

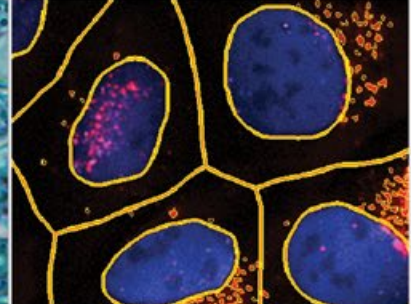
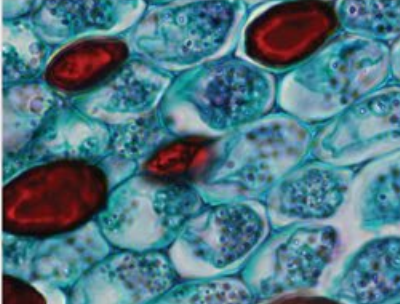
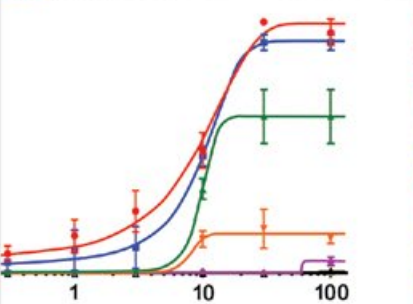
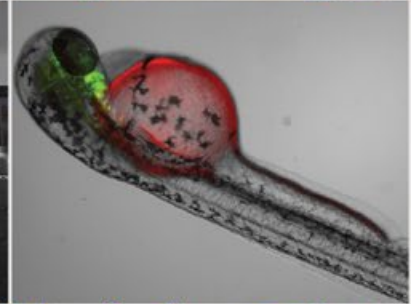
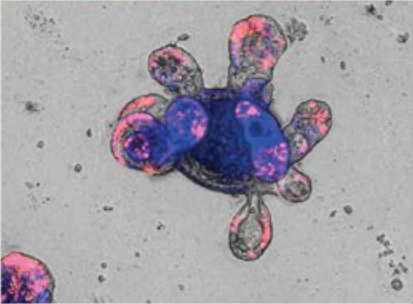
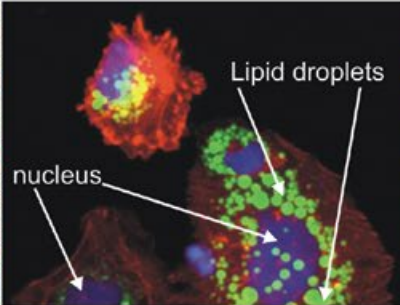
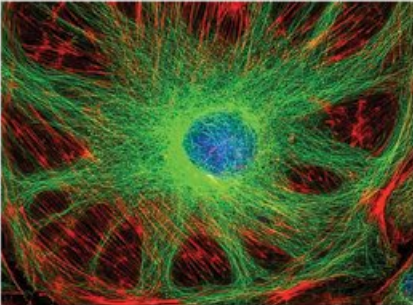
Imaging & Microscopy



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Twitter: @mscienceaustrz



A part of **Agilent**



BioTek

Imaging & Microscopy

Designed for a wide range of applications and budgets.

Lionheart Automated Microscopes offer powerful microscopy, and can easily be equipped with the environmental controls that are crucial for successful short- and long-term kinetic live cell imaging.

Cytation C10 Confocal Imaging Reader combines confocal, widefield and multimode detection in a single

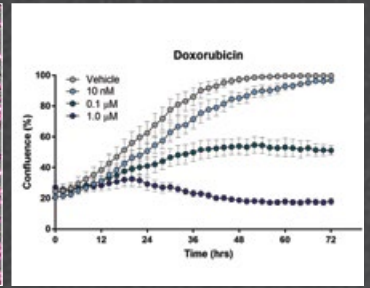
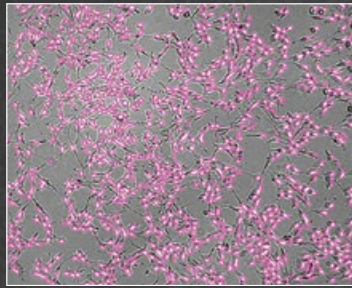
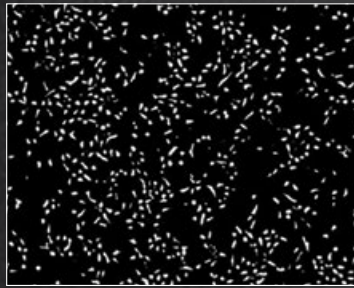
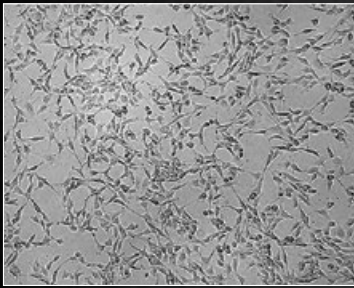
benchtop-sized platform, attainable for every laboratory. The Cytation Cell Imaging Multimode Readers offer an array of imaging and multimode capabilities, including digital widefield inverted microscopy, upright microscopy and environmental controls for live cell workflows.

Gen5 Microplate Reader and Imager Software includes functionality for easy image capture and analysis, for both qualitative and quantitative data.



Augmented Microscopy

BioTek's instrumentation and software together create the unique Augmented Microscopy experience; the integration and automation of all steps from image capture to publication-ready data. There's no need for other software – Gen5 does it all.

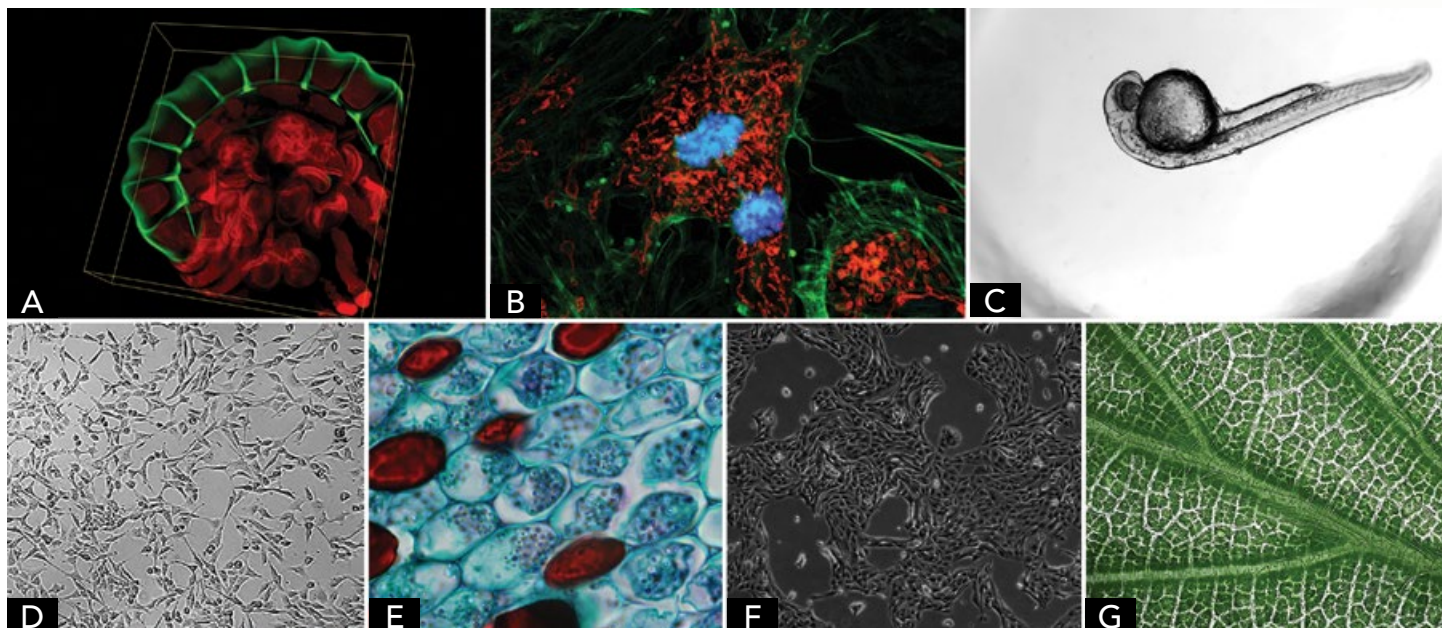


capture ► process ► analyze ► publish

Capture

The critical first step in a typical microscopy workflow is the efficient and precise acquisition of publication quality, high information images. Augmented Microscopy automates image capture for samples with powerful tools for endpoint and time lapse workflows.

Seven imaging modes



Powerful imaging for a wide range of applications, including live and fixed cell biologies: **A.** Confocal, **B.** Fluorescence, **C.** Brightfield, **D.** High contrast brightfield, **E.** Color brightfield, **F.** Phase contrast, **G.** Upright reflected and transmitted light.

Laser and image autofocus

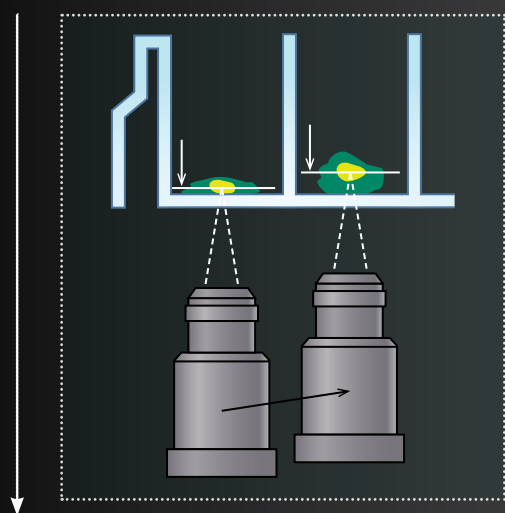
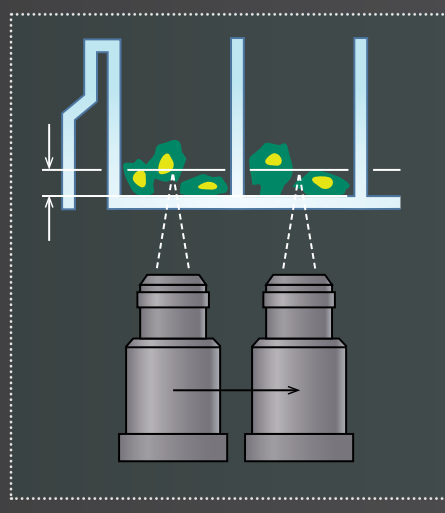


Image-based autofocus is available on all BioTek imaging systems. It focuses on the plane of highest contrast in the sample, including "shifting" biology within the well.

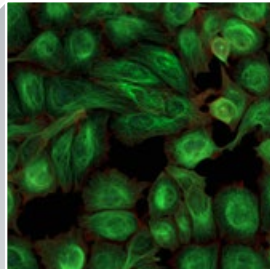
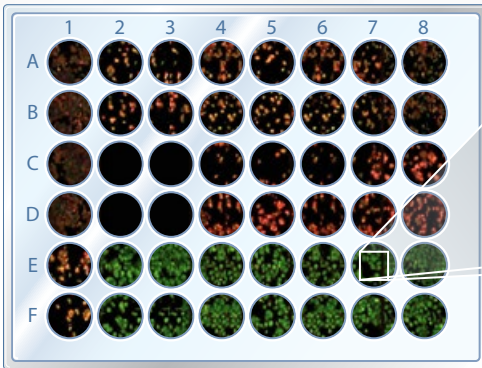


BioTek's patented laser autofocus uses the same focal offset from well to well and is typically faster. It works with dim fluorophores and helps prevent phototoxicity and photobleaching. Laser autofocus also offers better reproducibility and higher accuracy during long term kinetics.

