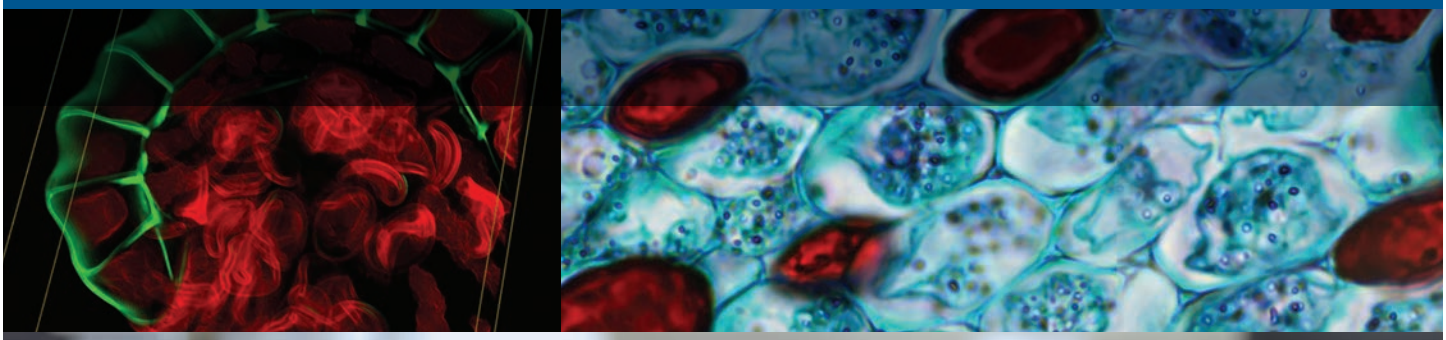
Agilent
Trusted Answers

Agilent BioTek Imaging and Microscopy

Ready for any assay



Agilent BioTek imaging and microscopy

Designed for a wide range of applications and budgets

Agilent BioTek automated cell imagers and microscopes bring your science to life, capturing spectacular images, Z-stacks, montages, and time-lapse sequences with ease. With up to 100x magnification, plus brightfield, color brightfield, phase contrast, and fluorescence channels, these instruments support a wide range of microscopy workflows including live cell kinetics. The compact, modular systems and Agilent BioTek Gen5 software for imaging and microscopy automate image capture, process, and analysis workflows to meet most laboratory budgets.

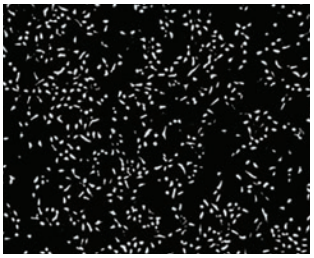
Agilent BioTek Cytation C10 confocal imaging reader.

Agilent BioTek Cytation 7 cell imaging multimode reader.

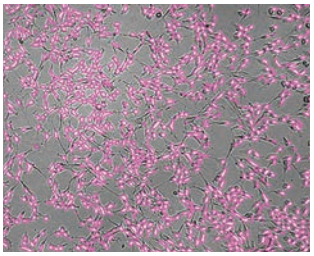
Agilent BioTek Lionheart FX automated microscope.

Augmented microscopy

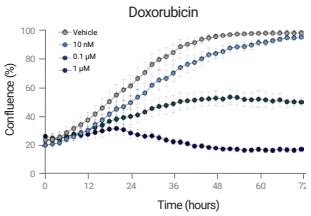
Capture



Process



Analyze



Publish

Agilent BioTek 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.

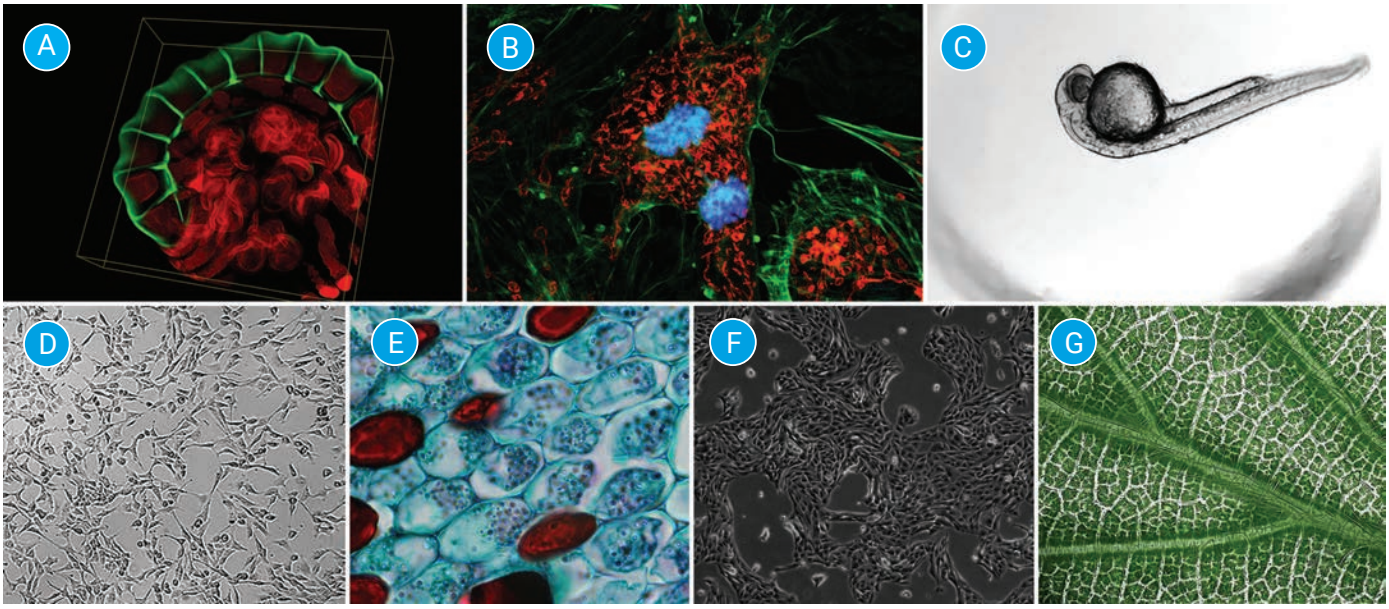
Capture

Capture modes in Gen5 provide simple, and efficient image capture, including live cell kinetic sequences.

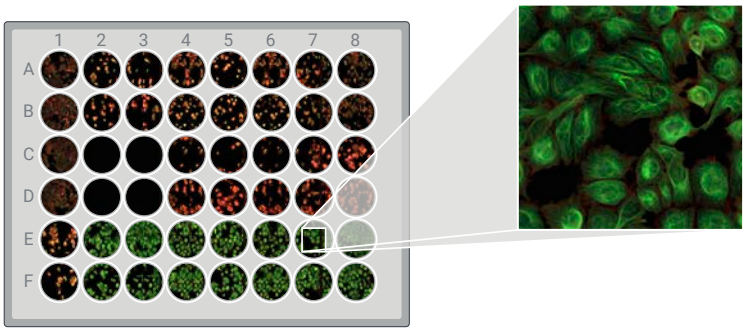
4x zebrafish embryo.

Capture

Seven imaging modes

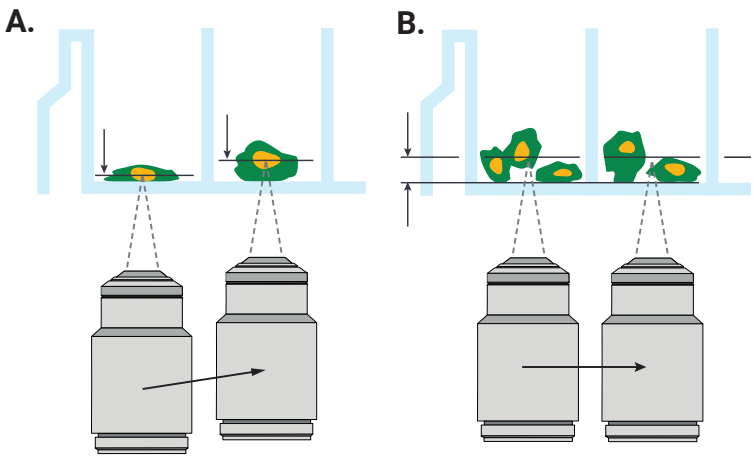


Powerful imaging for a wide range of applications, including live and fixed cell biologies: **A.** confocal, **B.** fluorescence, **C.** brightfield, **D.** high-contrast brightfield, **E.** color brightfield, **F.** phase contrast, **G.** upright reflected and transmitted light.



Batch mode

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

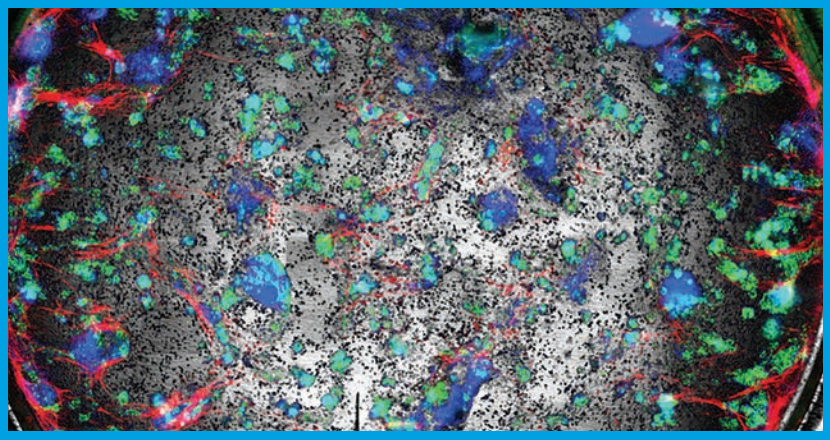


Laser and image autofocus

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

B. 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 kinetic imaging.

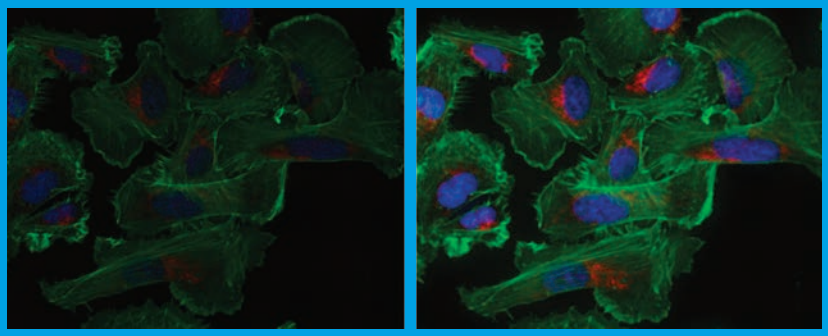
Capture



Up to four channel overlays

Four fluorescence channels plus brightfield provide maximum versatility.

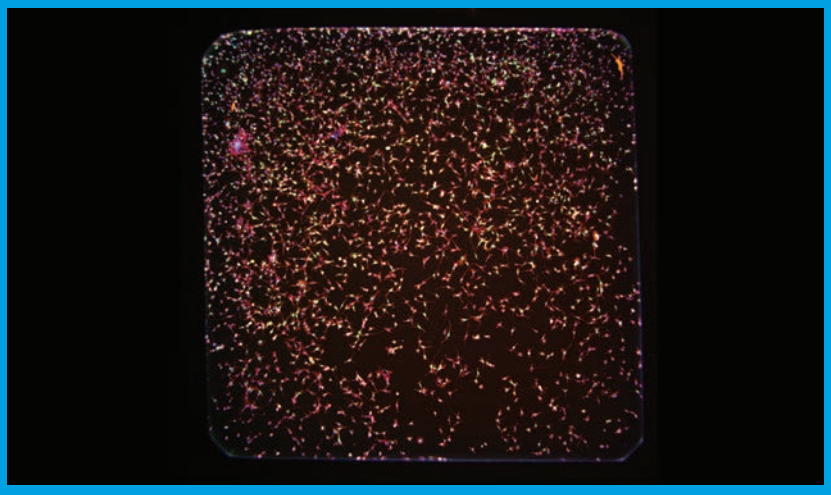
Choose from over 20 available LED/filter cube colors to cover a very broad range of fluorescent stains. AutoLED intensity ensures consistent capture for end-point and kinetic sequences. Each channel can be automatically adjusted and optimized–changes are easily saved.



