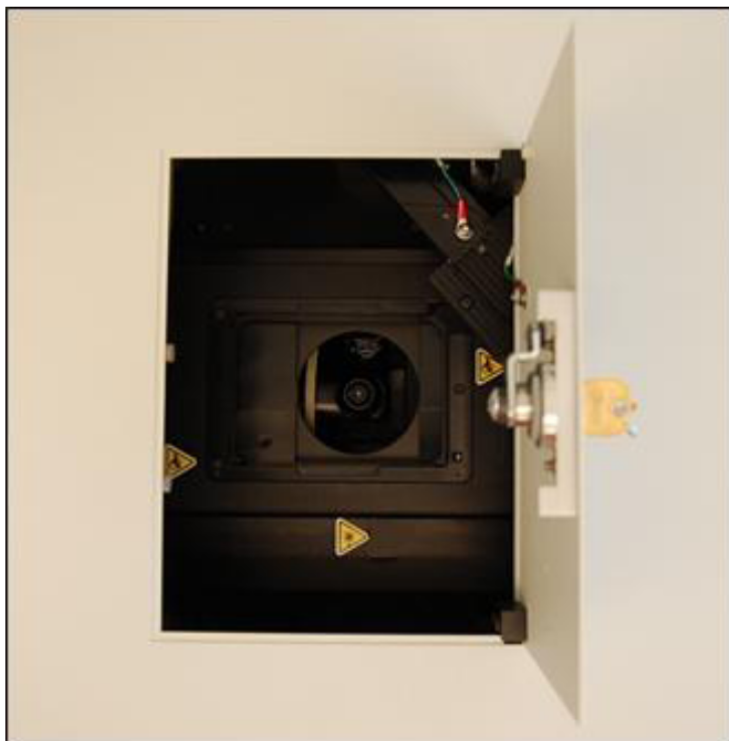


Figure 6 Top view of the CellInsight™ CX7 LZR Pro HCS Reader stage showing safety precaution labels.



Figure 7 Label placement – CellInsight™ CX7 LZR Pro HCS Reader interior

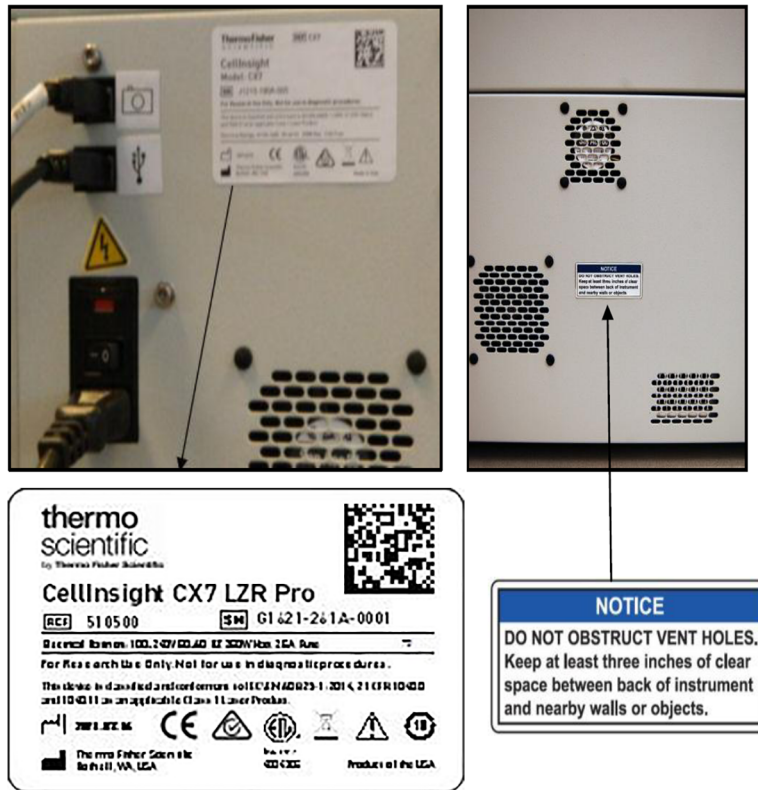


Figure 8 Label placement – CellInsight™ CX7 LZR Pro HCS Reader rear panel

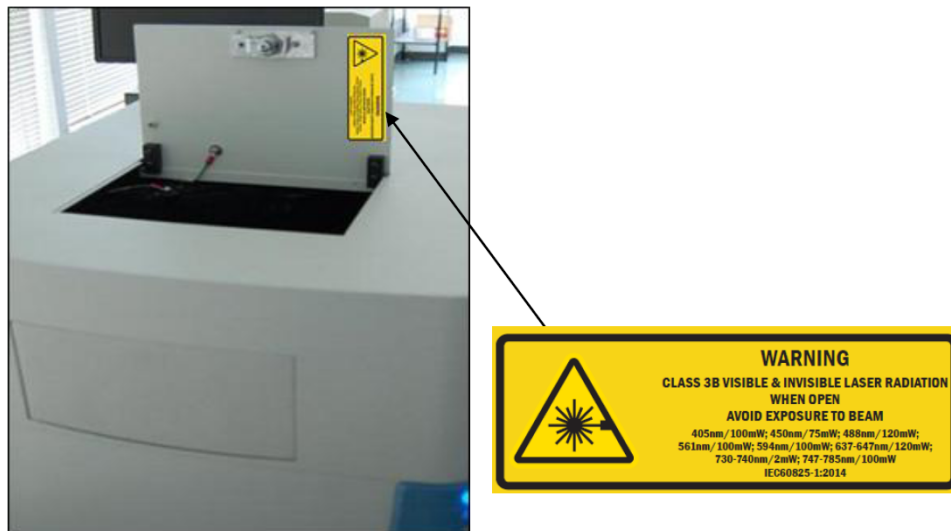


Figure 9 Label placement – CellInsight™ CX7 LZR Pro HCS Reader left side (service door open)

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.

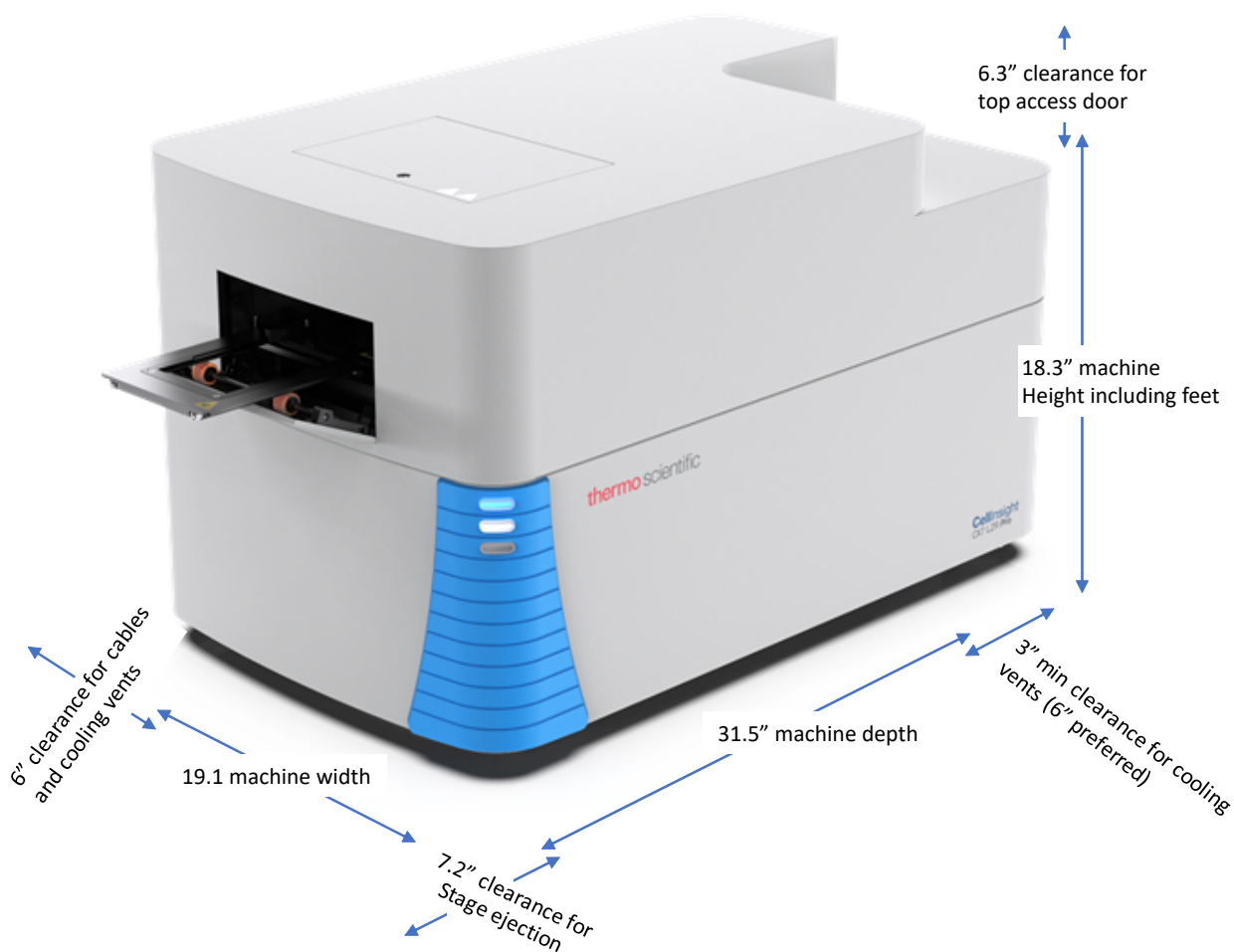


Figure 10 Dimensions and clearances

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 may be impaired. Please refer to the Chapter 2, “Safety precautions” ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please 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 may 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 LZR 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 LZR Pro HCS Reader contains voltages that are potentially hazardous. The CellInsight™ CX7 LZR Pro HCS Reader may 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 which does not have a protective ground wire. Make sure that the power voltage in use complies with specification. Using the equipment at any other voltage may cause a fire, electrical shock, and other problems.

If it is established that 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.



DANGER! ⚠️ CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

🧐 WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:

405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.



WARNING! ⚠ OVERCURRENT PROTECTIVE DEVICES

The CellInsight™ CX7 LZR 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: Check whether the local line voltage and the operating voltage of the CellInsight™ CX7 LZR Pro HCS Reader and all the other electrical components are identical. Incorrect voltage settings which do not meet the specifications may damage the instrument or impair its functions.

Note: The instrument is not intended for outdoor use and may therefore only be used in closed rooms. It may only be used in a laboratory environment which 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 LZR Pro HCS Reader operation and maintenance precautions

CellInsight™ CX7 LZR Pro HCS Reader



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.



DANGER! CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:
405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.



CAUTION! MOVING MECHANICAL PARTS!

The CellInsight™ CX7 LZR Pro HCS Reader incorporates moving parts. Please note the pinch hazard signs applied to potentially dangerous parts. DO NOT MANUALLY OPEN STAGE DOOR WHEN READER IS POWERED!



WARNING! **BIOHAZARD!**

Use proper laboratory practices when handling hazardous materials. Make sure that biohazards do not contaminate CellInsight™ CX7 LZR Pro HCS Reader components. In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.

The CellInsight™ CX7 LZR 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.



CAUTION! **Electrostatic Discharge**

CellInsight™ CX7 LZR Pro HCS Reader electronics are sensitive to electrostatic discharge (ESD) and transient voltage spikes.

To avoid permanently damaging the system, please 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.



WARNING! The CellInsight™ CX7 LZR 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 24. Do not obstruct the air vents of the CellInsight™ CX7 LZR 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.

Make sure 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.

Be sure to 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 LZR 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.

Note: Clean exterior of the unit with a water dampened cloth and simple detergent only.

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 may 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 LZR Pro HCS Reader weighs approximately 150 lbs. At least two people are required for transport. Take the proper lifting precautions to avoid injury and always lift the CellInsight™ CX7 LZR Pro HCS Reader by grasping the underside of the instrument frame. Do not attempt to lift the CellInsight™ CX7 LZR Pro HCS Platform 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 145.

Cleaning and decontaminating the instrument



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



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.

Refer to the “Sterilization procedure” on page 118 for more information.

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.

CellInsight™ computer

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.

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.

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.

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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.

Confocal imaging unit



WARNING! CONFOCAL IMAGING

There are no user repairable parts inside the unit. Contact Technical Support if you suspect the module is not operating properly.

System components

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

- Computer
- Monitor

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™ 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/



CellInsight™ CX7 LZR 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 Chapter 2, “Safety precautions” 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 may 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 LZR Pro HCS Reader startup

IMPORTANT! Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.

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

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



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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 are instructed to: DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.



DANGER! ⚠️ CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

⚠️ WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:

405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.

1. Turn on the CellInsight™ CX7 LZR Pro HCS Reader power switch located on the back of the instrument.
2. Turn on the CellInsight™ computer and the monitor.

The instrument status lights are shown in the following image.

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 in progress.

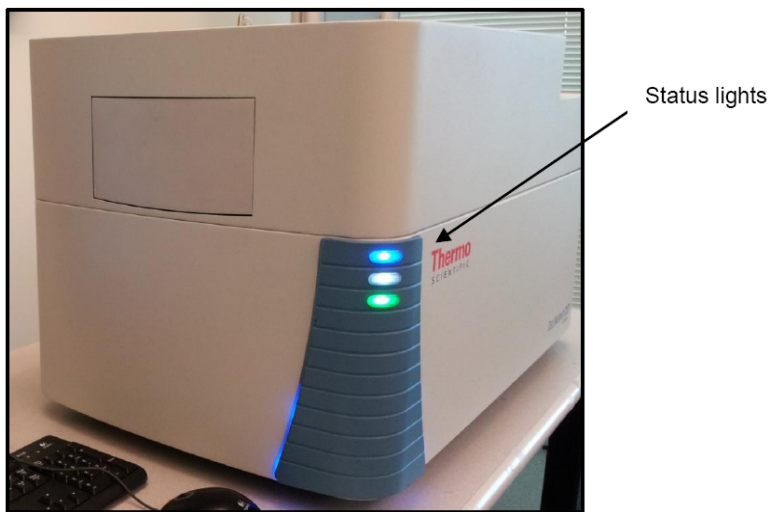
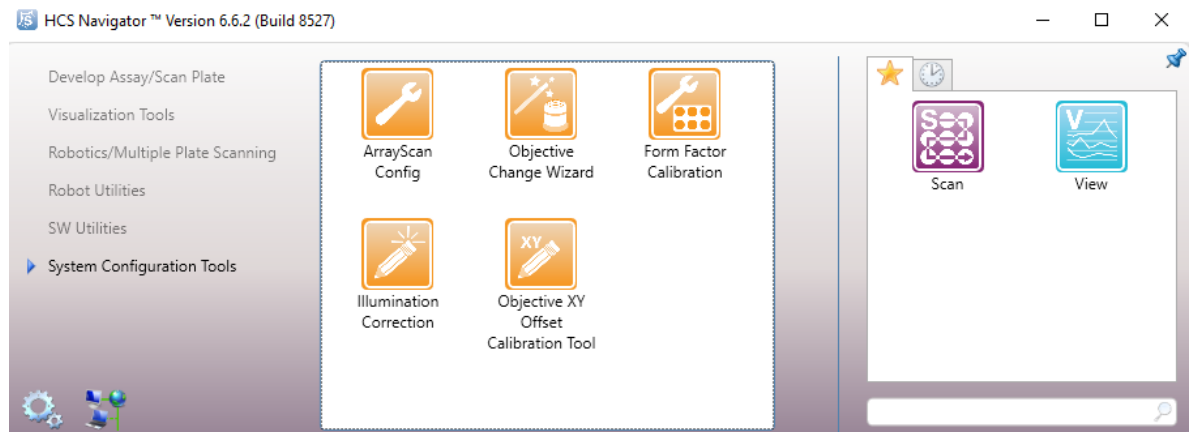


Figure 11 Instrument status lights

Starting 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.



Logging on to the CellInsight™ CX7 LZR Pro HCS Reader

1. Enter your **user name** and **password**. Refer to the *System Configuration Tool Administrator's Guide* for information on how to establish passwords.
2. Click **Login**. Refer to the online help for more information about the software features.

CellInsight™ CX7 LZR Pro HCS Reader startup troubleshooting

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

- Verify that all system components are plugged into their power sources. Refer to the Chapter 5, “CellInsight™ CX7 LZR Pro HCS Reader maintenance” in this guide for diagrams showing component connections.
- Check the CellInsight™ CX7 LZR Pro HCS Reader instrument fuse and change it if necessary. Refer to the Chapter 5, “CellInsight™ CX7 LZR Pro HCS Reader maintenance” in this guide for fuse replacement instructions.

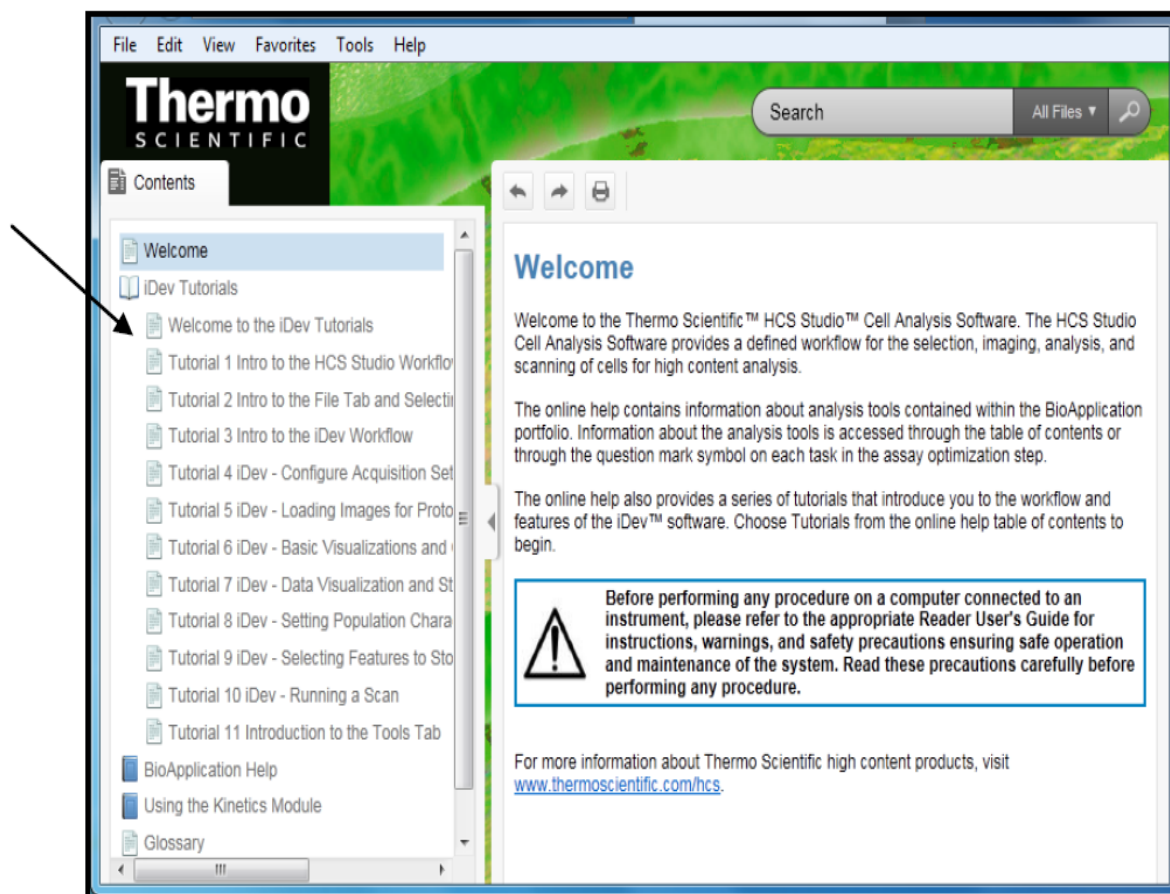
Platform setup procedures

Before scanning a microplate on the CellInsight™ CX7 LZR 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 “Labeling microplates” on page 43)
- Insert microplates in the CellInsight™ instrument (see “Inserting a microplate in the CellInsight™ CX7 LZR Pro HCS Reader stage” on page 44)
- Specify barcode settings (see “Specifying barcode settings” on page 46)
- Verify that desired objectives are installed (see “Objective inspection” on page 97)
- Install the desired objective and adjust correction collar, if needed (see “Changing the objective” on page 99)
- Verify that the Brightfield unit is in place and specify settings (see “Brightfield software setup procedures” on page 52)
- 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
- Set XY offsets 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 79)

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 49 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 58 and Help Tutorial 4)
- Set exposure and focus settings (see “Laser autofocus software setup” on page 49 and Help Tutorial 4)
- Contrast Stretching (see Online Help Tutorial 6)
- Use Image Projection, if desired (see “Creating an assay protocol using the confocal options” on page 58 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 LZR Pro HCS Reader, refer to your robotic plate handler user documentation for system setup procedures.