Capture

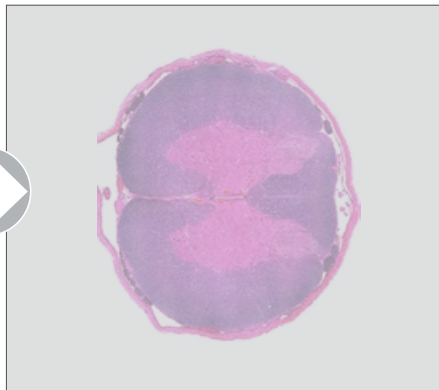
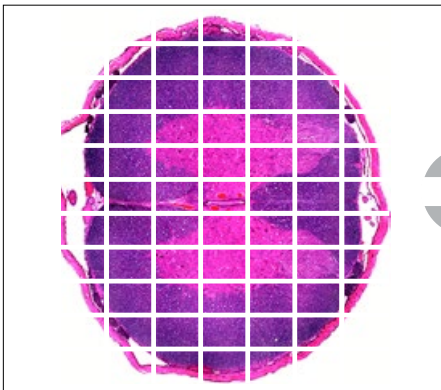


Batch mode



Capture multiple images in microplates, chamber slides and other multi-sample vessels automatically. BioTek's imagers can be used in manual mode to look at a few samples, or in full automation mode to capture endpoint images or extended kinetics over hours, days or weeks.

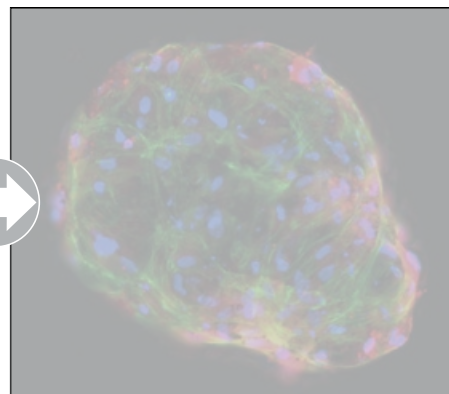
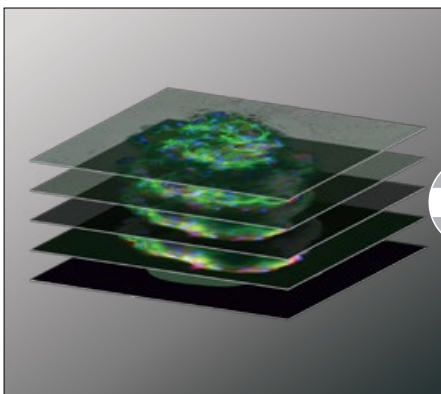
Montage



Capture large samples, like tissue sections (H&E), increase sample size for better data quality or to detect rare events. Montage image capture mode acquires up to 2000 tiled images per sample.

Each tile of a montage is saved as an individual, high resolution 16-bit TIFF. Stitching in Gen5 creates a seamless image.

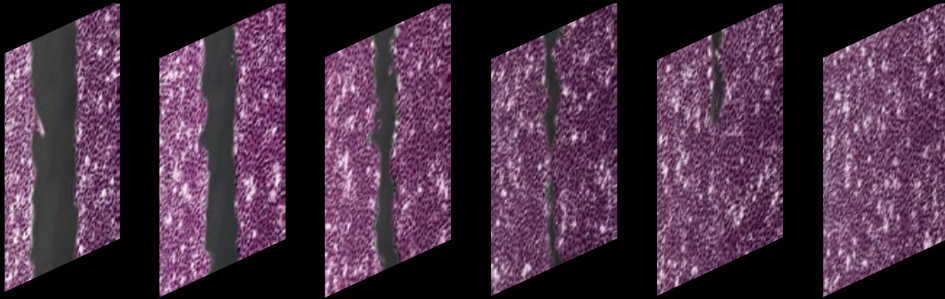
Z-stack



Gen5's z-stacking enables capture of up to 50 customizable slices – as thin as $0.1 \mu\text{m}$ – in a stack. The images can then be automatically z-projected. Z-stack capability is a critical requirement for imaging 3D samples, such as spheroids and tumoroids, along with samples that extend over multiple focal planes.

Capture

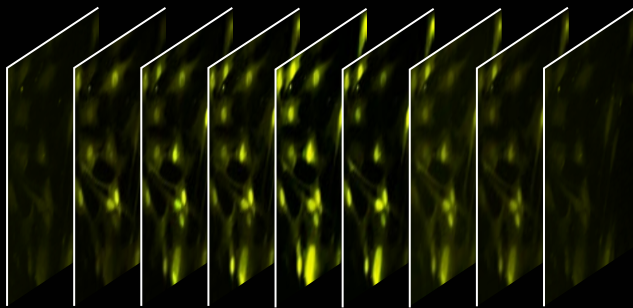
Time lapse imaging



TIME: days to weeks

Live cell kinetic assays such as wound healing and cell proliferation are imaged automatically over time, stored and ready to be published as a movie. Experiments can be run over days or weeks, and kinetic data is automatically plotted.

High speed imaging



TIME: seconds to hours

Very fast reactions like calcium flux kinetics are enabled with dual reagent injectors – images are automatically captured at up to 20 frames per second.

Beacons



Beacons are used to define custom x/y offsets for imaging in a well or vessel. Beacons are useful for monitoring specific regions of interests, as shown in this zebrafish example.

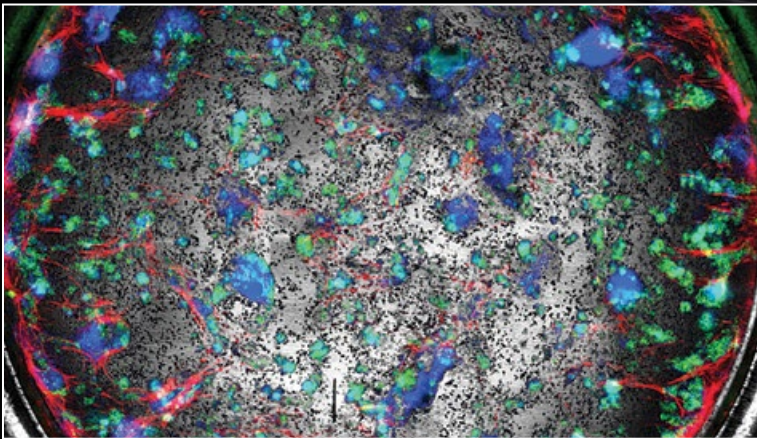
Capture

Live cell assay support



Temperature control, including the Condensation Control gradient, plus CO₂/O₂ control and humidity options provide the ideal environment for live cell assays. Observe label-free assays with brightfield and high contrast brightfield imaging, or fluorescence assays in up to 4 colors plus brightfield. Image time lapse sequences are easily, automatically compiled to video.

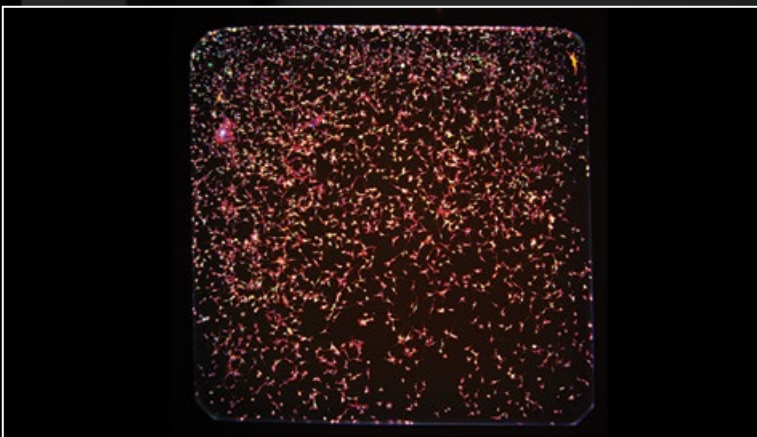
Up to 4 channel overlay



Four fluorescence channels plus brightfield provide maximum versatility.

Choose from nearly 20 available LED/filter cube colors to cover a very broad range of fluorescent stains. Gen5's auto LED intensity ensures consistent, high quality capture for end point and kinetic sequences. Each channel can be automatically adjusted and optimized – changes are easily saved.

Whole well imaging

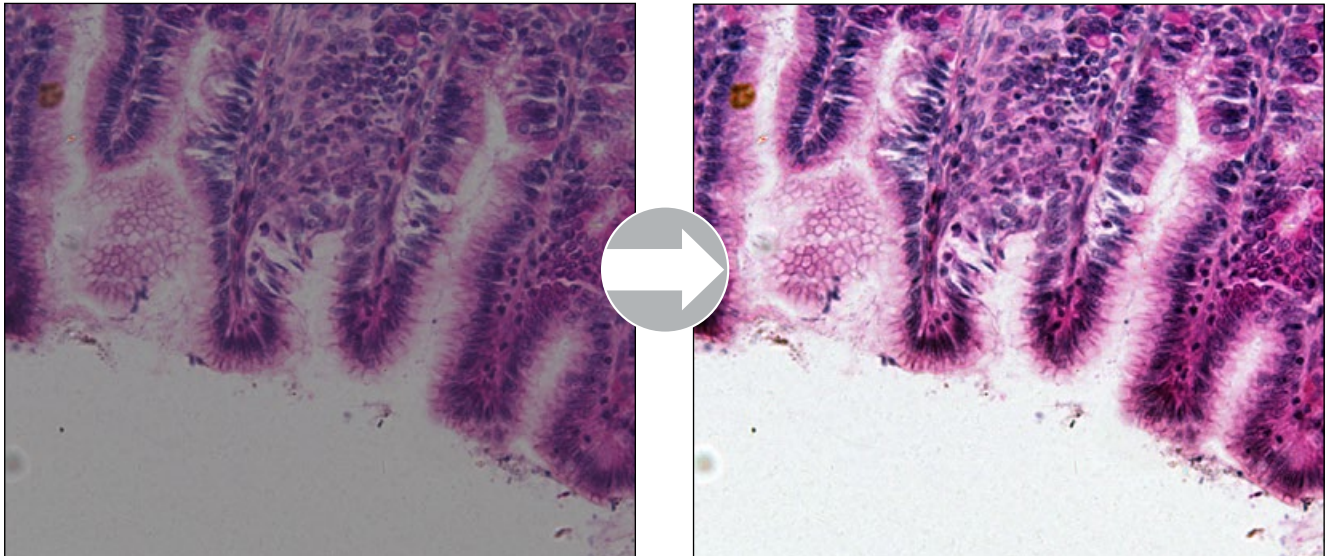


The wide field of view (wide FOV) camera available for Cytation 5 enables capture of an entire well of a 384-well plate. High resolution cellular screens can be captured much more rapidly, since multiple images aren't required. The wide FOV camera captures more cells in the field of view at higher magnification, providing a more statistically relevant population of cells in fewer images.

