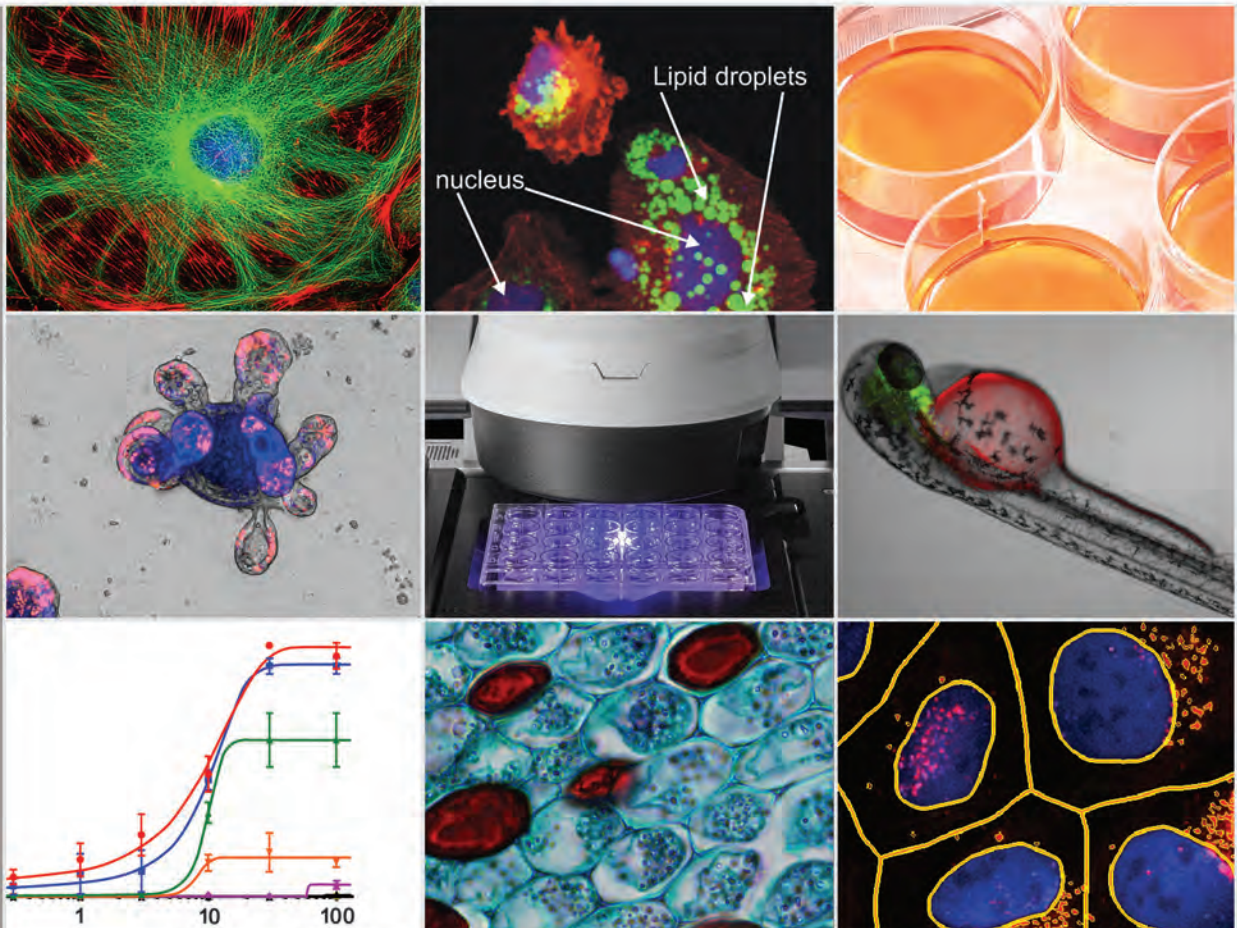


# Imaging & Microscopy

Think Possible

 **BioTek**<sup>®</sup>



CELEBRATING  
**50**  
YEARS  
OF PASSION AND  
INNOVATION

# BioTek

# Imaging & Microscopy

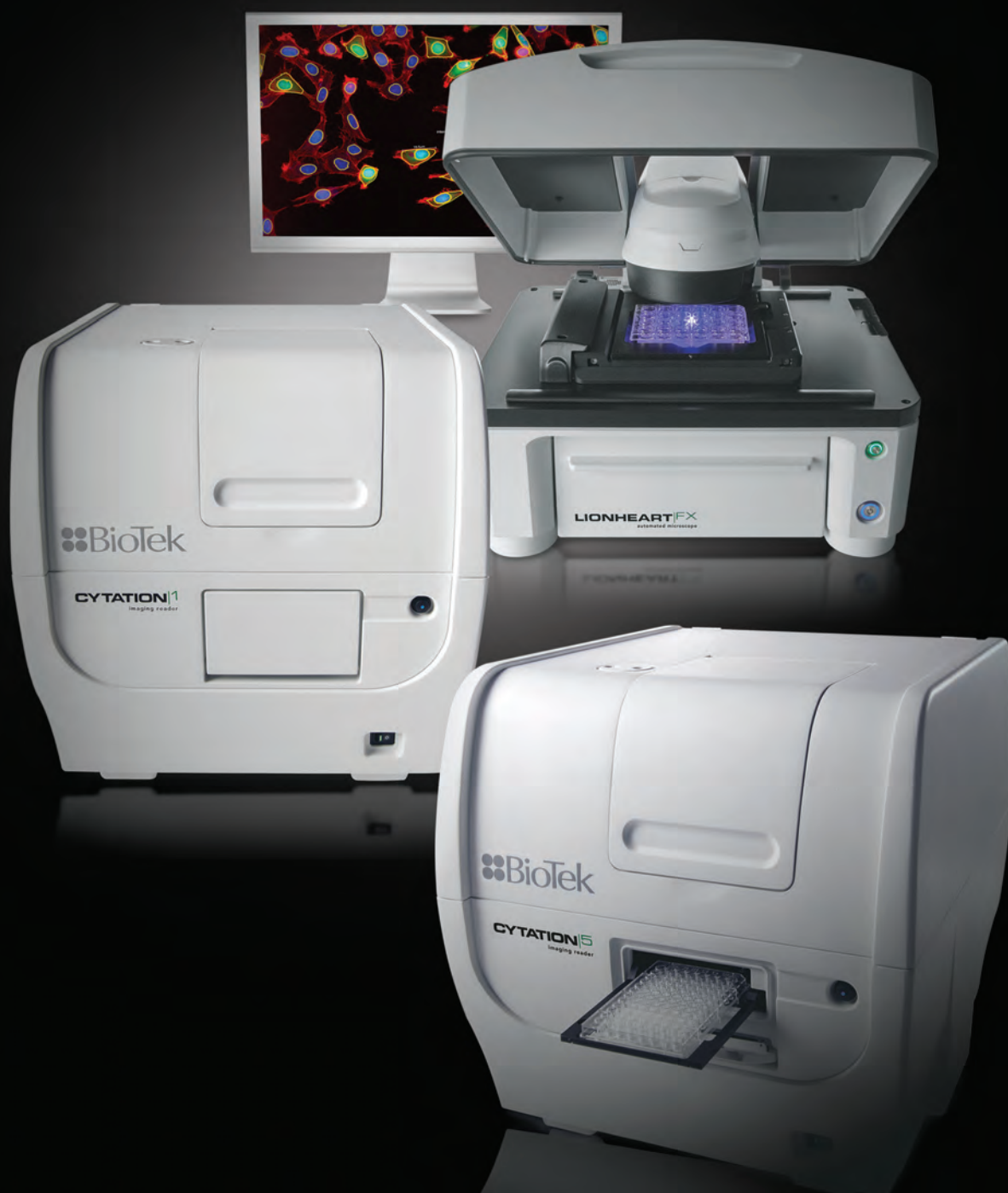
## Designed for a wide range of applications and budgets.

Lionheart™ FX Automated Microscope offers 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™ 5 and Cytation 1 Cell Imaging Multi-Mode Readers offer a unique, patented design that combines

automated microscopy with multi-mode detection, to capture both quantitative data and phenotypic information on a single platform. Cytation's modularity and upgradability provide the ability to expand the system as your laboratory's applications increase.

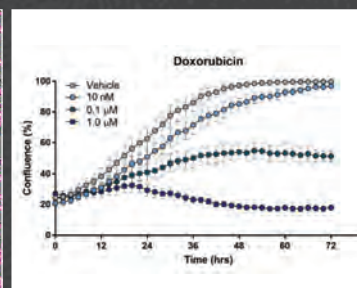
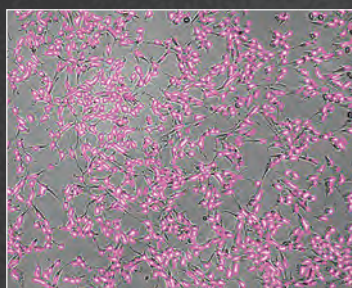
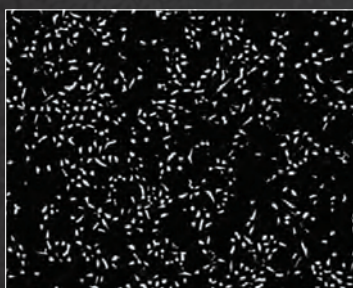
Gen5™ Microplate Reader and Imager Software includes functionality for easy image capture and analysis, for both qualitative and quantitative data.