Labeling 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 “Overall system specifications” on page 122 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.

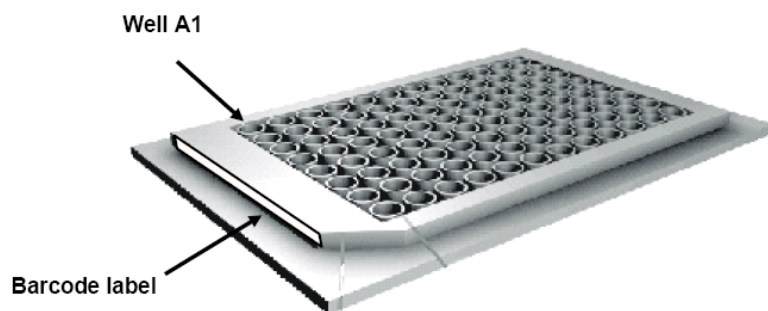


Figure 12 Barcode label placed on a 96-well microplate

Inserting a microplate in the CellInsight™ CX7 LZR Pro HCS Reader stage

IMPORTANT! Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.

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



WARNING! **BIOHAZARD!**

Use proper laboratory practices when handling hazardous materials.

Ensure that biohazards do not contaminate CellInsight™ CX7 LZR Pro HCS Reader components. In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.

The CellInsight™ CX7 LZR 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.



CAUTION! **MOVING MECHANICAL PARTS!**

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



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.



DANGER! ⚠ CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

🔒 WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:

405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.

To load a microplate in the Reader stage,

1. Refer to the Chapter 2, “Safety precautions”.
2. 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.



3. 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 and rotate the microplate so that well A1 is located in this corner.



Figure 13 Microplate placed in CellInsight™ CX7 LZR Pro HCS Reader stage

4. Insert the microplate into the stage.
5. Press down on the microplate to make sure it rests flat against the stage.
6. After the plate is inserted, press and hold the Ctrl key and click **Ctrl OK** in the **Plate Load/Unload** dialog.

Specifying 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.

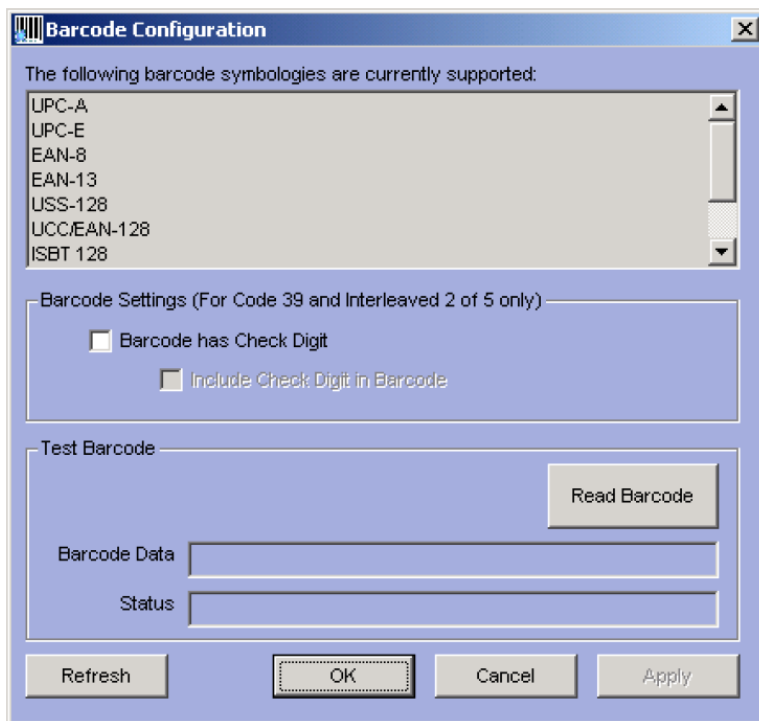
If you placed a barcode label on the plate for identification, you can specify barcode settings and test the barcode scan using the **Barcode Configuration** dialog.

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



CAUTION! MOVING MECHANICAL PARTS!

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



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.



DANGER! CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:
405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

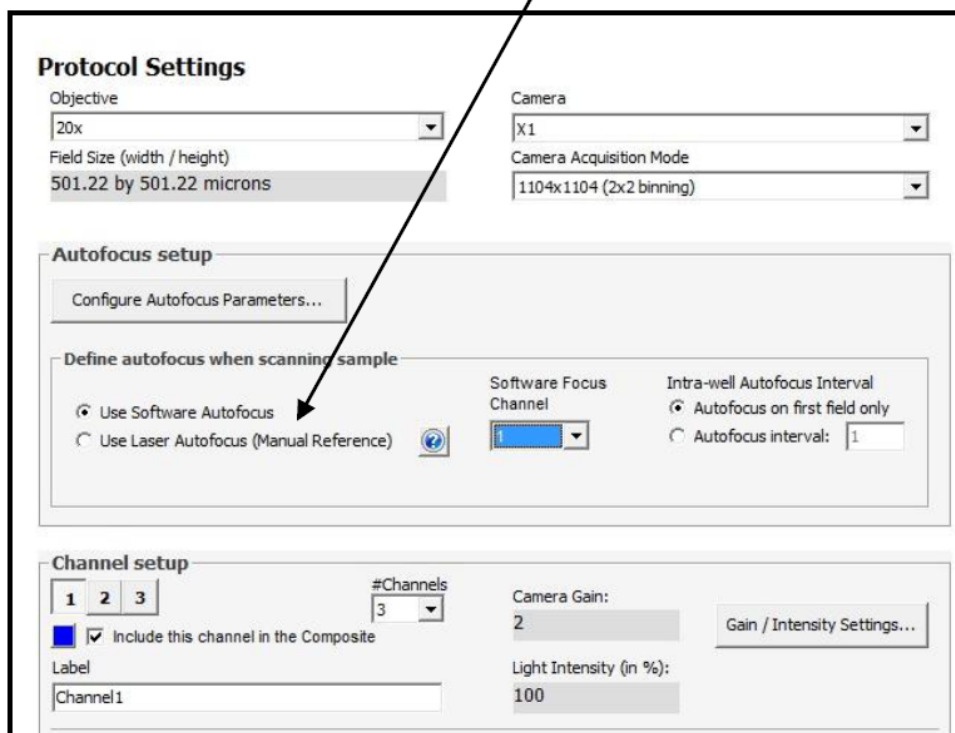
The CellInsight™ CX7 LZR Pro HCS Reader also contains a Class 3R laser device, conformant to IEC\EN 60825-1:2014. The wavelength of the laser is 720–740 nm. The output power is 2.0 mW Max. Avoid direct eye exposure!

Wear laser protective glasses with an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm.

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.



Protocol Settings

Objective: 20x
Field Size (width / height): 501.22 by 501.22 microns

Camera: X1
Camera Acquisition Mode: 1104x1104 (2x2 binning)

Autofocus setup

Configure Autofocus Parameters...

Define autofocus when scanning sample

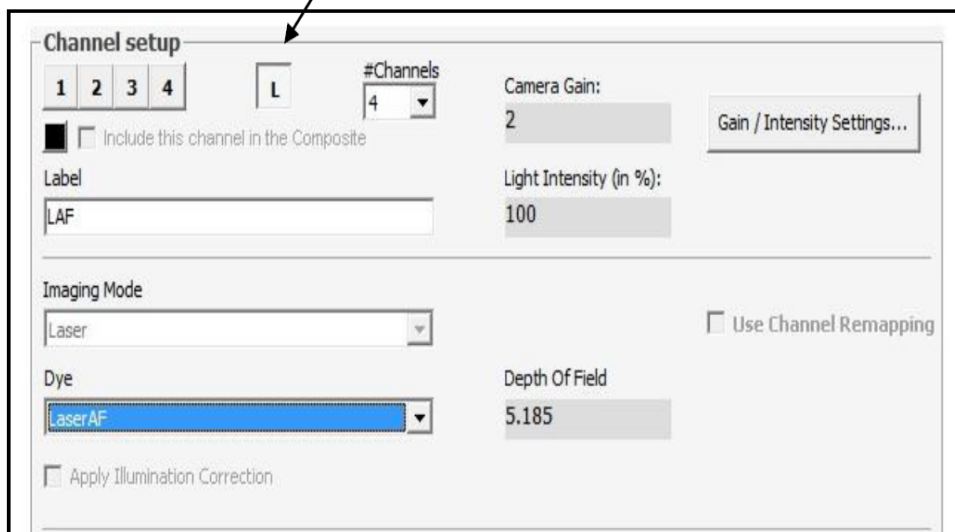
☒ Use Software Autofocus
☐ Use Laser Autofocus (Manual Reference)

Software Focus Channel: 1
Intra-well Autofocus Interval: ☒ Autofocus on first field only
☐ Autofocus interval: 1

Channel setup

1 2 3 #Channels: 3
☒ Include this channel in the Composite
 Label: Channel1
 Camera Gain: 2
 Light Intensity (in %): 100
 Gain / Intensity Settings...

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

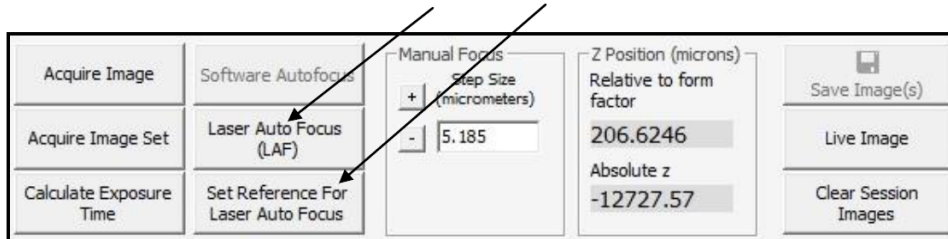


Channel setup

1 2 3 4 L #Channels: 4
☐ Include this channel in the Composite
 Label: LAF
 Camera Gain: 2
 Light Intensity (in %): 100
 Gain / Intensity Settings...

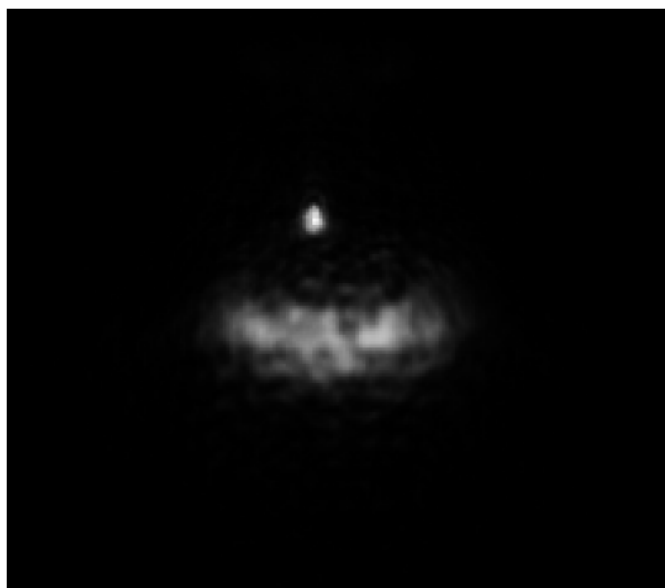
Imaging Mode: Laser
 Dye: Laser AF
 Depth Of Field: 5.185
☐ Apply Illumination Correction
☐ Use Channel Remapping

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.

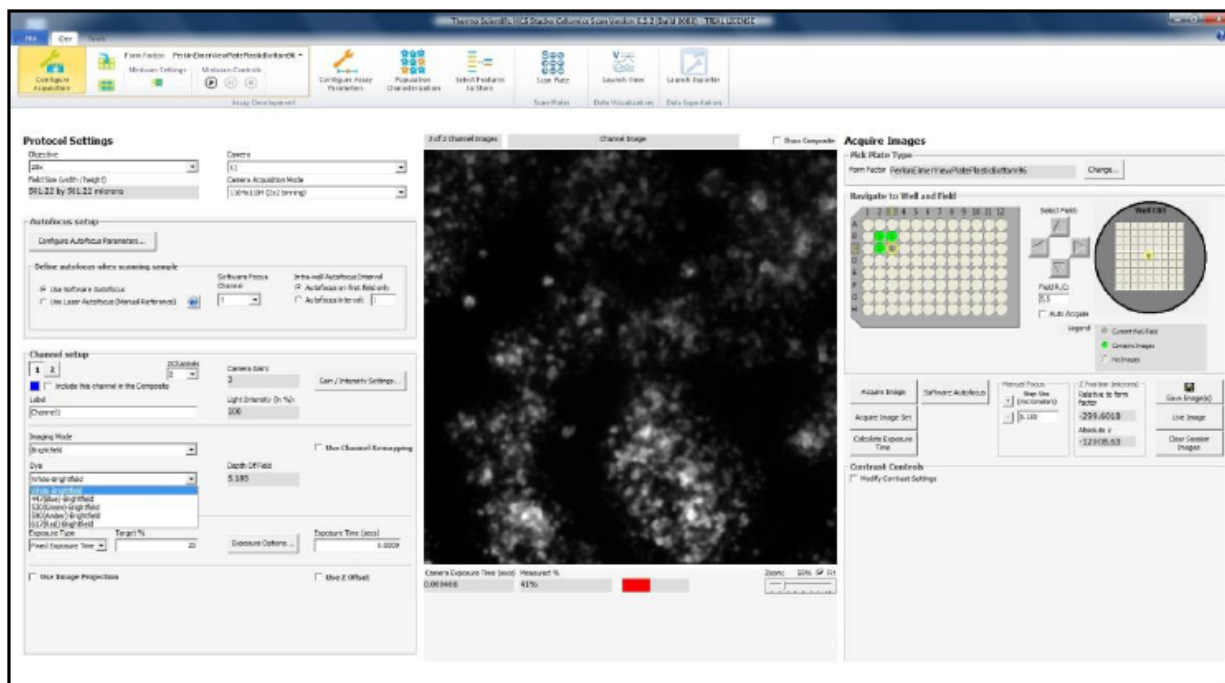
To verify that laser autofocus position is correct,

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

Settings for 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.



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 139.

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”.

Engaging 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 14.

Note: A key is required to open this door.



Figure 14 CellInsight™ CX7 LZR Pro HCS Reader service door

2. If the Brightfield arm is in place (engaged), it will be in the position as shown in Figure 15. If the arm is not in this position, follow the next step to engage the arm.

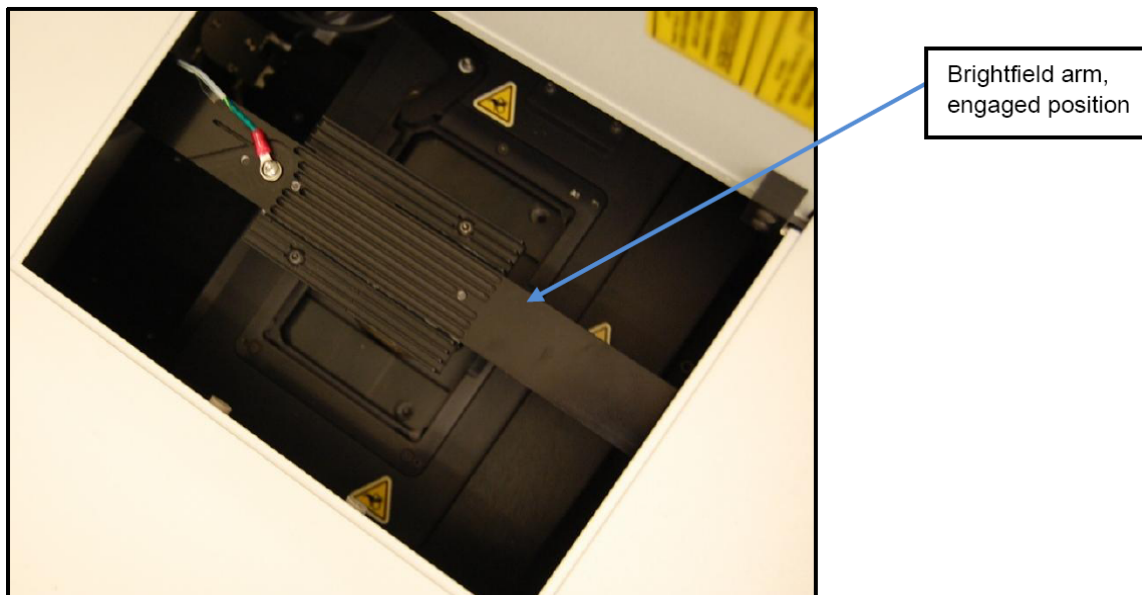


Figure 15 CellInsight™ CX7 LZR Pro HCS Reader open service door with engaged Brightfield arm.

3. If the Brightfield arm is not in the engaged position (Figure 16), you must physically move the arm to the engaged position. To do so, grasp the arm as indicated in the following image and move the arm until it is in the engaged position (as shown in Figure 15).

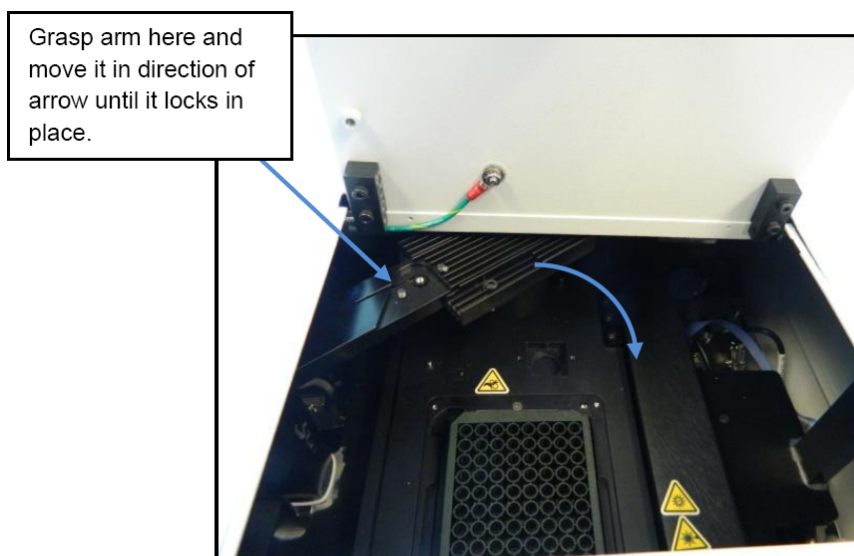


Figure 16 CellInsight™ CX7 LZR Pro HCS Reader open service door with disengaged Brightfield arm.

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.

Specifying the brightfield settings

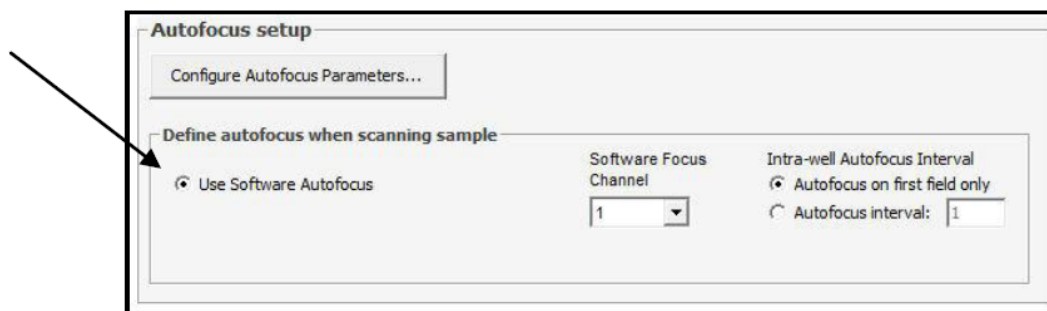
To specify Brightfield channels,

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.