Process

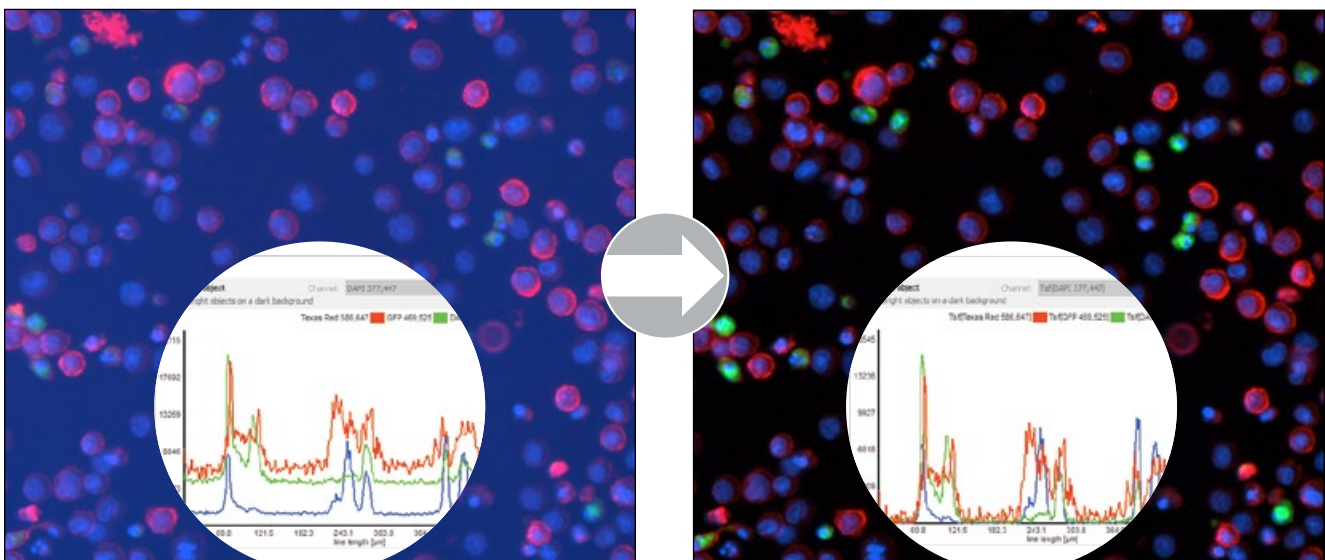
Image processing is essential for optimizing images prior to analysis. Gen5's tools provide exceptional processing capability to facilitate the analysis of complex biologies.

Powerful review tools



Quickly adjust brightness and contrast for better visualization. Gen5's measurement and annotation tools allow you to add information or highlight specific areas and objects of interest in the image.

Background flattening

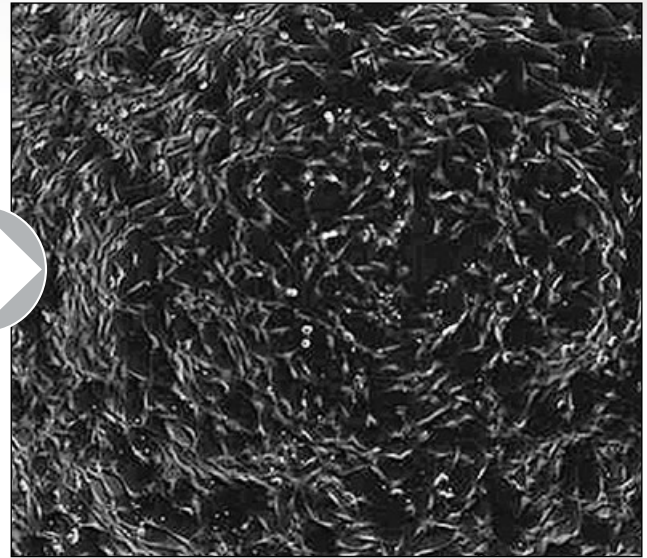
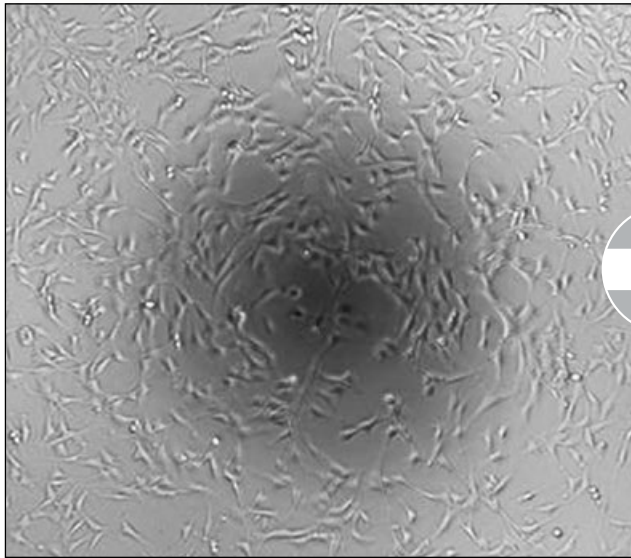


Background flattening using a rolling ball algorithm prepares the image for analysis by removing background artifacts and correcting for uneven illumination. Use the line profile tool to find recommended threshold values for image analysis.

Process

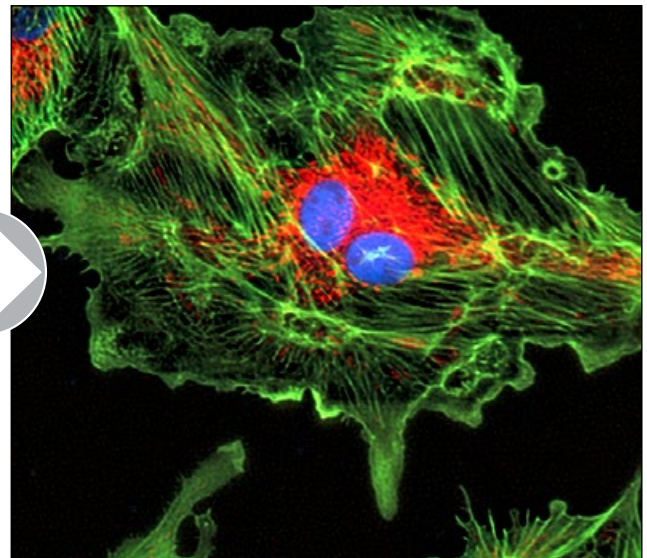
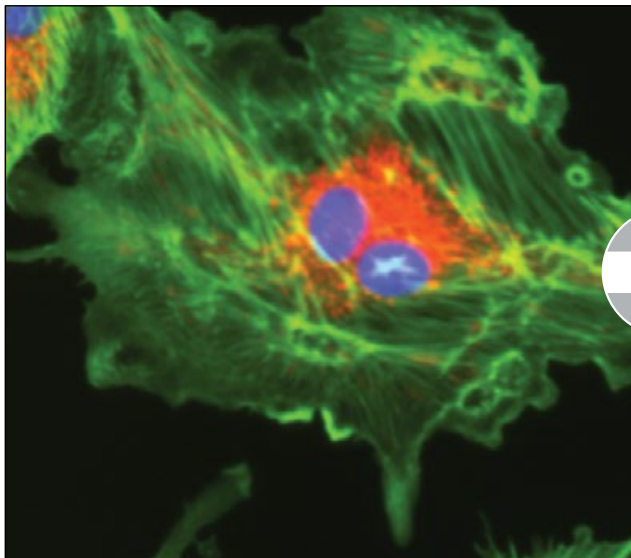


Digital phase contrast



Digital phase contrast improves brightfield contrast to correct for meniscus effect and other artifacts. The process enables clear visualization and easier analysis.

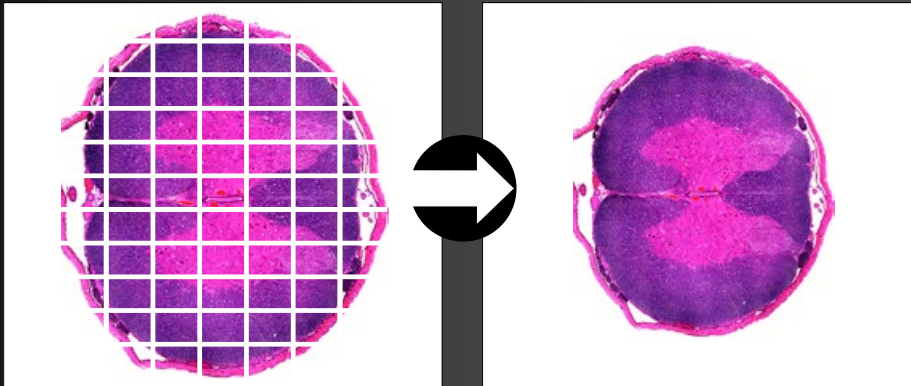
Deconvolution



Deconvolution reduces blur from out-of-focus light, commonly seen in widefield imaging. It improves image resolution, enabling better visualization of image details.

Process

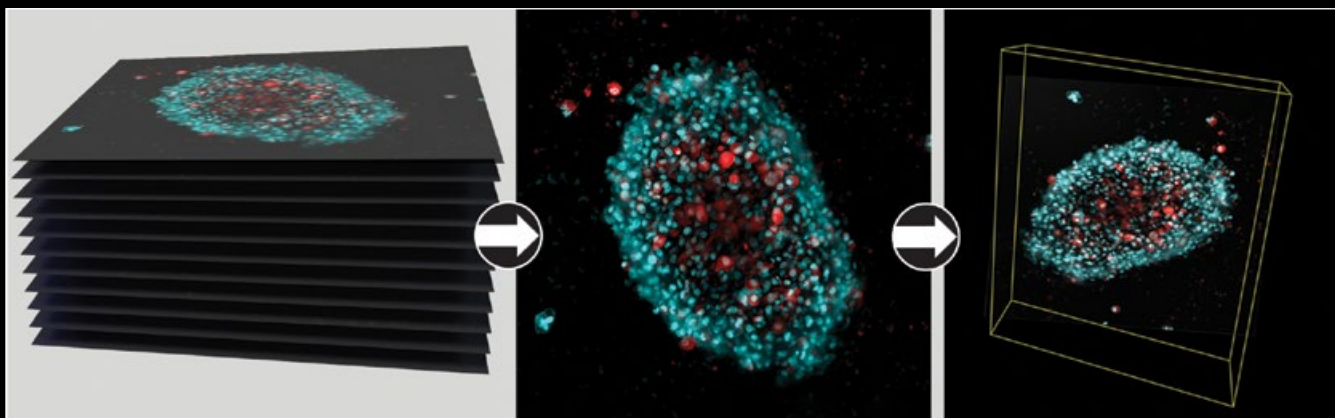
Stitching



After capturing a large field of view with an image montage, Gen5 automatically and precisely stitches the montage into a single uniform, high resolution image.

Gen5 can correct common artifacts seen with some montages, such as tiling effects. The stitching process automatically adjusts and corrects for a seamless image.