# 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.



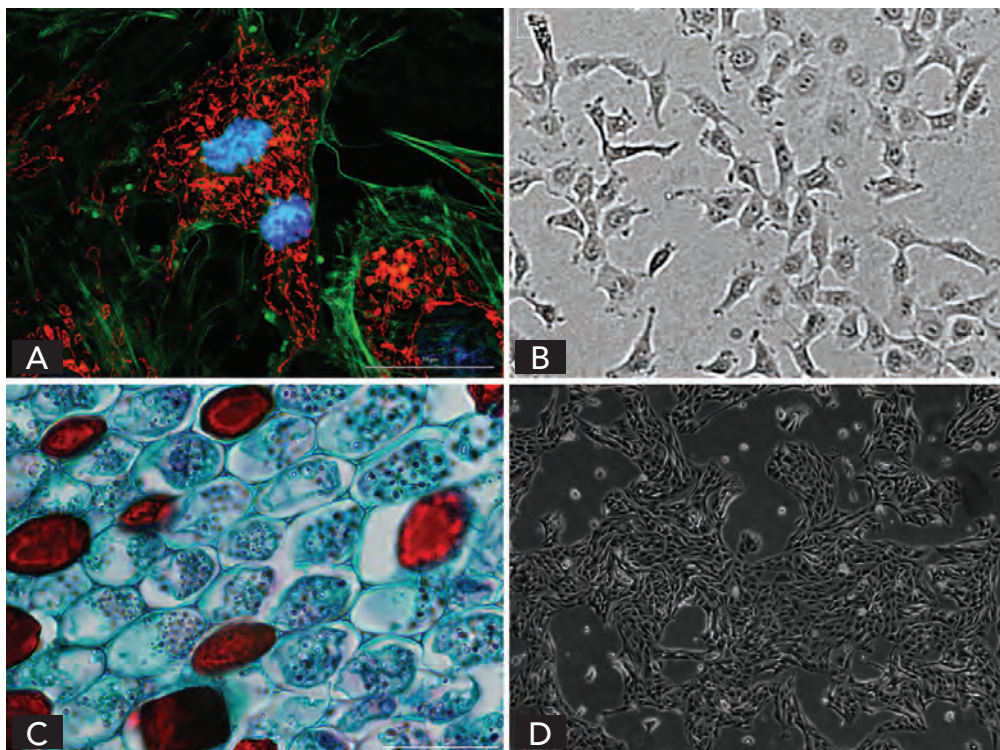
capture ► process ► analyze ► publish



# 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.

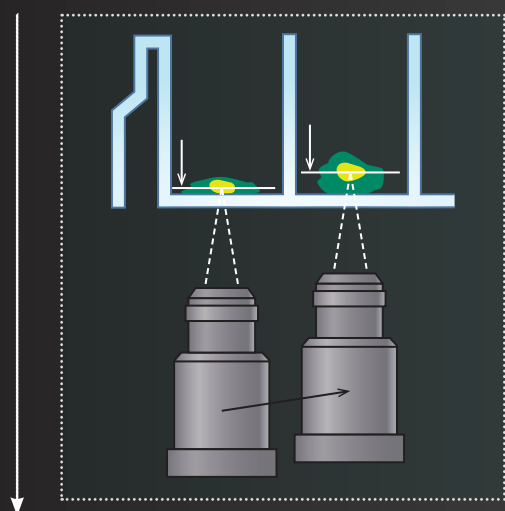
## Four imaging modes



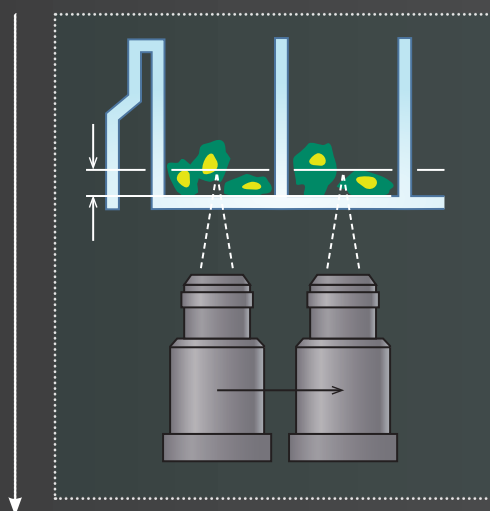
Powerful imaging for a wide range of applications, including live and fixed cell biologies.

- A. Fluorescence
- B. Brightfield
- C. Color brightfield
- D. Phase contrast

## 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.

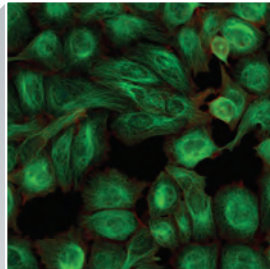
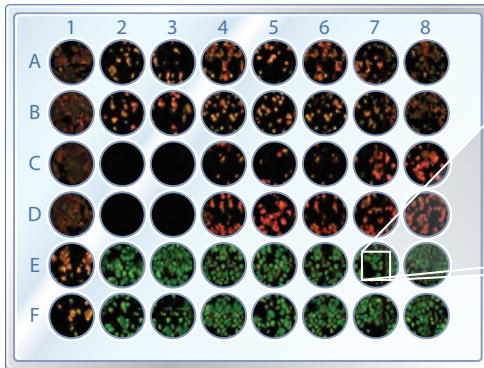


**BioTek's patented laser autofocus** uses the same focal offset from well to well and is typically faster. It works with dim fluorophores and helps prevent phototoxicity and photobleaching. Laser autofocus also offers better reproducibility and higher accuracy during long term kinetics.

# Capture

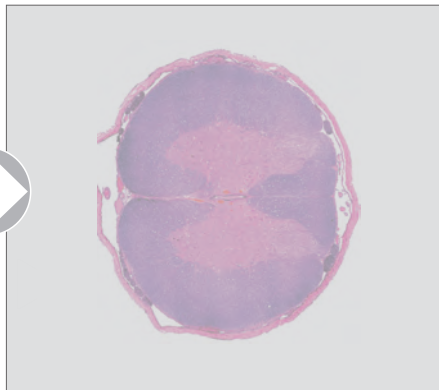
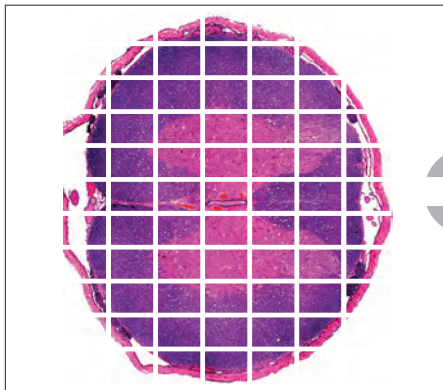


## Batch mode



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.

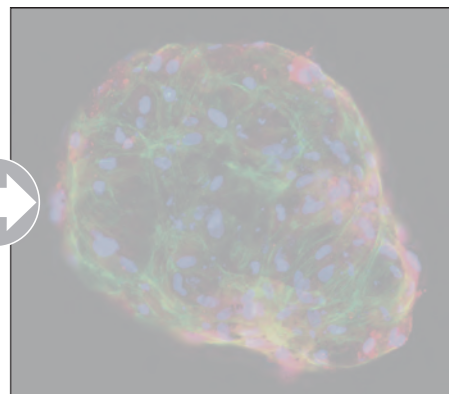
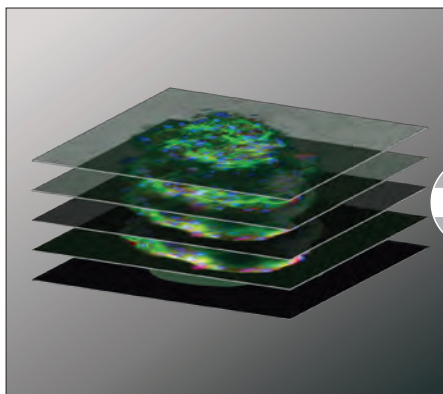
## Montage



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

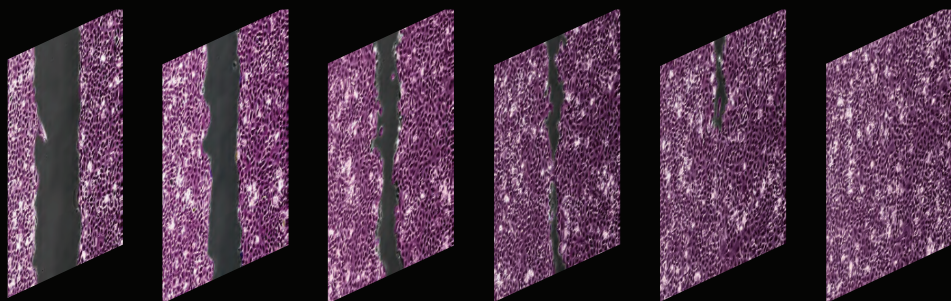


Gen5's z-stacking enables capture of up to 50 customizable slices – as thin as  $0.1 \mu\text{m}$  – in a stack. The images can then be automatically z-projected. Z-stack capability is a critical requirement for imaging 3D samples, such as spheroids and tumoroids, along with samples that extend over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



# Capture

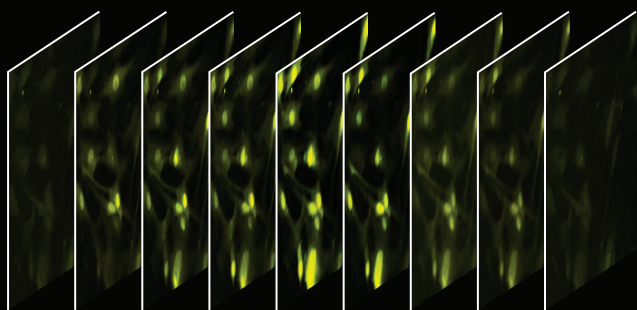
## Time lapse imaging



▶ **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.