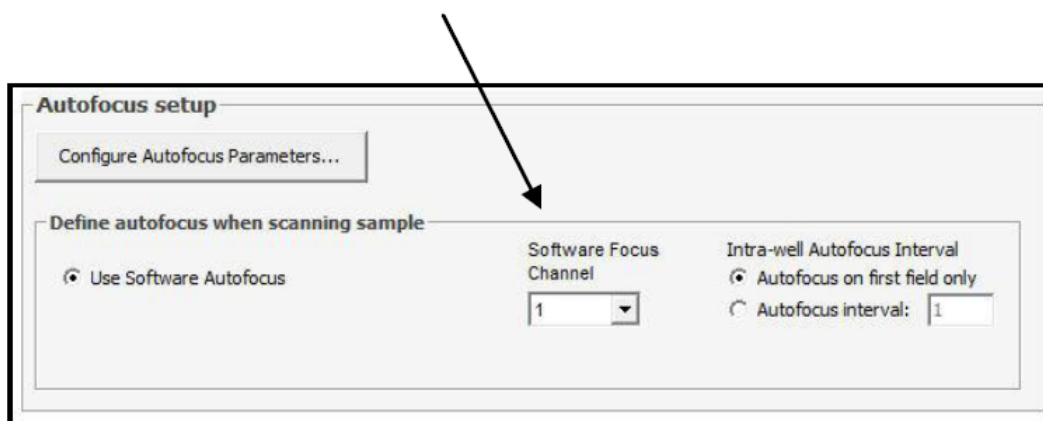
Note: To use software autofocus, continue with the following procedure. Otherwise, refer to “Laser autofocus software setup” on page 49 for instructions on using the laser autofocus option.

To specify a Brightfield focus channel,

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

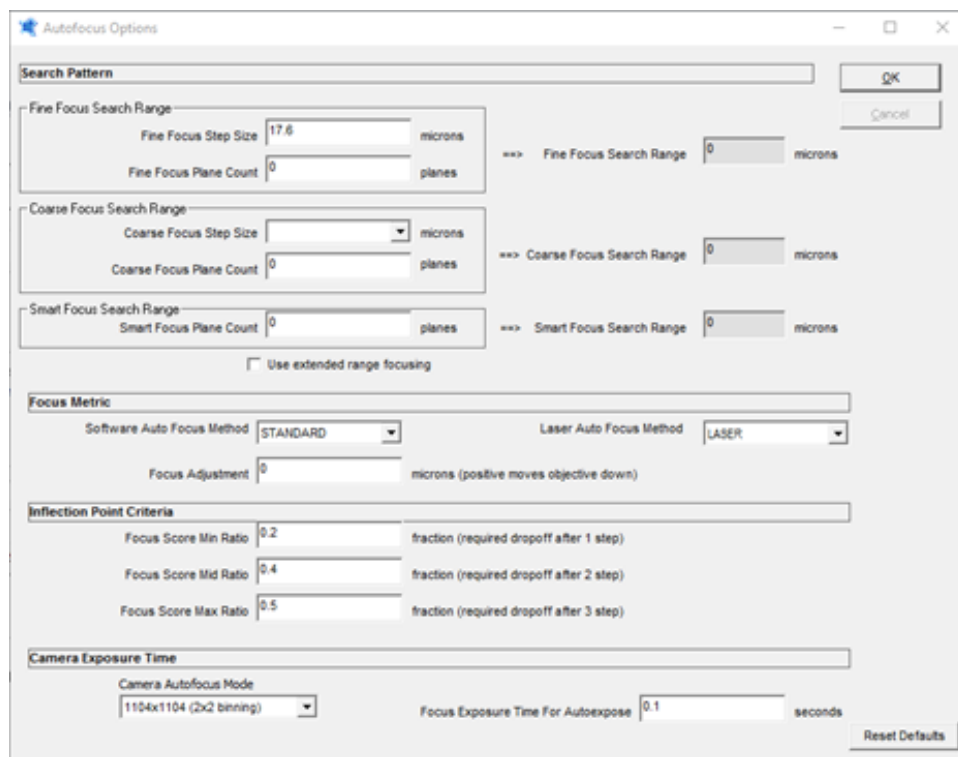


2. From the **Software Focus Channel** drop-down list, select the **Brightfield** channel to use for the Focus channel.



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.



The Autofocus Options dialog box contains the following sections and controls:

- Search Pattern:** A text input field with OK and Cancel buttons.
- Fine Focus Search Range:**
 - Fine Focus Step Size: 17.6 microns
 - Fine Focus Plane Count: 0 planes
 - Fine Focus Search Range: 0 microns
- Coarse Focus Search Range:**
 - Coarse Focus Step Size: (dropdown menu) microns
 - Coarse Focus Plane Count: 0 planes
 - Coarse Focus Search Range: 0 microns
- Smart Focus Search Range:**
 - Smart Focus Plane Count: 0 planes
 - Smart Focus Search Range: 0 microns
- ☐ Use extended range focusing
- Focus Metric:**
 - Software Auto Focus Method: STANDARD (dropdown menu)
 - Laser Auto Focus Method: LASER (dropdown menu)
 - Focus Adjustment: 0 microns (positive moves objective down)
- Inflection Point Criteria:**
 - Focus Score Min Ratio: 0.2 fraction (required dropoff after 1 step)
 - Focus Score Mid Ratio: 0.4 fraction (required dropoff after 2 step)
 - Focus Score Max Ratio: 0.5 fraction (required dropoff after 3 step)
- Camera Exposure Time:**
 - Camera Autofocus Mode: 1104x1104 (2x2 binning) (dropdown menu)
 - Focus Exposure Time For Autoexpose: 0.1 seconds
 - Reset Defaults button

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 LZR 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

Creating 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.

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 confocal options in HCS Studio™ Cell Analysis Software

1. Open the **Configure Acquisition** screen in the HCS Studio™ Cell Analysis Software. 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.

The screenshot shows the 'Channel setup' dialog box with the following settings:

- Channels:** 1, 2, 3 (Channel 1 is selected)
- #Channels:** 3
- Camera Gain:** 2
- Light Intensity (in %):** 100
- Imaging Mode:** Confocal (indicated by an arrow)
- Pin Hole Size:** 70
- Depth Of Field:** 10.255
- Dye:** 56 ILZR_BGRFR_BGRFR-Confocal
- Exposure Type:** Fixed Exposure Time
- Target %:** 25
- Exposure Time (secs):** 0.001

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: **405LZR_BGRFR_BGRFR-Confocal** refers to exciting the sample with light from the 405 nm laser, using a dichroic filter that passes blue (B), green (G), red (R), and far red (FR) light, and using an emitter filter that passes BGRFR 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.

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, you may consider using the 70 micrometer pinhole and 4x4 camera binning as the image resolution and detail under these settings may be sufficient for your needs. In addition, consider using the higher NA objectives for brighter signal – i.e., the 40x/0.95 or 20x/0.7 objectives.

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.

Autofocus setup

Configure Autofocus Parameters...

Define autofocus when scanning sample

☒ Use Software Autofocus
☐ Use Laser Autofocus (Manual Reference)

Software Focus Channel: 1

Intra-well Autofocus Interval

☒ Autofocus on first field only
☐ Autofocus interval: 1

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.

☒ Use Image Projection

Projection Direction: Centered

Projection Method: Maximum

Number Of Steps: 3

Step Size: 5.185

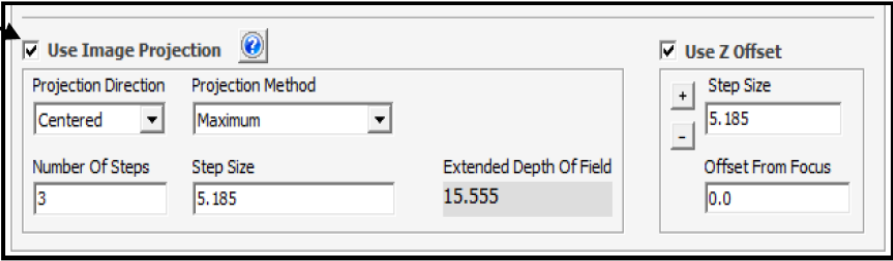
Extended Depth Of Field: 15.555

☒ Use Z Offset

Step Size: 5.185

Offset From Focus: 0.0

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



☒ Use Image Projection

Projection Direction: Centered
Projection Method: Maximum

Number Of Steps: 3
Step Size: 5.185
Extended Depth Of Field: 15.555

☒ Use Z Offset

Step Size: 5.185
Offset From Focus: 0.0

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 <i>Offset From Focus</i> 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. <u>None:</u> No projection <u>Above:</u> Z-stack starts at <i>Offset From Focus</i> position and extends higher into the well (negative Z direction) <u>Center:</u> Z-stack is centered about the <i>Offset From Focus</i> position <u>Below:</u> Z-stack starts at <i>Offset From Focus</i> 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 <i>Step Size</i>. 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: <u>Maximum:</u> 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) <u>Nearest Neighbor Deblurring (NND):</u> 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. Note: The NND method is implemented for the Maximum Projection only.

(continued)

Setting	Description
Projection Method	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 than 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 <i>Depth of Field</i> .

Z - Stack Projections

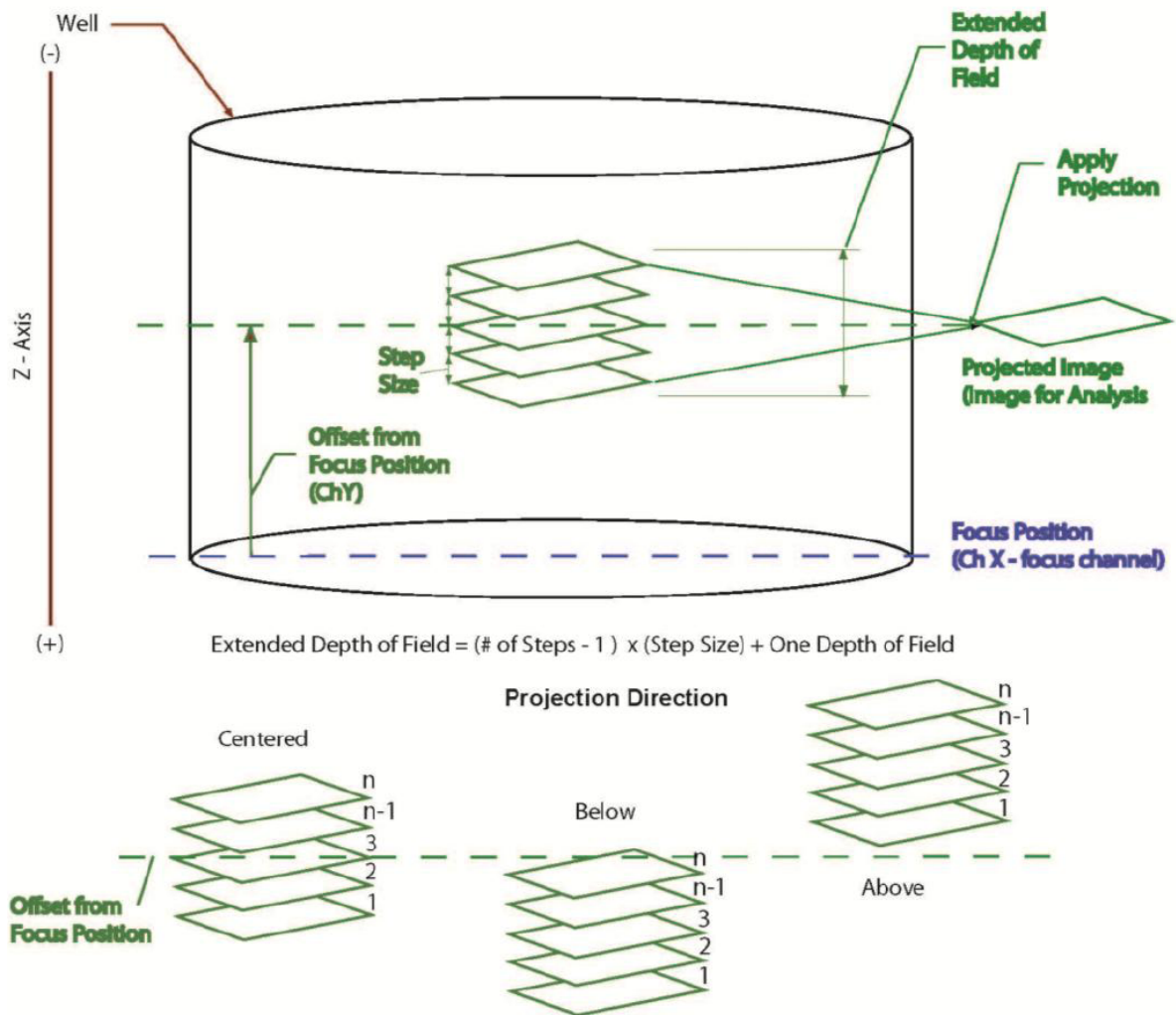


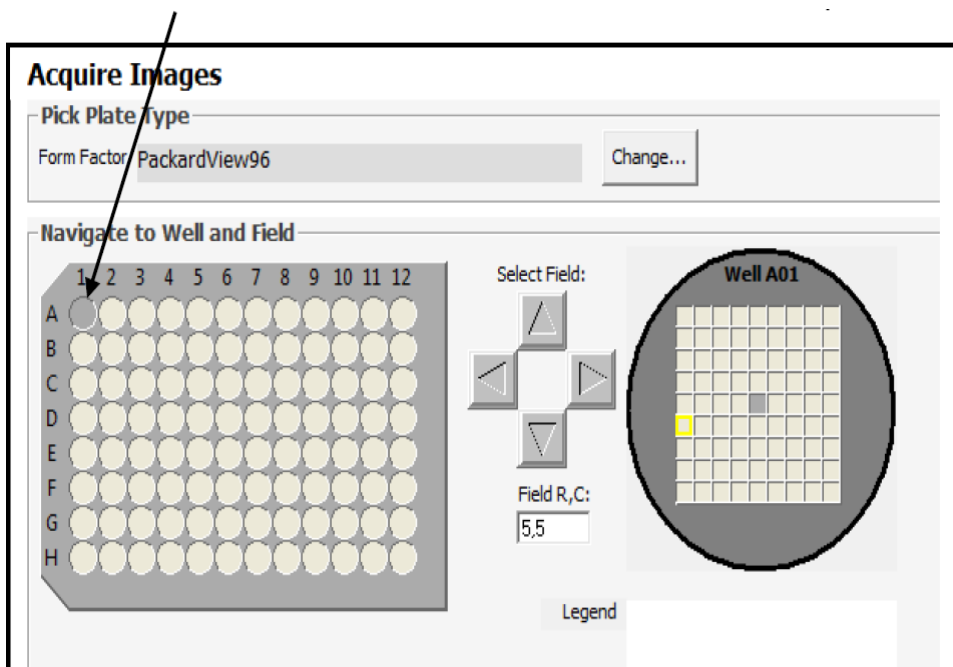
Figure 17 Z-Stack Projections

Acquiring 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,

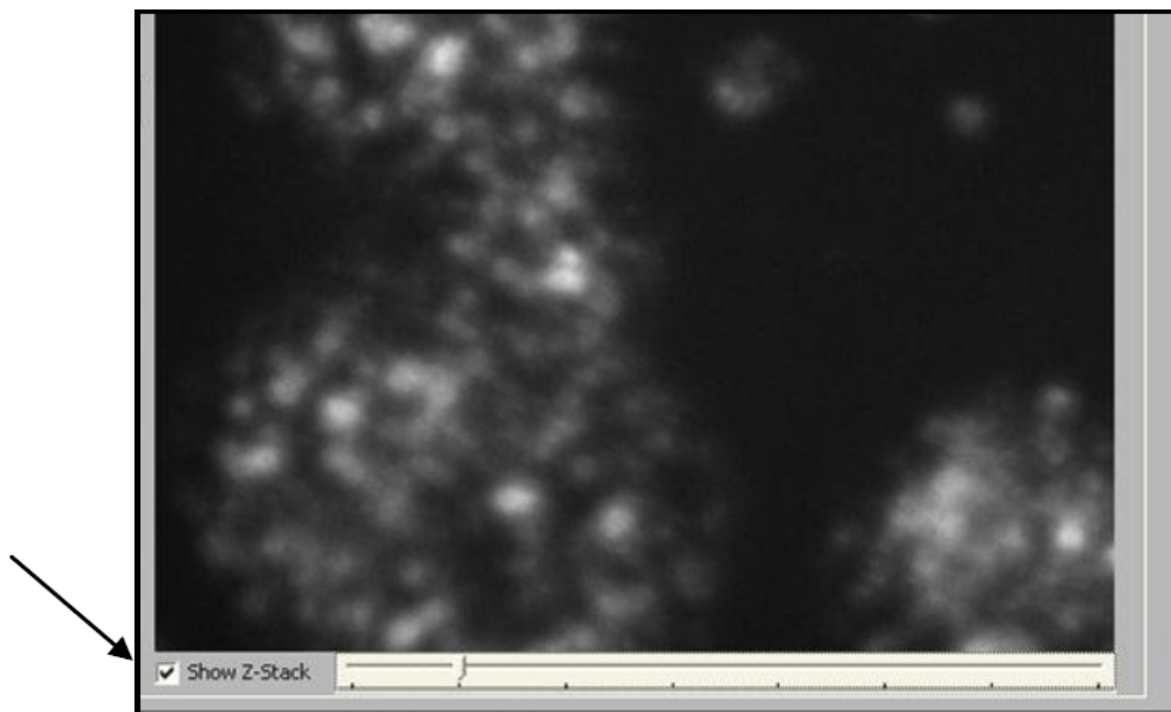
1. Open the **Configure Acquisition** screen. Refer to the software tutorials in the HCS Studio™ Cell Analysis Software online help for instructions.
2. Select the **Form Factor**.
3. Specify the **Confocal Detection Mode** options as described before.
4. From the plate representation, click on the desired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

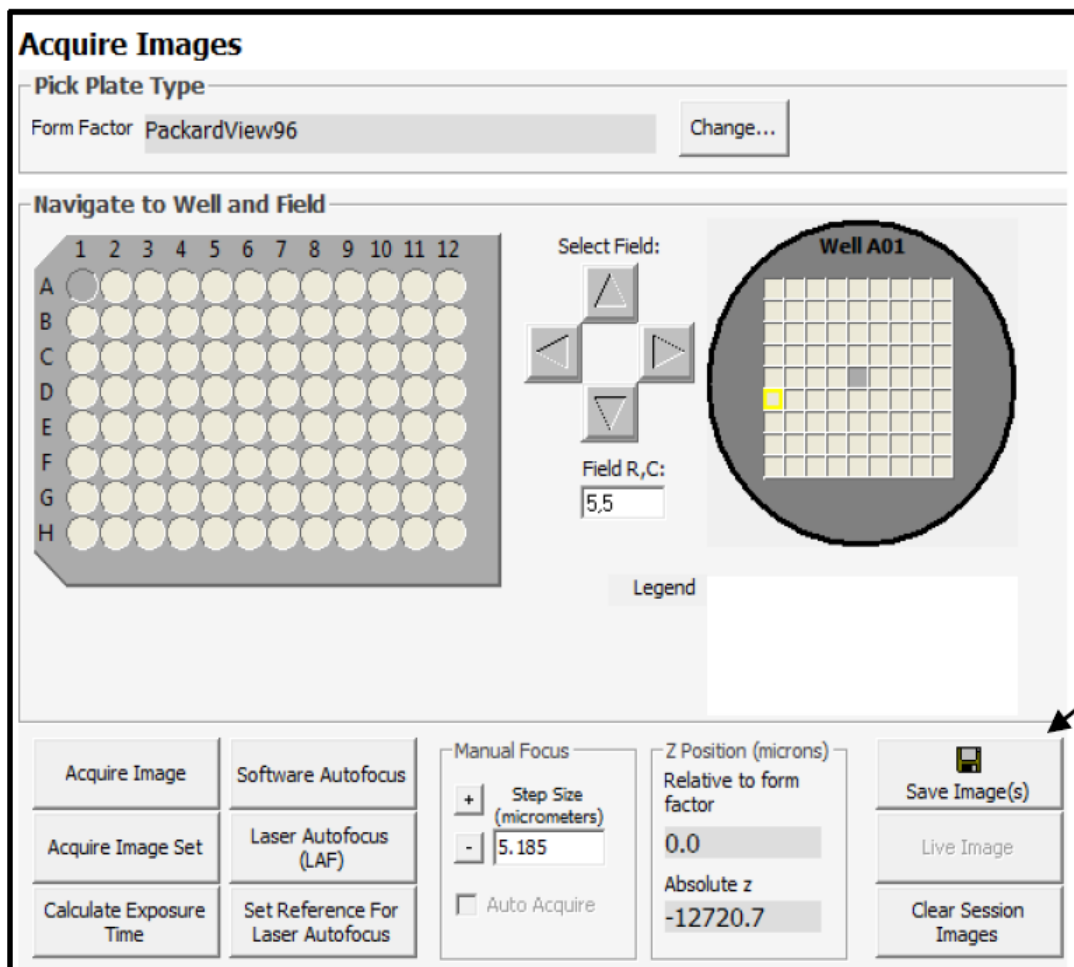


2. When the Z-stack option is selected, you can view each image of the Z-stack by clicking on the slider bar or using the up/down arrow keys on your keyboard.