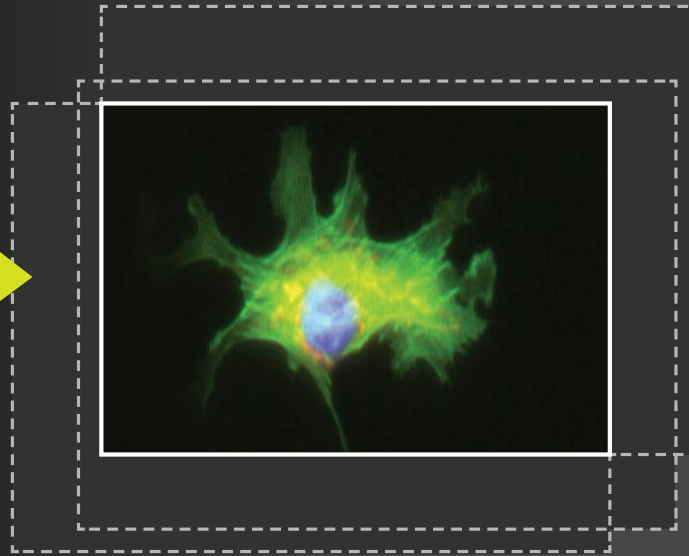
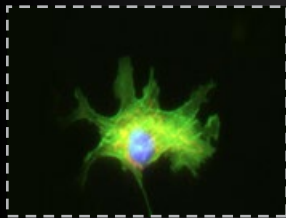
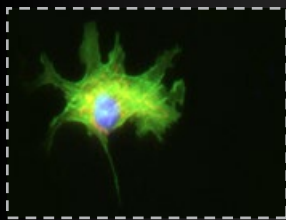
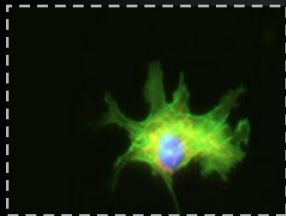
Z-projection with 3D rendering



A z-stack is captured, then Gen5 processes the stack of images into the spheroid. The 3D viewer is used to explore the sample in greater detail.

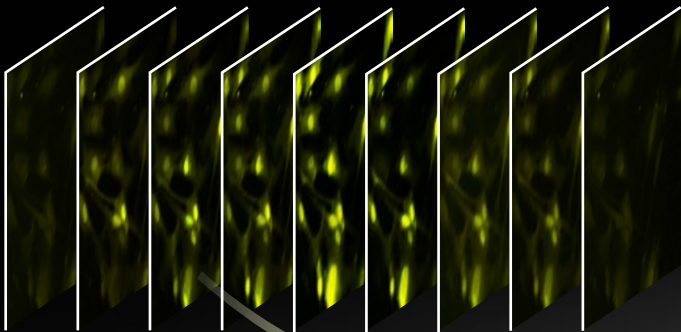
Process

Kinetic image alignment



During live cell kinetic imaging, samples can sometimes shift slightly. Gen5 automatically adjusts for positional differences, keeping the region of interest fully stabilized, even during long term imaging.

Movie file creation

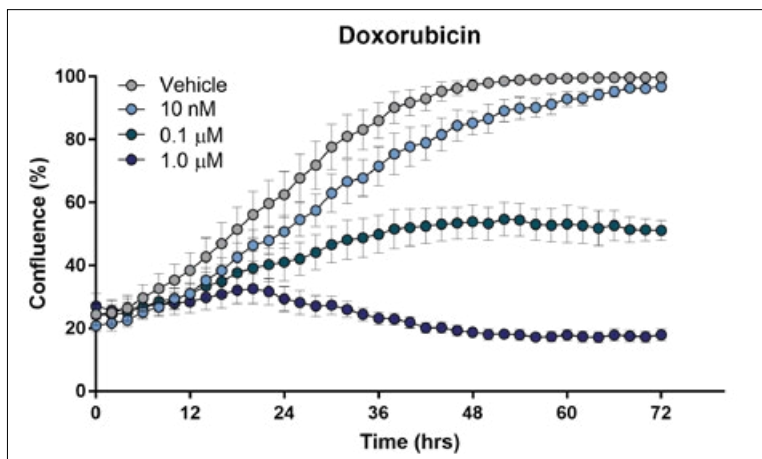
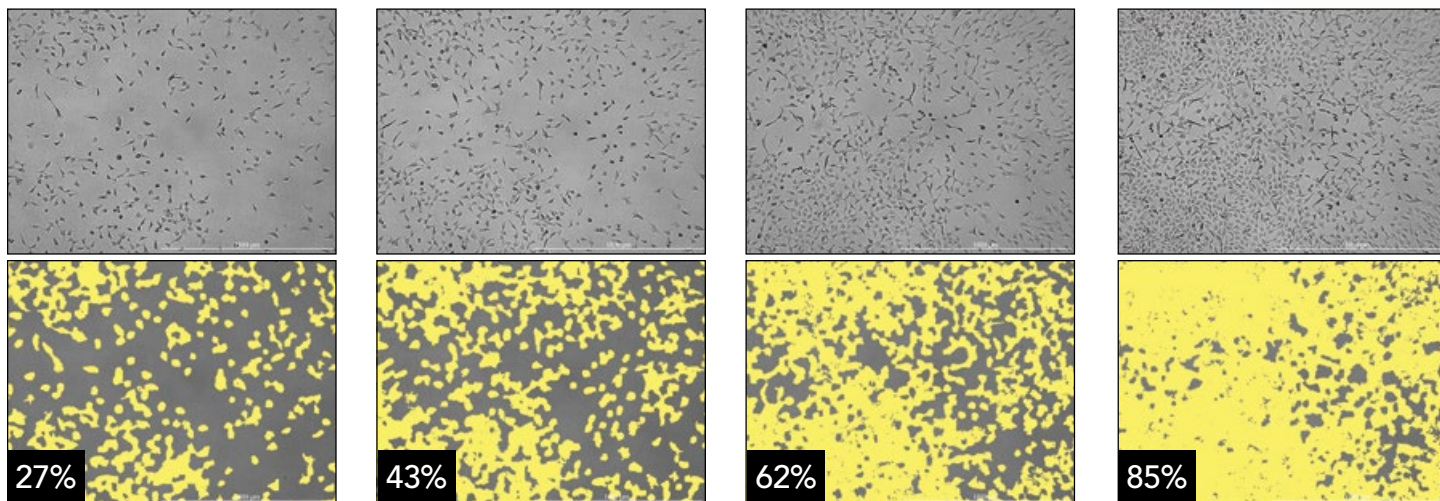


Gen5 quickly compiles time lapse images into time-stamped .wmv or .mp4 files.

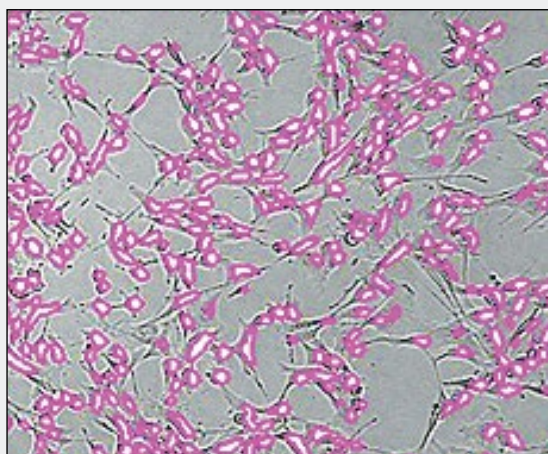
Analyze

Captured, processed images are ready for analysis. Image analysis tools in Gen5 cover a very broad range of application requirements, and are both powerful and easy to use. Analysis functions in Gen5 extend to quantitative data as well.

Confluence



Confluence measurements quickly and accurately identify and mask cells. In cell growth, health and proliferation studies, % confluence is an important measurement.

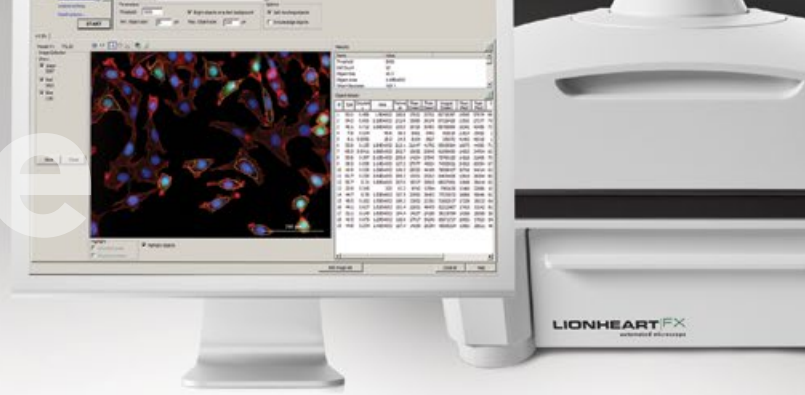


Label-free cell count

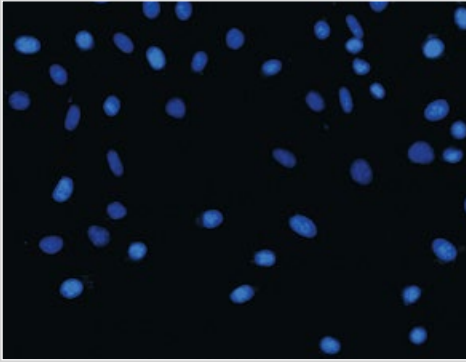
Fluorescent stains can sometimes interfere with cellular functions, so label-free methods are increasingly being used for cell counting.

Along with label-free confluence measurements, label-free cell counts are performed efficiently using high contrast brightfield. Cell counts are essential to cell growth, health and proliferation studies. Gen5 efficiently identifies highly confluent cells without dyes.

Analyze



Nuclei count and analysis



Original image

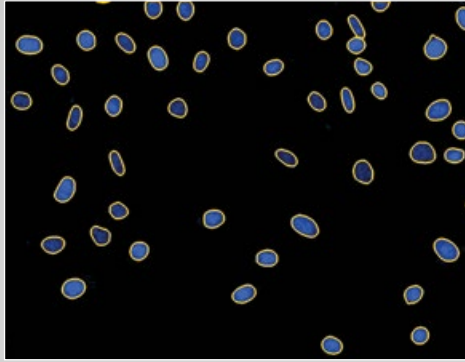
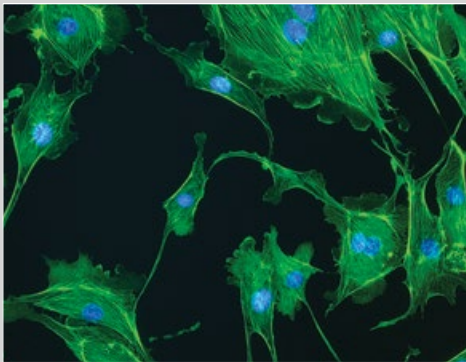


Image with mask applied

Gen5 automatically identifies cell nuclei for rapid cell counts. Applications include cell proliferation, cell cycle and toxicity analysis. This primary mask can also be used to count non-mammalian cells, spores and bacteria.

Cytoplasm analysis



Original image

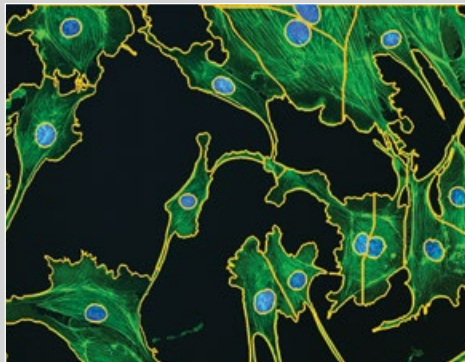
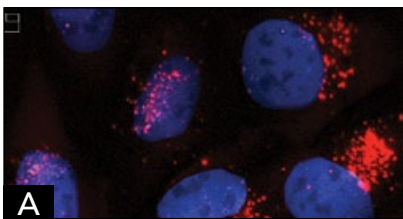


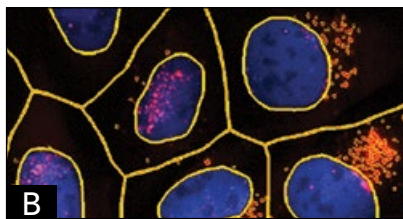
Image with mask applied

A secondary mask enables observation and characterization of cytoplasm size, shape, intensity, and other morphological changes, which are common to a broad range of applications.