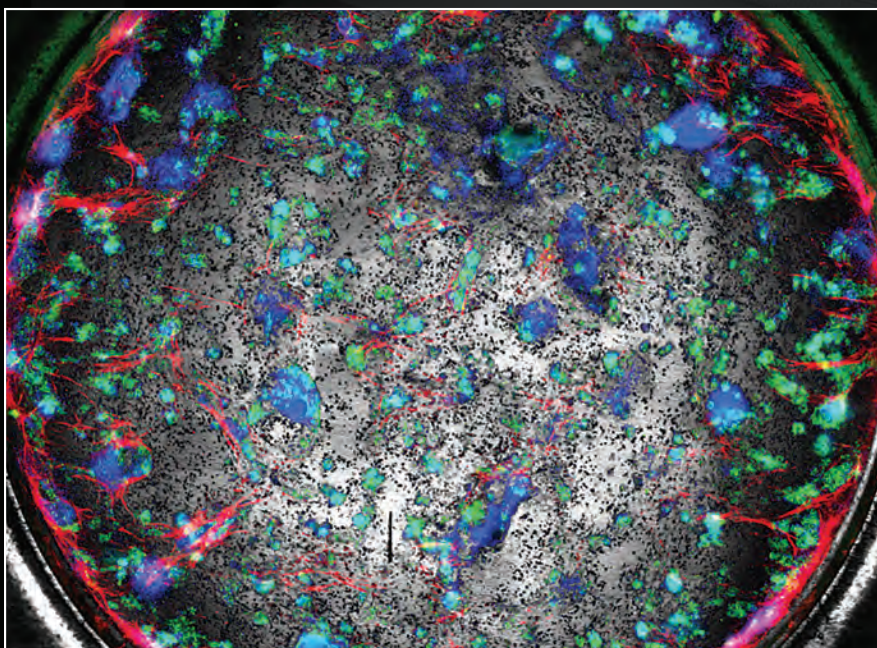
# Capture

## Live cell assay support



Temperature control, including the Condensation Control™ gradient, plus CO<sub>2</sub>/O<sub>2</sub> 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.

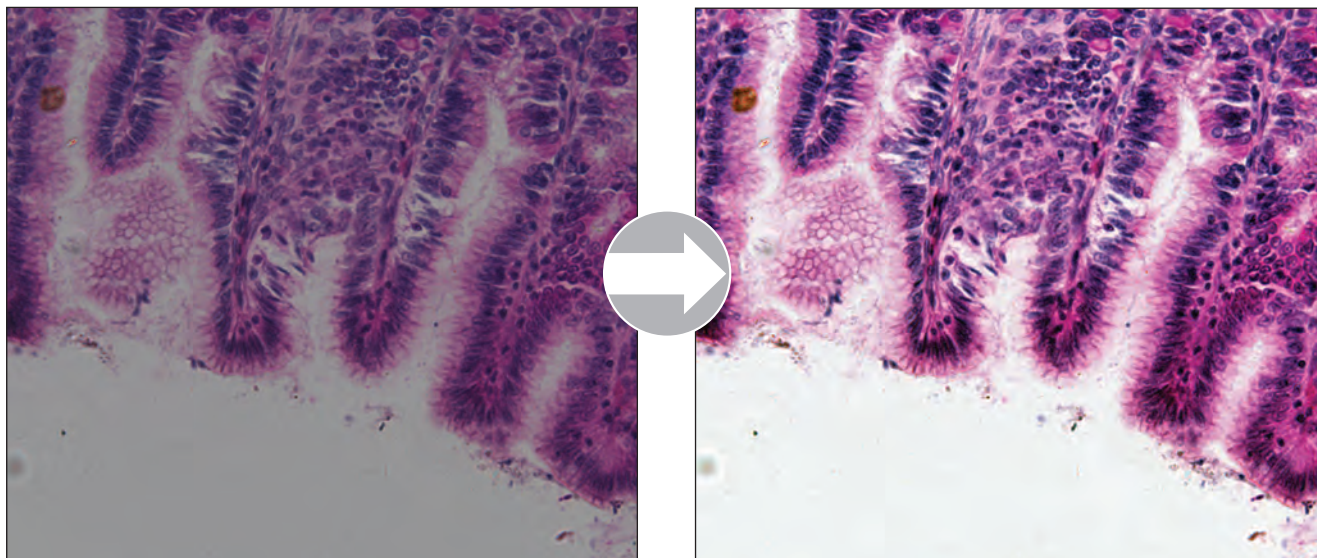
Choose from nearly 20 available LED/filter cube colors to cover a very broad range of fluorescent stains. Gen5's auto LED intensity ensures consistent, high quality capture for end point and kinetic sequences. Each channel can be automatically adjusted and optimized – changes are easily saved.



# Process

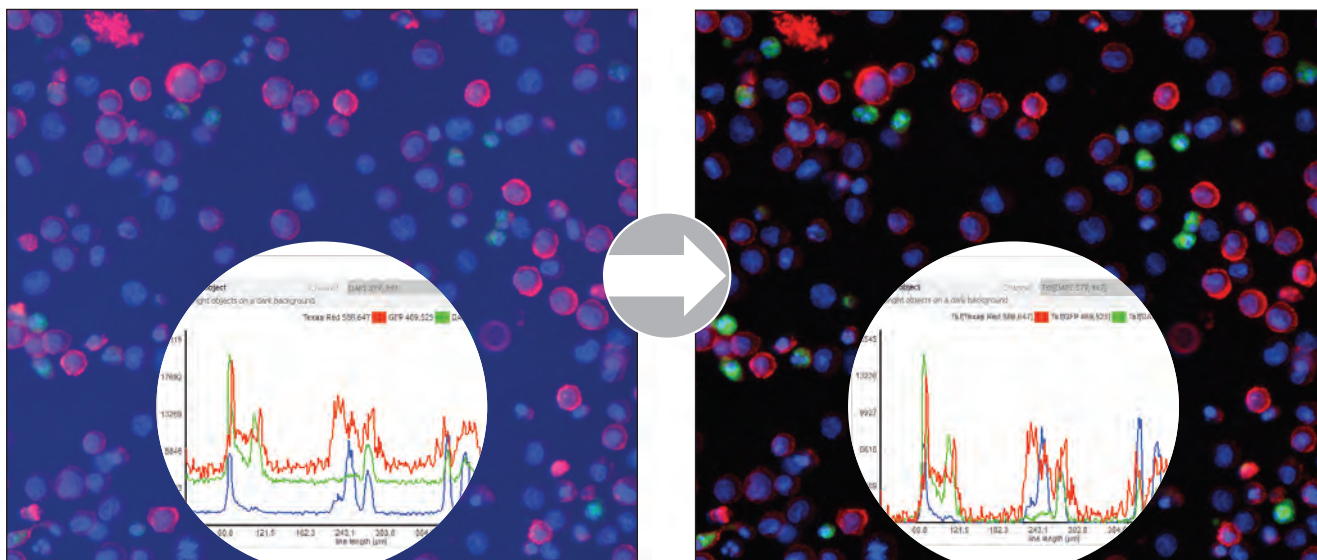
Image processing is essential for optimizing images prior to analysis. Gen5's tools provide exceptional processing capability to facilitate the analysis of complex biologies.