Water immersion objectives decrease exposure times

Water immersion objectives capture more light with lower exposure times, reducing the potential for phototoxicity and photobleaching in live cells. The increased signal enables more detailed analyses without increasing gain and noise.

Cells captured with 40x air (left), 40x water (right) using identical exposure settings.

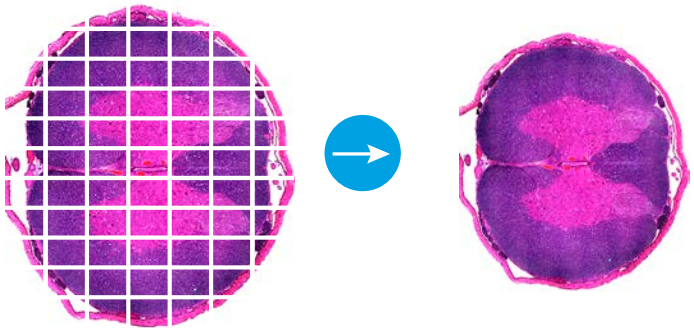


Whole-well imaging

The wide field of view (WFOV) camera available on the Cytation and Lionheart platforms enables capture of an entire well of a 384-well plate in a single image. High-resolution cellular screens can be captured much more rapidly, since multiple images are not required. The WFOV camera captures more cells in the field of view at higher magnification, providing a more statistically relevant population of cells in fewer images.

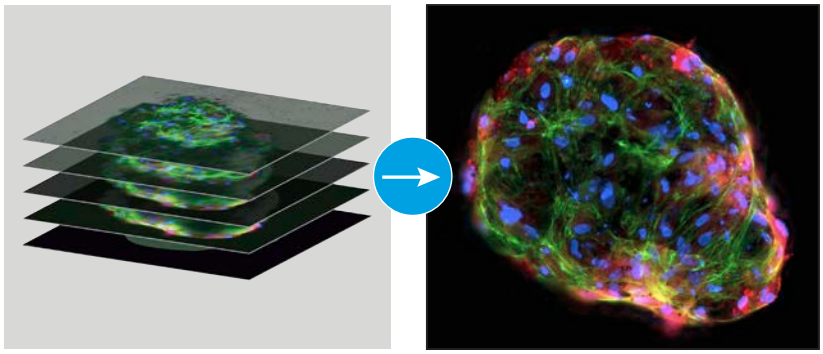
Capture

Montage



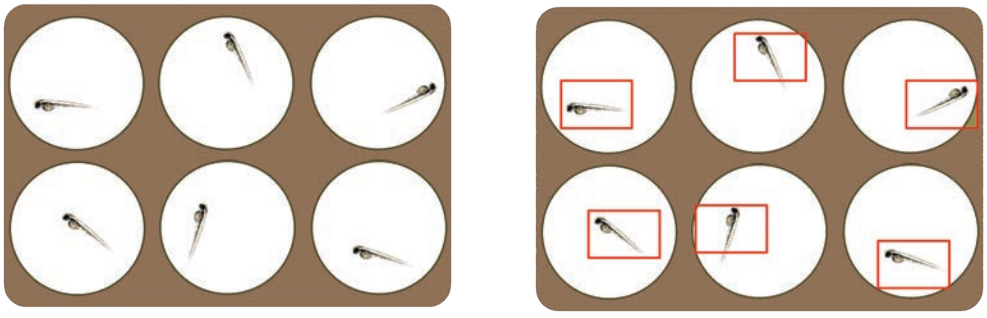
Capture large samples like tissue sections (H&E); increase sample size for better data quality or to detect rare events. Montage image capture mode acquires up to 2,000 tiled images per imaging channel. Each tile of a montage is saved as an individual, high-resolution 16-bit TIFF. Stitching in Gen5 software creates a seamless image.

Z-stack



Z-stacking in Gen5 software enables capture of up to 200 customizable slices–as thin as 0.1 µm–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.

Beacons



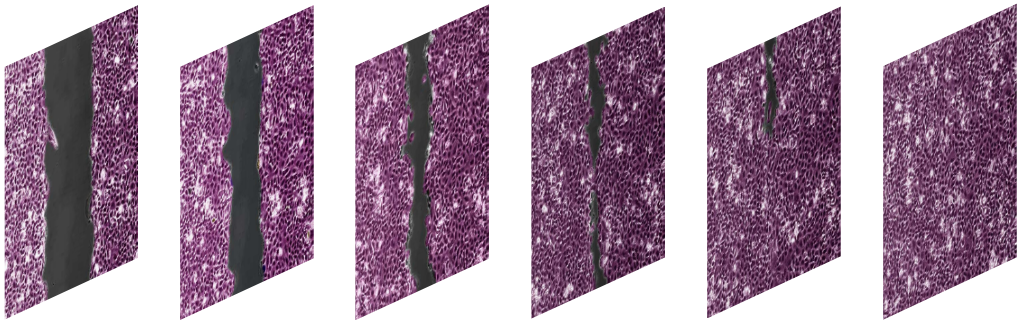
Beacons are used to define custom X/Y offsets for imaging in a well or vessel. Beacons are useful for monitoring regions of interest specific to each well or area to be imaged, as shown in this zebrafish example.

Capture

Live cell assay support

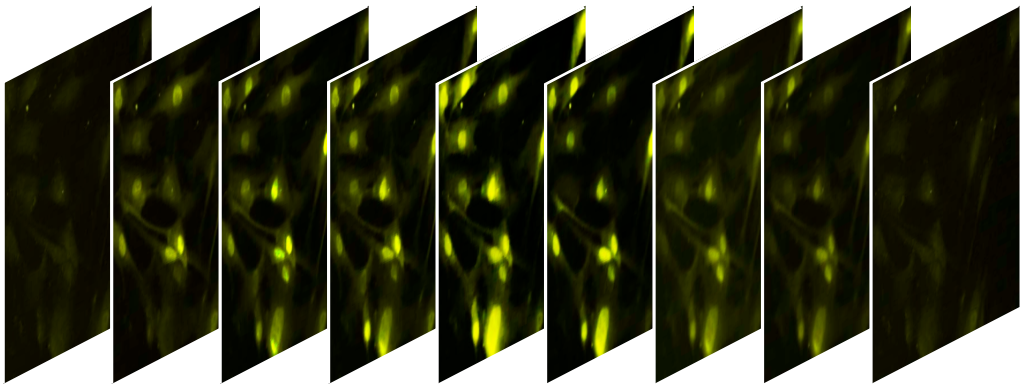
Temperature control, including the Agilent BioTek Condensation Control gradient, plus CO_2/O_2 control and humidity options, provide the ideal environment for live cell assays. Observe label-free assays with brightfield and high-contrast brightfield imaging, or fluorescence assays in up to four colors plus brightfield. Time-lapse image sequences are easily, automatically compiled into video.

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.

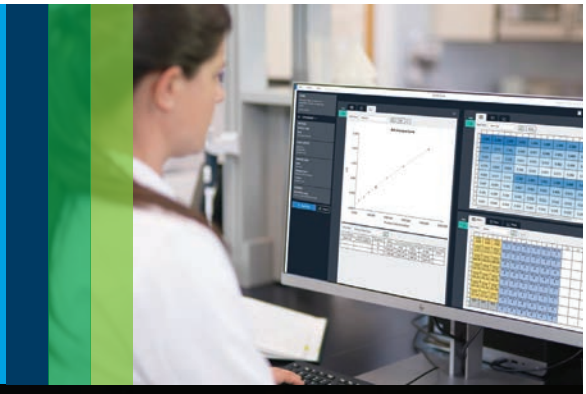
High-speed imaging

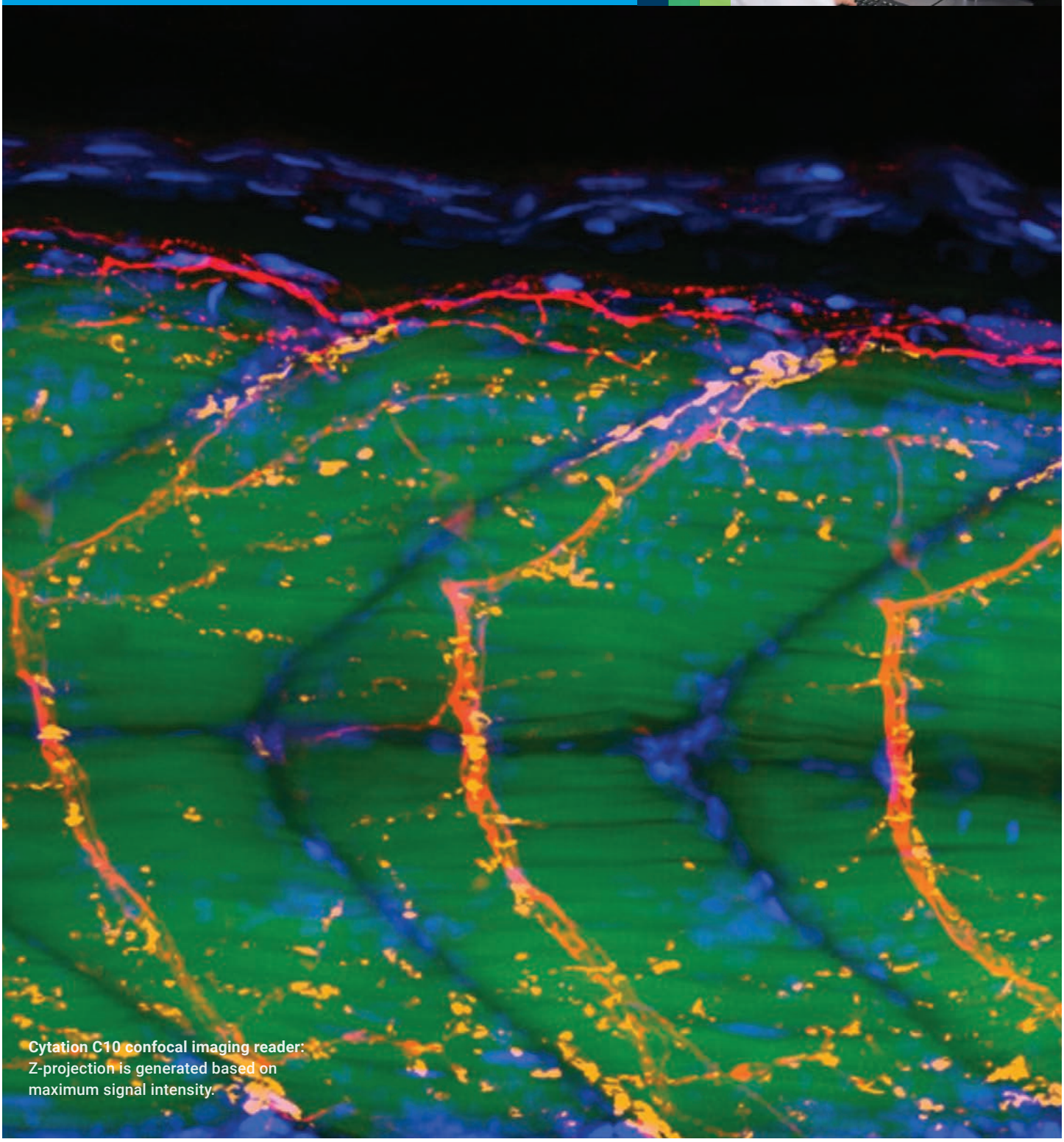


Time: Seconds to hours. Very fast reactions such as calcium flux kinetics are enabled with dual-reagent injectors–images are automatically captured at up to 20 frames per second.