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,

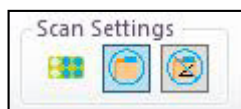
1. Display the desired image in the **Image Display** window.
2. Click **Save Image(s)**.



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

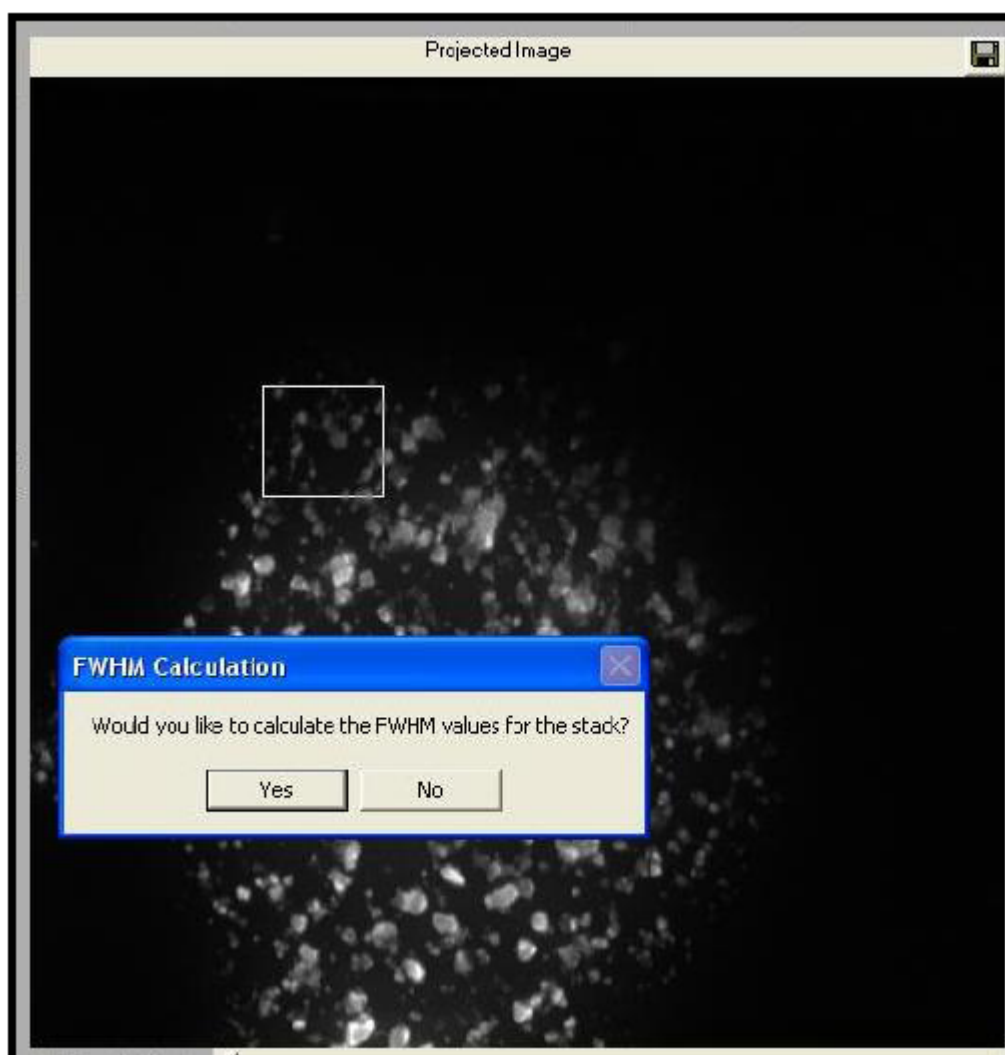
Setting	Description
Z-position	Z-position, in micrometers, of the image within the Z-stack, relative to the focus position
Total Intensity	Sum of the pixel intensity values within the selected rectangular region of the image, for a specific Z-position
Peak Total Intensity	Largest Total Intensity value 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. <i>This value is useful for defining the Z-resolution of the optics.</i>
Percent Area In Tails	The area of the curve outside the “full width” relative to the total area of the curve. <i>This value is useful for defining the sharpness of the cutoff of out-of-focus light.</i>

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.

Scanning 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 requiring confocal imaging, autofocus should be performed on a conventional Widefield image rather than a confocal image. It is performed on the channel set for autofocusing. Typically this is Channel 1 using the Widefield Detection Mode.
- For channels configured for Confocal, autoexpose is performed 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.

Running 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.

Removing a microplate from the CellInsight™ CX7 LZR Pro HCS Reader stage

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.

 If you are using a robotic plate handler with the CellInsight™ CX7 LZR Pro HCS Reader, refer to your robotic plate handler user documentation for instructions on inserting 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 LZR Pro HCS Reader components. In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.

The CellInsight™ CX7 LZR 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.



CAUTION! MOVING MECHANICAL PARTS!

The CellInsight™ CX7 LZR Pro HCS Reader incorporates moving parts. Please note the pinch hazard signs applied to potentially dangerous parts. **DO NOT MANUALLY OPEN STAGE DOOR WHEN READER IS POWERED!**



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.



DANGER! ⚠ CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

🔒 WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:
405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.

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

1. Refer to the Chapter 2, “Safety precautions” above.
2. 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.
3. When the plate is in the unload position, remove the plate from the stage.
4. After the plate is removed, click **Ctrl OK** in the **Plate Load/ Unload** dialog. This command places the stage inside the CellInsight™ CX7 LZR Pro HCS Reader.

Manually removing a microplate from the stage

Under normal operating conditions, refer to “Confocal imaging software setup procedures” on page 58 for instructions on removing a single microplate from the CellInsight™ CX7 LZR Pro HCS Reader stage.

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

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.



WARNING! **BIOHAZARD!**

Use proper laboratory practices when handling hazardous materials. Make sure that biohazards do not contaminate CellInsight™ CX7 LZR Pro HCS Reader components. In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.

The CellInsight™ CX7 LZR 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.



CAUTION! **MOVING MECHANICAL PARTS!**

The CellInsight™ CX7 LZR Pro HCS Reader incorporates moving parts. Please note the pinch hazard signs applied to potentially dangerous parts. **DO NOT MANUALLY OPEN STAGE DOOR WHEN READER IS POWERED!**



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.



DANGER! ⚠ CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

🔒 WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:

405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.

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

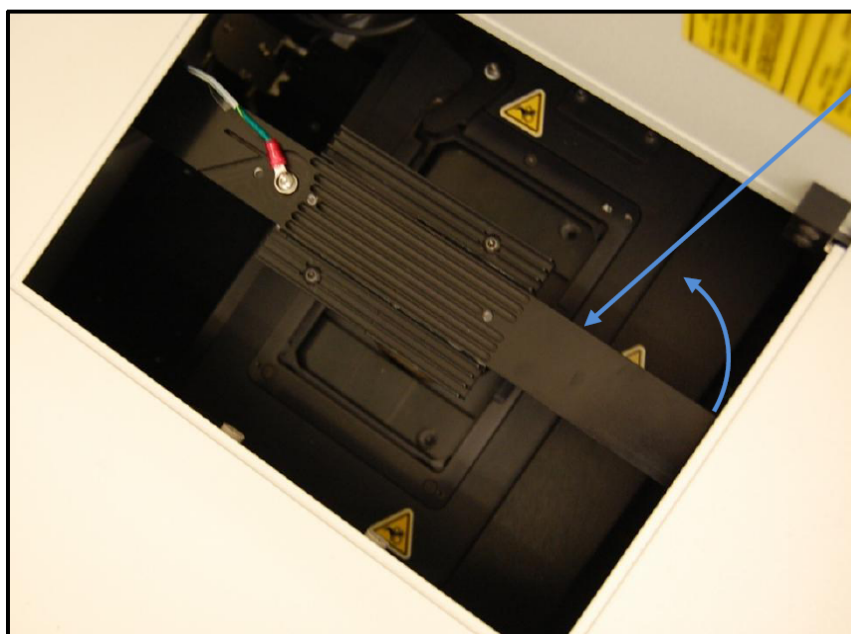
1. In the event of a power failure, turn off the CellInsight™ CX7 LZR Pro HCS Reader main power switch.
2. Turn power off to terminal strip and remove terminal strip power cord from outlet. If terminal strip is not used, remove CellInsight™ CX7 LZR Pro HCS Reader power cord from outlet.
3. Open the service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

Note: A key is required to open this door.



Figure 18 CellInsight™ CX7 LZR 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 figure below and moving the arm to the disengaged position (see following two figures). When the arm is in the disengaged position, you will feel a magnet holding the arm in place.



Grasp arm here
and move it in
direction of arrow
to disengaged
position until it
locks in place.

Figure 19 CellInsight™ CX7 LZR Pro HCS Reader open service door with engaged Brightfield arm.

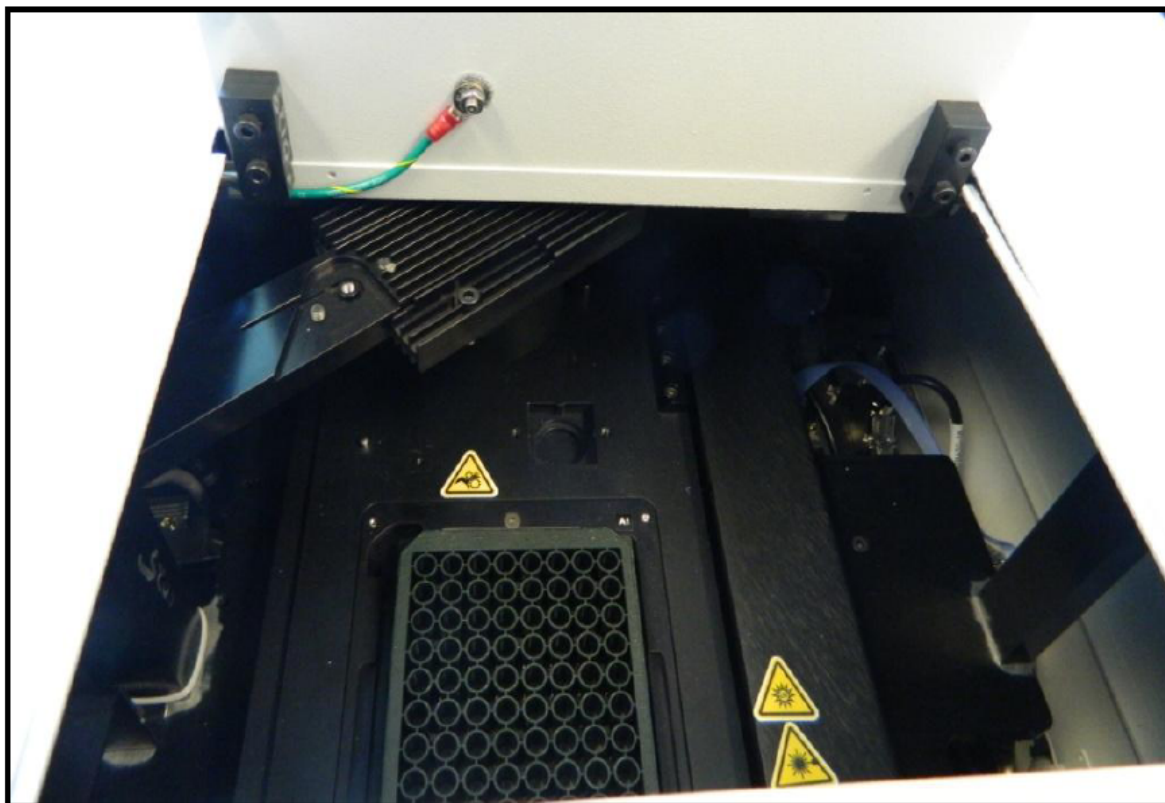


Figure 20 CellInsight™ CX7 LZR Pro HCS Reader open service door with disengaged Brightfield arm.

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 LZR Pro HCS Reader shutdown

Note: If you are using a robotic plate handler with the CellInsight™ CX7 LZR 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 LZR 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 LZR Pro HCS Reader power switch located on the back of the instrument.

4

Using the illumination correction tool

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.



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. Please refer to the Chapter 2, “Safety precautions” ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please 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 may 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.

Illumination Correction on the CellInsight™ CX7 LZR 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 may 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 may impact the extraction of spatially resolved data.

The illumination correction wizard on the CellInsight™ CX7 LZR 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 illumination 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 field illumination correction (see below). This dark current subtraction effectively improves the contrast in the image.

The CellInsight™ CX7 LZR 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 may 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 LZR 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 LZR Pro HCS Platform
- Utilizing 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 LZR 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 LZR Pro HCS Platform will now automatically perform 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.

Thermo Fisher Scientific recommends using the Illumination Correction feature for all channels of all BioApplications when intensity variations (COV) are high (see the status message table on page 90). You have the option of selecting this option for one or more channels (when optical performance is passed).

The Image Display Mode is set to Image Stretch Max 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 will also show gradients of unevenness even though the range of pixel intensity is minimal. It is recommended that Image Stretch Max be maintained 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 21 and Figure 22.

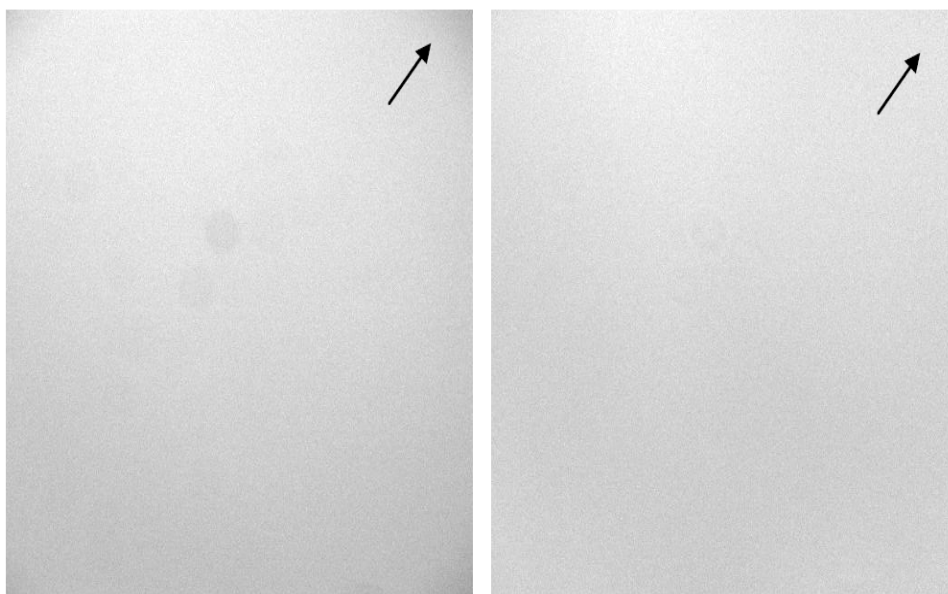


Figure 21 The Illumination Correction image in the left panel shows the subtle shading on a Contrast Stretch Max displayed image that is uncorrected with a COV = 7.2%. The right panel has been corrected and now appears more uniform with a COV = 5.2%.

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 22). It is visually apparent that in this particular case, using a 5× objective, the Illumination Correction Tool had a noticeable effect in reducing spatial heterogeneity (correction removed the darker corners of the uncorrected initial image).

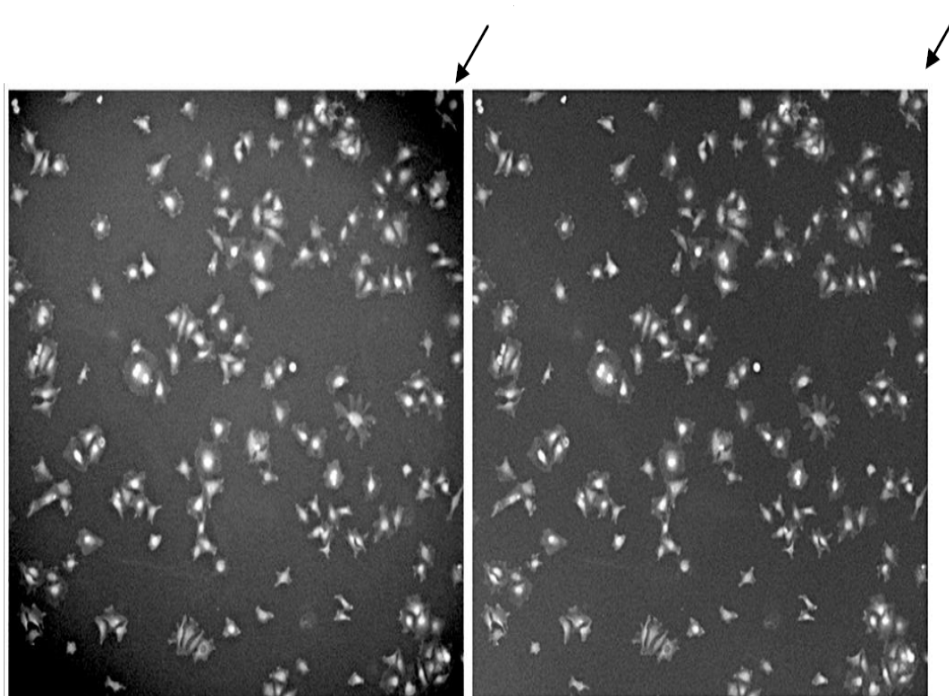


Figure 22 The image on the left shows an uncorrected 5×,TRITC image. The image on the right shows an illumination corrected 5×,TRITC image.

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 5×, 10×, and 20× objectives. Approximately 5000 cells were measured. One of the cellular features extracted, the average nuclear intensity of NF- κ B, 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 5× 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 10× or 20× objective did not require any cellular image values to be corrected more than 13%.

Preparing the illumination correction plate



WARNING! The Illumination Correction Solution contains fluorescent reagents in a DMSO solution. It is necessary to follow all safety procedures given in the MSDS for DMSO.

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

Once the OptiTracker™ II plate is available, you are ready to use the Illumination Correction Image Acquisition Wizard.

Running 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.

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.

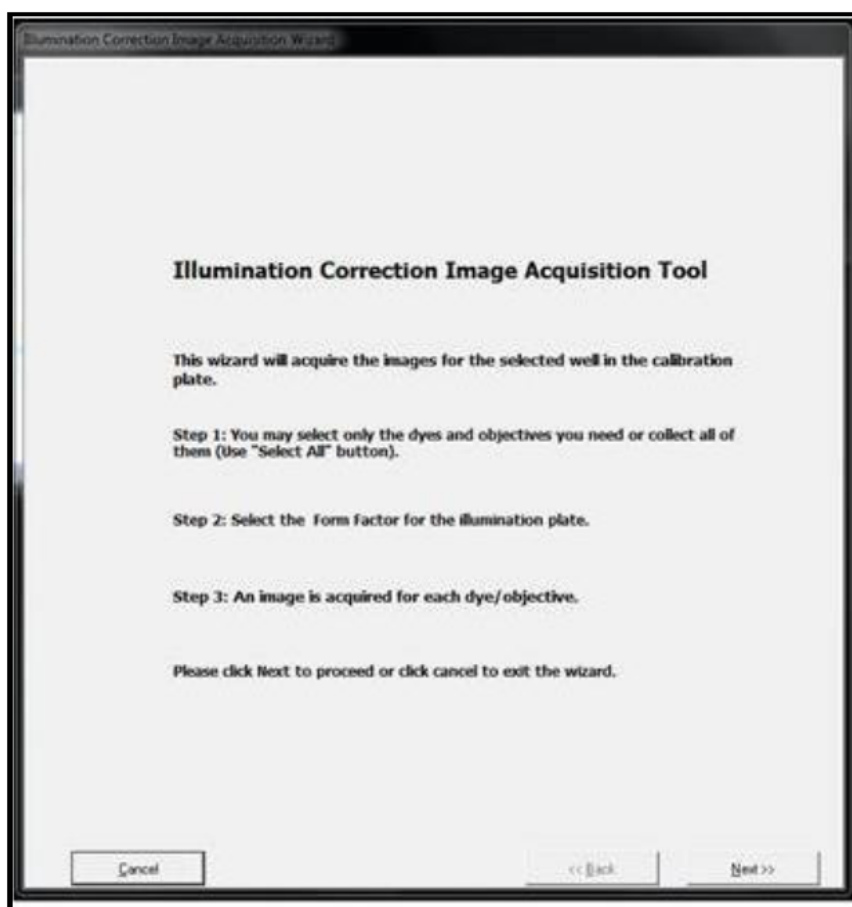


Figure 23 Illumination Correction Image Acquisition Wizard

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



Figure 24 Warning message

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: You can use the **Select All** feature if you are using a single dilution of the cocktail. If you require multiple dilutions of the cocktail, you must run the Wizard for each dilution.