## 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

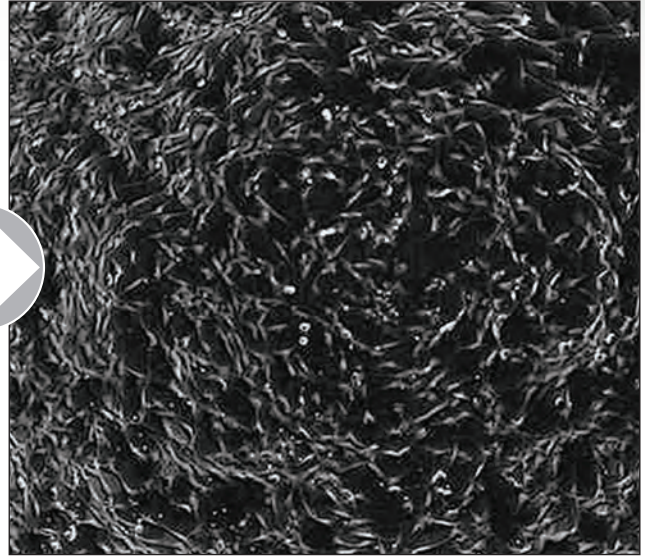
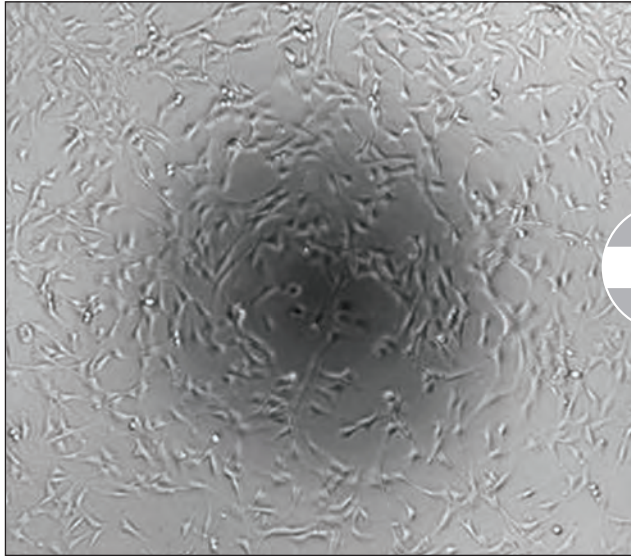


Background flattening using a rolling ball algorithm prepares the image for analysis by removing background artifacts and correcting for uneven illumination. Use the line profile tool to find recommended threshold values for image analysis.



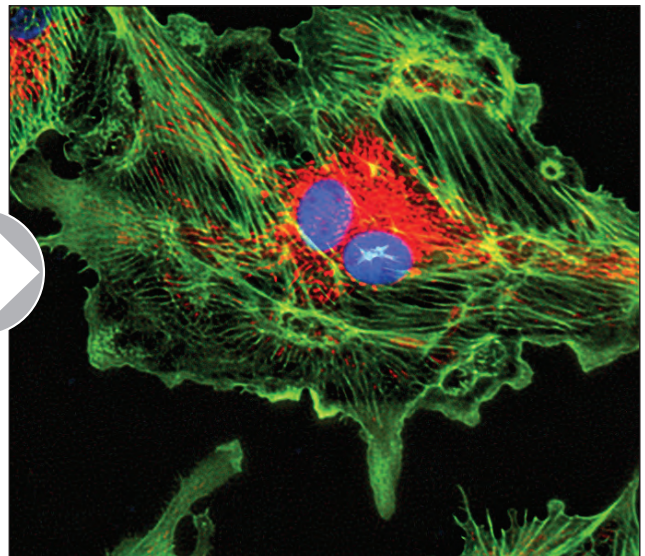
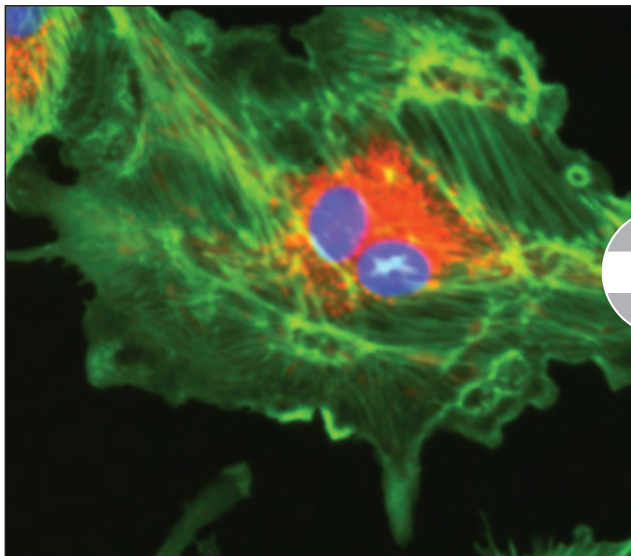
# Process

## 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

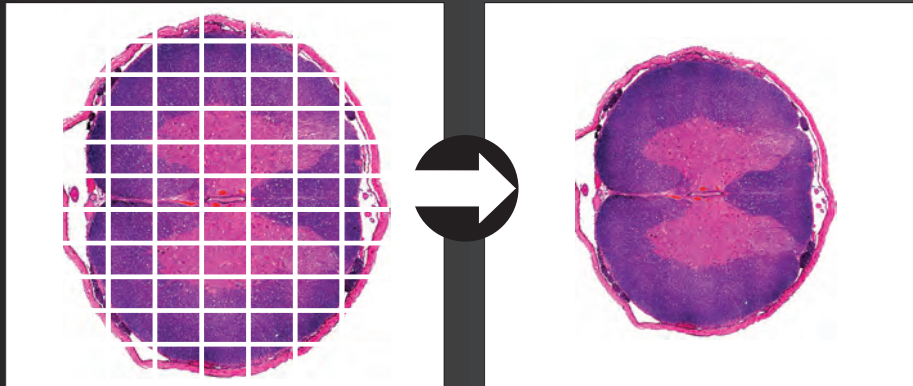


Deconvolution reduces blur from out-of-focus light, commonly seen in widefield imaging. It improves image resolution, enabling better visualization of image details.



# Process

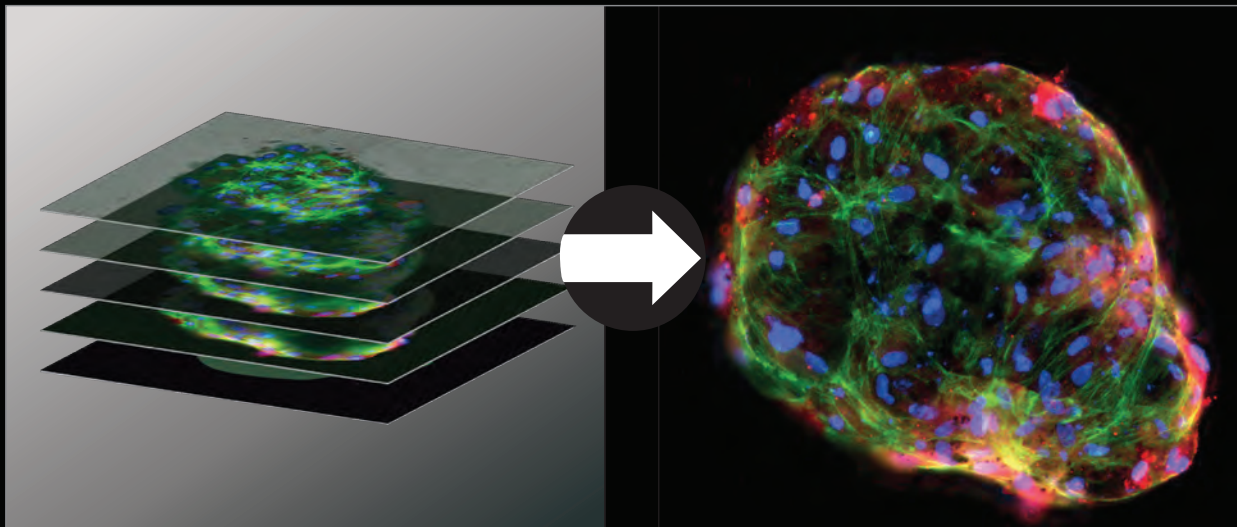
## Stitching



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

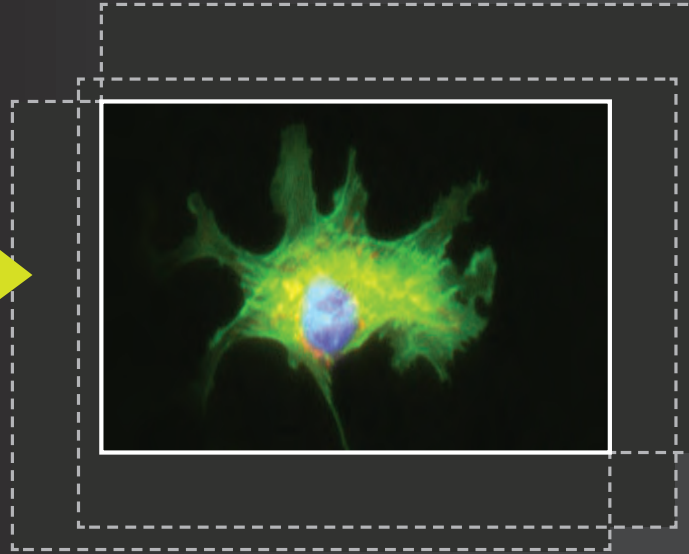
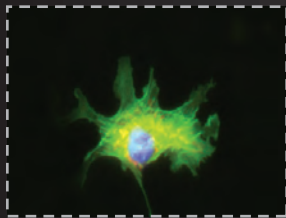
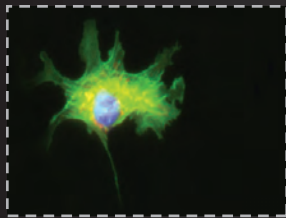
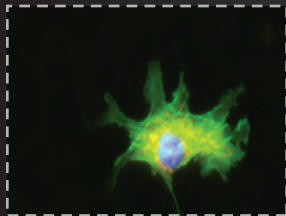


Captured z-stacks are automatically z-projected with a single click. Z-projection enables a fully focused view of challenging biology such as 3D spheroids, tissues and other thick samples.



