IN Cell Analyzer 6500*HS*

IN Cell Analyzer 6500*HS* is a fully-automated confocal cell imaging system from GE Healthcare. Building on the capabilities of earlier IN Cell Analyzer systems, it provides a platform to deliver advances in speed, image quality, and throughput. When combined with intuitive workflows provided by new IN Carta analysis software, IN Cell Analyzer 6500*HS* enables users with all levels of experience to perform powerful analysis as part of a complete high-content analysis (HCA) solution from assay to answer.

IN Cell Analyzer 6500*HS* delivers:

- **Ease-of-use:** Collect data with ease and confidence through flexible protocol design in an intuitive user interface.
- **Versatility:** Configure your system with a range of optional modules to perform fixed or live-cell assays using multi-well plates, culture dishes, glass slides, or chambered cover glass.
- **Speed:** Achieve fast acquisition times with four color laser illumination, advanced scientific CMOS camera, and precise motorized stage.
- **Sensitivity:** Image even the most delicate live-cell assays without sacrificing physiological relevance or image quality.
- **High throughput:** Design assays that utilize the large field-of-view sCMOS camera and numerous automation options to image hundreds of plates per day.

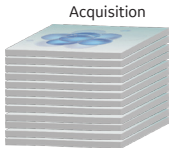
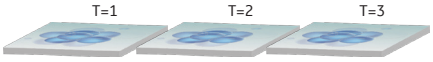
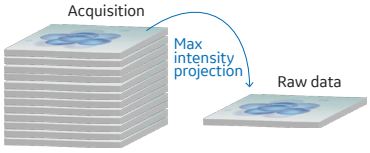
Fig 1. IN Cell Analyzer 6500*HS* system is an automated confocal imaging system for HCA.

- **High resolution:** Resolve detail in cellular and subcellular structures down to 300 nm for high quality images.
- **Quantitative data:** Adjust confocal settings to optimize imaging for any assay, magnification or wavelength and preserve signal across images with flat illumination.

Imaging modes

Multiple imaging modes give you the flexibility to image samples using a broad range of techniques. IN Cell Analyzer 6500*HS* utilizes laser illumination for fluorescence imaging in either IRIS confocal, widefield (open aperture confocal mode), or EDGE confocal mode and is equipped with transmitted light imaging. Images can be acquired in a single plane (2D), in multiple stacked planes (3D), or as a maximum intensity projection. Time series acquisition in 2D and 3D is also enabled.

Table 1. Imaging modes

Modes	Benefit	Schematic
2D	Collects a single 2D image for fastest scan times or when using an objective with large depth-of-field.	
3D	Collects a series of 2D images through the z-axis, enabling visualization of sample volume.	
Time series	Images are captured at user-defined time intervals to capture and analyze kinetic events.	
Maximum intensity projection	Projects all objects of interest from a 3D volume into a single 2D image to reduce data volume and analysis time.	

IRIS and EDGE confocal

Realizing results on the most challenging samples, from wash-free assays with high background signal to 3D spheroids with densely packed structures, requires maximal image resolution and contrast to ensure robust segmentation. IRIS, an adjustable width line-scanning confocal technology, enables optimized imaging of any magnification level, wavelength, assay or experiment. For the greatest improvement in image contrast and resolution in all three dimensions*, EDGE confocal quantitatively removes out-of-focus light to improve visualization and segmentation of structures in thick samples (Fig 2).

*Compared with standard confocal imaging.