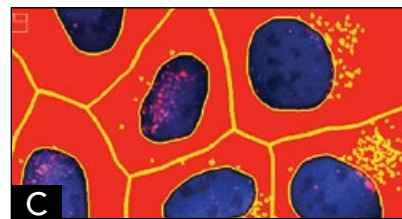
Organelle analysis



A



B



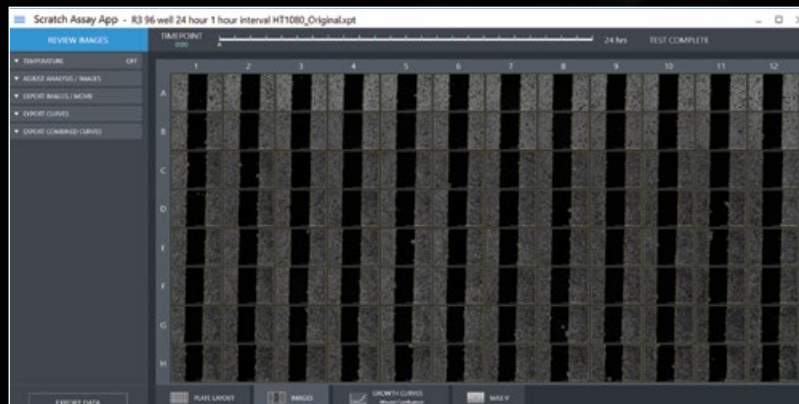
C

Gen5's add-on Spot Count module enables in-depth analysis of intracellular objects of interests ("spots"), within either a primary or secondary mask. Measurements include spot count, average spot size and area, total spot area per cell, mean, standard deviation and integral spot intensity. The module is a useful tool for applications including analysis of steatosis, autophagosomes, liposomes, micronuclei, viral infections and many assays with punctate biology.

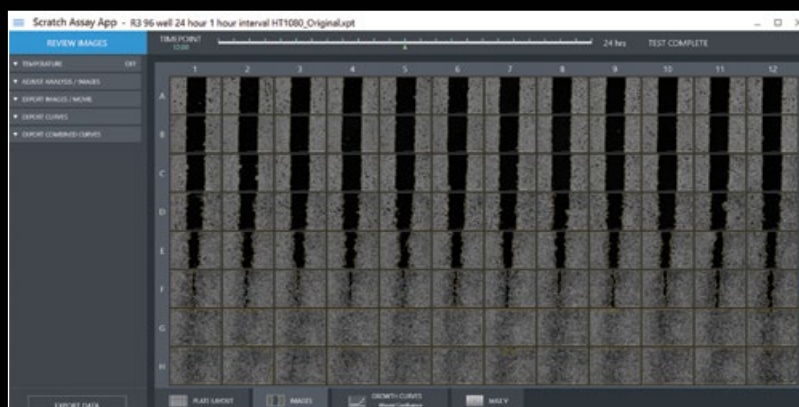
- A. Shows nuclei and objects for counting
- B. Spot counting of objects within primary and secondary masks
- C. Spot counting with secondary mask filled for better visualization

Analyze

Cell migration/scratch wound assays

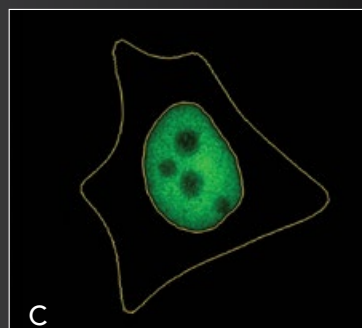
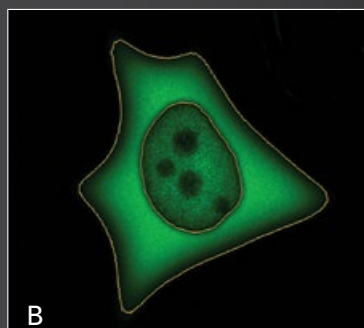
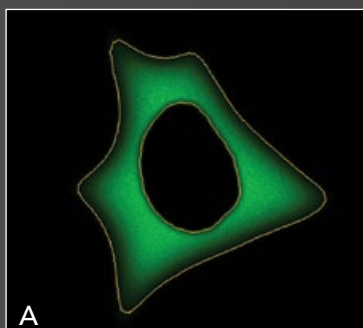


Cell migration and invasion assays such as scratch wound healing are kinetic processes easily handled in Gen5 software. Migration analyses and measurements include wound width, % confluence, maximum healing rate (max V) and spheroid size.



BioTek's Scratch Assay Starter Kit provides an "out-of-the-box" implementation of automated scratch wound assays. (See Automation Accessories)

Signal translocation

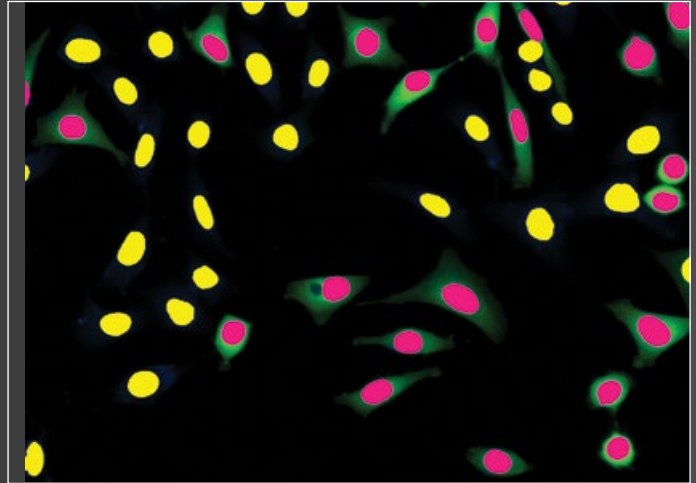


Monitoring molecular movement between cellular compartments, typically referred to as translocation, requires advanced cell analysis tools. This response is seen in many assays, including transcription factor activation and caspase cascade events (apoptosis), as shown. Using a nuclear mask and a cytoplasmic mask, Gen5 automatically quantitates translocation events.

- A.** Protein (caspase-3) in a resting state stays within the cytoplasm
- B.** Upon activation, caspase-3 begins translocating to the nucleus
- C.** Caspase-3 has completely translocated, eliciting the desired cellular effect, such as apoptosis

Analyze

Subpopulation analysis

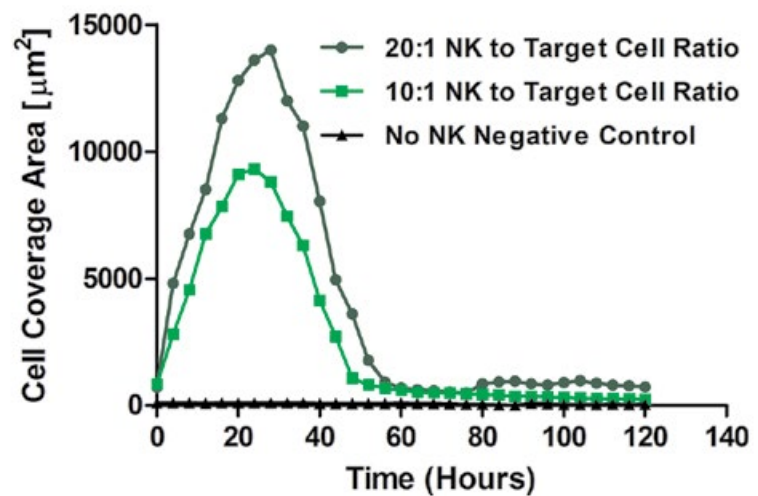


Cell populations rarely have homogenous responses. Subpopulation analysis is a powerful tool to identify various response levels or outliers within the population. Typical applications include rare event detection, transfection efficiency calculation, viral infection, among many others.

Kinetic analysis

Any cellular measurement can be plotted over time to better visualize real-time cellular dynamics.

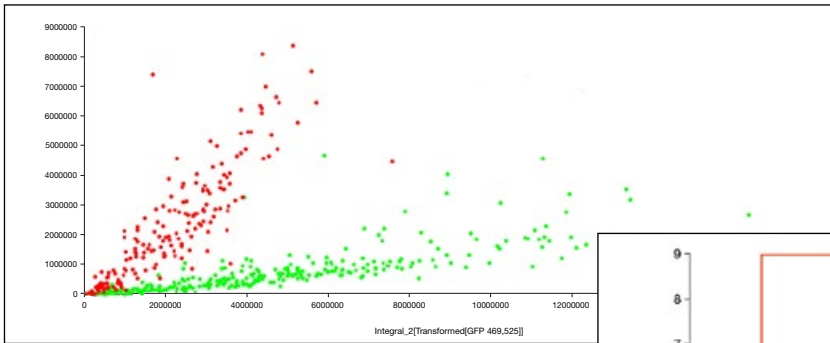
Kinetic calculations include rate of change, min/max signal, lag time, peak response.



Publish

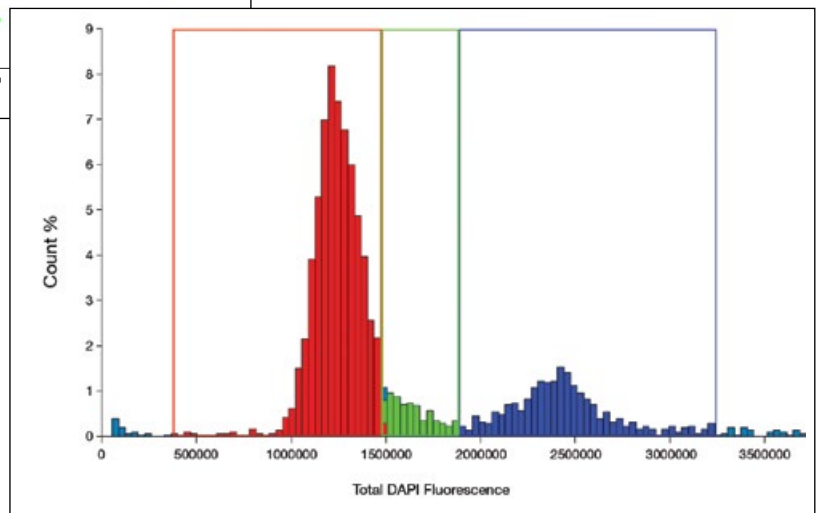
Augmented Microscopy tools include the ability to create publication-ready images, graphs and data using the functions in Gen5 software. There is no need to export images or data to external software.

Scatter plots & histograms

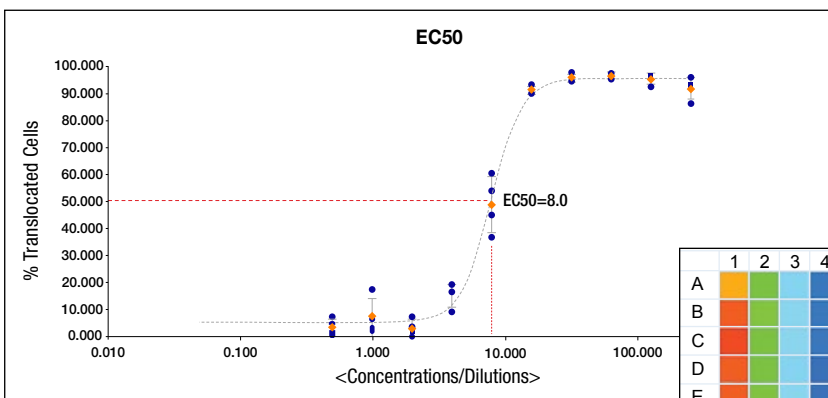


Commonly used in flow cytometry, scatter plots are a powerful tool to visualize variations within large cell populations. This scatter plot shows two distinct populations, responders are in red, normal population is in green.