Note: If your instrument has a 7-color LED light source, 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. 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.

Note: If your instrument has a laser light source, we do not recommend selecting any of the dyes that start with 450LZR, such as the 450LZR_C_C or the 450LZR_C_Y. The dye solution that is used to obtain illumination correction images does not have a dye that can be excited with the 450 nm wavelength laser line.

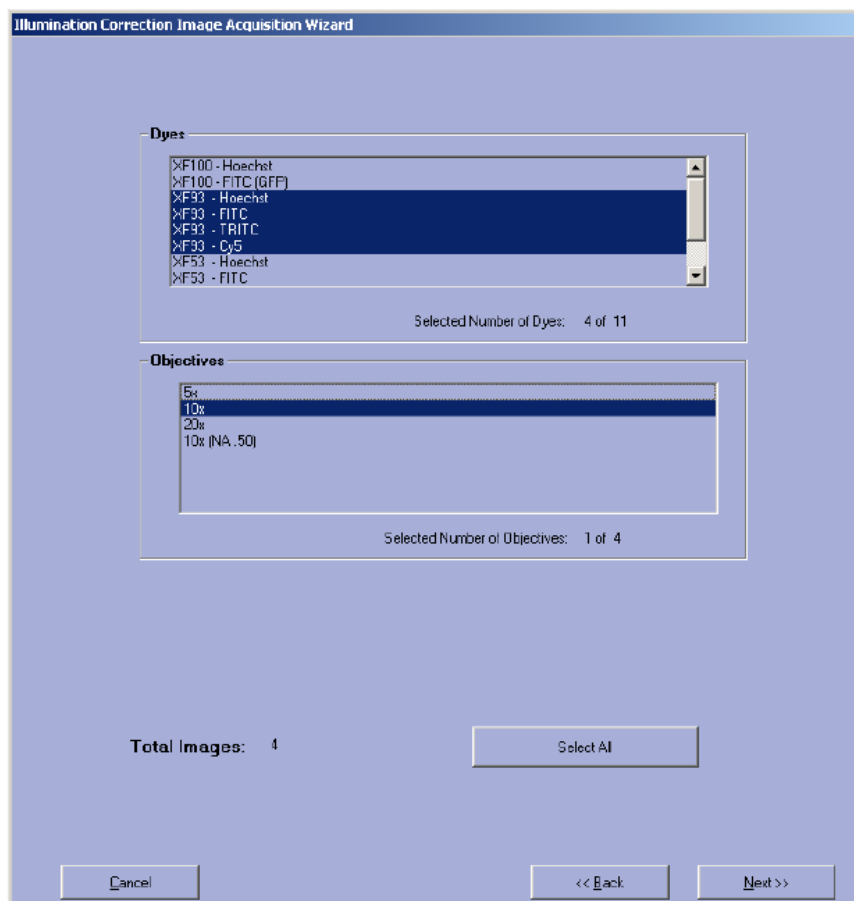


Figure 25 Illumination Correction Image Acquisition Wizard: Selecting dyes and objectives. In this example, the user has selected all four filters associated with the XF-93 filter-dichroic set and the 10x objective.

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.

Illumination Correction Image Acquisition Wizard

Form Factor Selection

Manufacturers List:

Plate Type:

Please select a well

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Buttons: Cancel, << Back, Next >>

Figure 26 Illumination Correction Image Acquisition Wizard: Selecting plate manufacturer, plate type, and well selection.

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.

[illegible]

Figure 27 Illumination Correction Image Acquisition Wizard: Option setting summary

8. After the correction process has completed, the Correction Done screen appears. In this case, 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 in this chapter for a description of all other status messages.

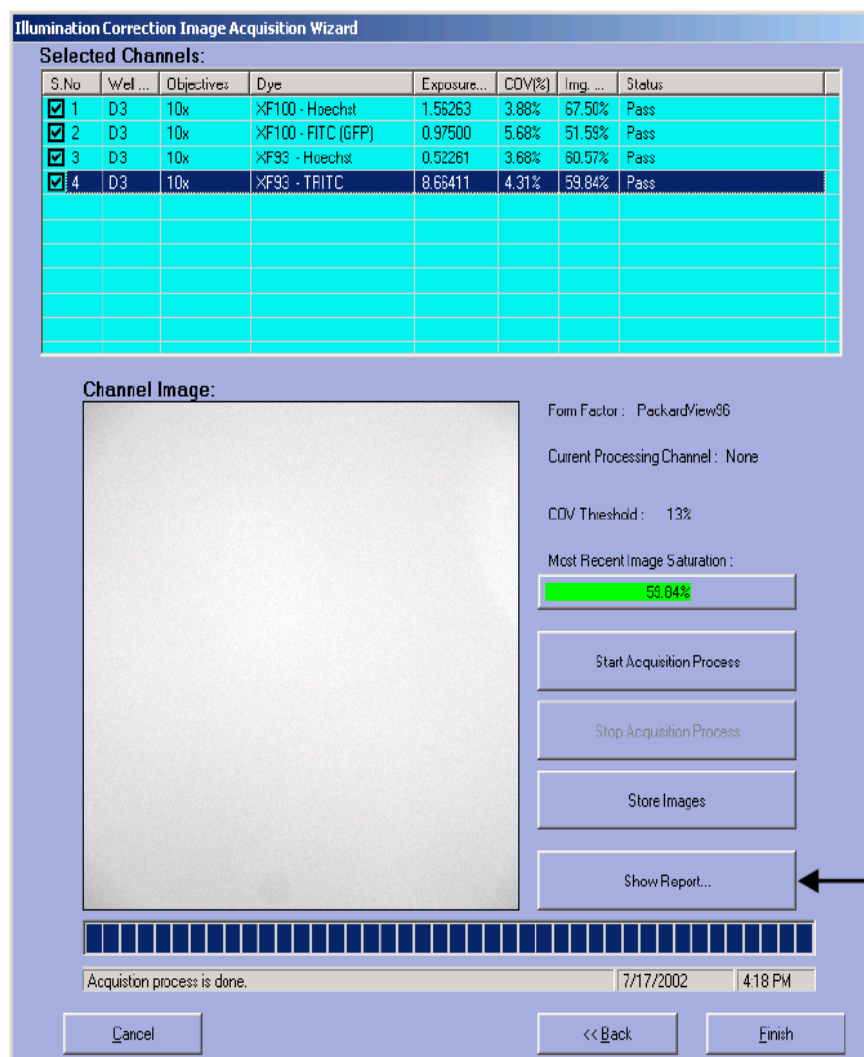


Figure 28 Illumination Correction Image Acquisition Wizard: Processing complete

9. If a printed report is required, click **Show Report**.

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

Illumination Correction Image Acquisition Report								
Operator:					Images Acquired on: 6/9/2002 11:01:13 AM			
S.No	Well Name	Objective	Dye	Camera Mode	Exposure Time	% COV	%Img. Saturation	Result(COV < COVThreshold[8%])
1	D5	10x	XP93 - Hoechst	Standard	0.43105	3.86%	62.33%	Passed
2	D5	10x	XP93 - FITC	Standard	1.89507	4.62%	66.50%	Passed
3	D5	10x	XP93 - TRITC	Standard	3.54448	6.44%	64.40%	Passed
4	D5	10x	XP93 - Cy5	Standard	2.23622	5.68%	66.16%	Passed

Figure 29 Illumination Correction Image Acquisition Report. The % COV describes the intensity variation across the image of the fluorescent standard and is a quantitative description of optical performance.

11. Click **Store Images** if no additional correction image acquisition is required.
12. The Images Stored message appears. Click **OK** to continue.

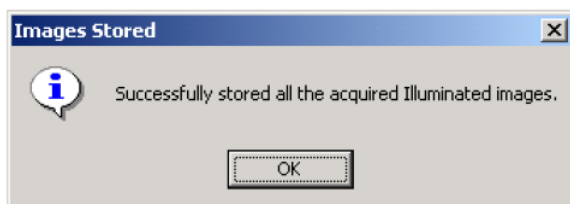


Figure 30 Message box: Storing Images

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

Using the illumination correction feature in your scans

1. Insert an assay cell plate into the stage.



WARNING! Refer to the Chapter 2, “Safety precautions” and instructions appearing on “Equipment safety labels” on page 17.

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 plate as usual. All images processed through the Store Image and Database Management Software from the 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, a warning appears asking 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 LZR Pro High Content Screening (HCS) Platform, or if you should contact Technical Support for assistance. You can operate your CellInsight™ CX7 LZR 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 may continue to operate your CellInsight™ CX7 LZR Pro HCS Platform. We recommend that you review the Diagnostic Checklist to return the COV back to 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 indicating a Pass/Illumination Correctable (COV >13%–25%) condition or Fail/Illumination Issue (COV >25%), refer to the following “Diagnostic checklist” on page 91 to assist you in troubleshooting or restoring the CellInsight™ CX7 LZR 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.

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 the use of the OptiTracker™ plate

- 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 at least 20 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.

- Thermo Fisher Scientific recommends that you use illumination correction for all channels in your assay.
- 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 to do today?	No, the CellInsight™ CX7 LZR Pro HCS Platform prompts you to collect a new set of images before scanning a plate if greater than 48 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 10–15 minutes. For systems with automated objectives and filters, you can walk away and return later, click Store Images, then start your scan. Note: This function is possible if the specified filters and objectives require a single dilution of the cocktail. If you require multiple dilutions of the cocktail, you must run the Wizard for each dilution.
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 LZR Pro HCS Platform?	We recommend that you use 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. • If the COVs are above 25% for filters and objectives in use, do not use your instrument. Contact Technical Support for assistance.



CellInsight™ CX7 LZR Pro HCS Reader maintenance

This chapter describes routine maintenance and cleaning procedures.

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.



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. Please refer to the Chapter 2, “Safety precautions” ensuring safe operation and maintenance of the system. Read these precautions carefully before performing the procedures outlined in this document. In addition, please 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 may 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.

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

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.

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

- Periodically check all power cords and data cables for secure connection and proper condition. Refer to “System schematics” on page 109.
- Keep the CellInsight™ CX7 LZR 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, as well as to mechanical shocks.
- Avoid jarring the unit, especially while it is operating.

CellInsight™ CX7 LZR Pro HCS Reader cleaning



WARNING! Ensure that the CellInsight™ CX7 LZR 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 instrument.

Dust and dirt can impair the performance of optics and moving parts. When performing any maintenance procedure, do not leave the instrument doors open for an extended period.



WARNING!  BIOHAZARD!

Use proper laboratory practices when handling hazardous materials.

Make sure that biohazards do not contaminate CellInsight™ CX7 LZR Pro HCS Reader components. In the event that system contamination occurs, contact Technical Support for applicable cleanup procedures.

The CellInsight™ CX7 LZR 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 LZR Pro HCS Reader, perform the following cleaning procedures:

- Clean the outer surfaces of the CellInsight™ CX7 LZR 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 LZR Pro HCS Reader fuse replacement

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.

If the CellInsight™ CX7 LZR 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 LZR Pro HCS Reader power cord is plugged firmly into the terminal strip and firmly attached to the back of the unit.



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 LZR 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. The fuse holder contains two fuse locations.

Fuse

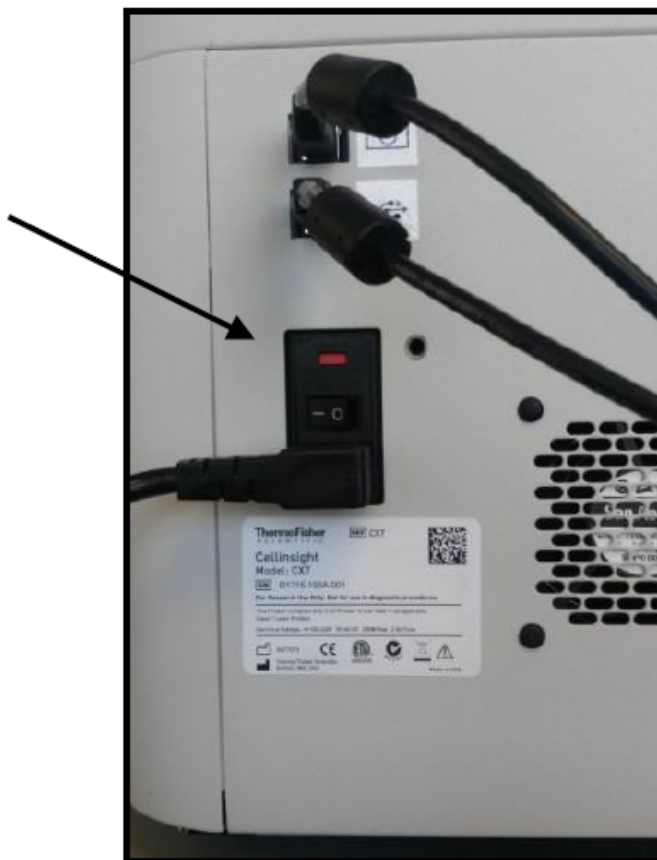


Figure 31 Fuse holder on back of the instrument

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

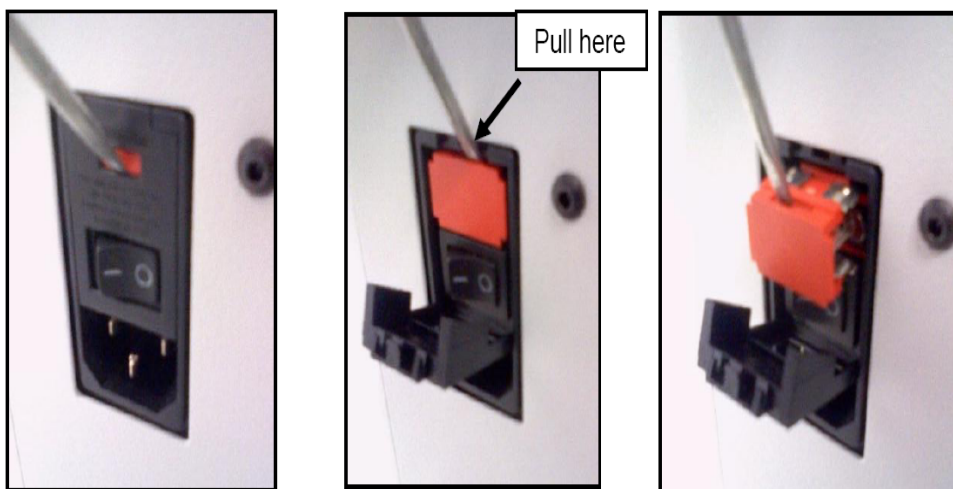


Figure 32 Removing fuse holder from back of the instrument

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: Slow Blow 2.5A@250VAC Cartridge Fuse, 5 mm × 20 mm

220–240V: Slow Blow 2.5A@250VAC Cartridge Fuse, 5 mm × 20 mm

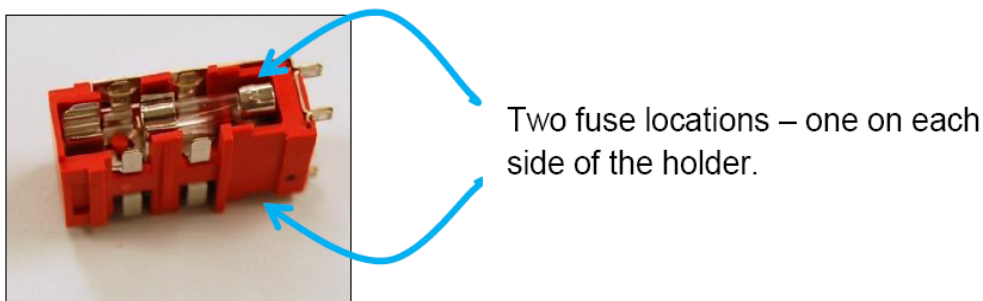


Figure 33 Fuse locations

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

Objective inspection

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 LZR Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. **DO NOT MANUALLY OPEN STAGE DOOR WHEN READER IS POWERED!**



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.



DANGER! ⚠️ CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

☠️ WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:

405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.

To inspect the objective,

1. If a microplate is inserted in the stage, follow the procedures on “Confocal imaging software setup procedures” on page 58 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 LZR 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.

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 LZR Pro HCS Reader service door.
9. If you are unable to clean the objective in this way, contact Technical Support for assistance.

Changing 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 LZR Pro HCS Reader incorporates moving parts. Note the pinch hazard signs applied to potentially dangerous parts. **DO NOT MANUALLY OPEN STAGE DOOR WHEN READER IS POWERED!**



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.



DANGER!  **CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!**

 **WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!**

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:

405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

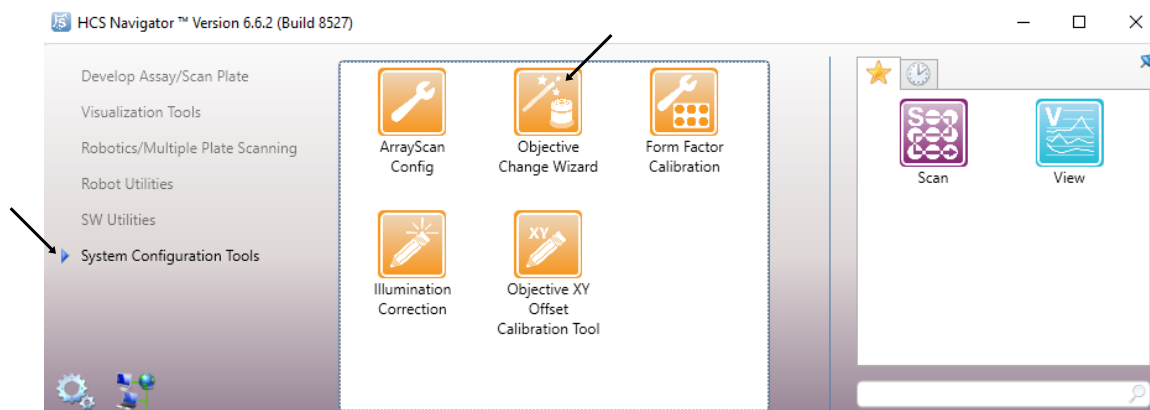
Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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!

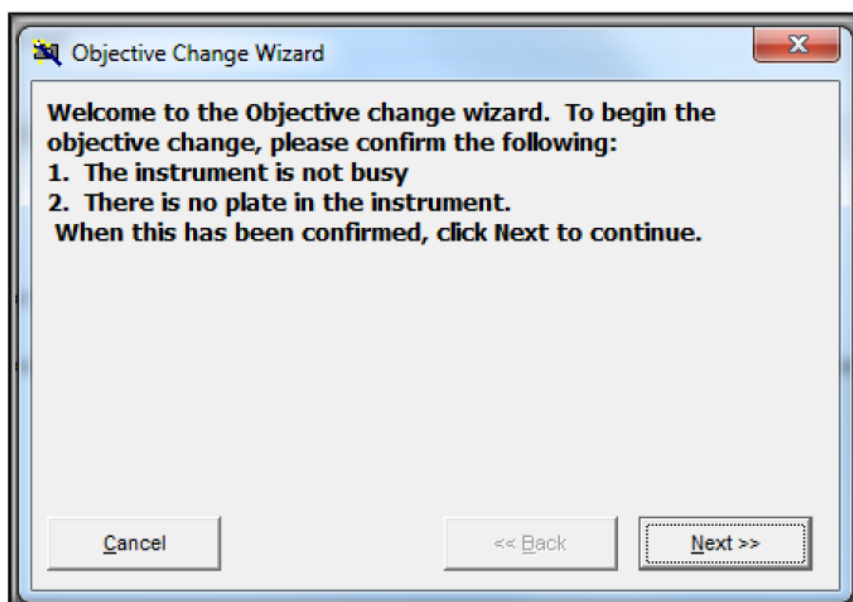
Wear laser protective glasses with an optical density greater than 7 (OD > 7) for wavelengths at 720–810 nm.

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 58 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.