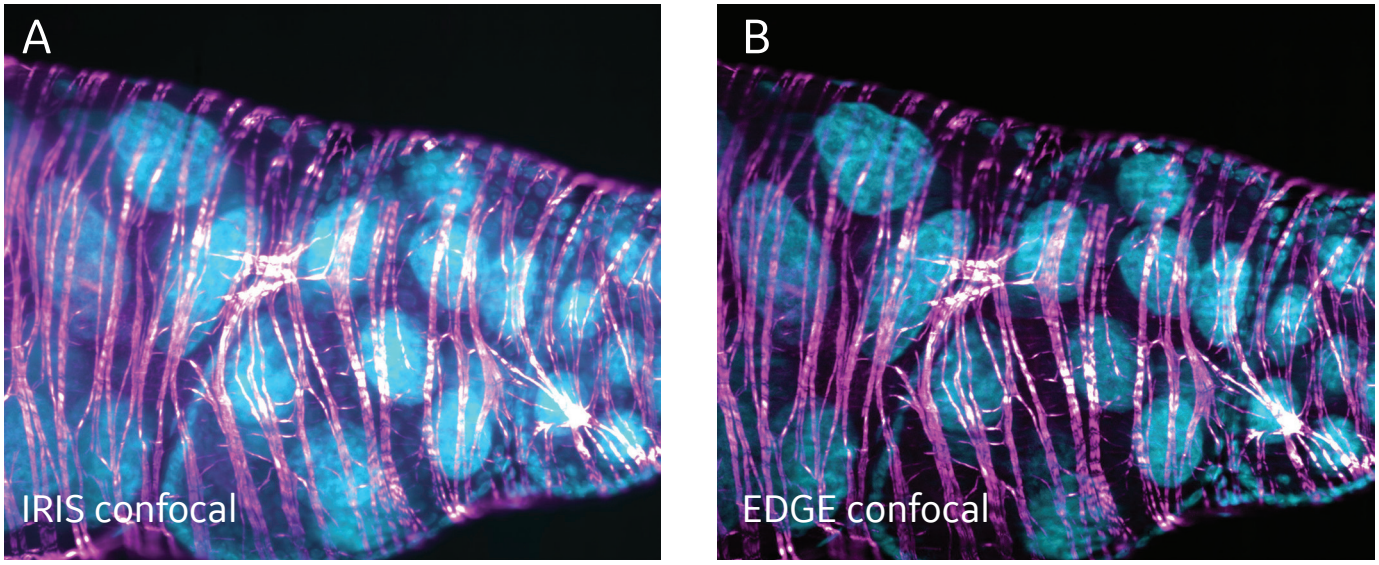


Fig 2. A single z-slice of a *Drosophila* oocyte acquired with (A) IRIS confocal illumination and with (B) EDGE confocal enhancement applied. The improvement in image contrast and resolution in the z-dimension (axially) is clearly visible in image B with EDGE enhancement.

3D Imaging

3D models, such as spheroids, organoids, and 3D cell cultures are becoming essential due to the biologically relevant data and context they provide. IN Cell Analyzer 6500*HS* offers several tools to make volumetric data acquisition easy. Set up z-stack parameters graphically using ***3D Frame*** to ensure all relevant volumetric data is captured or utilize the ***Smart Scan*** toolkit including ***Spheriod Finder***, an automated target finding workflow, to optimize data collection and minimize data volume. When 3D information is required but localization is not, use the ***Maximum Intensity Projection*** imaging modes to collect 3D information in a single 2D image.

High quality optics

A highly optimized optical system reduces exposure times and maximizes speed while delivering publication quality images from a wide range of sample types.

Flexible objective selection

IN Cell Analyzer 6500*HS* is supplied with a 10× objective that can be used for a wide range of high-content assays. In addition, alternative objectives are available to suit your imaging needs (Table 3). The instrument has a four-position motorized turret that accommodates up to two objectives with automated spherical aberration collars (ASAC) to maximize signal brightness and contrast.

Table 2. Specifications of available objectives

Magnification	NA	Optical corrections	WD (mm)	ASAC
2×	0.1	CFI Plan Apo Lambda	8.5	No
4×	0.2	CFI Plan Apo Lambda	20	No
10×*	0.45	CFI Plan Apo Lambda	4.0	No
20×	0.45	CFI S Plan Fluor ELWD	8.1–7.0	Yes
20×†	0.75	CFI Plan Apo Lambda	1.0	No
40×	0.6	CFI S Plan Fluor ELWD	3.7–2.7	Yes
40×†	0.95	CFI Plan Apo Lambda	0.20–0.11	Yes
60×	0.7	CFI S Plan Fluor ELWD	2.1–1.5	Yes
60×†	0.95	CFI Plan Apo Lambda	0.2–0.11	Yes
100×†	0.85	CR L EPI Plan CRA	1.2–0.85	No

*Supplied as standard with IN Cell Analyzer 6500*HS*
†High NA objectives for high resolution imaging

Key
NA: Numerical aperture
WD: Working distance
ASAC: Automated spherical aberration adjustment collar
CFI: Chromatic aberration free infinity
Plan: Flat field correction
Apo: Apochromat, indicating color correction
S Plan: Super Plan
Fluor: Fluorite
ELWD: Extra-long working distance
EPI: Epifluorescence
CR: Correction Ring
L: Long

Automatic spherical aberration collar (ASAC)

Custom-built motors drive the spherical aberration collars on ASAC objectives to compensate for differences in plate bottom thickness. Adjustment of the collar setting is performed automatically based on predefined plate parameters and requires no user intervention. Optimization of the correction collar is critical to obtaining the sharpest image contrast (Fig 3).