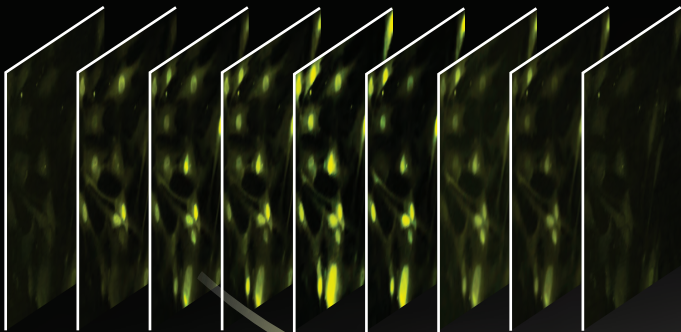
# Process

## 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

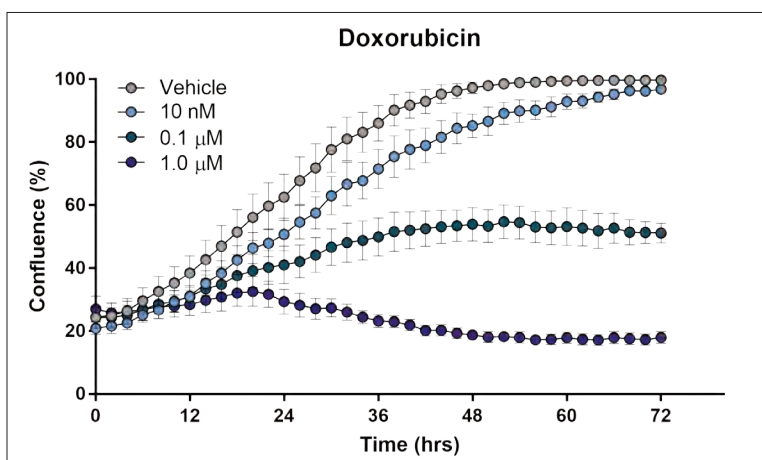
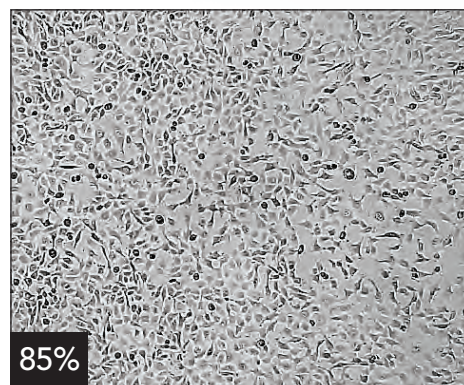
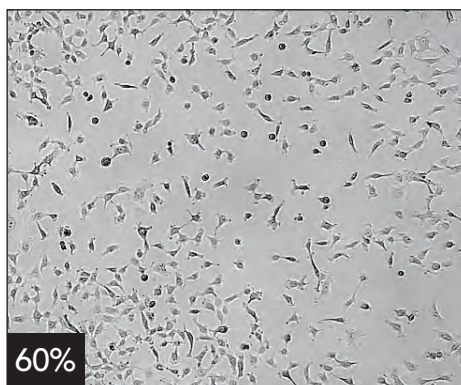
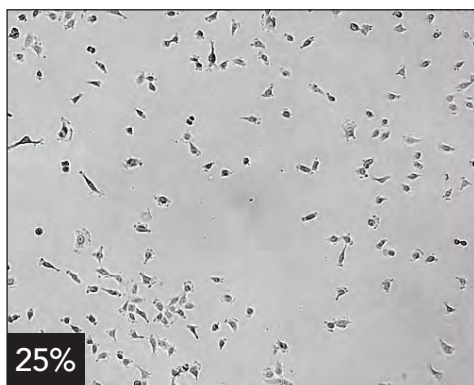


Gen5 quickly compiles time lapse images into time-stamped .wmv or .mp4 files.

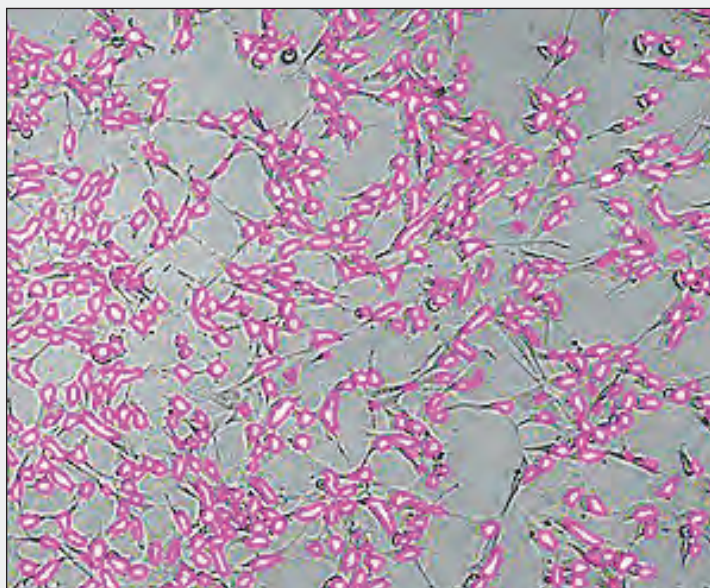
# Analyze

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.



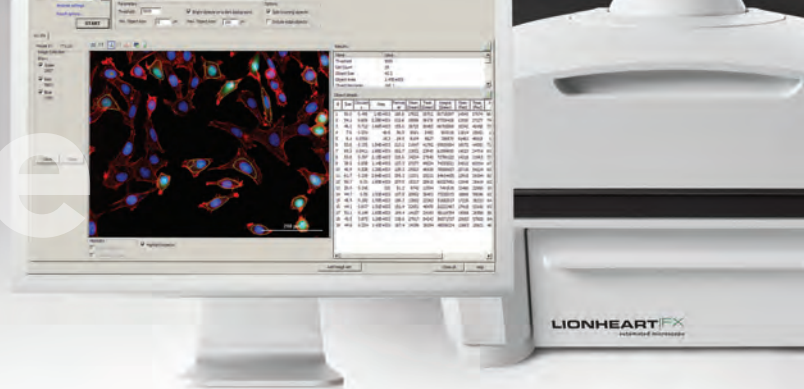
## Label-free cell count

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

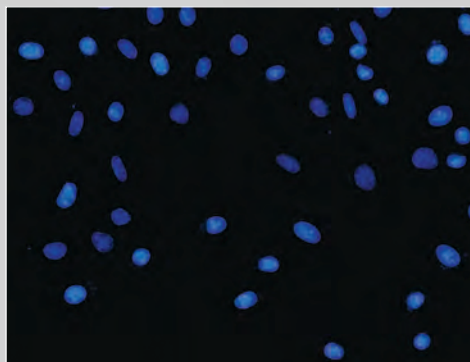
Along with label-free confluence measurements, label-free cell counts are performed efficiently using high contrast brightfield. Cell counts are essential to cell growth, health and proliferation studies. Gen5 efficiently identifies highly confluent cells without dyes.



# Analyze



## Nuclei count and analysis



Original image

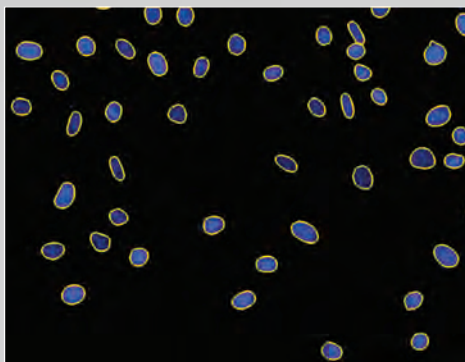
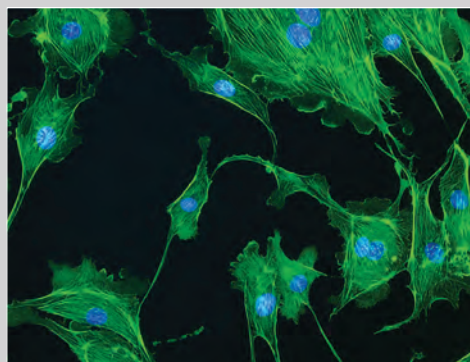


Image with mask applied

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

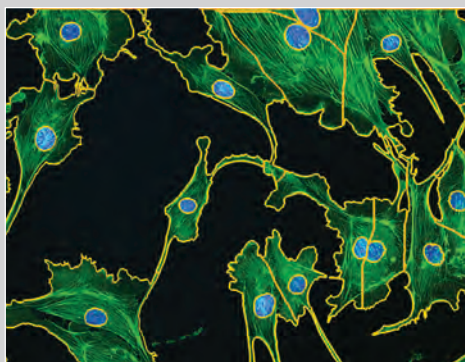
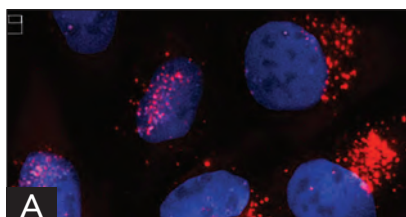


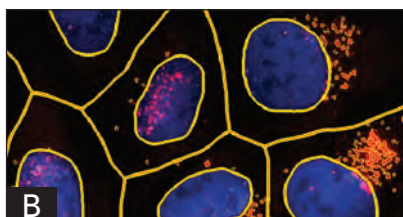
Image with mask applied

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.