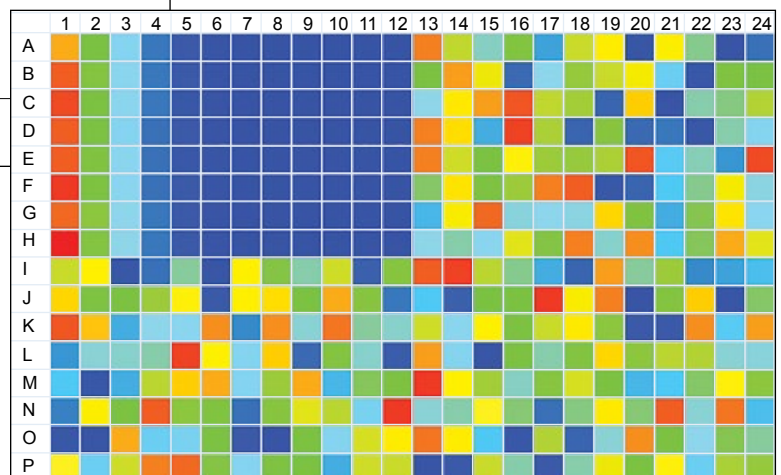
The histogram shows subpopulations of object total DAPI fluorescence.



Data analysis



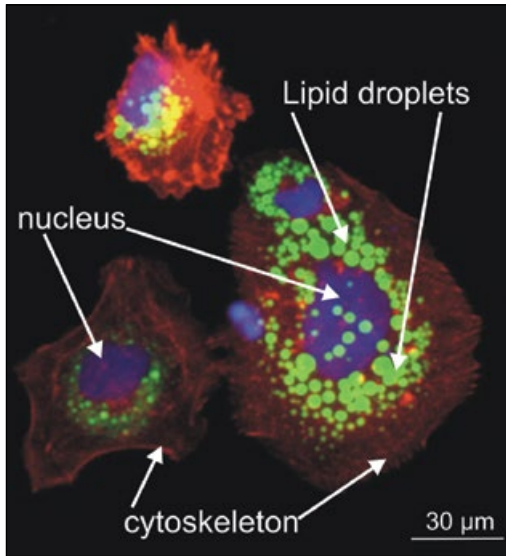
Gen5 includes data analysis tools that are often handled in third party software, enabling a complete workflow within one platform. Powerful analyses such as EC_{50} , parallel line analysis, statistical analysis, heat maps and custom calculations are all built-in to Gen5.



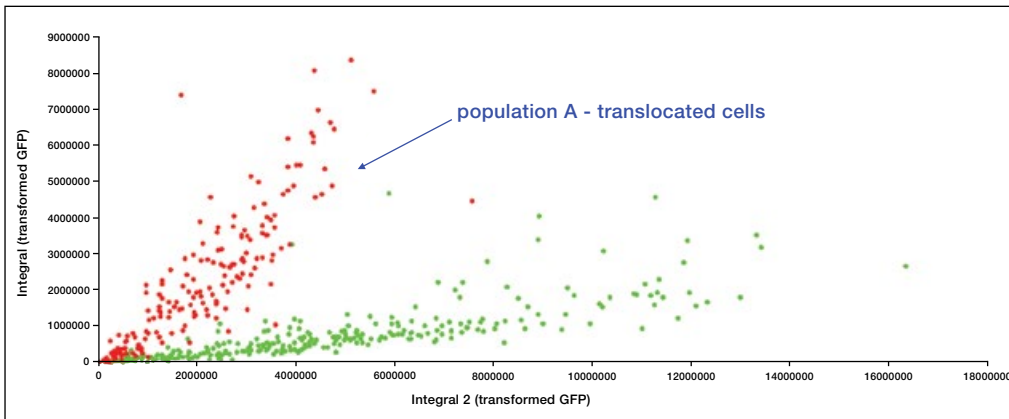
Publish



Image & graph annotation



Use the annotation tools in Gen5 to highlight important elements of an image or graph. Add text, measurement lines, callouts, shapes and grids to an image – they are saved along with the image or video, ready for publication.



Locked raw image



Copy for editing

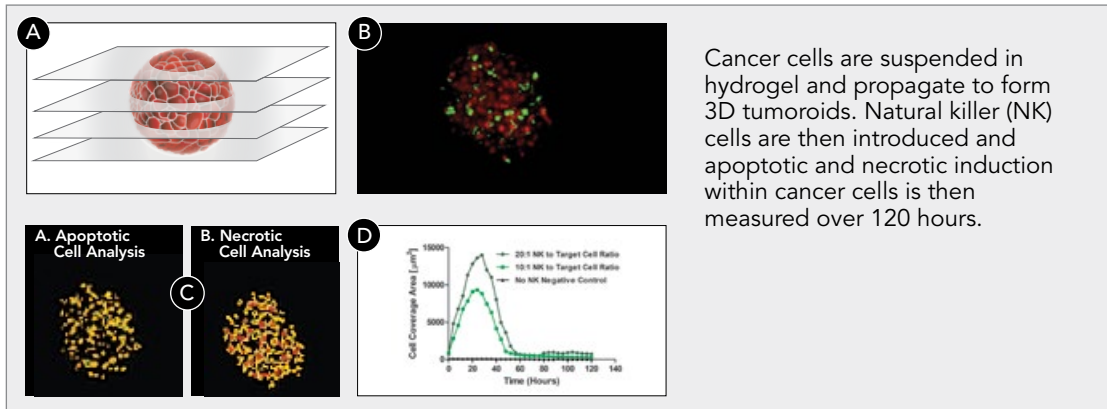
Raw image retention

Before processing or analyzing images, Gen5 makes a copy of the raw data and the raw data is retained as a separate file. Gen5 protects raw images and provides traceability from the raw through the modified images.

Select Applications

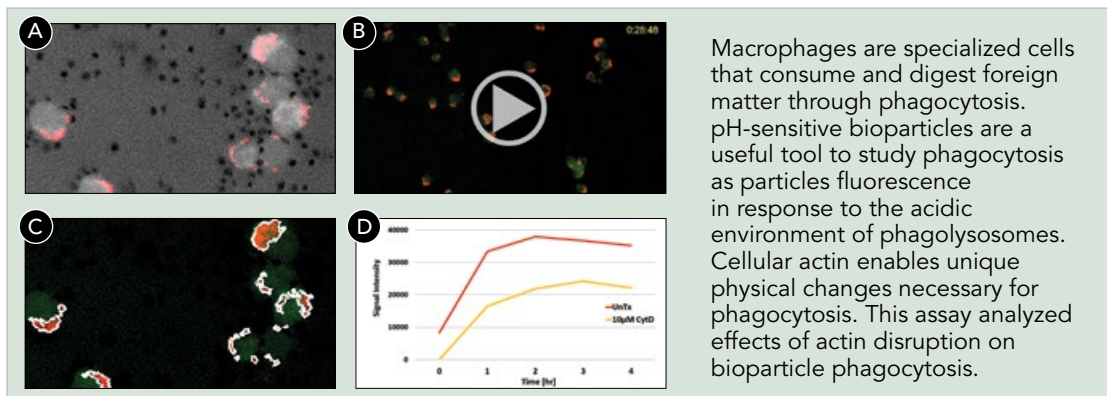
BioTek's imaging and microscopy instruments, along with Gen5 software, are capable of automating a broad range of application workflows. Augmented Microscopy tools guide users through the four major steps of microscopy: **capture** ► **process** ► **analyze** ► **publish** across a broad range of applications. In this section are just a few examples of important applications easily managed with BioTek's imagers and Gen5 software.

3D NK cell cytotoxicity



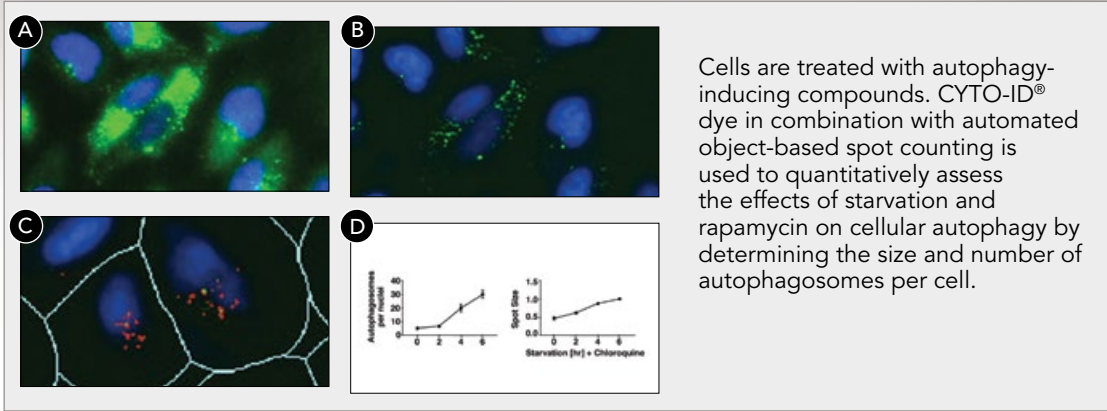
- A. Capture:** Three color Z-stacked images are captured of tumoroids in each well over 120 hours.
- B. Process:** Each set of z-stacked images is z-projected at each time point for analysis of apoptosis (green fluorescence) or necrosis (red fluorescence).
- C. Analyze:** Image analysis quantifies apoptosis (green fluorescence) and necrosis (red fluorescence).
- D. Publish:** Apoptotic and necrotic induction are plotted over time for each condition.

Phagocytosis assay



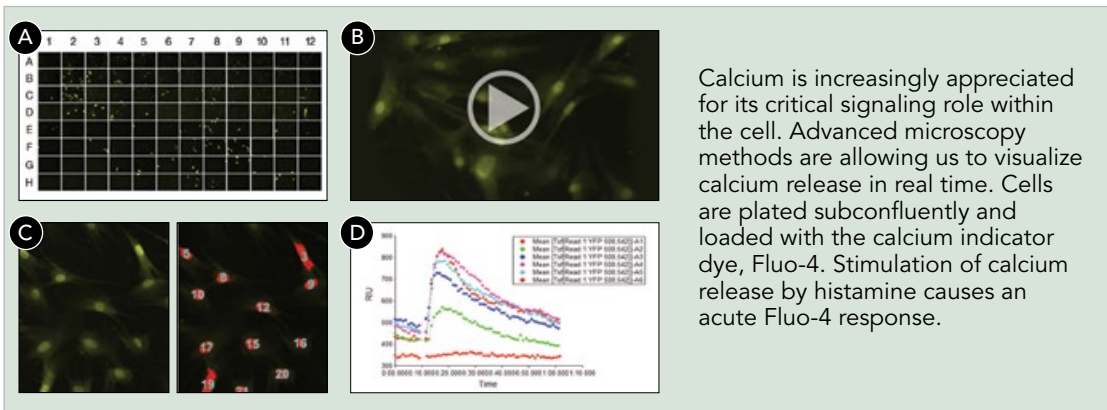
- A. Capture:** A two channel image at one kinetic timepoint shows black extracellular bioparticles in contrast to the red fluorescence of phagocytized bioparticles (RFP).
- B. Process:** A time-stamped movie is generated of kinetic images showing an increase in bioparticle phagocytosis over time (orange).
- C. Analyze:** A primary mask on bioparticle phagocytosis is applied to all kinetic images.
- D. Publish:** Compared to untreated macrophages (red) actin disruption causes decreased bioparticle phagocytosis (yellow).