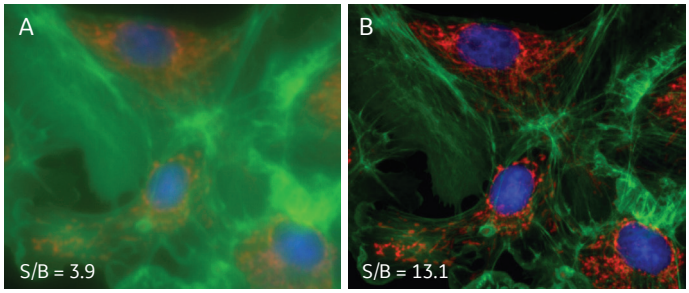


Fig 3. The same field-of-view is captured with (A) a non-optimized, and (B) optimized spherical aberration collar setting for comparison. The improvement in image contrast is easily seen with the correct collar adjustment. **S/B:** signal/background ratio.

High numerical aperture objectives

Accurate segmentation of subcellular structures requires optimization of both contrast and resolution. Resolution of a microscopy image is dependent on the numerical aperture (NA) of the objective. For applications requiring maximal resolution, IN Cell Analyzer 6500*HS* offers several high NA objectives at various magnification levels (Fig 4).

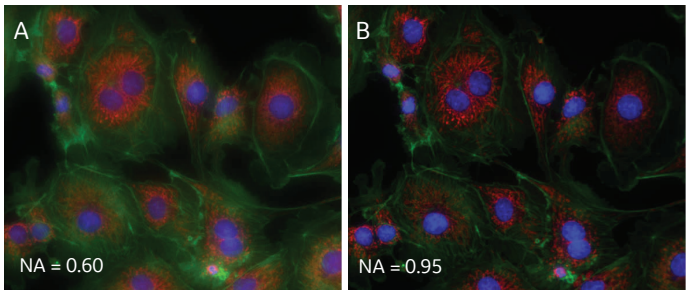


Fig 4. The same field-of-view is captured with (A) a 40x objective with low NA (0.60) and (B) a 40x objective with high NA (0.95). The improvement in resolution and image contrast (brightness) is easily seen with the increased NA.

Fluorescence imaging

A four-color laser engine for fluorescence imaging provides extremely bright, stable, and long-lasting illumination enabling fast multicolor assay acquisition. Specialized polychroic mirrors and emission filters designed for optimal light throughput enable imaging of dyes and fluors across the visible spectrum (UV to far-red).

Transmitted light imaging

An integrated white light emitting diode (LED) can be used for brightfield imaging. An optional Phase Contrast and Differential Interference Contrast (DIC) Module includes computational algorithms to generate phase contrast and DIC reference images without additional optics.

Focus maintenance

Hardware and software autofocus features are selectable through the user interface. The laser based hardware autofocus utilizes a 780-nm laser to determine the interface between air and the bottom of the plate as well as the interface between the plate and sample medium. Software autofocus captures multiple images in different z-planes and determines the focus position with maximal contrast. Hardware and software autofocus can be used independently, in combination, or turned off.

Throughput and productivity

IN Cell Analyzer 6500*HS* is designed to maximize productivity beyond what is possible with standard manual workflows. It achieves this by maximizing throughput and speed plus the application of robotic automation.

High throughput

Leverage IN Cell Analyzer 6500*HS* acquisition tools to maximize throughput while minimizing data volume.

- **Smart scan:** Automatically identify x/y locations with relevant biology and perform follow-up scans at higher magnification or in 3D.
- **Count cells:** Ensure adequate population statistics during acquisition without oversampling.
- **Maximum intensity projection:** Use this mode to capture 3D information in a 2D image.

Speed

The IN Cell Analyzer 6500*HS* stage balances speed with accuracy and repeatability to enable precise imaging without sacrificing throughput. Combined with an sCMOS camera and quad band emission filter it delivers ultra-fast frame rates for fast multichannel time-series imaging. To capture dynamic responses, utilize ***Burst Mode*** for ultra-fast kinetic imaging within a well or field-of-view.