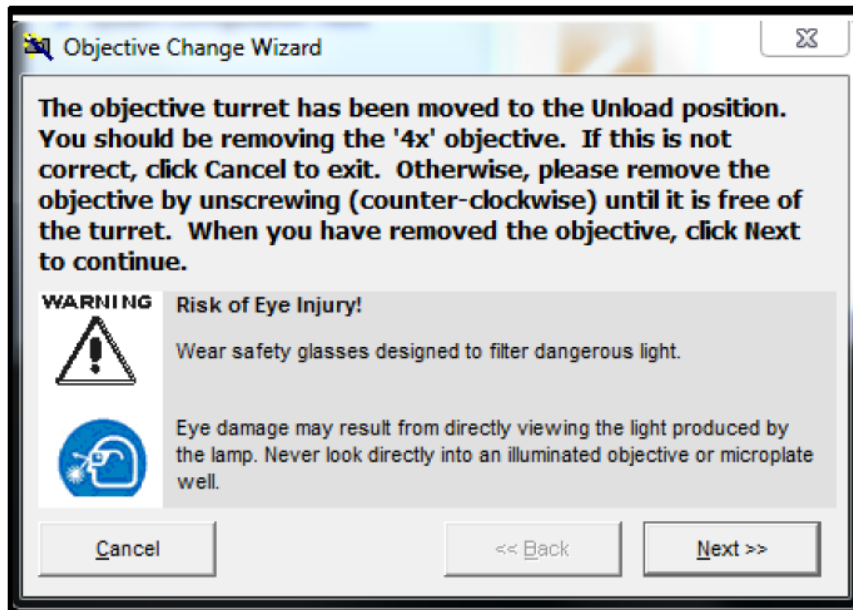
Figure 34 CellInsight™ CX7 LZR Pro HCS Reader service door

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**.

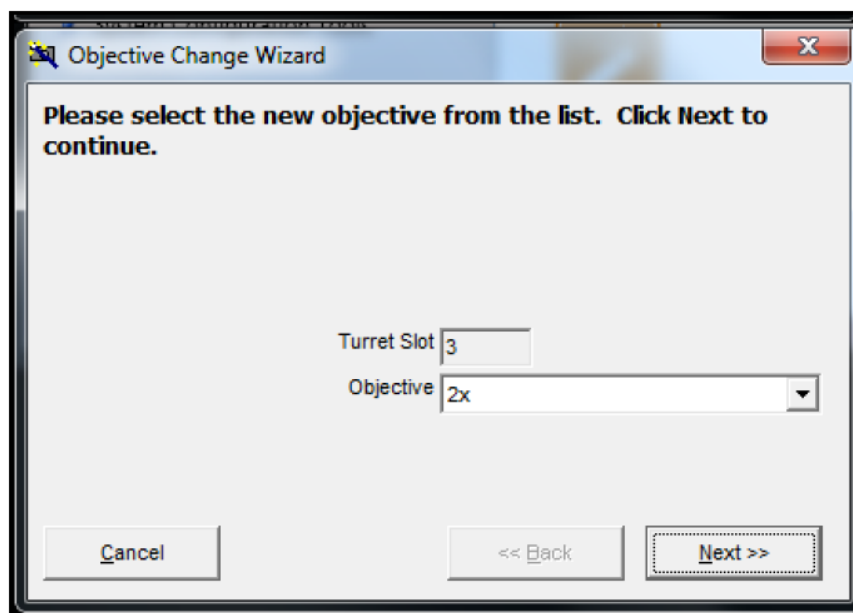


CAUTION! To avoid system damage, uninstall and install objectives only in the Load/Unload Position.

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.

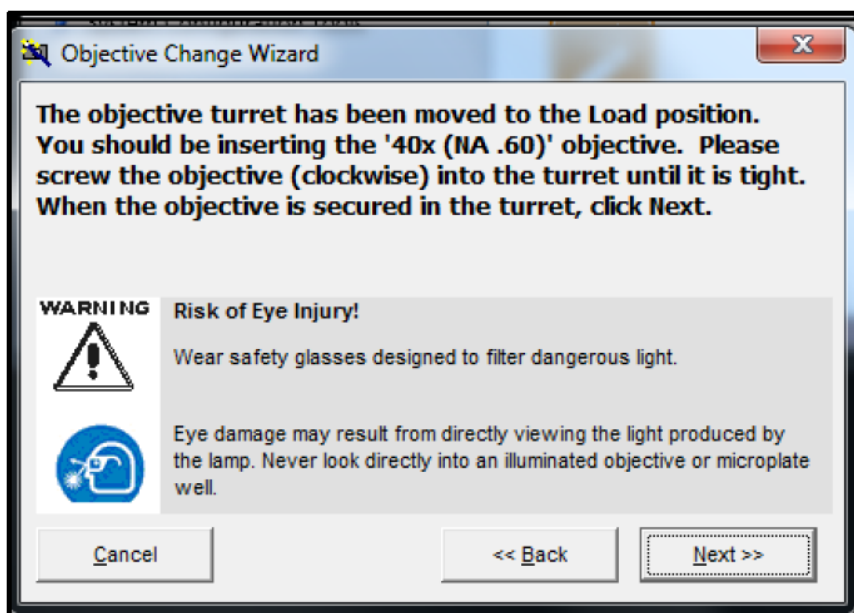


9. From the Objective list, select the objective that you are installing, then click **Next**.

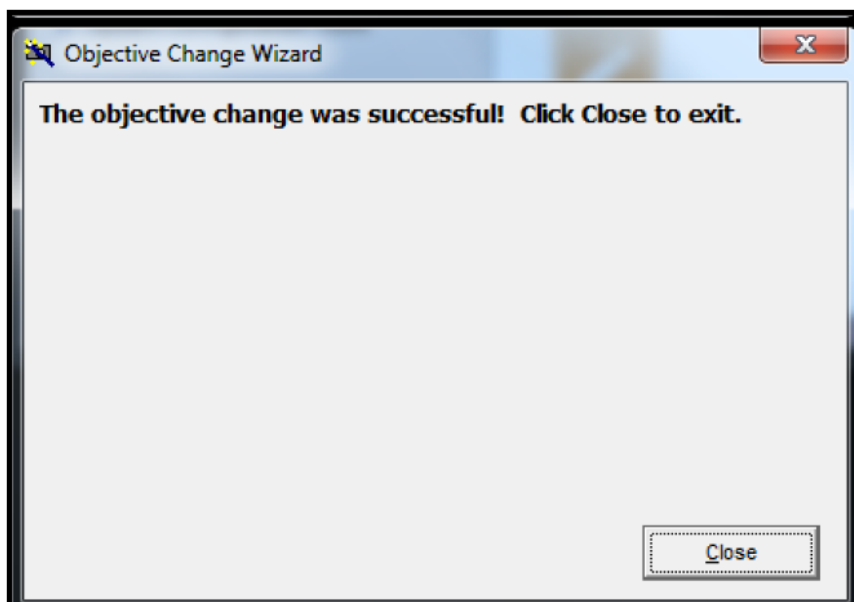


10. The following screen appears. Insert the new objective in the Load/Unload Position by following the instructions on screen. Close and lock the service door. Click **Next** to continue.

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 LZR Pro HCS Reader.

Adjusting 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 most optimal position for the particular plate type, the images can appear soft and slightly out of focus. Rotating the collar can correct for thick culture vessels. The purpose is to eliminate spherical aberration and thus provide the highest quality image.



CAUTION! MOVING MECHANICAL PARTS!

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



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 for instrument use. These glasses will filter infrared wavelengths.

- Eye damage may 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 may 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.



DANGER! CLASS 3B and 3R LASERS – VISIBLE & INVISIBLE LASER RADIATION WHEN OPEN – AVOID EXPOSURE TO BEAM!

WEAR THE REQUIRED LASER PROTECTIVE EYEWEAR!

The CellInsight™ CX7 LZR Pro HCS Reader contains a Class 3B laser device, conformant to IEC\EN 60825-1:2014. The wavelength and output power of the lasers are as follows:

405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW;
647 nm/120 mW; 785 nm/100 mW

Avoid direct eye exposure!

The CellInsight™ CX7 LZR 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 7 (OD > 7) for wavelengths at 720–810 nm.

To adjust the correction collar,

1. Refer to the Chapter 2, “Safety precautions” above.
2. 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.
3. 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 35 CellInsight™ CX7 LZR Pro HCS Reader service door

4. 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

5. 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 6.
6. 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.
7. Repeat step 1–step 5 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.
8. Once the collar position has been determined, record the collar position and use this whenever the same type of plate is used.
9. Close and lock the service door.

System schematics

Internal AC system diagram – power A/C

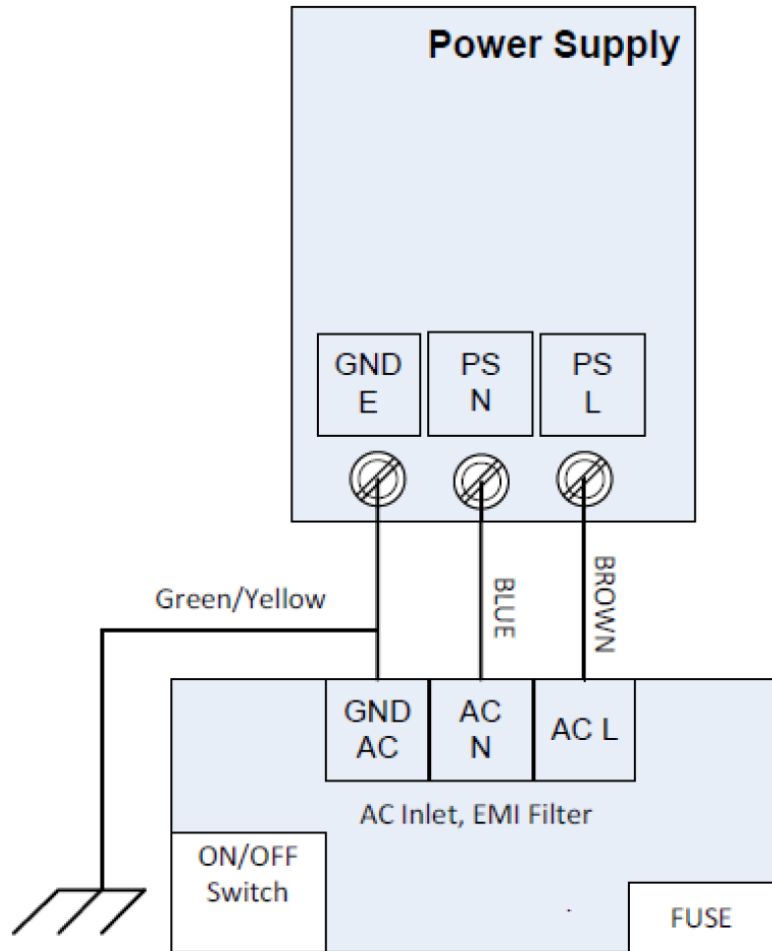


Figure 36 CellInsight™ CX7 LZR Pro HCS Reader System Connection Diagram – Power A/C and D/C

System connection diagram – communication layout

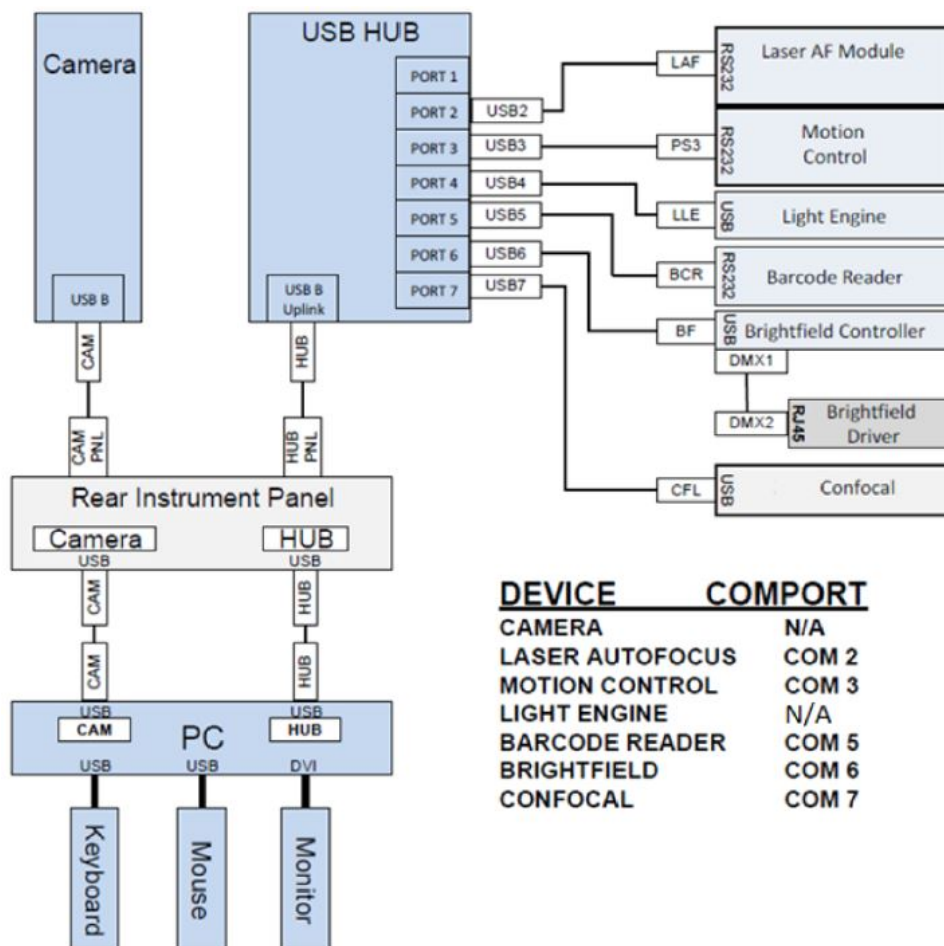


Figure 37 CellInsight™ CX7 LZR Pro HCS Reader Connection Diagram – Communication Layout

Power connection diagram – 110–120VAC

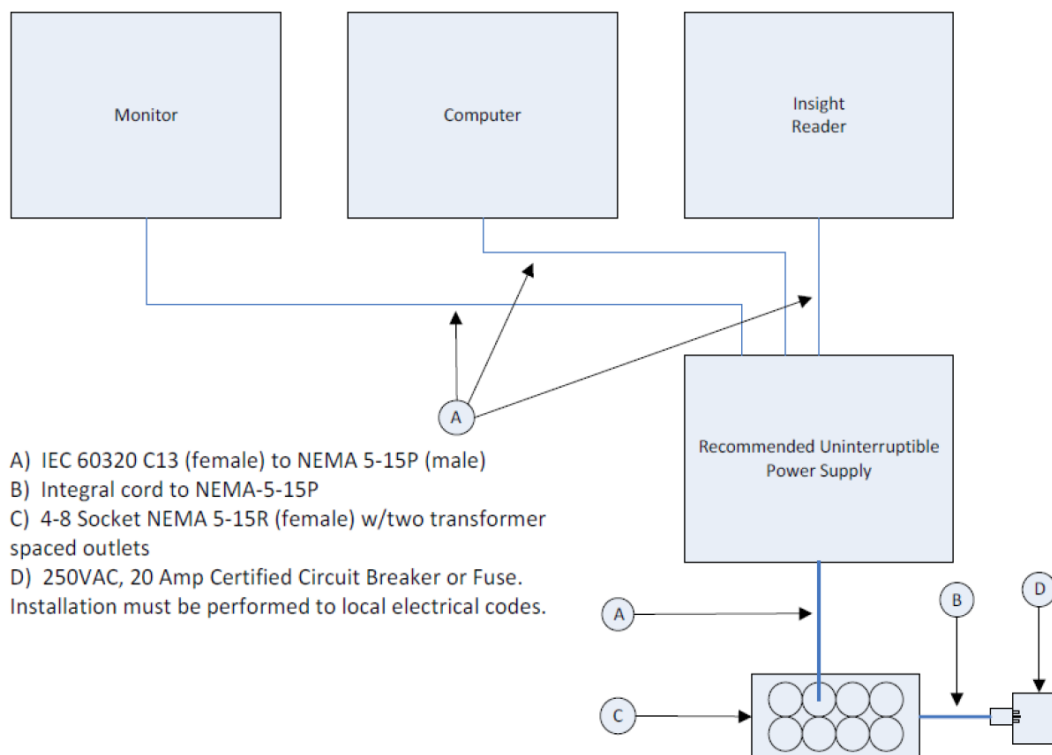


Figure 38 CellInsight™ CX7 LZR Pro HCS Reader System Connection Diagram – Power – 110–120V

Power connection diagram – 220–240VAC

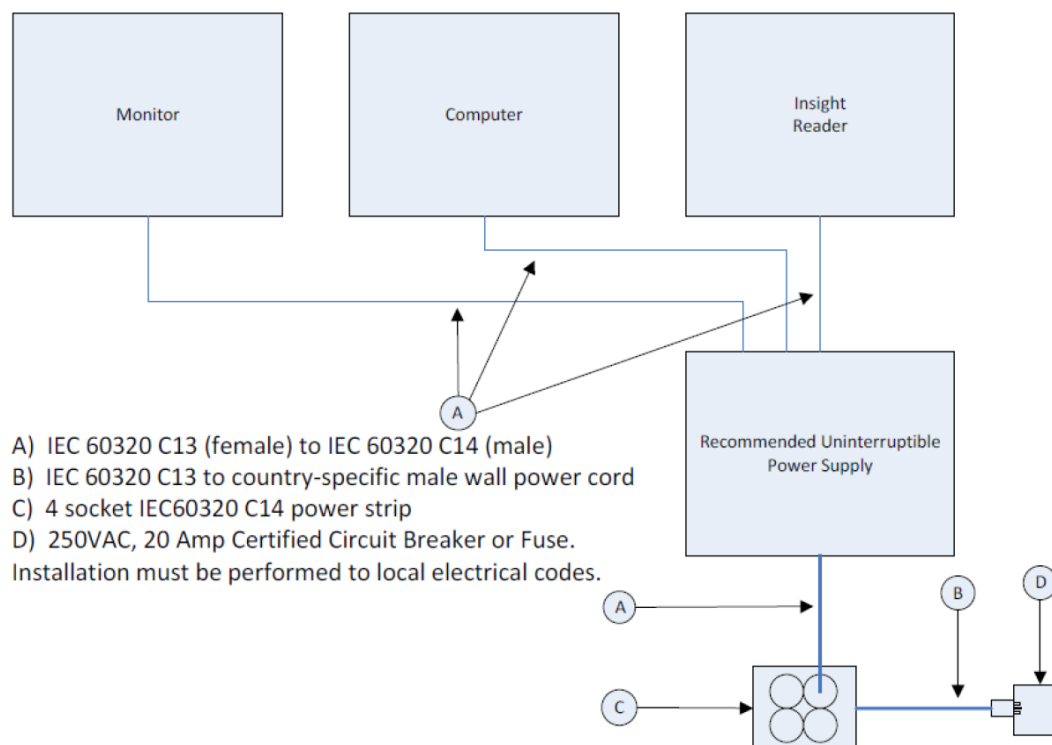


Figure 39 CellInsight™ CX7 LZR Pro HCS Reader System Connection Diagram – Power – 220–240V

Connection diagram – optional onstage incubator

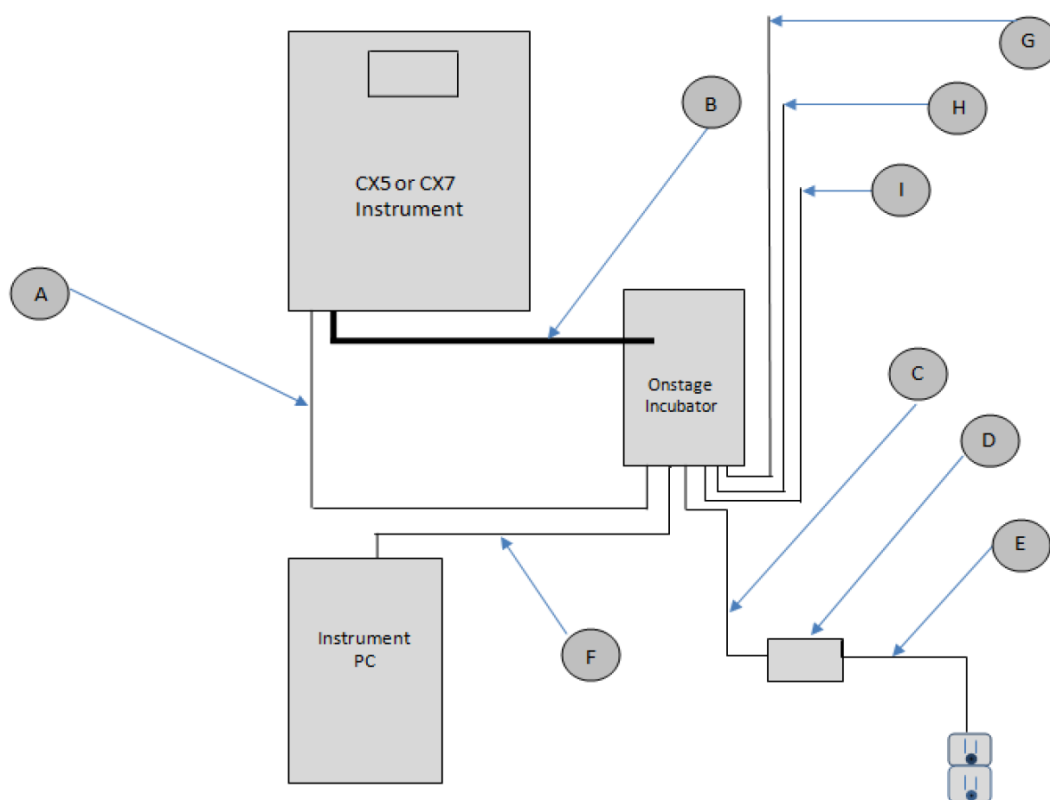


Figure 40 CellInsight™ CX7 LZR Pro HCS Reader System Connection Diagram – CellInsight™ Onstage Incubator

- 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: CO₂ Inlet Line
- H: N₂ Inlet Line
- I: Compressed Air Inlet Line

Computer and monitor

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.