Process

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

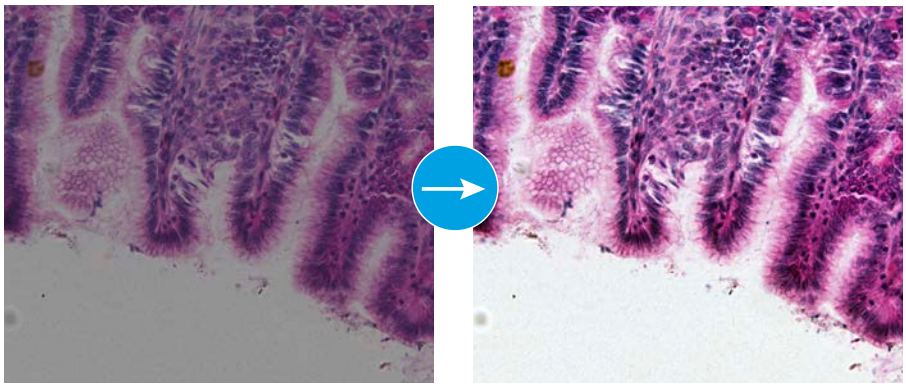




Cytation C10 confocal imaging reader: Z-projection is generated based on maximum signal intensity.

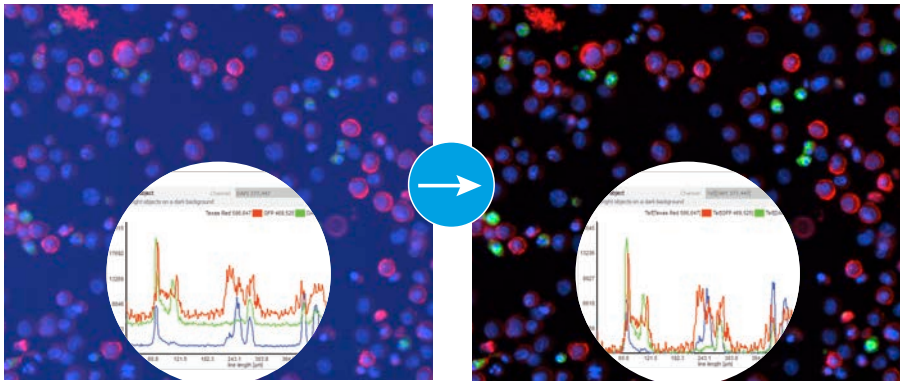
Process

Agilent BioTek Lionheart FX automated microscope shown with CO_2/O_2 gas controller and dual-reagent injector.



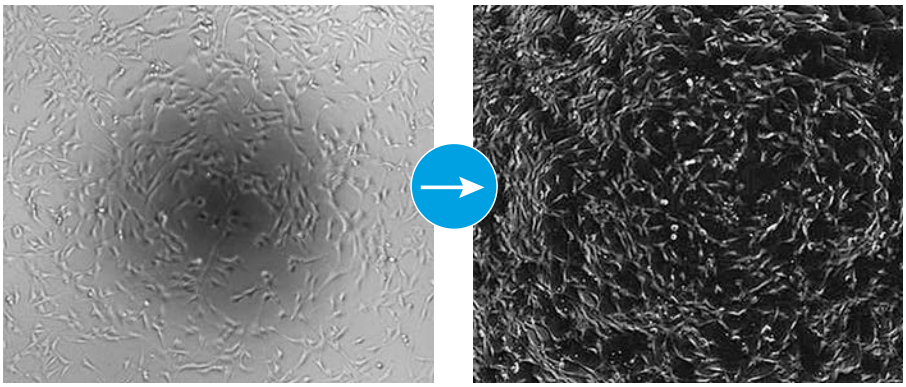
Powerful review tools

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



Background flattening

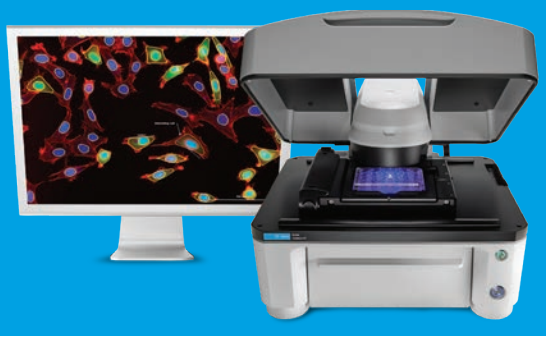
Background flattening using a rolling-ball algorithm prepares the image for analysis by removing background artifacts and correcting for uneven illumination. Use the line profile tool to find recommended threshold values for image analysis.

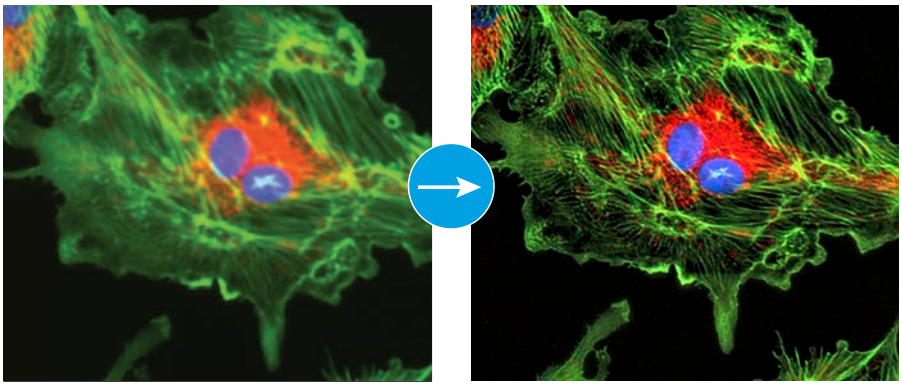


Digital phase contrast

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

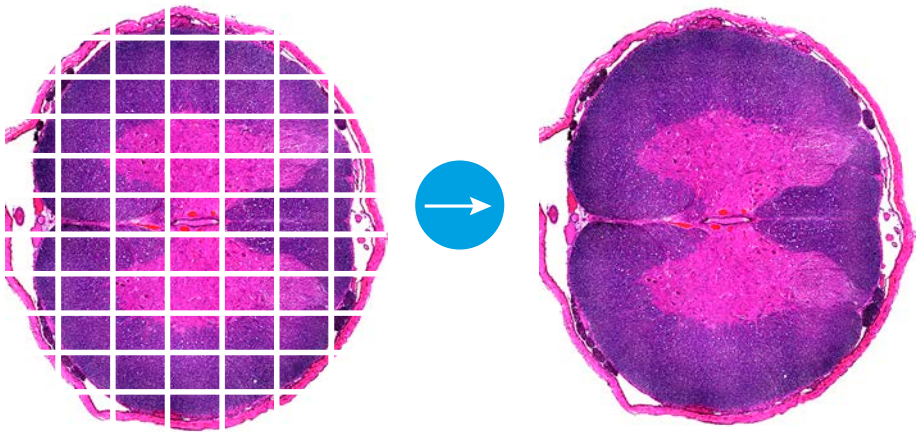
Process





Deconvolution

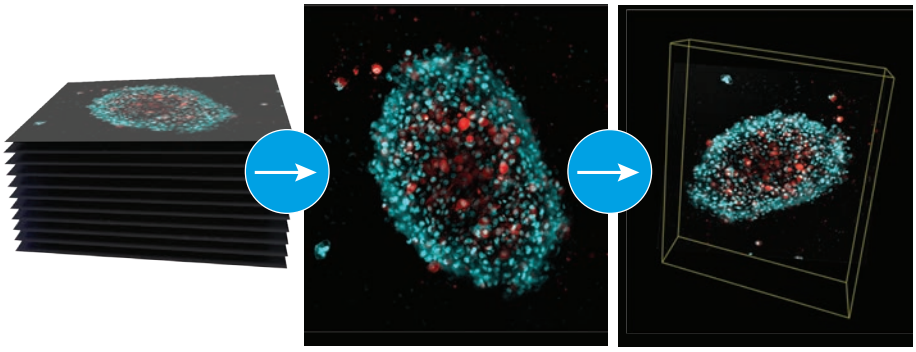
Deconvolution reduces blur from out-of-focus light, commonly seen in widefield imaging. It improves image resolution, enabling better visualization of image details.



Stitching

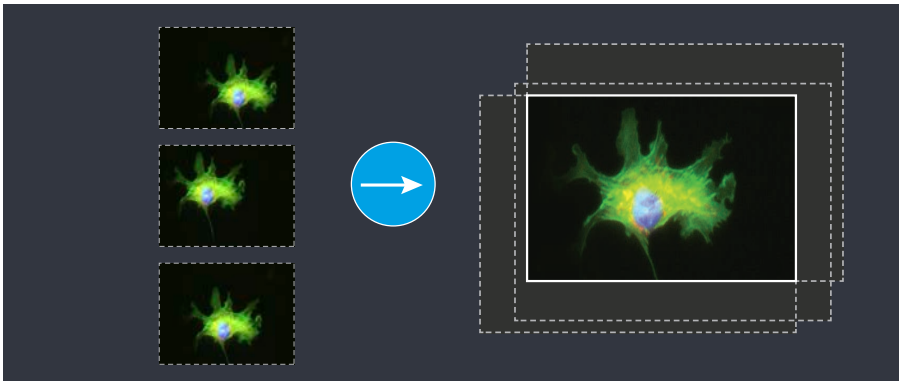
After capturing a large field of view with an image montage, Gen5 software automatically and precisely stitches the montage into a single uniform, high-resolution image. Gen5 can correct common artifacts seen with some montages, such as tiling effects. The stitching process automatically adjusts and corrects for a seamless image.

Process



Z-projection with 3D rendering

Following capture of a stacked set of images, Gen5 creates a final image containing the most in-focus information from each slice. The 3D viewer is used to explore the sample in greater detail.



Kinetic image alignment

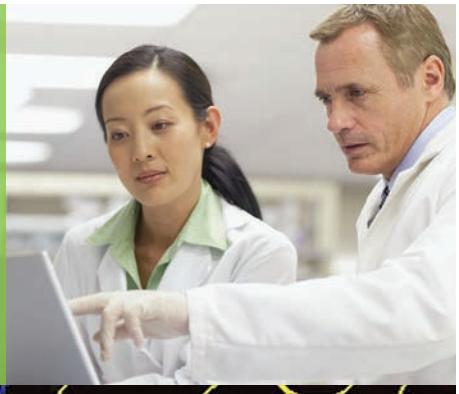
During live cell kinetic imaging, samples can sometimes shift slightly. Gen5 automatically adjusts for positional differences, keeping the region of interest fully stabilized, even during long-term imaging.

Movie file creation

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

Analyze

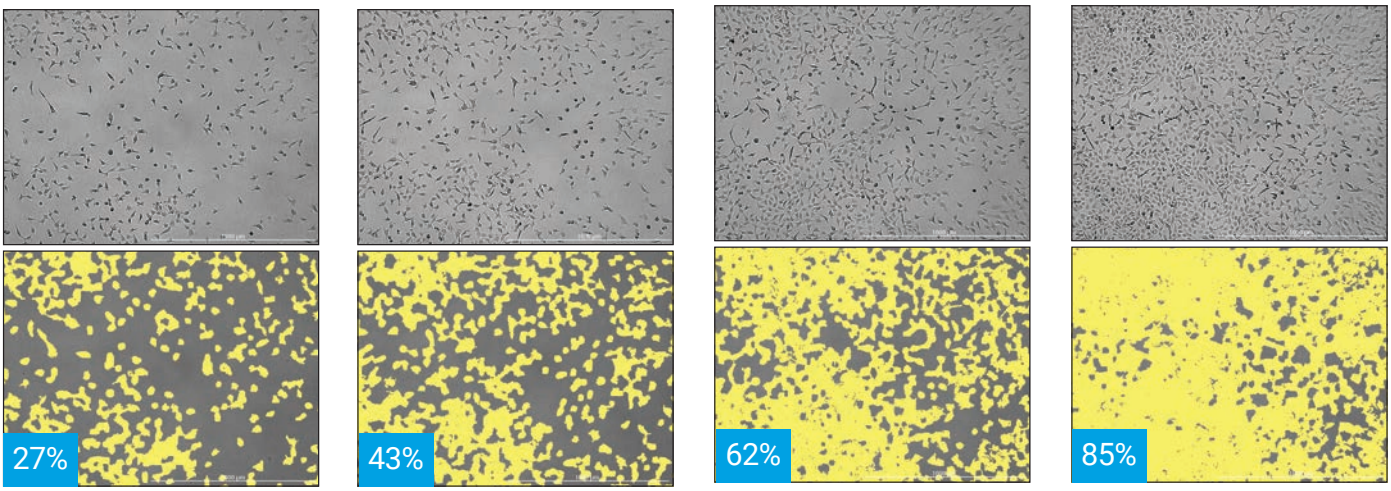
Captured, processed images are ready for analysis. Image analysis tools in Gen5 cover a very broad range of application requirements, and are both powerful and easy to use. Analysis functions in Gen5 extend to quantitative data as well.