Autophagy (spot count)



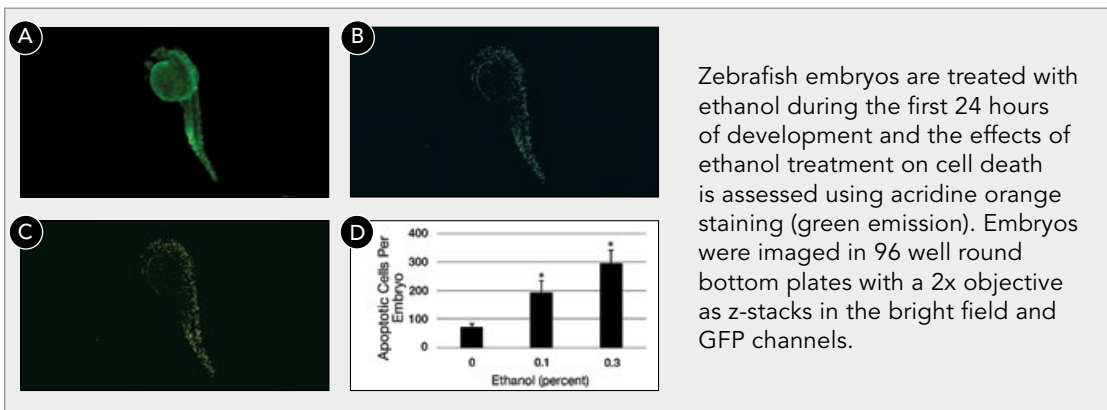
- A. Capture:** Each well is automatically imaged at 20x.
- B. Process:** Images are processed in order to better separate individual autophagosomes.
- C. Analyze:** The pre-processed image is analyzed, and each individual autophagosome is counted as a per-cell object.
- D. Publish:** Consistent and precise measurement of spot count per cell (top) and spot size (bottom).

Calcium Kinetics



- A. Capture:** Kinetic images are captured every second for 2 minutes in each well of a 96-well plate.
- B. Process:** Time-stamped movies are generated from these images showcasing calcium release and recovery.
- C. Analyze:** Cell counts are performed at the time point of peak signal for data normalization.
- D. Publish:** Overlaid kinetic curves highlight the impact of experimental substrates on inhibition of calcium release.

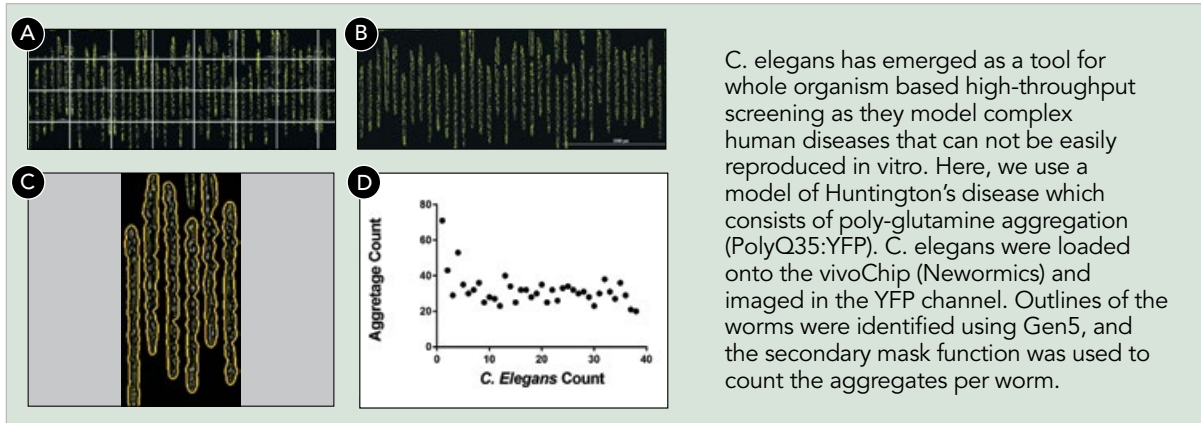
Measuring apoptosis in zebrafish treated with ethanol



- A. Capture:** Each well is automatically imaged as a 2x z-stack.
- B. Process:** Images are z-projected then pre-processed in order to better separate individual positive cells.
- C. Analyze:** The pre-processed images are analyzed, and each individual GFP positive cell is automatically identified and counted.
- D. Publish:** The effect of ethanol treatment on the number of apoptotic cells per embryo can be graphed for publication.

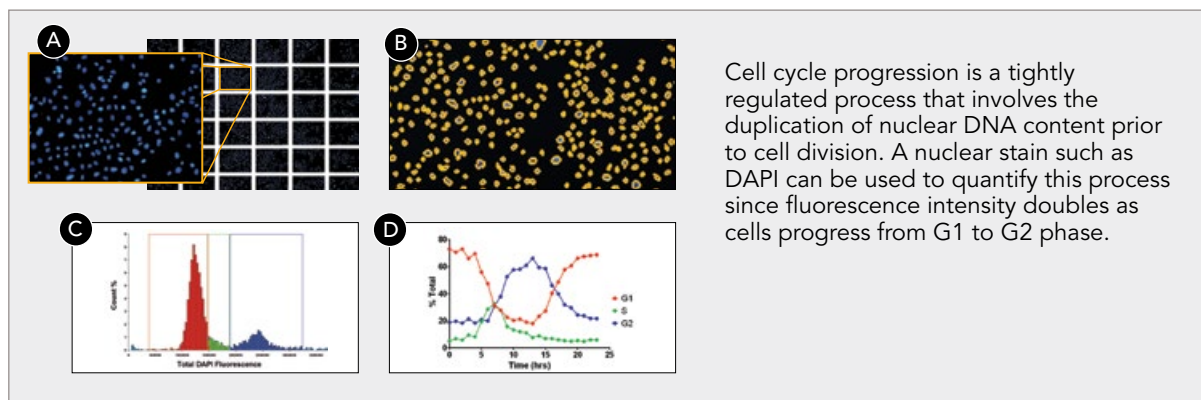
Select Applications

Quantifying poly-glutamine aggregates in *C. elegans* using vivoChip



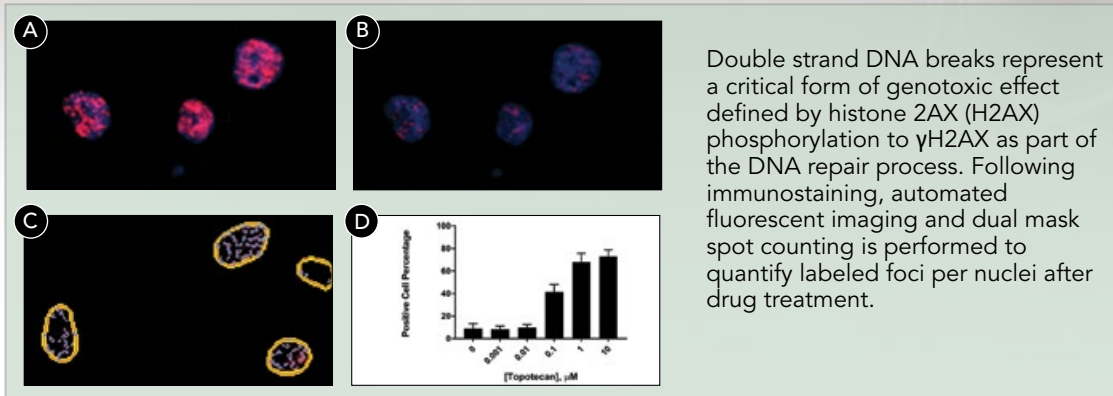
- A. Capture:** Each vivoChip is automatically imaged at 10x as a 4x8 montage and z-stack in brightfield and YFP channels.
- B. Process:** Image tiles are stitched together then z-projection and background subtraction are applied.
- C. Analyze:** The primary mask function in Gen5 identifies each individual worm and the secondary mask function identifies the polyQ aggregates per worm.
- D. Publish:** Aggregate numbers can be quantified for publication.

Cell cycle analysis using a nuclear stain



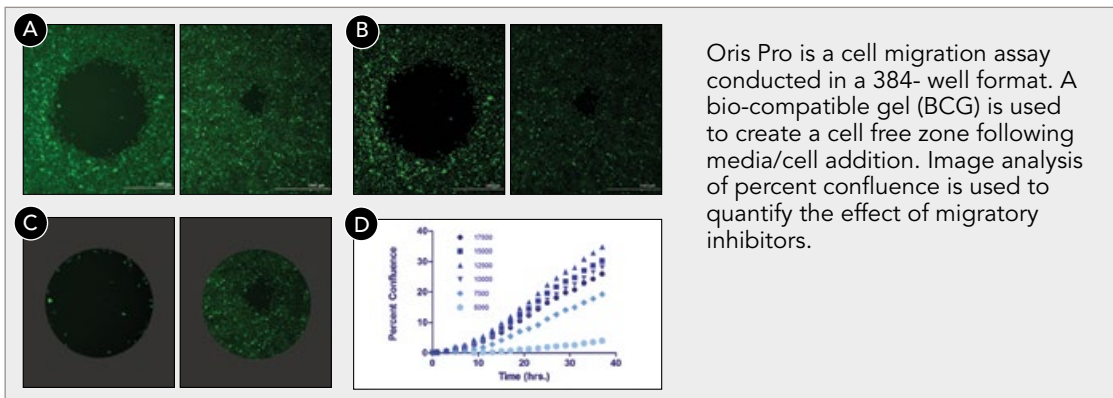
- A. Capture:** DAPI montage (6 x 6) image using 10x objective (one tile expanded).
- B. Process:** Stitched and background subtracted, montage image with cell nuclei identified (zoom shown, about 3,000 cells per well counted on final montage).
- C. Analyze:** Determination of G1, S, and G2 subpopulations using histogram analysis of object total DAPI fluorescence.
- D. Publish:** Cell cycle progression of synchronized PC-3 cells.

γ H2AX foci spot counting as a determinant of genotoxicity



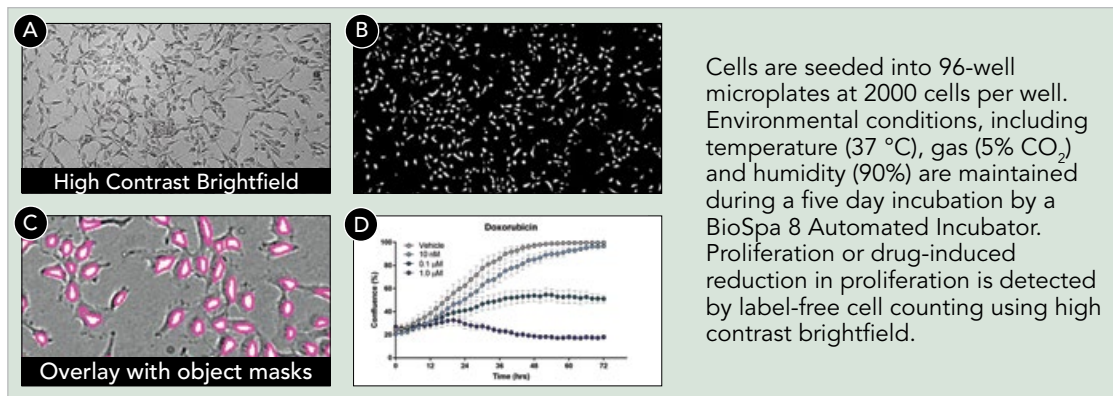
- A. Capture:** Images captured of DAPI stained nuclei and fluorescent antibody labeled γ H2AX signal.
- B. Process:** Pre-processing eliminates background signal revealing actual labeled γ H2AX spots.
- C. Analyze:** Secondary spot counting capability allows quantification of spots per nuclei.
- D. Publish:** Statistical determination of minimum spots per nuclei enables calculation of induced DNA damage per treatment.