Table 3. Plate scan times

			Imaging time (s)*	
Plate format	**No. of channels**	**Exposure (ms)**	**Single bandpass EM**	**Quad bandpass EM**
96 well	1	35	73.9	N/A
96 well	2	35	92.3	88.5
96 well	3	35	109.8	96.8
96 well	4	35	127.1	104.2
1536 well	1	35	813.4	N/A

*Imaging times based on utilizing hardware autofocus, 10× objective, thin-bottom plate, full frame images, IN Cell Analyzer acquisition software v7.0

Table 4. Frame rate specifications

	Frame rate (fps)*	
Mode	**1 channel**	**2 channels**
Full frame ***Burst Mode***	19.3	5.5
256 × 256 ***Burst Mode***	99.1	6.9

*Frames per second (fps) based on 0.035s exposure, 10× objective, IN Cell Analyzer acquisition software v7.0, and no autofocus.

Robotic automation

Speed up screening processes by automating plate loading and acquisition. IN Cell Analyzer 6500*HS* can be integrated with many commercially available automation/ robotic systems.

Table 5. Preferred automation vendor and models

Preferred vendor	PAA
Preferred model	KiNEDx™

Liquid Handling Module

For automated addition of compounds or reagents the optional Liquid Handling Module is equipped with a single syringe and needle to transfer liquid to the sample plate. The module supports aspiration and dispensing of volumes between 10 and 100 µL from 96- or 384-well microplates. To prevent cross-contamination, the aspiration function is separate from the dispensing function.

Intuitive acquisition software

Image acquisition is controlled with IN Cell Analyzer Acquisition Software v7.0 or higher. In addition to the previously mentioned acquisition tool options, this software package enables the following functionality:

Item	Details
Find sample	• Preloaded and customizable plate maps • ***Focus Finder*** tool to easily locate focal plane • ***Preview Scan*** to quickly identify objects of interest
Setup protocol	• Auto detection of channel offsets • Flexible field placement options including the ability to create point lists • Support for multiple frame sizes, frame averaging, and binning • **3D Frame** tools to graphically setup 3D z-scan parameters
Acquire quantifiable data	• EDGE confocal imaging enables better image quality in thick samples • Flat illumination eliminates the need for separate flat field correction
Review and export data	• Extensive data review tools including image exporting with annotations, autofocus diagnostics, synchronized multiple well comparison, movie generation • Advanced thumbnail viewing with adaptive resolution and montage capabilities • ***Report Generator*** allowing easy import into CellProfiler • Stack merger feature allows the user to fuse data points after acquisition which enables other users to work on the system in between time points

Live Cell Imaging Modules

Proper environmental control is essential for biologically relevant live-cell imaging studies. For applications that require a regulated environment, two Live Cell Imaging Modules are available. Both the CO_2 Module and CO_2/O_2 Module do not require premixed CO_2/O_2 as an input and control temperature and humidity at the sample. A heated lid is fitted to the microplate and distributes gas mixtures evenly across the wells with minimal evaporation to ensure long-term cell viability.

Table 6. Live Cell Module specifications

Parameter	Specification
Supported temperature range	Temperature control from ambient to 42°C
CO_2 input requirement	100%
N_2 input requirement*	100%
Background gas (air) input requirement	Clean, dry air
Supported CO_2 range	0% to 20%
Supported O_2 range	0% to 20%
Humidity percentage range	> 70% relative humidity

*N_2 only used for CO_2/O_2 Live Cell Module to control O_2 levels.

Image analysis

Next generation IN Carta software enables high-content analysis of images acquired on IN Cell Analyzer systems. Powerful analytics combined with an intuitive interface simplifies workflows for a user-friendly experience.

Fig 5. IN Carta software screen shots showing a heat map and a scatter plot in Interactive mode.

System specifications

IN Cell Analyzer 6500*HS* is fully enclosed and can be installed on a bench in a standard laboratory setting. The enclosure provides isolation from vibration and air flow fluctuations to create a stable imaging environment.