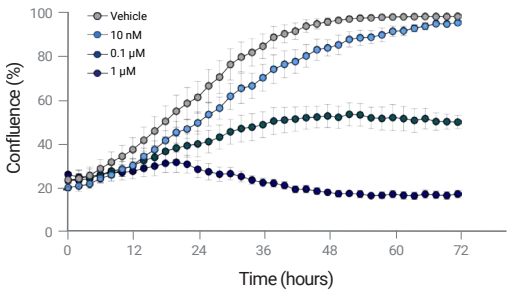


Analysis of induced apoptotic and necrotic activity in a cell population.

Analyze

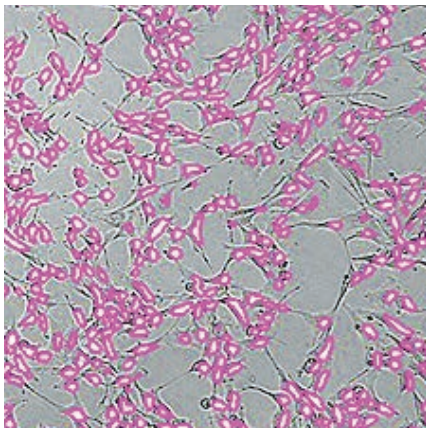
Primary analysis–confluence





Confluence measurements quickly and accurately identify areas of an image meeting designated criteria. In cell growth, health and proliferation studies, % confluence is an important measurement.

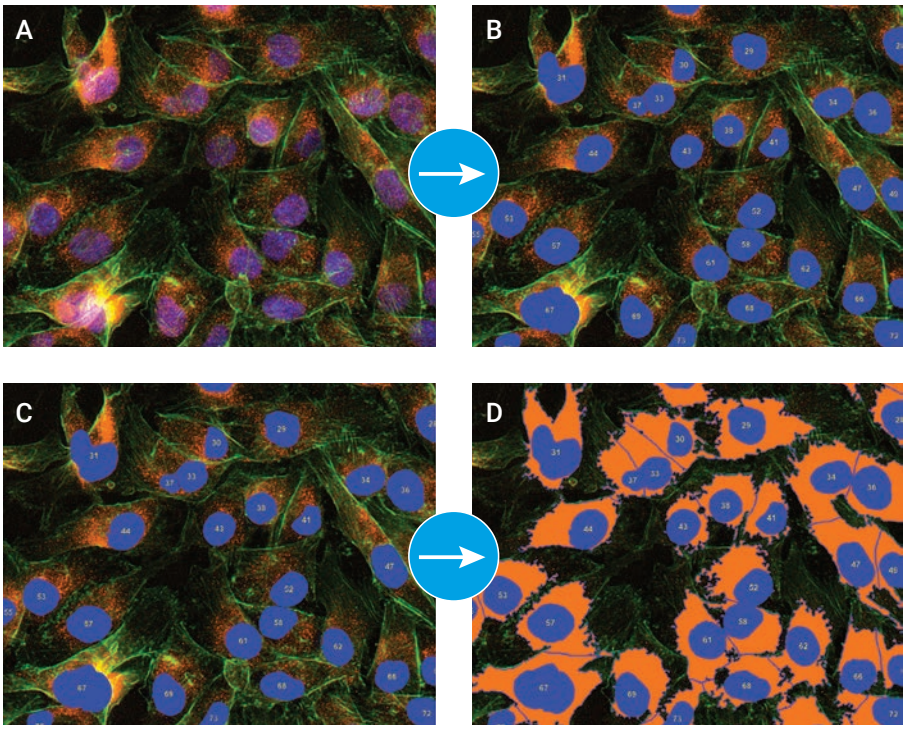
Primary analysis–label-free cell count



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

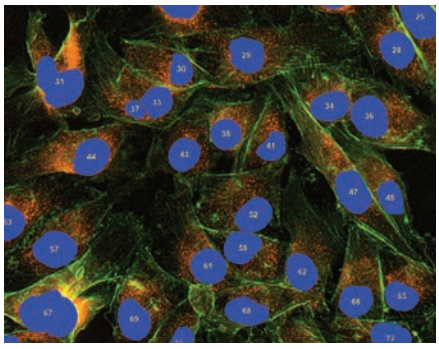
Along with label-free confluence measurements, label-free cell counts are performed efficiently using high-contrast brightfield. Cell counts are essential to cell growth, health, and proliferation studies. Gen5 efficiently identifies highly confluent cells without dyes.

Analyze



Automated cell identification, counting, and analysis

The primary cell analysis capabilities in Gen5 enable automated confluence determination and object counting, ranging from whole organisms to subcellular structures (A and B). Each can be done using transmitted light or fluorescence imaging channels. The secondary cellular analysis capabilities enable masking of a second area around the primary mask to gain additional information specific to that object (C and D). This can be critical for assays such as signal translocation, DNA damage, tumor invasion, evaluation of cytoplasmic structures, and others.



Original image.

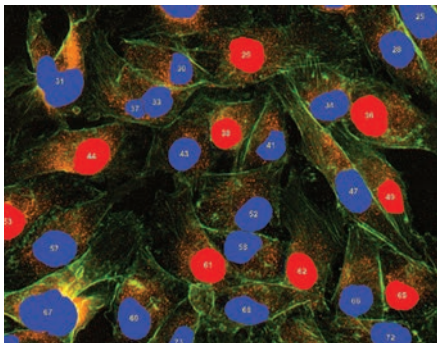
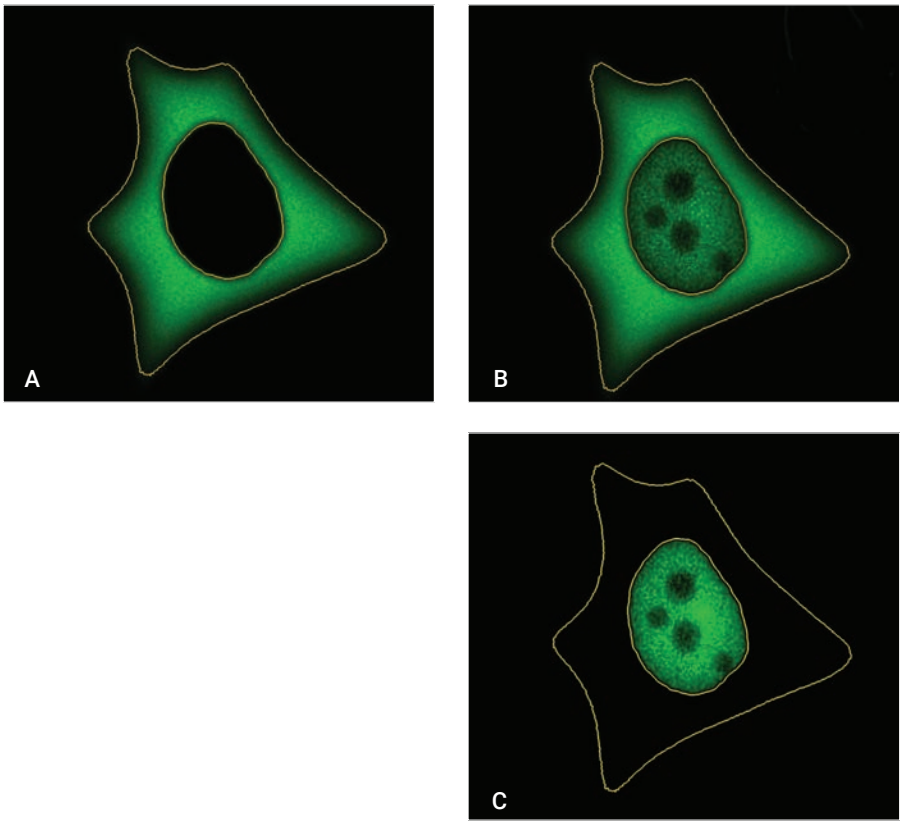


Image with mask applied.

Subpopulation analysis

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

Analyze



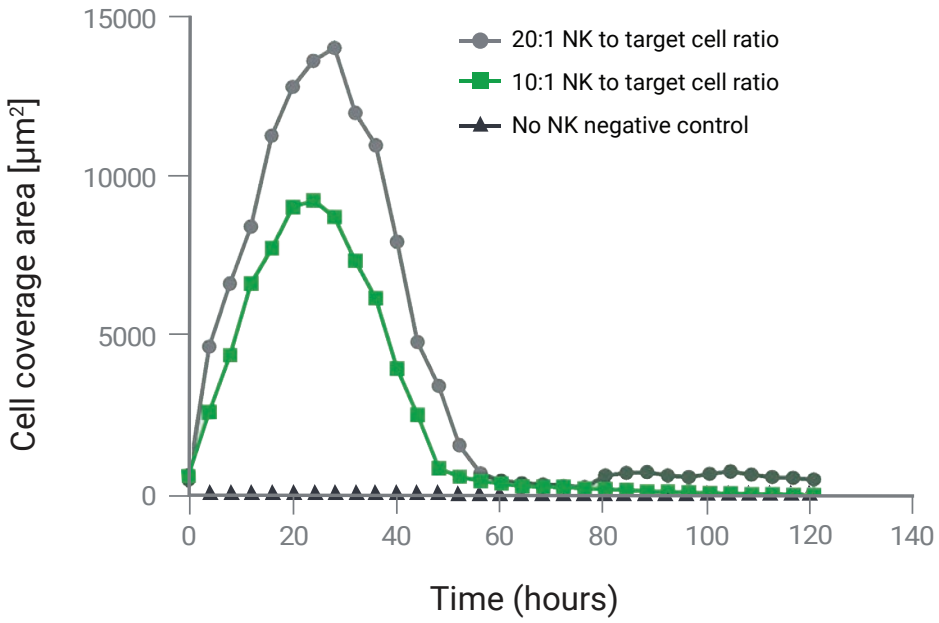
Signal translocation

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.



Kinetic analysis

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

Kinetic calculations can include cell count, rate of change, minimum/maximum signal, lag time, peak response, and many others.

Advanced Gen5 image analysis modules

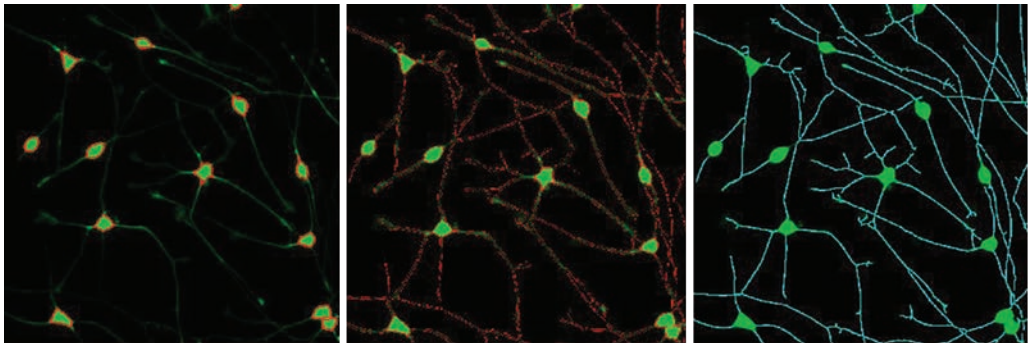
Beyond the powerful analysis features in Gen5 software, specialized add-on modules expand method-specific analyses to automate processes and generate advanced metrics.