High throughput cell migration assay



- A. Capture:** Cell migration into the detection zone is monitored kinetically.
- B. Process:** Background flattening is applied to facilitate image analysis.
- C. Analyze:** A disc-shaped "plug" is applied to determine percent confluence within the cell-free zone.
- D. Publish:** Kinetic and endpoint dose responses can quantify potency of migratory inhibitors.

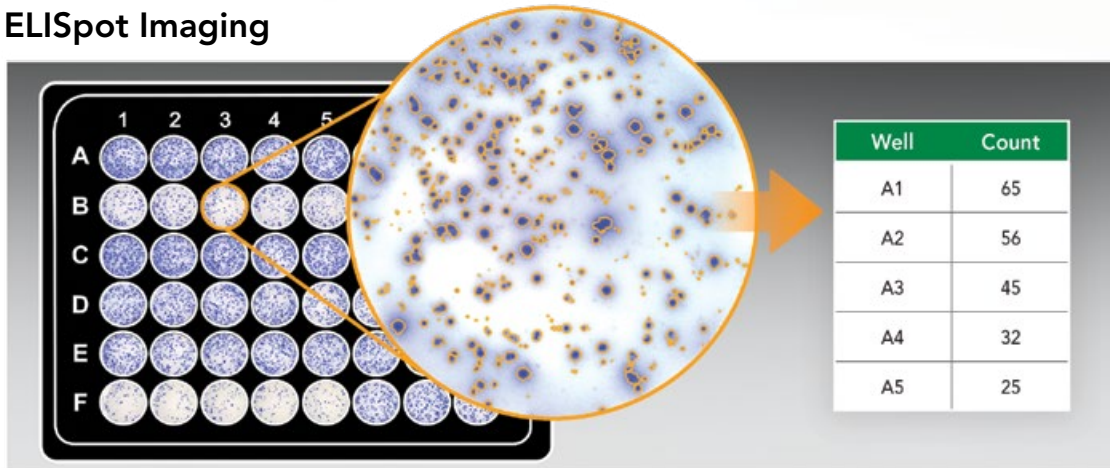
Label-free cell proliferation



- A. Capture:** Each well is kinetically monitored every 2 hours using high contrast brightfield.
- B. Process:** All images are processed to maximize contrast of cells over background.
- C. Analyze:** The processed image is analyzed, cell objects are identified using intensity and size thresholds.
- D. Publish:** Anti-proliferative agent pharmacology can be published.

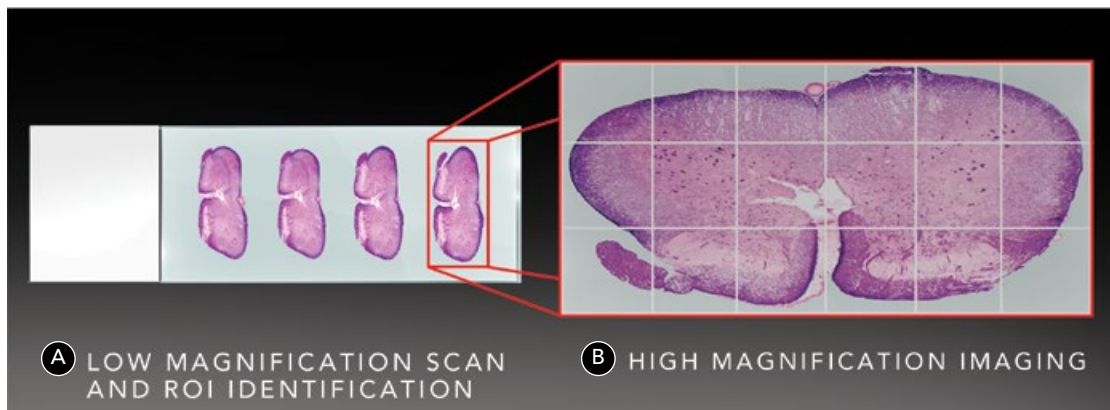
Select Applications

ELISpot Imaging



Cytation's upright imaging module can be used to automate assays such as ELISpot, in which cell secretions are rendered visible through the use of a colorimetric reaction. Cytation fully automates image acquisition, processing, image analysis and object count.

AutoROI identification feature



The AutoROI feature in Gen5 automates the identification of ROIs at low magnification, and then automatically images the ROIs at a higher magnification.

Hardware Features, Automation, Accessories

Applications for BioTek's imaging & microscopy instrumentation are enabled and enhanced through automation solutions and a wide variety of labware adapters and other accessories.

Automation

For higher volume processing or long-term workflows, Cytation Cell Imaging Multi-Mode Readers integrate to BioTek's automation solutions.



Cytation integrated with BioSpa 8 Automated Incubator

The BioSpa Automated Incubator has environmental controls and labware handling capabilities to facilitate long term live cell kinetic imaging processes, for up to 8 microplates.



Cytation C10 integrated with BioStack

BioStack manages up to 50 microplates for automated imaging or multi-mode operations, including de-lidding and re-lidding of microplates used with cell-based assays.



Cell Count & Viability Starter Kit

BioTek's Cell Count & Viability Starter Kit includes everything a researcher needs to count

cells and measure viability in a mammalian cell suspension using BioTek's Cytation 5 Cell Imaging Multi-Mode Reader or Lionheart FX Automated Imager. Automates the process of mammalian cell counting to improve data quality and save time.



Scratch Assay Starter Kit

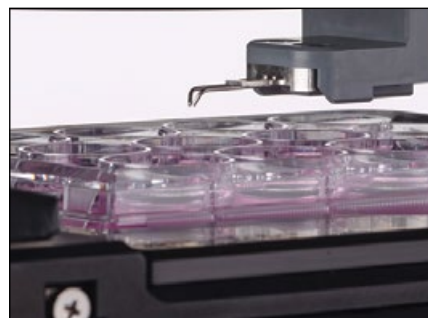
The Scratch Assay Starter Kit includes everything a researcher needs to implement automated,

kinetic cell migration and scratch wound assays. Use the kit along with a BioTek Lionheart Automated Microscope or Cytation Cell Imaging Multi-Mode Reader for kinetic image capture, processing and data analysis.

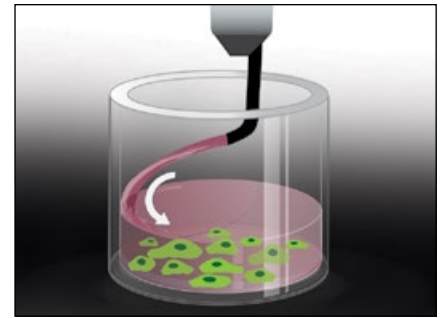
CO₂/O₂ control and reagent injectors



The compact gas controller maintains control of CO₂ and O₂ levels for live cell assays. The gas controller is for use with Lionheart FX and Cytation systems.



The dual reagent injector module for Lionheart FX and Cytation allows fast cellular reactions to be imaged or detected.



Angled injector tips protect cell monolayers from shear stress during injection.

High quality objectives

BioTek uses the highest quality optical components, including objectives for standard and phase contrast imaging. Objective magnifications range from 1.25x to 100x oil immersion. 20x and greater objectives have correction collars to adjust for variations in sample vessel bottom thickness. Lionheart, Cytation C10, Cytation 7 and Cytation 5 have an automated 6-objective turret, Cytation 1 has an automated 2-objective turret.



Imaging filter cubes

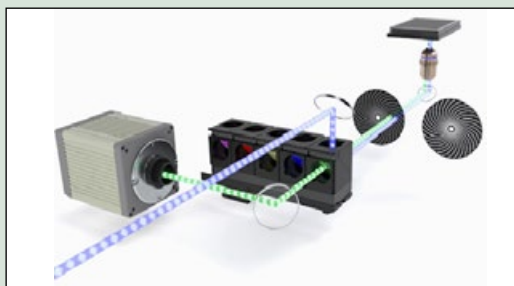
Using the 6-line laser light engine, the confocal cubes for Cytation C10 use high quality, deep blocking filters for optimum intensity and image resolution.

The filter/LED cubes used for widefield imaging use high powered, low maintenance LEDs to provide full control over light intensity and to reduce phototoxicity.



Confocal hardware

The Cytation C10 confocal microscope uses a Hamamatsu sCMOS camera, along with 60 μm and 40 μm Nipkow spinning disks. The high quality components ensure the highest quality resolution for spheroids and thick biologies.



Humidity control

The unique humidity chamber for Lionheart FX helps maintain cell viability during kinetic imaging sessions.



Labware adapters

From microscope slides, cell culture dishes and chamber slides to microplates T75 flasks and hemocytometers, BioTek's range of labware adapters support many imaging workflows.

Gen5 software includes a database of pre-defined plate and other vessels. To quickly define a new microplates type, just take a photo with your phone and import the image to Gen5 for final definition – no need for cumbersome measurements.



Clockwise from upper left: Dual slide adapter, T25 flask holder, multi-vessel adapter, hemocytometer adapter, dual 35 mm and 100 mm cell culture dish holder.

Imaging & Microscopy Product Comparison

	Lionheart FX	Lionheart LX	Cytation C10	Cytation 7	Cytation 5	Cytation 1
General						
Microplate types	6- to 1536-well plates					
Other labware	Slides, cell culture dishes & flasks, hemocytometers, chamber slides					
Incubation	to 40 °C		to 45 °C	to 45 °C	to 65 °C	to 45 °C
Joystick controller	•	•	•	•	•	
BioStack compatible			•	•	•	•
BioSpa compatible				•	•	•
Agilent BenchCel compatible				•	•	•
Multi-mode plate reading			•	•	•	•
Imaging						
Widefield fluorescence	•	•	•	•	•	•
Confocal fluorescence			•			
High contrast brightfield	•	•	•	•	•	•
Brightfield	•		•	•	•	
Color brightfield	•	•	•	•	•	
Phase contrast	•		•		•	
Magnifications available - air	1.25x, 2.5x, 4x, 10x, 20x, 40x, 60x					
Magnifications available - oil	60x, 100x					
Inverted microscope	•	•	•	•	•	•
Upright microscope				•		

Think Possible with BioTek



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Imaging & Microscopy

About BioTek

Since the launch of the Cytation Cell Imaging Multi-Mode Reader in 2013, BioTek has become a leading manufacturer of high quality imaging and microscopy instrumentation and software. Our **Think Possible** approach leads to innovative and robust solutions that are designed to meet a broad range of applications and instrumentation budgets.

Please visit www.biotek.com to learn about other BioTek Life Science Instrumentation.



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