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.

Computer monitor cleaning

To clean the monitor screen,

1. Make sure 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. Make sure 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.

Computer case exterior cleaning

Make sure 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

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.

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

The CellInsight™ CX7 LZR 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.



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 LZR Pro HCS Reader weighs approximately 68 kg (150 lbs). At least two people are required for transport. Take the proper lifting precautions to avoid injury and always lift the CellInsight™ CX7 LZR 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 LZR 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” appendix 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, an elevator is recommended.

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 LZR Pro HCS Reader for a short move

1. Stop any current plate scan and, if present, remove the microplate from the CellInsight™ CX7 LZR 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 LZR Pro HCS Reader. See “CellInsight™ CX7 LZR Pro HCS Reader shutdown” on page 76 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 that 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 LZR Pro HCS Reader is approximately 68 kg (150 lbs). At least two persons are required for transport. Take the proper lifting precautions to avoid injury. Lift the CellInsight™ CX7 LZR 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 LZR Pro HCS Reader requires careful handling. Do not attempt to pack and ship the system without contacting Technical Support.

Long-term shutdown

 Read and understand all safety precautions appearing in this chapter AND the Safety Precautions chapter of this guide before performing these procedures.

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

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

1. Remove the plate, if present, from the CellInsight™ CX7 LZR Pro HCS Reader.
2. Follow the shutdown procedures described in “CellInsight™ CX7 LZR Pro HCS Reader shutdown” on page 76.
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 LZR 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 35 and contact Technical Support for applicable cleanup procedures.**



CAUTION! IMPORTANT!

Do **not** subject the CellInsight™ CX7 LZR 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 35.

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 LZR 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.

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

1. Turn OFF the CellInsight™ CX7 LZR 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 LZR Pro HCS Reader with paper towels or Kimwipes™ tissues dampened with 70% ethanol or 4,000 ppm hydrogen peroxide (H₂O₂).



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 LZR Pro HCS Reader equipment

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

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



Technical specification

Note: Technical specifications are subject to change without notice.

CellInsight™ CX7 LZR Pro HCS Reader instrument

Power consumption	250 W max
Frequency range	50–60 Hz
AC voltage	100–240 V
Current	6 A maximum
Supported microplates	- Compatible with clear bottom 96-, 384- and 1536-well microplates - Slideport™ Slide Inserts for up to 4 glass microscope slides
Stage travel	79.4 mm × 329 mm
Focus resolution	Sub-micron step size with advanced micro stepping and double stepping capabilities
Wavelength range	Specific wavelengths between 405 nm–785 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™ LAPAO 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™ 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)
Optics	Infinity corrected system

Image compatibility	JPG, BMP, GIF, PNG, TIF
Dimensions	80 (D) × 48.5 (W) × 46.5 (H) cm 31.5 (D) × 19.1 (W) × 18.3 (H) in. Height includes the feet which the instrument sits on. The feet should not be removed.
Weight	68 kg (150 lbs)
Optical filters	Refer to Appendix B, “Automated spectral selection for fluorescence imaging” for detailed information.

Robotic plate handler

Refer to the appropriate robot manufacturer documentation for detailed specifications.

Light source

The system is equipped with a 7-color laser light source for fluorescence excitation. The Light Engine houses seven class 3B lasers (405 nm/100 mW; 450 nm/75 mW; 488 nm/120 mW; 561 nm/100 mW; 594 nm/100 mW; 647 nm/120 mW; 785 nm/100 mW).

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.

Overall 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 61326-1:2006
Line frequency	50/60 Hz
Line voltage	110–120 VAC $\pm$ 10% (220–240 VAC $\pm$ 10%)
Maximum elevation of site above sea level	2000 m
Noise immunity	Conforms to EN 61326-1:2006
Sound pressure Lp	< 70 dB (A)
Overall space required for entire system	414 mm (W), 372 mm (H), 594 mm (D)
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 consumption	250 W
Power line fuses	110–120V: Slow Blow 2.5A@250VAC Cartridge Fuse, 5 mm $\times$ 20 mm 220–240V: Slow Blow 2.5A@250VAC Cartridge Fuse, 5 mm $\times$ 20 mm
Protection class	I
Weight of instrument	68 kg (150 lbs) plus computer/monitor weight



Automated spectral selection for fluorescence imaging

This appendix describes the key spectral elements of the 7-color CellInsight™ CX7 LZR Pro High Content Screening (HCS) Platform that enables 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 41). Two options are available for fluorescence imaging: widefield and confocal fluorescence imaging. The CellInsight™ CX7 LZR 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 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:

1. “Instrument configurations” on page 124
2. “Light source for fluorescence excitation” on page 125
3. “Basic configurations for laser light source, dichroic mirrors, and emission filters” on page 126
4. “Advanced configurations for light source, dichroic mirrors, and emission filters” on page 132
5. “General guidelines to optimize scan speeds” on page 134

Instrument configurations

Widefield – confocal configuration

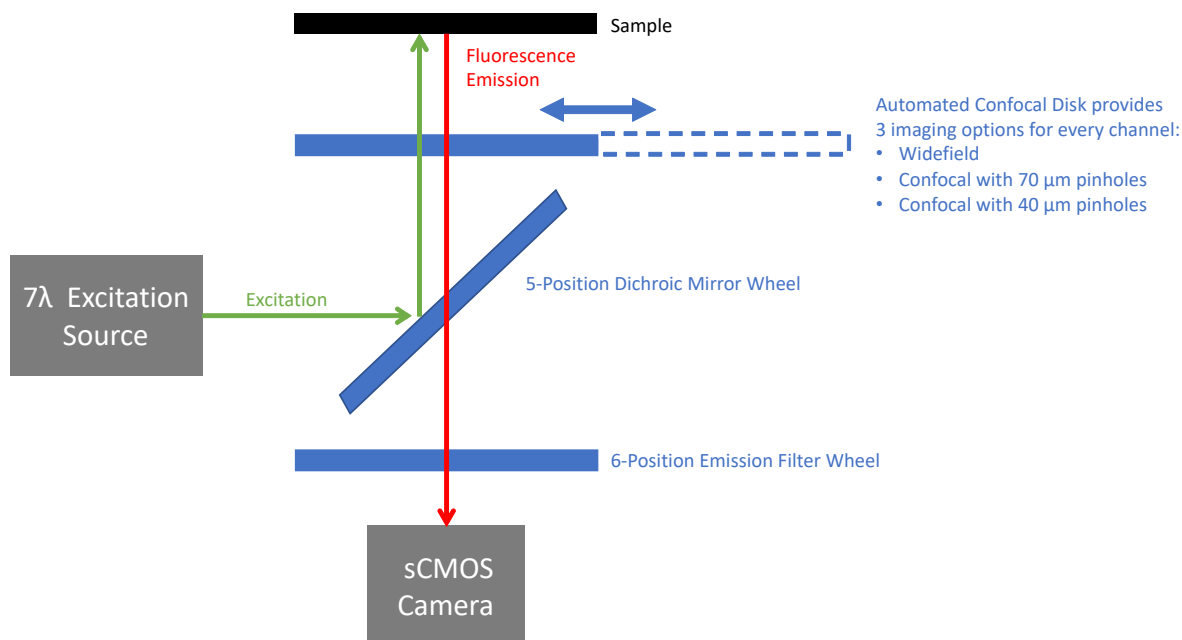


Figure 41 The Widefield – Confocal configuration for the 7-color CellInsight™ CX7 LZR Pro HCS Platform

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 containing seven separate continuous-wave solid-state lasers. 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 42 shows the seven wavelengths emitted from the laser light source (the spectral power output for each laser line is normalized to 100% in this figure). These seven colors can excite a broad range of fluorescent probes, giving you many options and flexibility in choosing fluorescent dyes (Figure 43).

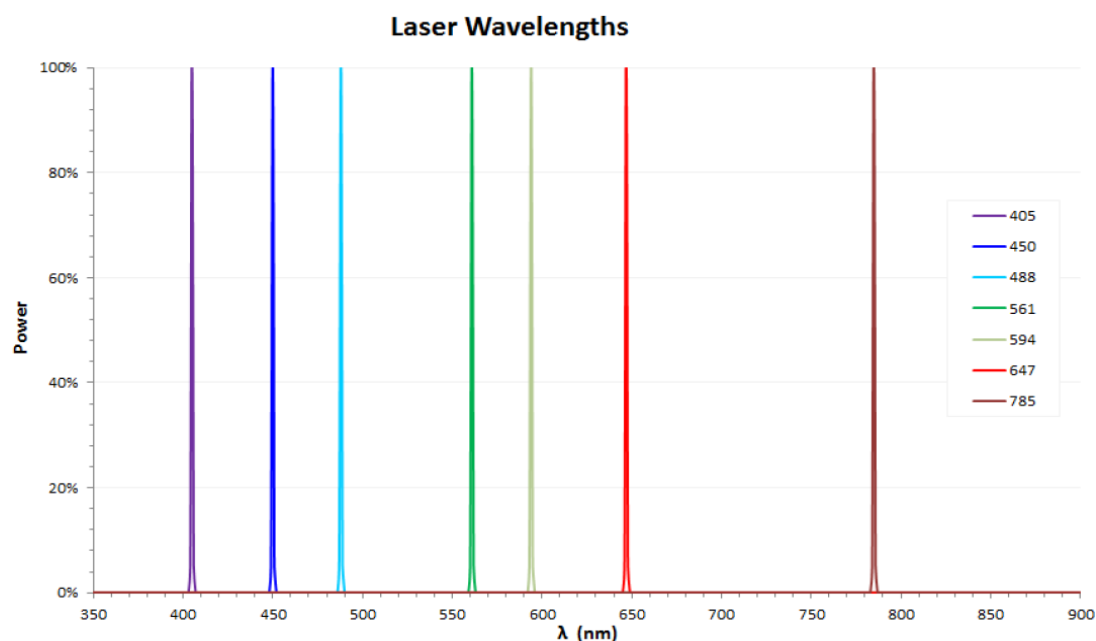


Figure 42 Laser wavelengths from the 7-color excitation laser light source

Basic configurations for laser 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 to be used. The excitation sources and filters enabling imaging are available in two modes: Basic and Advanced.

- **Basic mode:** Enables measuring the fluorescence 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 43. The naming convention specifies the laser wavelength, dichroic mirror, and emission filter combination. For example, **405LZR_BGRFR_BGRFR**, refers to exciting the sample with the 405 nm laser line, and using the quad-band dichroic mirror along with the quad-band emission filter that transmits blue (B), green (G), red (R), and far red (FR) light. Likewise, **450LZR_C_Y** refers to exciting the sample with the 450 nm laser line, 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 43 summarizes this instrument's basic spectral capability; where for each of the seven colors, it shows the excitation wavelength, 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 wavelength, 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 43, is laid out in Figure 44. Figure 45-Figure 47 show the normalized excitation wavelengths 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:

- **BGRFR:** The quad-band (i.e., 4-color) BGRFR dichroic mirror and emission filter enable the multiplexed imaging of up to four different colors (Figure 44-Figure 45).
- **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 BGRFR quad-band set (Figure 44-Figure 46).
- **RS:** Additionally, there is a broad RS (red scarlet) single band emission filter which can be used with the BGRFR dichroic mirror. With 561 nm excitation, the RS emitter enables brighter detection of red and scarlet dyes than with the BGRFR emitter. The 488 nm laser 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 47):

- **C:** The matched C (cyan) dichroic mirror and emission filter, which with 450 nm excitation, enables excitation of versions of CFP (cyan fluorescent protein).
- **Y:** The matched Y (yellow) dichroic mirror and emission filter, which with 488 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 785 nm laser excitation, enables excitation of versions of fluorescent probes that fluoresce in the near infrared range.

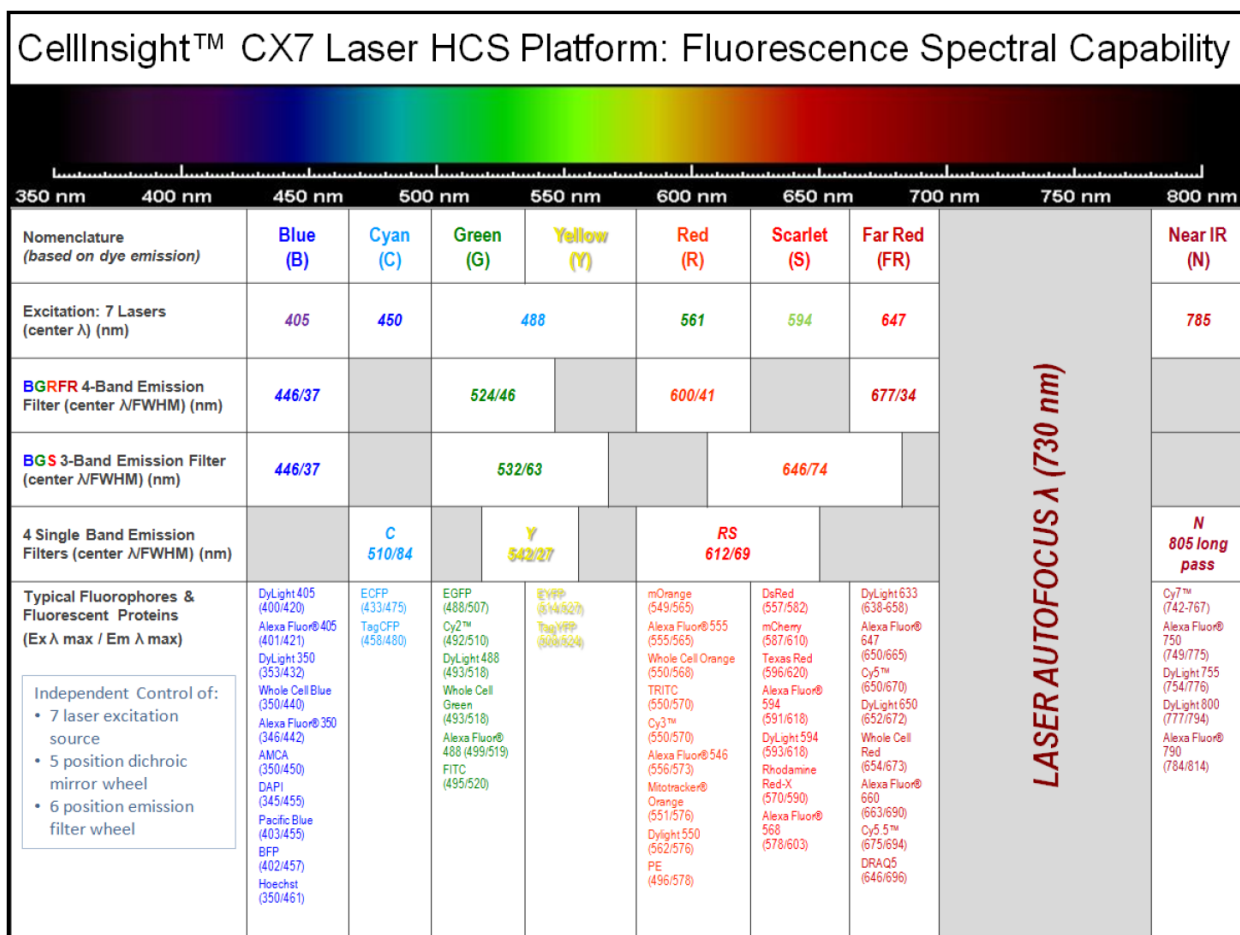


Figure 43 7-color basic spectral capability of the CellInsight™ CX7 LZR Pro HCS Platform

CellInsight™ CX7 Laser HCS Platform: Basic Fluorescence Configuration

Excitation Laser Wavelengths (nm)	5 Position Dichroic Mirror Wheel	6 Position Emission Filter Wheel	Use
405 488 561 647	BGRFR	BGRFR	4λ multiband dichroic & emitter covering visible spectrum to simultaneously detect 4 colors (Blue, Green, Red or Scarlet, & Far Red), for speed - Uses 4 of the 7 lasers available for excitation
		RS	Green/Red-Scarlet FRET with 488 nm excitation & RS emitter
405 488 594	BGS	BGS	- Optimized 3λ multiband for Blue, Green, & Scarlet dyes - Brighter detection of Green, & Scarlet dyes than with BGRFR set
450	C (Cyan)	C (Cyan)	Enables detecting CFP
488	Y (Yellow)	Y (Yellow)	Enables detecting YFP
785	N (Near IR)	N (Near IR)	Dedicated Near IR/Near IR channel for optimized detection of near IR dyes

Figure 44 Basic configuration for excitation light source, dichroic mirrors, and emission filters

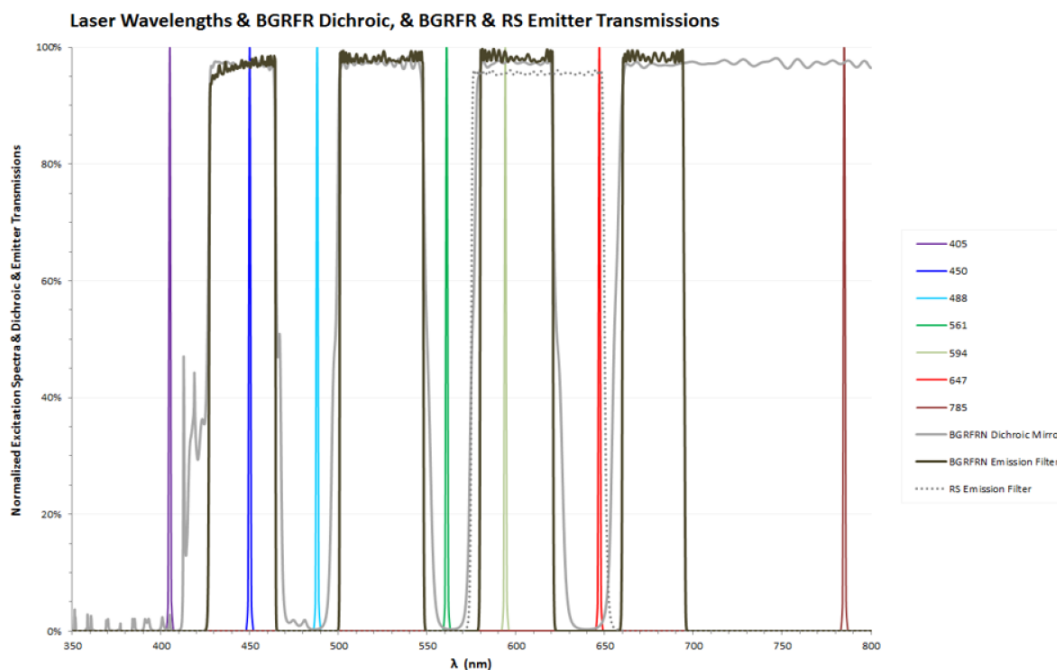


Figure 45 Quad-band BGRFR dichroic mirror and emission filter transmission spectra, overlaid with the normalized wavelengths from the 7-laser excitation light source

Also shown is the single band RS emission filter's transmission. Only excitation wavelengths 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 wavelengths that are inside a transition band (e.g., 450 nm and 594 nm for this set) will cause bleed through, and thus should be avoided.