Neurite outgrowth

The neurite outgrowth module for the Agilent BioTek Gen5 software enables the analysis of neuronal cell metrics and growth using fluorescent or label-free methods. The neurite outgrowth analysis capabilities within the Gen5 module facilitate research into normal development and regeneration, neurotoxicity, and neurological disorders.

Assessing neurite outgrowth

The Agilent BioTek Gen5 neurite outgrowth module accurately quantifies neuronal cell metrics and provides masking options including soma and neurite masks, along with skeletonized images. Calculated metrics include soma and neurite counts, total neurite length, neurite thickness, total neurite area, and total neurite branches, among others.

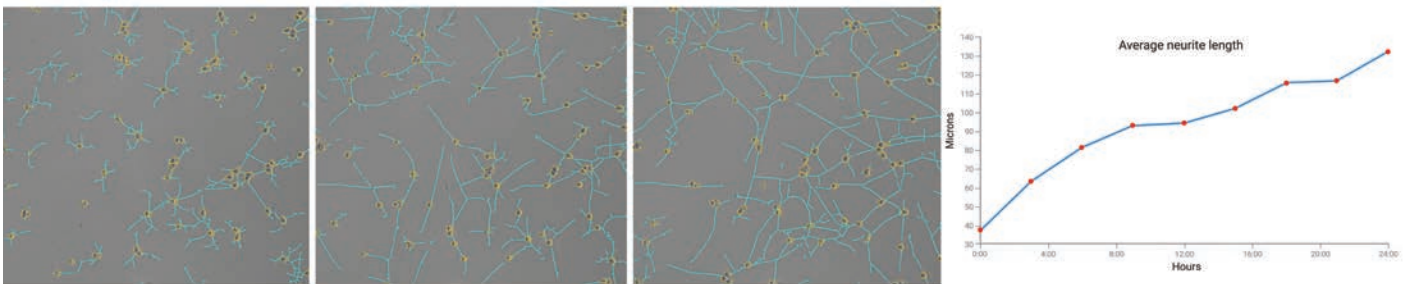


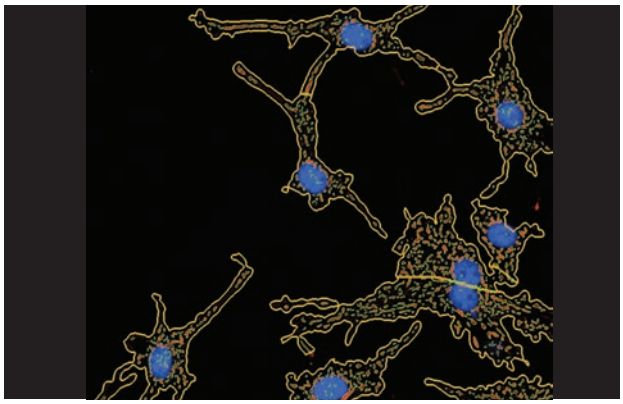
Soma mask | Neurite mask | Skeletonized neurite image

Label-free kinetic neurite outgrowth

The Gen5 neurite outgrowth module accurately detects neuronal outgrowth in kinetically monitored, unlabeled live cells. The following images show iCell GlutaNeurons in an iPSC-derived neuron culture. The cells were imaged after plating for 24 hours at 20x magnification in widefield, with phase contrast. Time points shown, left to right, are at 3, 12, and 24 hours.

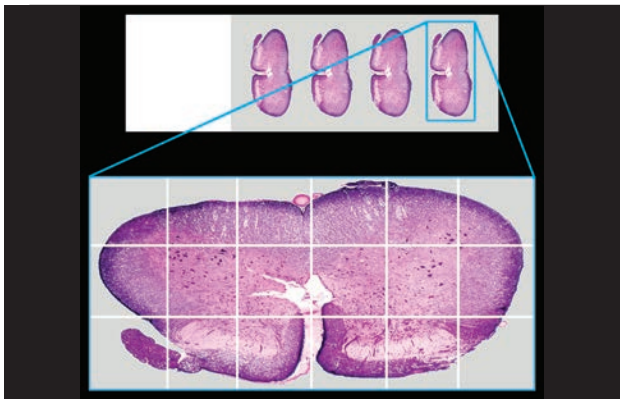
3 hours | 12 hours | 24 hours





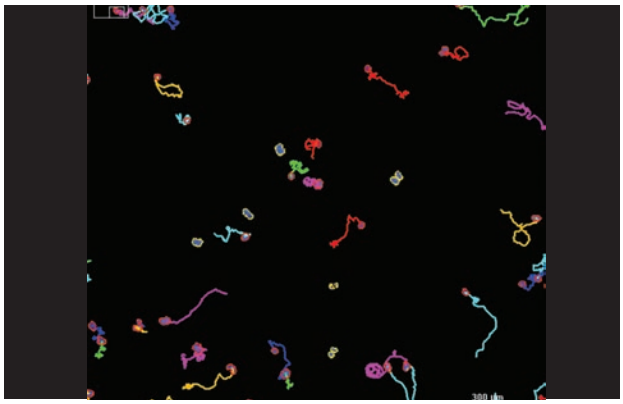
Spot counting

The Agilent BioTek Gen5 spot counting module enables users to gain information about a second set of objects within primary and/or secondary mask compartments, which are tied to the original primary mask data.



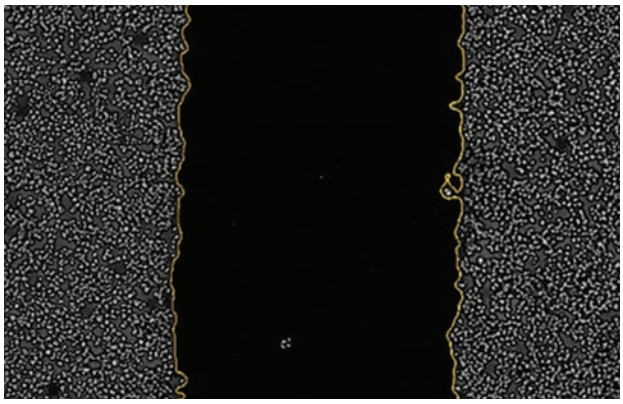
Automatic region of interest

The automatic region-of-interest (AutoROI) module is a three-step process to eliminate superfluous image capture. A low magnification step quickly images the entire area. The regions of interest are automatically identified, and then imaged at high magnification.



Single-object tracking

The Agilent BioTek Gen5 object tracking module provides the ability to track single objects over time. Relative motility can be visualized by selecting single cells or entire populations within an image. Calculated metrics include total distance, Euclidean distance, and mean, median, and maximum object velocity.

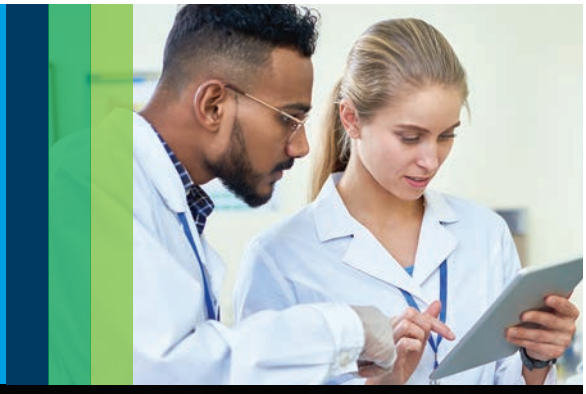


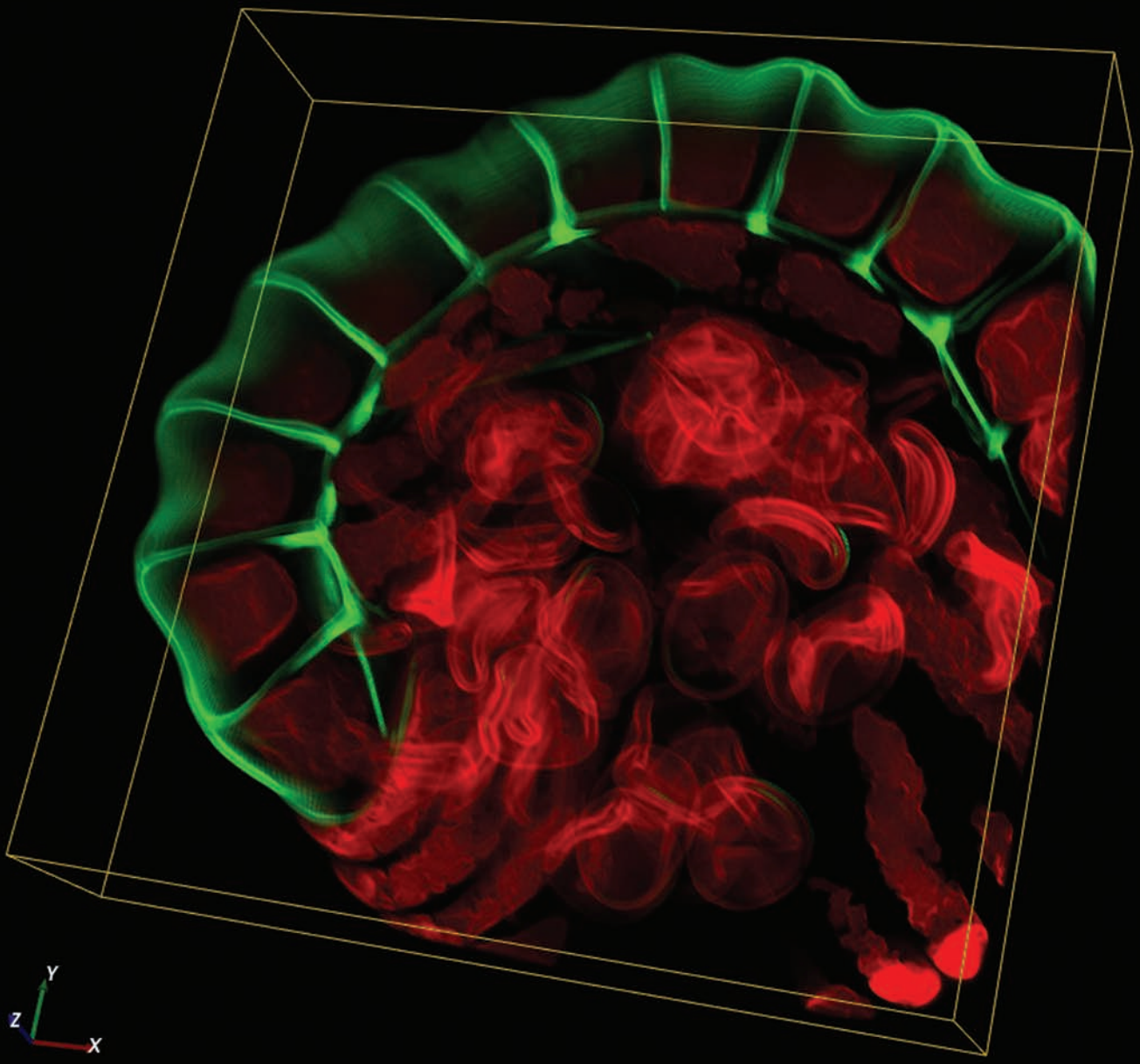
Scratch wound healing assays

The Agilent BioTek Scratch Assay app provides an integrated workflow to capture, process, and analyze images from 2D scratch-wound healing assays. Predefined protocols for 24- and 96-well plates include automated processing and analysis to calculate average wound width, percent wound confluence, and maximum wound healing rate.