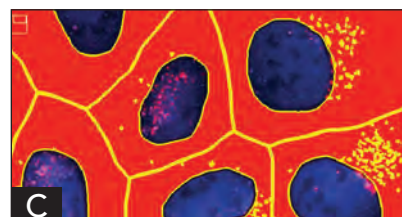
## Organelle analysis



A



B



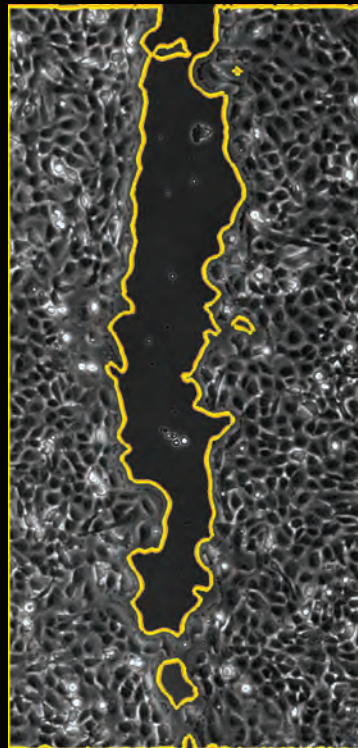
C

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

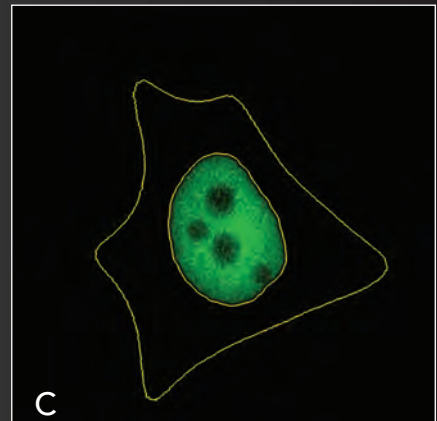
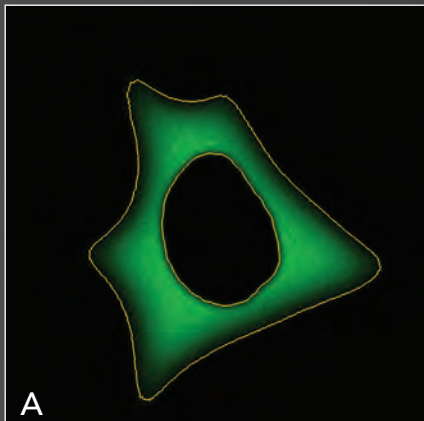
# Analyze

## Cell migration



Cell migration and invasion assays such as wound healing and spheroid formation are kinetic processes easily handled in Gen5 software. Analysis includes migration measurements, such as wound width or spheroid size.

## Signal translocation



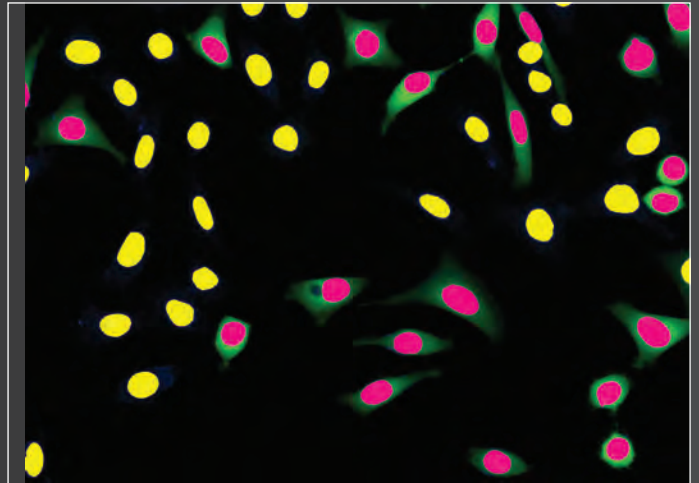
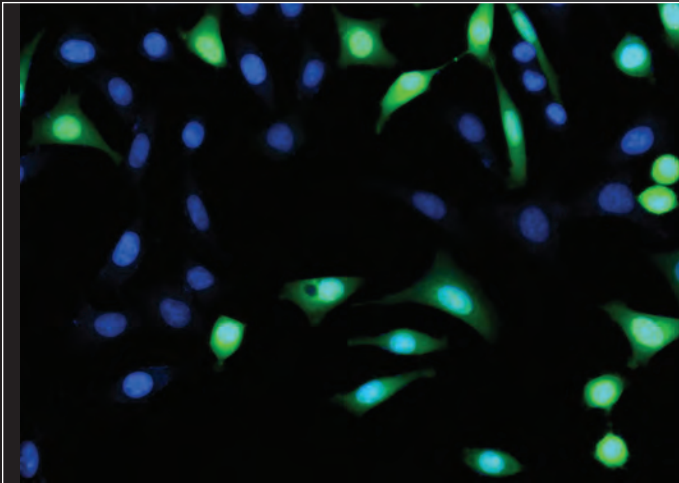
Monitoring molecular movement between cellular compartments, typically referred to as translocation, requires advanced cell analysis tools. This response is seen in many assays, including transcription factor activation and caspase cascade events (apoptosis), as shown. Using a nuclear mask and a cytoplasmic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

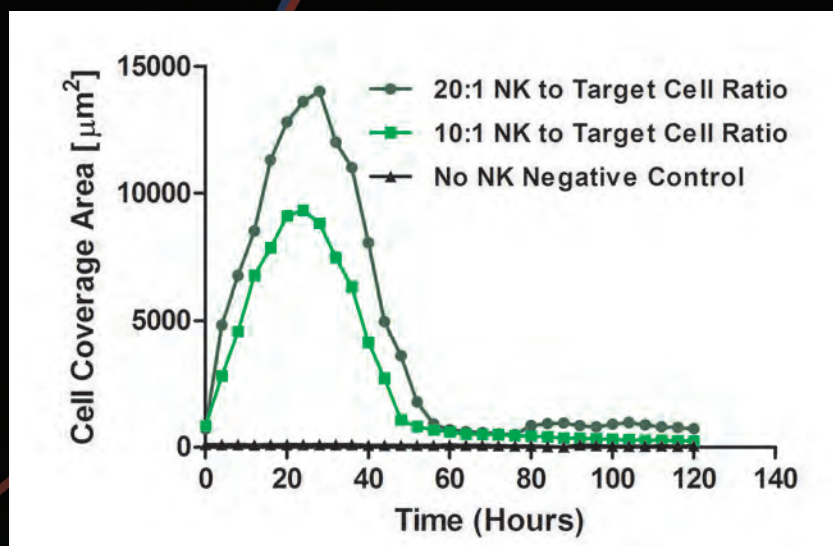


Cell populations rarely have homogenous responses. Subpopulation analysis is a powerful tool to identify various response levels or outliers within the population. Typical applications include rare event detection, transfection efficiency calculation, viral infection, among many others.

## Kinetic analysis

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

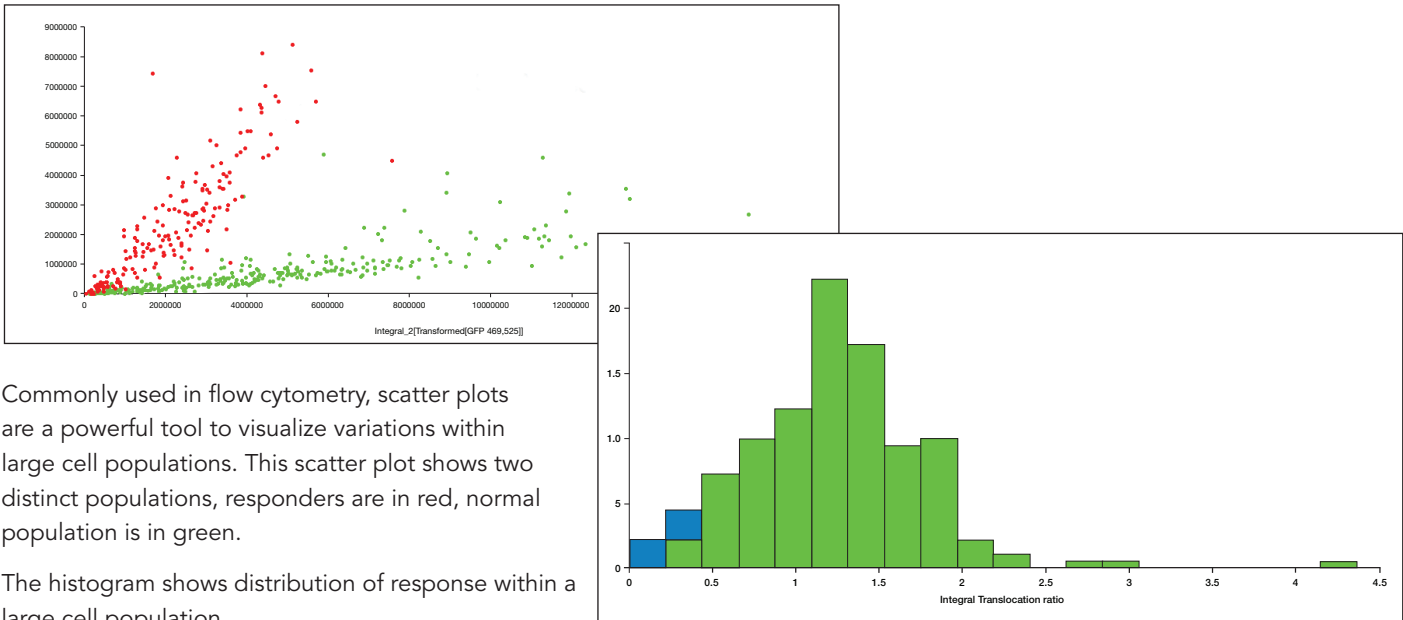
Kinetic calculations include rate of change, min/max signal, lag time, peak response.