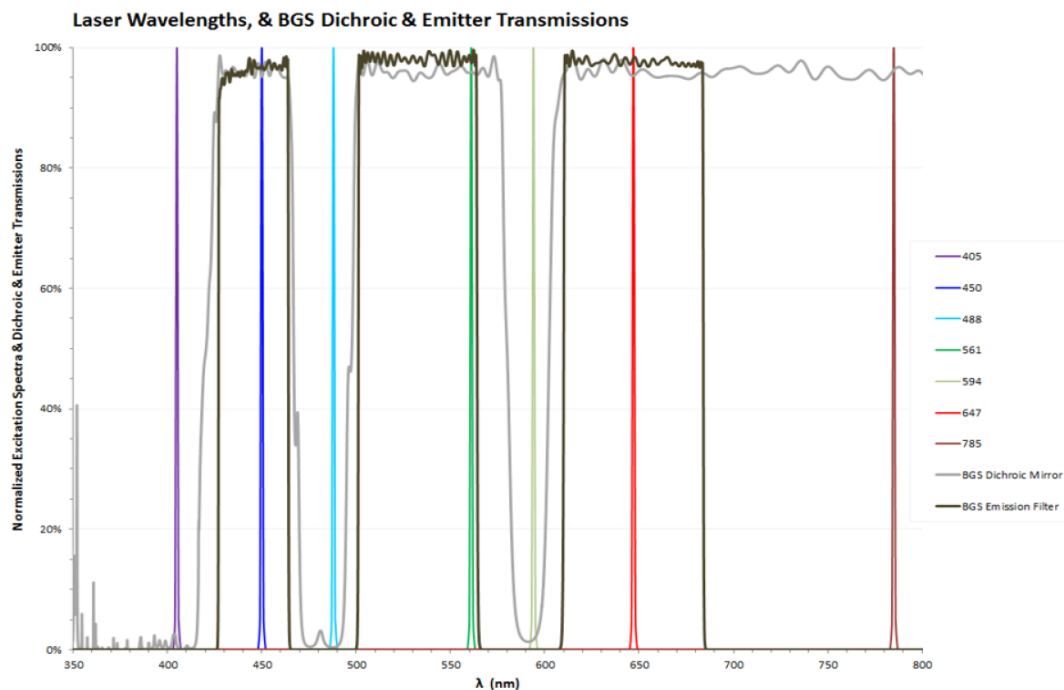


Figure 46 Tri-band BGS dichroic mirror and emission filter transmission spectra, overlaid with the normalized wavelengths from the 7-laser excitation light source

Only excitation wavelengths 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 wavelengths that are inside a transition band (e.g., 450 nm and 647 nm for this set) will cause bleed through, and thus should be avoided.

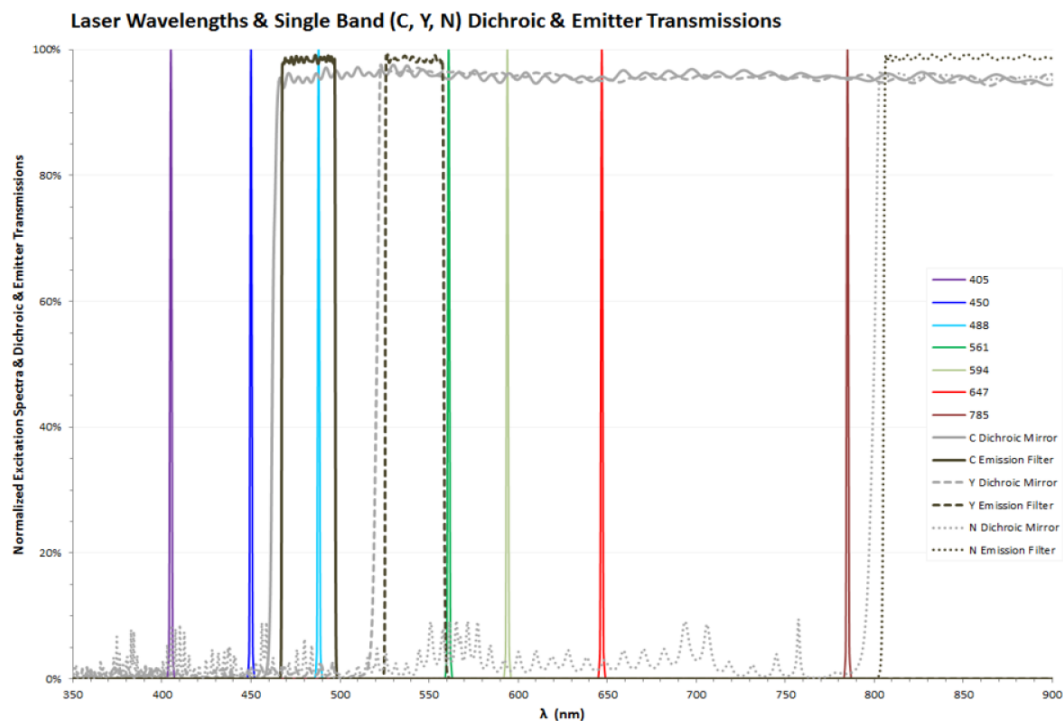


Figure 47 Single band matched C, Y, and N dichroic mirrors and emission filters transmission spectra, overlaid with the normalized wavelengths from the 7-laser excitation light source

Note: The C Emission filter has been updated and has a 510/84 curve, rather than 482/25.

Advanced configurations for 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, and then either 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 to enable FRET of different donor-acceptor pairs.
- **Quantum Dots:** Quantum Dots (QDot) are excited in the blue or UV, and can emit over a wide spectral range, depending on the specific Qdot™ probe. Figure 48 shows the exciter, dichroic mirror, and emitter combinations to 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.

CellInsight™ CX7 Laser HCS Platform: Advanced Fluorescence Configuration

Excitation Laser Wavelengths (nm)	5 Position Dichroic Mirror Wheel	6 Position Emission Filter Wheel	Use
405	BGRFR or BGS	Y (Yellow)	- Qdots 525, 565 - BFP/GFP FRET
		RS	Qdots 565, 585, 605, 625, or 655
450		Y (Yellow)	- Qdots 525, 565 - Single band green detection
		RS	Qdots 565, 585, 605, 625, or 655
488		Y (Yellow)	- GFP/YFP FRET - Single band green detection
		RS	Green/Red-Scarlet (e.g. GFP/RFP) FRET
405	C (Cyan)	C (Cyan)	Detection of cyan dyes
		Y (Yellow)	Qdots 525, 565
450			CFP/YFP FRET & BRET
488			GFP/YFP FRET & BRET
405 450	Y (Yellow)	Y (Yellow)	- Qdots 525, 565 - BFP/GFP FRET - GFP/YFP FRET
405	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.



Automated spectral selection for transmitted light imaging

The CellInsight™ CX7 LZR 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 LZR 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 5-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

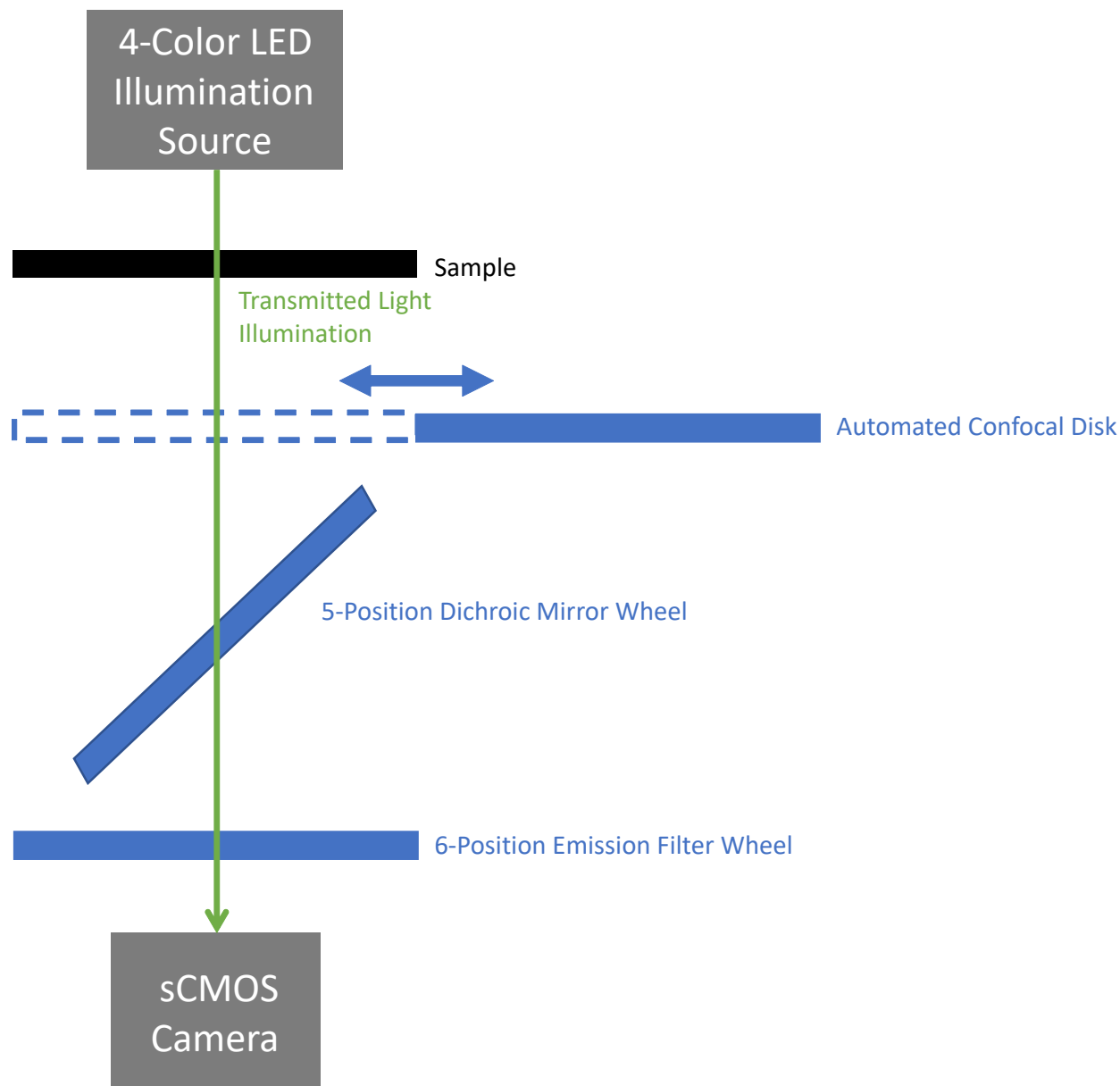


Figure 49 The Transmitted light configuration for the CellInsight™ CX7 LZR Pro HCS Platform

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 quad-band BGRFR 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 (Haematoxylin 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 quad-band BGRFR 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 quad-band dichroic mirror and emission filter.

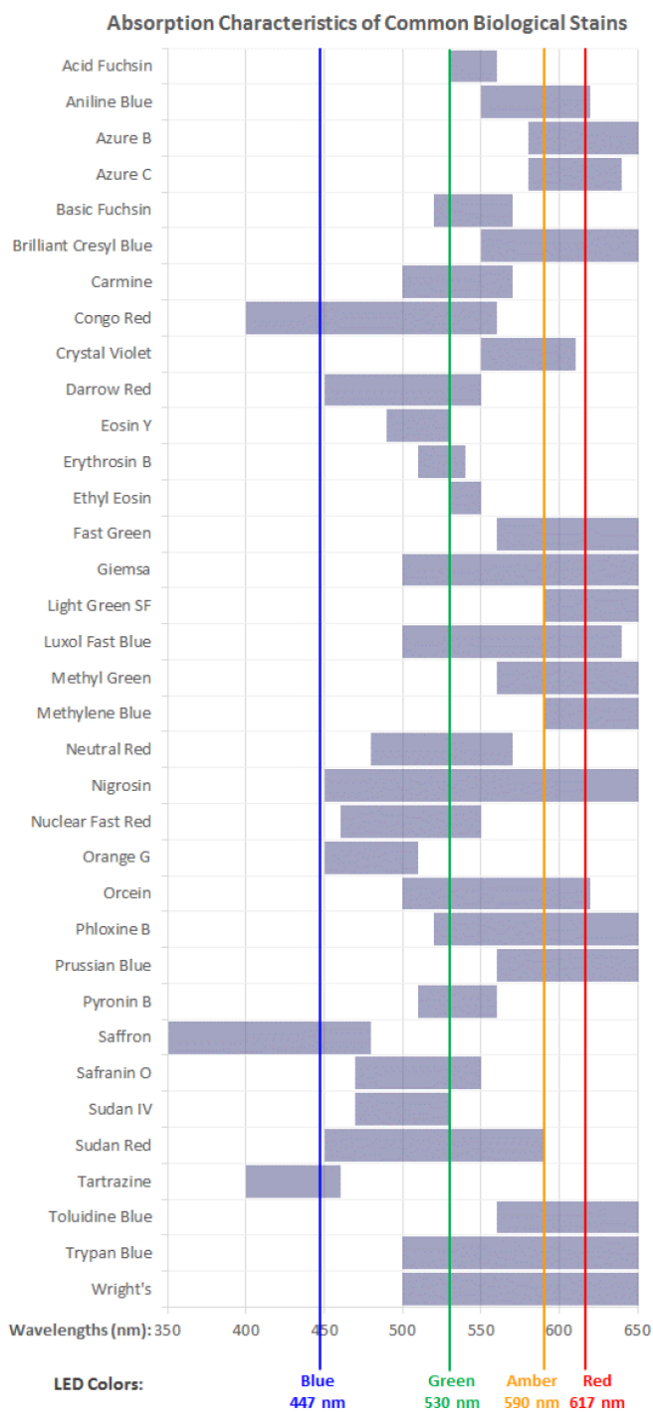


Figure 50 Absorption ranges of common biological stains, overlaid by the four LED wavelengths from the transmitted light source on the CellInsight™ CX7 LZR Pro HCS Platform

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

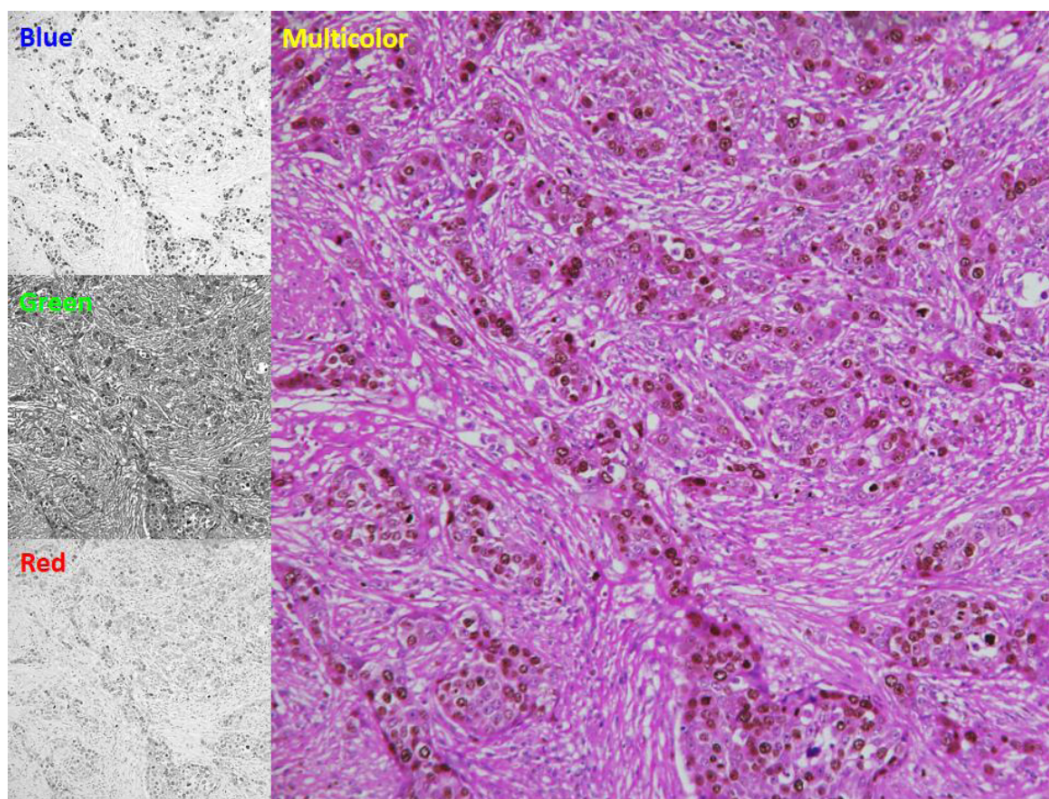


Figure 51 Human breast cancer tissue stained with the proliferation marker KI67-DAB and counterstained with H&E (Haemotoxylin and Eosin)

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

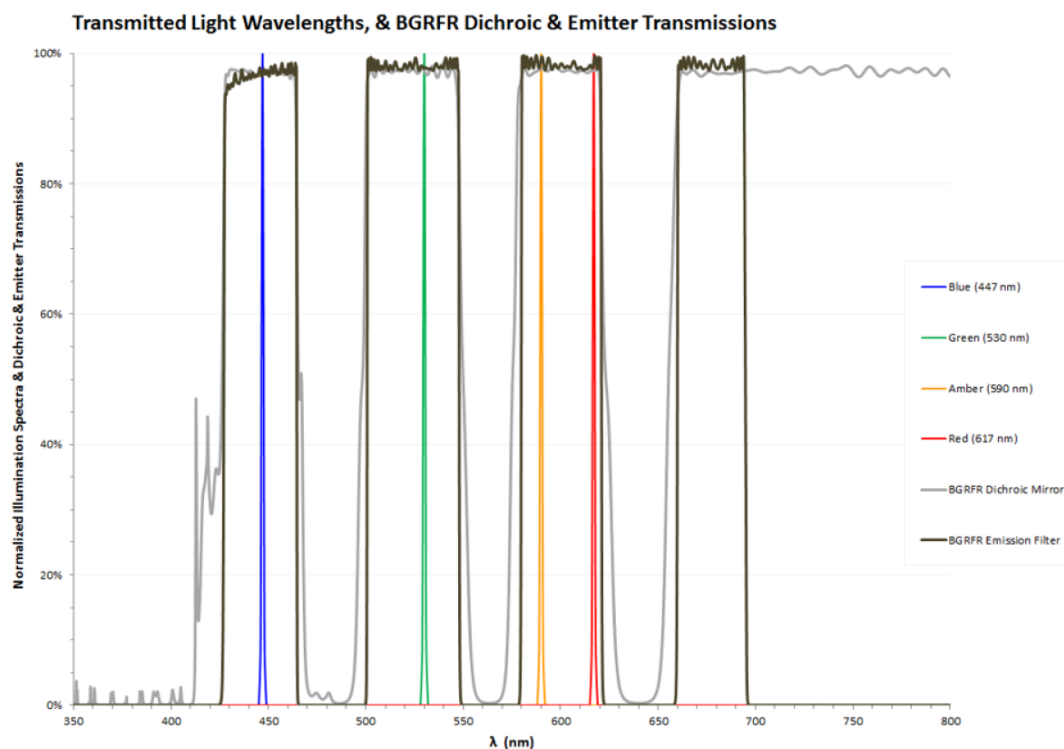


Figure 52 Quad-band BGRFR dichroic mirror and emission filter transmission spectra, overlaid with the normalized spectral power distribution from the transmitted light source's four illumination colors

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.



Replacement parts

All consumable parts can be purchased through customer support. To order replacement parts, contact your local sales representative or contact Technical Support directly.

CellInsight™ instrument

Part Name	Part Description	Part Number
Power cord	Power cord, United Kingdom, Ireland 230V	AMEP4645
Power cord	Power cord, Japan 15A	AMEP4783
Power cord	Power cord, Continental Europe, 10A	AMEP4646
Power cord	Power cord, China 10A	AMEP4782
Power cord	Power cord, North America 115V	AMEP4644
Stage Lock Kit	CellInsight™ stage locks needed when shipping instrument	CI00023



Safety



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, see the “Documentation and Support” section in this document.

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- 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. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of 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.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the 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.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf>
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311

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.