Publish

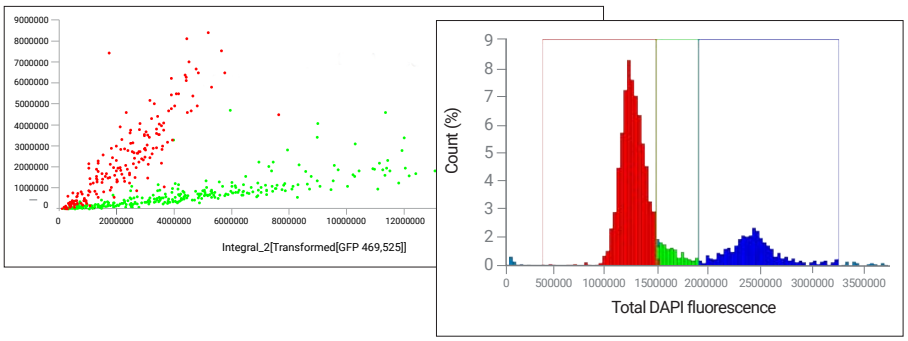
Agilent BioTek Augmented Microscopy tools include the ability to create publication-ready images, graphs, and data using the functions in Gen5 software. There is no need to export images or data to external software.





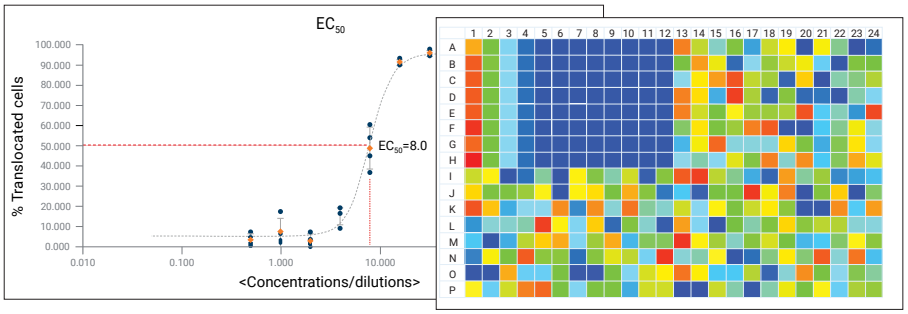
Fern sporangium imaged using Cytation C10 confocal imaging reader displayed in Agilent BioTek Gen5 software 3D viewer.

Publish



Scatter plots and histograms

Commonly used in flow cytometry, scatter plots are a powerful tool for visualizing 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 software 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 into Gen5.

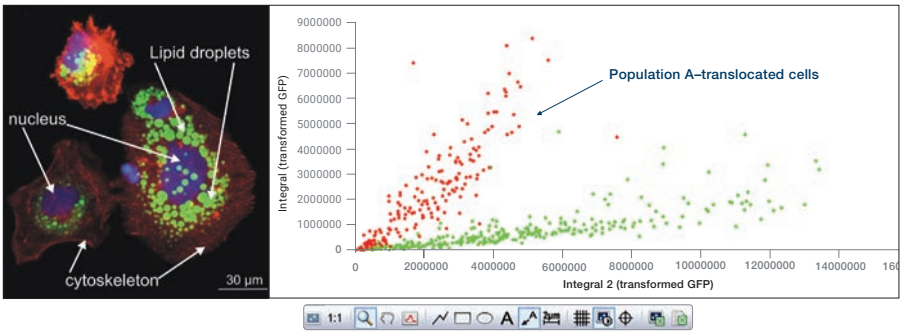
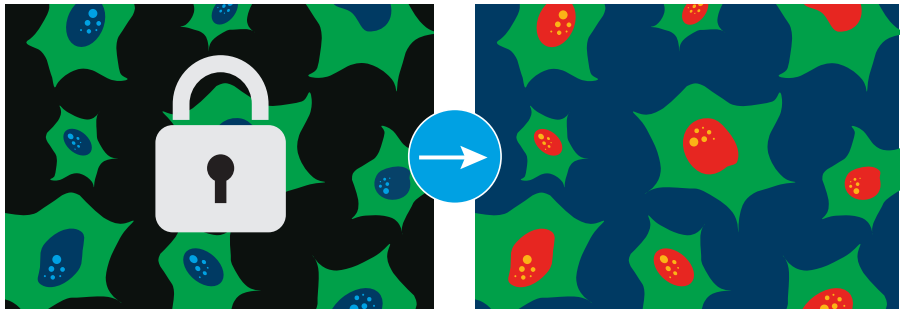


Image and graph annotations

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.



Raw image retention

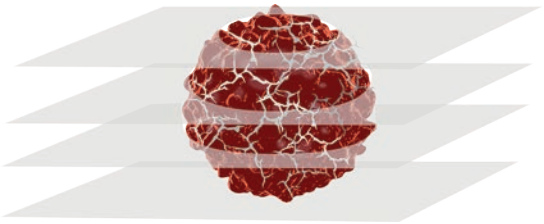
Before processing or analyzing images, Gen5 makes a copy of the raw data and the raw data is retained as a separate file. Gen5 protects raw images and provides traceability from the raw to the modified images.

Select applications

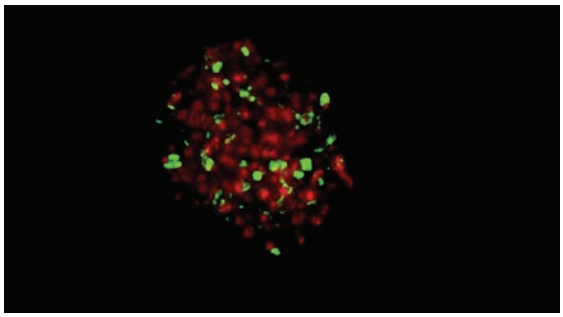
Agilent BioTek imaging and microscopy instruments, along with Gen5 software, are capable of automating a broad range of application workflows. Augmented Microscopy tools guide users through the four major steps of microscopy–capture, process, analyze, and publish across a broad range of applications.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

1. Three-dimensional natural killer cell cytotoxicity

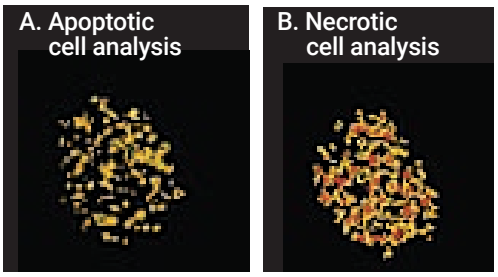
Cancer cells are suspended in hydrogel and propagate to form 3D tumoroids. Natural killer cells are then introduced and apoptotic and necrotic induction within cancer cells is then measured over 120 hours.



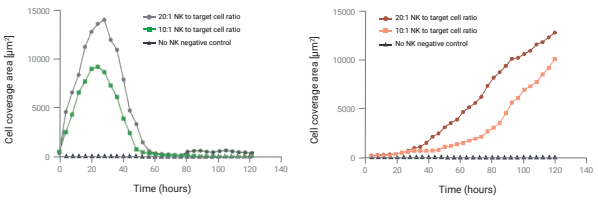
Capture → Three-color Z-stacked images are captured of tumoroids in each well over 120 hours.



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

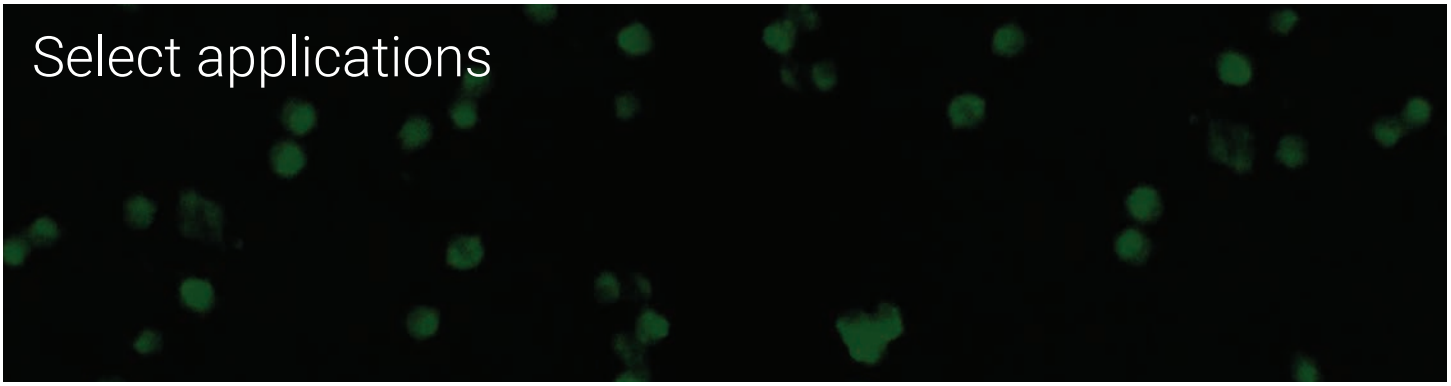


Analyze → Image analysis quantifies apoptosis (green fluorescence) and necrosis (red fluorescence).



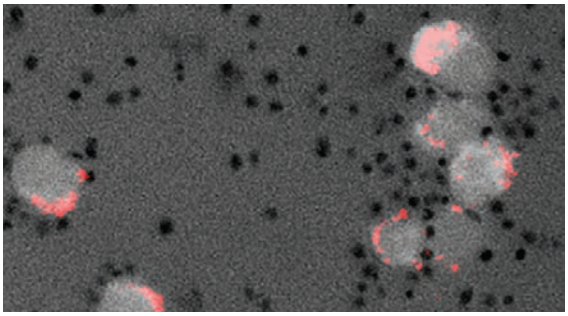
Publish → Apoptotic and necrotic induction are plotted over time for each condition.

Select applications

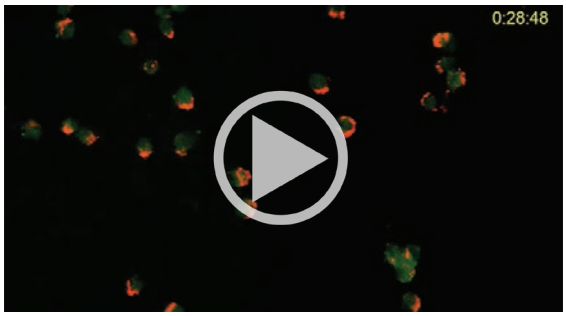


2. Phagocytosis assay

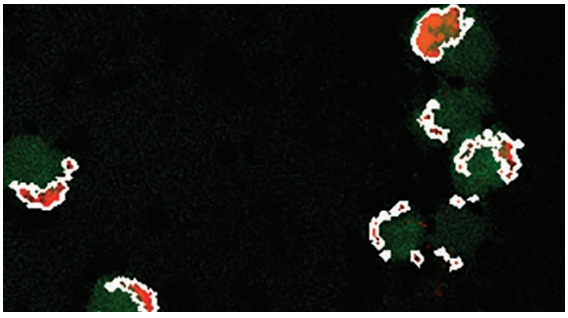
Macrophages are specialized cells that consume and digest foreign matter through phagocytosis. pH-sensitive bioparticles are a useful tool for studying phagocytosis, as particles fluoresce in response to the acidic environment of phagolysosomes. Cellular actin enables unique physical changes that are necessary for phagocytosis. The following assay analyzed effects of actin disruption on bioparticle phagocytosis.



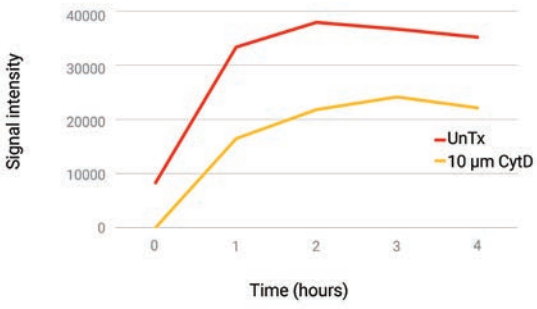
Capture → A two-channel image at one kinetic time point shows black extracellular bioparticles in contrast to the red fluorescence of phagocytized bioparticles (red).



Process → A time-stamped movie is generated from static images, showing an increase in bioparticle phagocytosis over time (orange).