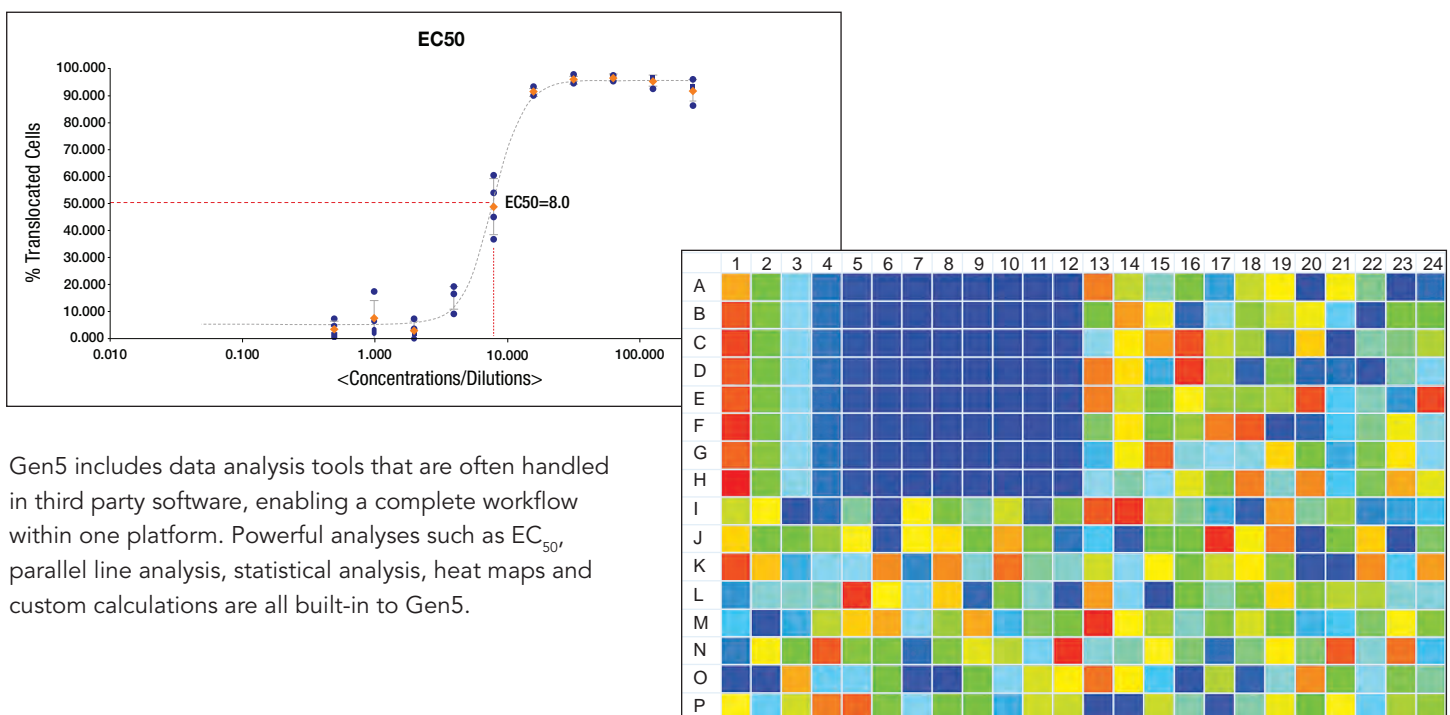
# Publish

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



## Data analysis



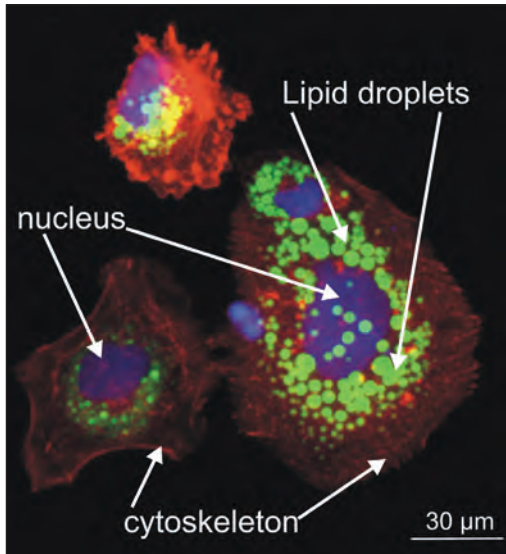
Gen5 includes data analysis tools that are often handled in third party software, enabling a complete workflow within one platform. Powerful analyses such as EC<sub>50</sub>, parallel line analysis, statistical analysis, heat maps and custom calculations are all built-in to Gen5.



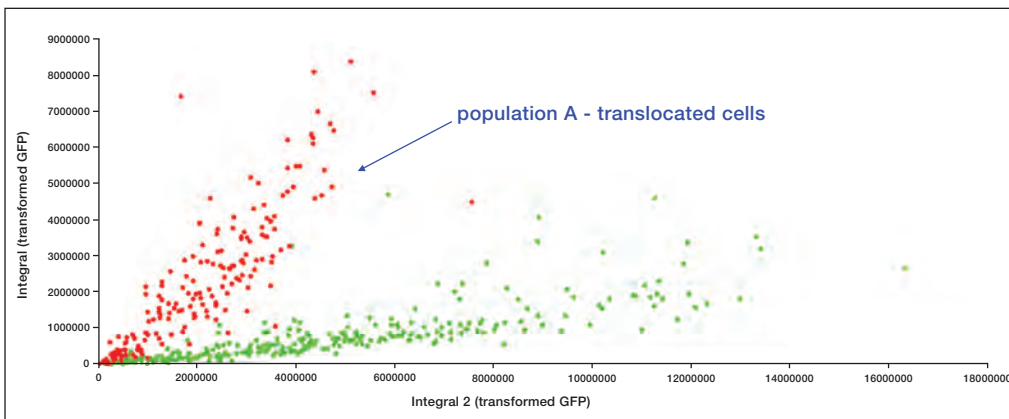
# Publish



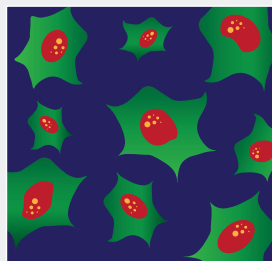
## Image & graph annotation



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.

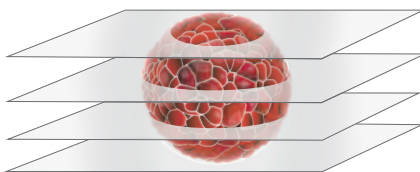
# Applications

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:

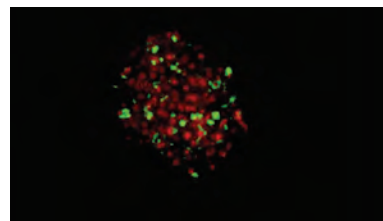
**capture ► process ► analyze ► publish** across a broad range of applications. Below are just a few examples of important applications easily managed with BioTek's imagers and Gen5 software.

## 3D NK cell cytotoxicity

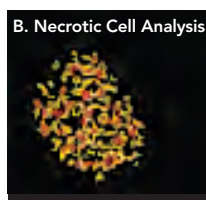
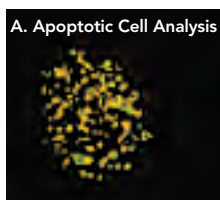
Cancer cells are suspended in hydrogel and propagate to form 3D tumoroids. Natural killer (NK) cells are then introduced and apoptotic and necrotic induction within cancer cells is then measured over 120 hours.