Table 7. System specifications

Parameter	Specification
Illumination modalities	IRIS confocal fluorescence
	EDGE enhanced confocal fluorescence
	Widefield fluorescence*
	Transmitted light
Excitation Lasers (nm)	405, 488, 561, 642
CDRH Class	Class IIIb
Standard supported dyes/ fluorophores	Blue (DAPI, Hoescht, CF™405M)
	Green (GFP, Cy™2, AlexaFluor™ 488, ATTO-488, CellTracker™ Green, Calcein AM)
	Red (mCherry, mKate2, AlexaFluor 568, CellTracker™ Red)
	Far Red (Cy5, AlexaFluor 647, To-Pro™-3, SiR)
Standard objective lens	10× 0.45 NA CFI Plan Apo Lambda
Camera	sCMOS detector
	2040 × 2040 imaging array
	6.5 x 6.5 µm pixels
	16 bit dynamic range
	272.3 MHz readout speed
	0.9 e- (rms) readout noise
Standard supported sample types	SBS footprint Multi-well plates (6, 24, 96, 384, 1536 well)
Optionally supported sample types	Microscope slides (75 × 25mm)
	35mm dishes
	2, 4 or 8-chambered coverglass (24 × 60mm)
	2, 4, or 8-chamberd microscope slides (75 × 25mm)
Dimensions (W × H × D)	112.8 × 66 × 63.5 cm (44.4 × 26 × 25 in)
Weight	107.5 kg (237 lbs)
Temperature	Ambient temperature 15°C to 33°C (59°F to 91.5°F)
Humidity	Relative humidity 10% to 80%, noncondensing
Workstation	Windows® 10 Pro 64-bit OS
	16 GB RAM
	3 TB 7200 RMP SATA hard drive
Operating conditions	Ambient temperature 15°C to 33°C (59°F to 91.5°F)
Power requirements	100–127/200–240 VAC, 5/2.5 A 50–60 Hz
Power consumption (max)	500 W
Heat output	500 W
Overvoltage category	Category II
Manufacturing site	GE Healthcare, Issaquah, WA, USA

*Open aperture confocal fluorescence

Key
NA: Numerical aperture
CFI: Chromatic aberration free infinity
Plan: Flat field correction
Apo: Apochromat, indicating color correction

Warranty information

IN Cell Analyzer 6500*HS* includes a one-year warranty that includes all base system components and optional accessories, subject to terms and conditions.

Ordering Information

Description	Product code
IN Cell Analyzer 6500*HS* System	29240358
IN Carta Software	29220275
IN Carta Workstation	29239111
4× 0.2 NA CFI Plan Apo Lambda	28953477
20× 0.45 NA CFI S Plan Fluor ELWD (ASAC)	29051872
20× 0.75 NA CFI Plan Apo Lambda	28953478
40× 0.6 NA CFI S Plan Fluor ELWD (ASAC)	29051871
40× 0.95 NA CFI Plan Apo Lambda (ASAC)	29051876
60× 0.7 NA CFI S Plan Fluor ELWD (ASAC)	29051873
60× 0.95 NA CFI Plan Apo Lambda (ASAC)	29051877
100× 0.85 NA CR L EPI Plan CRA	29136081
Slide Handling Module	28954475
IN Cell Analyzer Sample Adapter Kit*	29254209
Live Cell Imaging Module – Temperature, Humidity, and CO_2 regulation	29251177
Live Cell Imaging Module – Temperature, Humidity, CO_2, and O_2 regulation	29251074
Liquid Handling Module	29248665
Phase Contrast and DIC Module	28953487
KiNEDx robot	28922202

*IN Cell Analyzer Sample Adapter Kit contains a Slide Adapter, µSlide Adapter, Chambered Coverglass Sample Holder, and 35 mm Dish Sample Holder.

GE Healthcare Bio-Sciences Corp.
100 Results Way
Marlborough MA 01752,
USA

www.gelifesciences.com

GE Healthcare Bio-Sciences AB, Björkgatan 30, SE-751 84 Uppsala, Sweden
GE Healthcare UK Ltd, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
GE Healthcare Bio-Sciences Corp. 100 Results Way, Marlborough, MA 01752, USA
GE Healthcare Dharmacon, Inc., 2650 Crescent Dr., Lafayette, CO 80026, USA
HyClone Laboratories, Inc., 925 W 1800 S, Logan, UT 84321, USA
GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Japan Corporation, Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan
For local office contact information, visit: www.gelifesciences.com/contact

29255121AA 04/2017