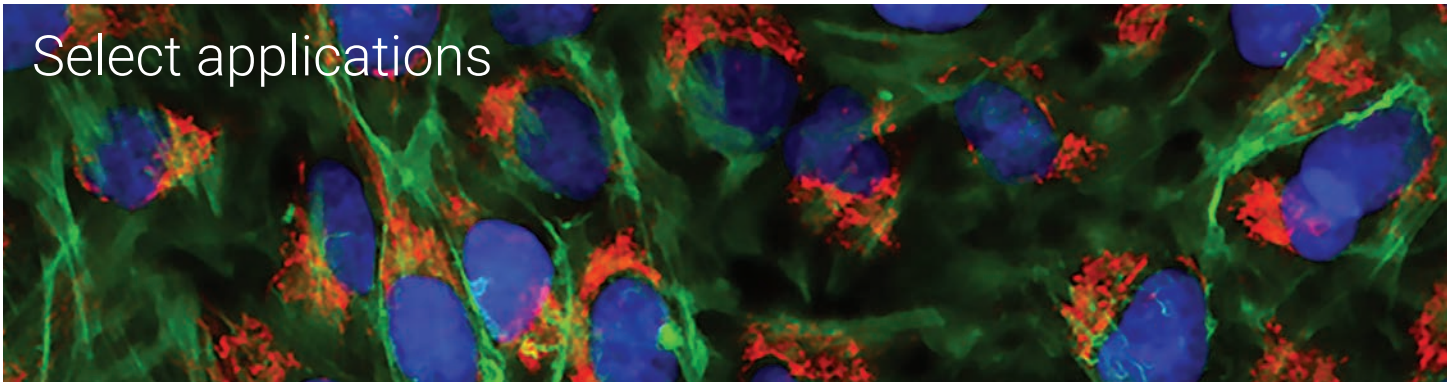


Analyze → A primary mask on bioparticle phagocytosis is applied to all kinetic images.



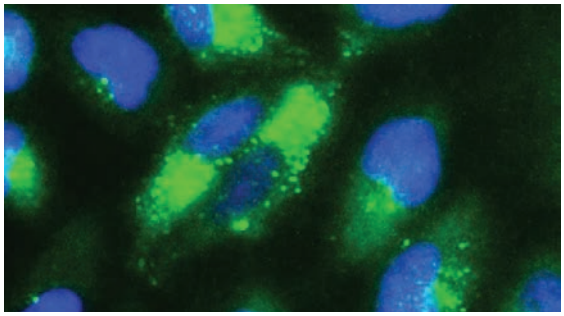
Publish → Compared to untreated macrophages (red), actin disruption causes decreased bioparticle phagocytosis (yellow).

Select applications

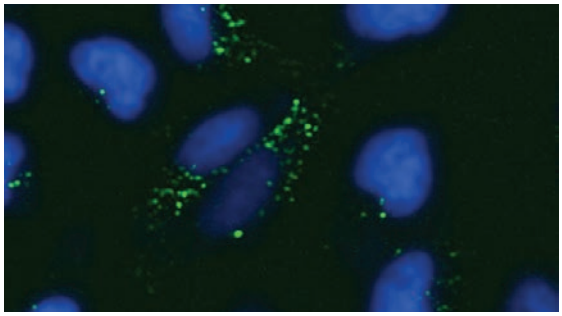


3. Autophagy (spot count)

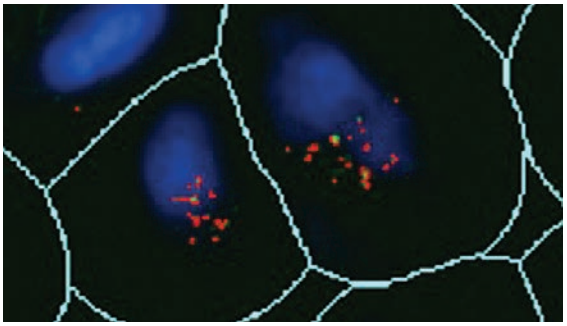
Cells are treated with autophagy-inducing compounds. CYTO-ID dye in combination with automated object-based spot counting is used to quantitatively assess the effects of starvation and rapamycin on autophagy by determining the size and number of autophagosomes per cell.



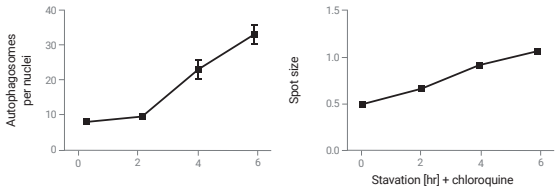
Capture → Each well is automatically imaged at 20x.



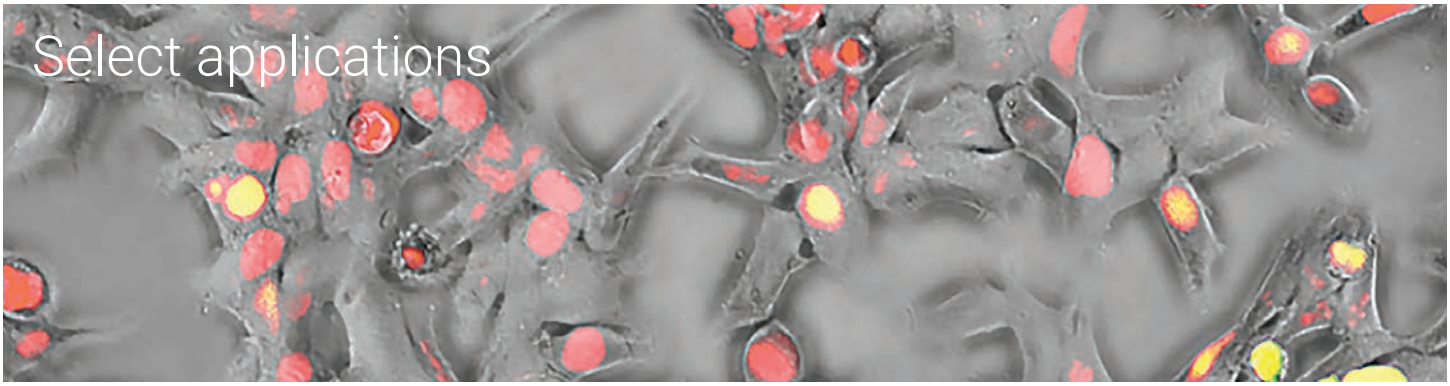
Process → Images are processed in order to better separate individual autophagosomes.



Analyze → The preprocessed image is analyzed, and each individual autophagosome is counted as a per-cell object.

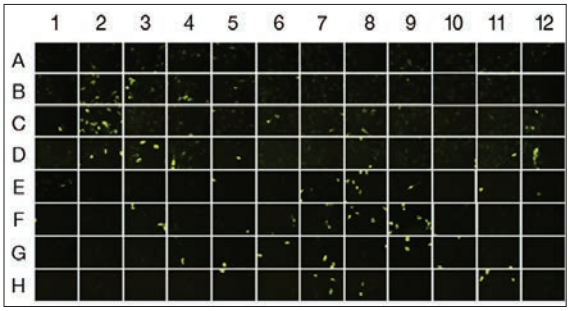


Publish → Consistent and precise measurement of spot count per cell (left) and spot size (right).

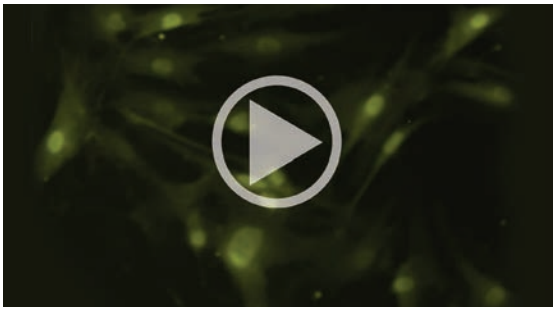


4. Calcium kinetics

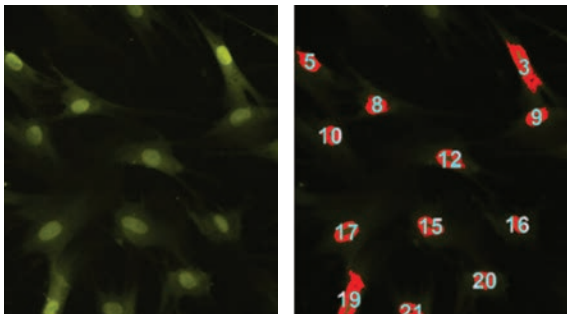
Calcium is increasingly appreciated for its critical signaling role within the cell. Advanced microscopy methods allow us to visualize calcium release in real time. Cells are plated subconfluently and loaded with the calcium indicator dye, Fluo-4. Stimulation of calcium release by histamine causes an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



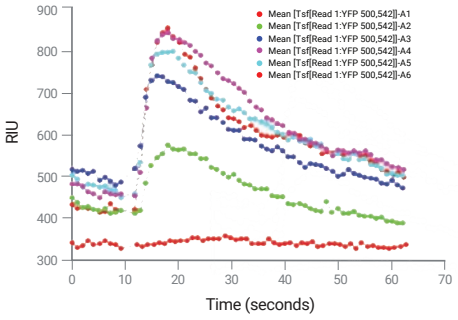
Capture → Kinetic images are captured every second for 2 minutes in each well of a 96-well plate.



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

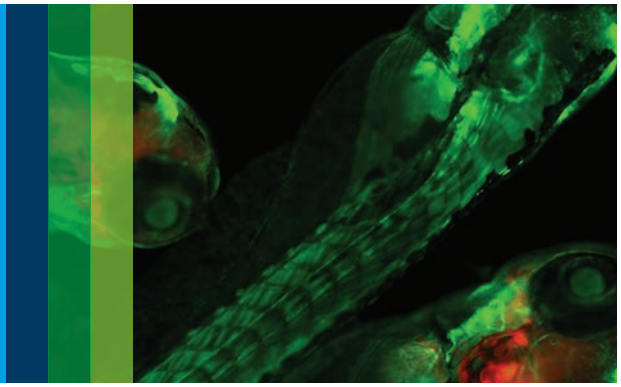


Analyze → Cell counts are performed at the time point of peak signal for data normalization.



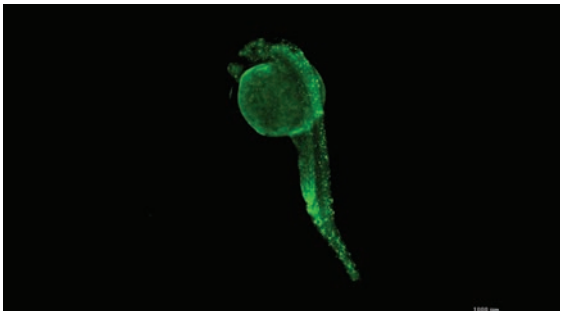
Publish → Overlaid kinetic curves highlight the impact of experimental substrates on inhibition of calcium release.

Select applications

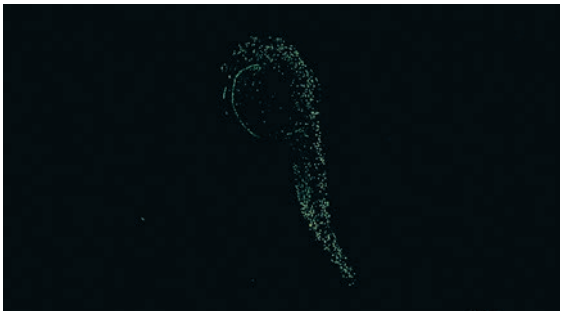


5. Measuring apoptosis in zebrafish treated with ethanol

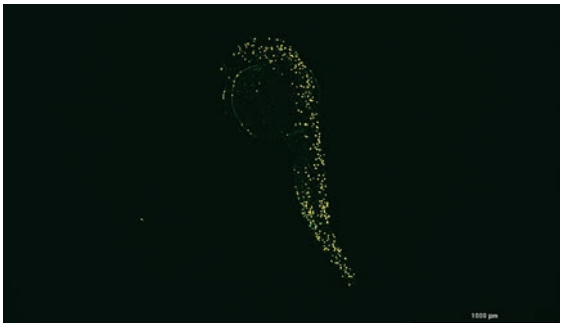
Zebrafish embryos are treated with ethanol during the first 24 hours of development, and the effect of ethanol treatment on cell death is assessed using acridine orange staining (green emission). Embryos were imaged in 9-well round-bottom plates with a 2x objective as Z-stacks in the brightfield and GFP channels.