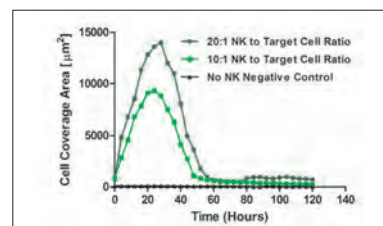
**Capture:** Three color Z-stacked images are captured of tumoroids in each well over 120 hours.



**Process:** Each set of z-stacked images is z-projected at each time point for analysis of apoptosis (green fluorescence) or necrosis (red fluorescence).



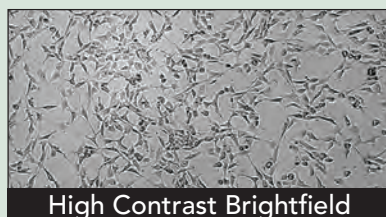
**Analyze:** Image analysis quantifies apoptosis (green fluorescence) and necrosis (red fluorescence).



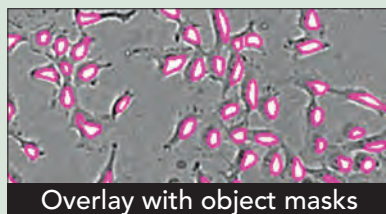
**Publish:** Apoptotic and necrotic induction are plotted over time for each condition.

## Label-free cell proliferation

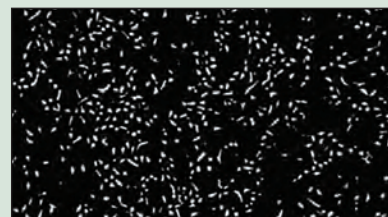
Cells are seeded into 96-well microplates at 2000 cells per well. Environmental conditions, including temperature (37 °C), gas (5% CO<sub>2</sub>) and humidity (90%) are maintained during a five day incubation by a BioSpa™ 8 Automated Incubator. Proliferation or drug-induced reduction in proliferation is detected by label-free cell counting using high contrast brightfield.



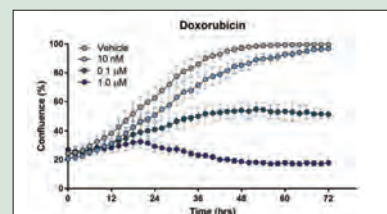
**Capture:** Each well is kinetically monitored every 2 hours using high contrast brightfield.



**Analyze:** The processed image is analyzed, cell objects are identified using intensity and size thresholds.



**Process:** All images are processed to maximize contrast of cells over background.



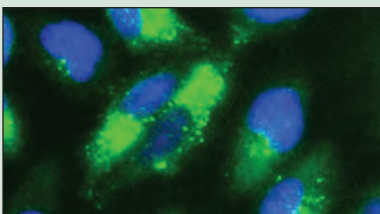
**Publish:** Anti-proliferative agent pharmacology can be published.



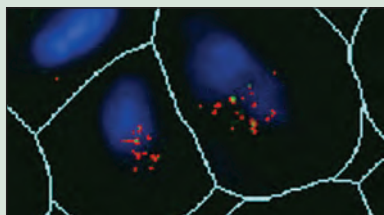
# Applications

## Autophagy (spot count)

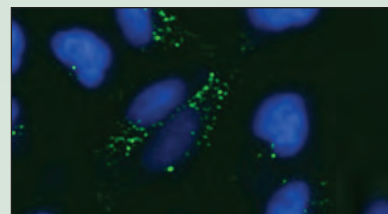
Cells are treated with autophagy-inducing compounds. CYTO-ID® dye in combination with automated object-based spot counting is used to quantitatively assess the effects of starvation and rapamycin on cellular autophagy by determining the size and number of autophagosomes per cell.



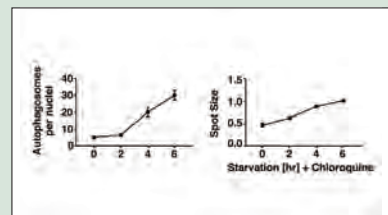
**Capture:** Each well is automatically imaged at 20x.



**Analyze:** The pre-processed image is analyzed, and each individual autophagosome is counted as a per-cell object.



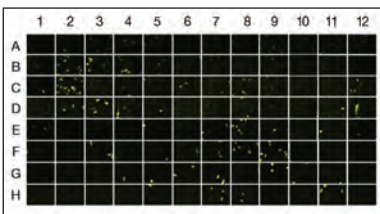
**Process:** Images are processed in order to better separate individual autophagosomes.



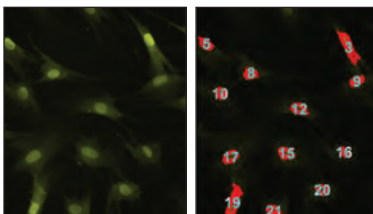
**Publish:** Consistent and precise measurement of spot count per cell (top) and spot size (bottom).

## Calcium Kinetics

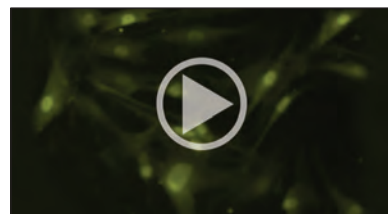
Calcium is increasingly appreciated for its critical signaling role within the cell. Advanced microscopy methods are allowing us to visualize calcium release in real time. Cells are plated subconfluently and loaded with the calcium indicator dye, Fluo-4. Stimulation of calcium release by histamine causes an acute Fluo-4 response.



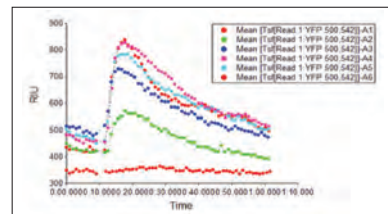
**Capture:** Kinetic images are captured every second for 2 minutes in each well of a 96-well plate.



**Analyze:** Cell counts are performed at the time point of peak signal for data normalization.



**Process:** Time-stamped movies are generated from these images showcasing calcium release and recovery.



**Publish:** Overlaid kinetic curves highlight the impact of experimental substrates on inhibition of calcium release.

# 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 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.



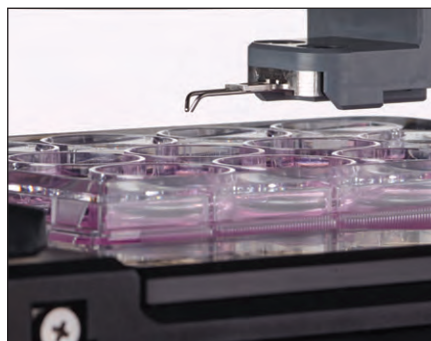
*Cytation integrated with BioSpa 8 Automated Incubator*

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.

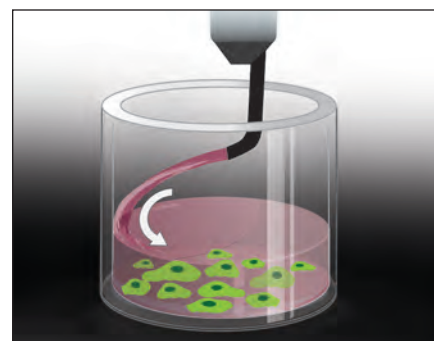
## CO<sub>2</sub>/O<sub>2</sub> control and reagent injectors



The compact gas controller maintains control of CO<sub>2</sub> and O<sub>2</sub> levels for live cell assays. The gas controller is for use with Lionheart FX and Cytation systems.



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



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



## Humidity control

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



## 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 FX and Cytation 5 have an automated 6-objective turret, Cytation 1 has an automated 2-objective turret.



## LED/Filter cubes

BioTek's imaging LED/filter cubes include high power LEDs and deep blocking filters. This design significantly reduces maintenance costs and provide full control over light intensity to increase image resolution or reduce phototoxicity when required. BioTek's design also includes a reference photodiode that controls a positive feedback loop - this ensures that illumination intensity is constant over time-lapse imaging experiments, making kinetic data truly quantitative.



## 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	Cytation 5	Cytation 1
<b>Imaging Methods</b>			
Fluorescence	•	•	•
High contrast brightfield	•	•	•
Brightfield	•	•	
Color brightfield	•	•	
Phase contrast	•	•	
Air	1.25x, 2.5x, 4x, 10x, 20x, 40x, 60x		
Phase	4x, 10x, 20x, 40x		
Oil Immersion	60x, 100x		
<b>General</b>			
Microplate type	6- to 1536-well plates		
Other labware	Slides, cell culture dishes & flasks, hemocytometers, chamber slides		
Incubation	to 40 °C	to 65 °C	to 45 °C
Humidity control available	•		
Joystick controller	•	•	
Multi-mode detection capable		•	•
BioStack and BioSpa compatible		•	•





Think Possible with BioTek



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## 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. Founded 50 years ago, we are the only life science instrumentation company with corporate headquarters, manufacturing, research and development, applications and service in the USA.

Please visit [www.biotek.com](http://www.biotek.com) to learn about other BioTek Life Science Instrumentation.

Think Possible



BioTek Instruments, Inc.

Phone: (802) 655-4040 • Toll-Free: (888) 451-5171 • Outside the USA: (802) 655-4740

[www.biotek.com](http://www.biotek.com)

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