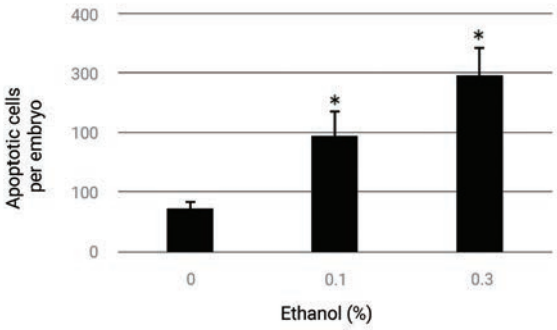
Capture → Each well is automatically imaged as a 2x Z-stack.



Process → Images are Z-projected then preprocessed in order to better separate individual positive cells.



Analyze → The preprocessed images are analyzed, and each individual GFP-positive cell is automatically identified and counted.

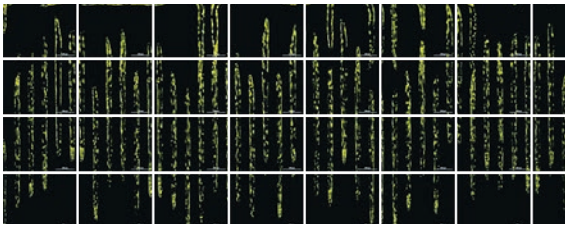


Publish → The effect of ethanol treatment on the number of apoptotic cells per embryo can be graphed for publication.

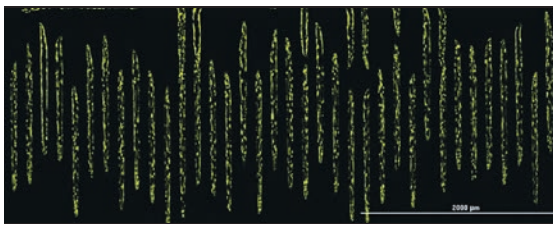
Select applications

6. Quantifying polyglutamine aggregates in *C. elegans* using vivoChip

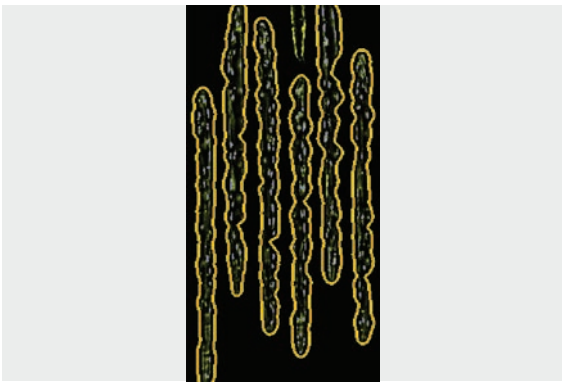
C. elegans have emerged as a tool for whole-organism-based high-throughput screening as this organism models complex human diseases that cannot be easily reproduced in vitro. Here, we use a model of Huntington's disease, which consists of polyglutamine aggregation (PolyQ35:YFP). *C. elegans* were loaded into a vivoChip platform (Newormics) and imaged in the YFP channel. Outlines of the worms were identified using Gen5 software, and the secondary mask function was used to count the aggregates per worm.



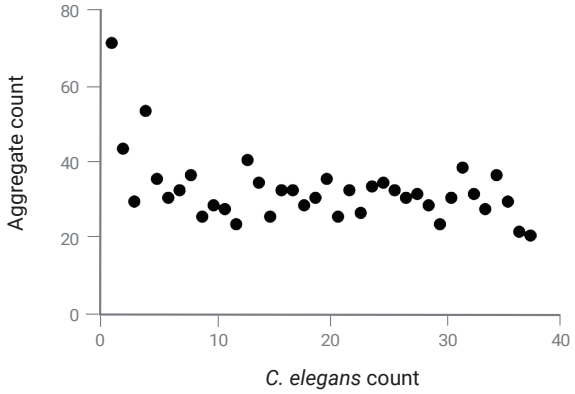
Capture → Each vivoChip is automatically imaged at 10x as a 4 x 8 montage and Z-stack in brightfield and YFP channels.



Process → Image tiles are stitched together, then Z-projection and background subtraction are applied.

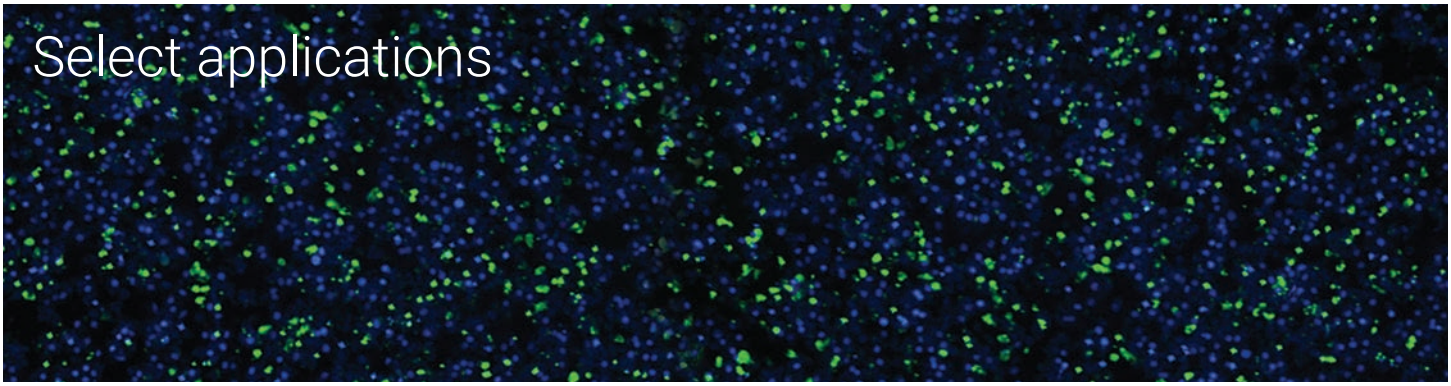


Analyze → The primary mask function in Gen5 identifies each individual worm, and the secondary mask function identifies the polyQ aggregates per worm.



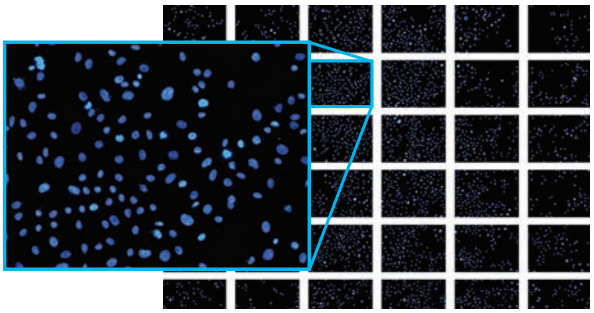
Publish → Aggregate numbers can be quantified for publication.

Select applications

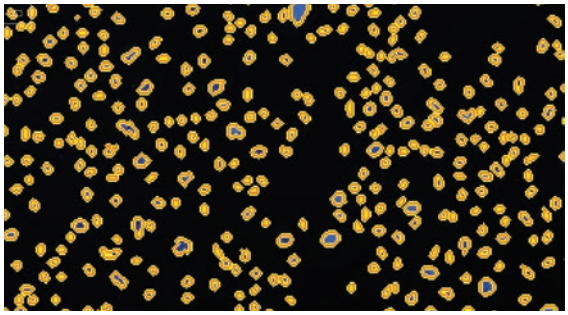


7. Cell cycle analysis using a nuclear stain

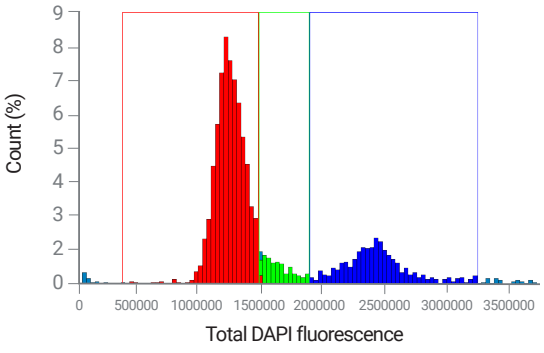
Cell cycle progression is a tightly regulated process that involves the duplication of nuclear DNA content prior to cell division. A nuclear stain such as DAPI can be used to quantify this process, since fluorescence intensity doubles as cells progress from phase G1 to G2.



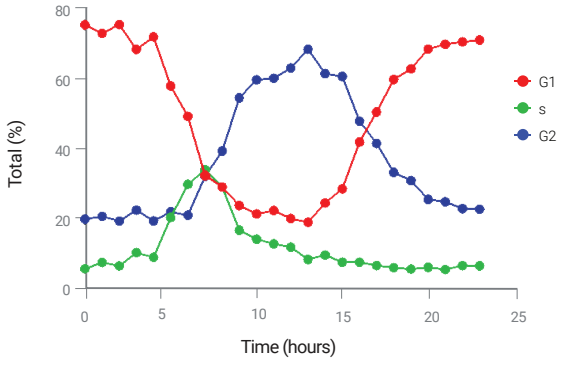
Capture → DAPI montage (6 x 6) image using 10x objective (one tile expanded).



Process → Stitched and background-subtracted, montage image with cell nuclei identified (zoom shown, about 3,000 cells per well counted on final montage).

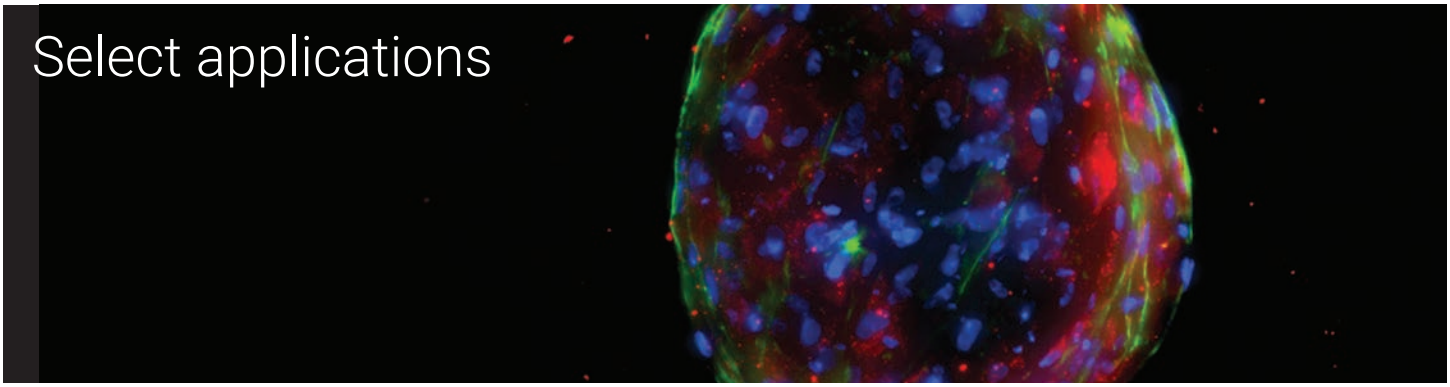


Process → Determination of G1, S, and G2 subpopulations using histogram analysis of object total DAPI fluorescence.



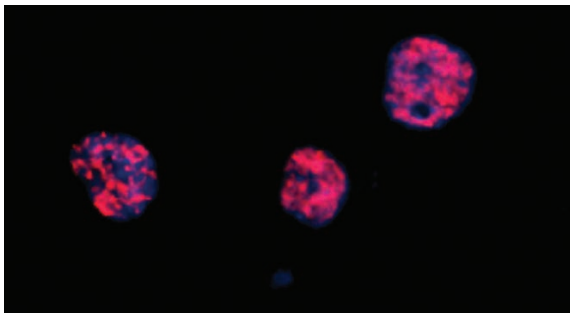
Publish → Cell cycle progression of synchronized PC-3 cells.

Select applications

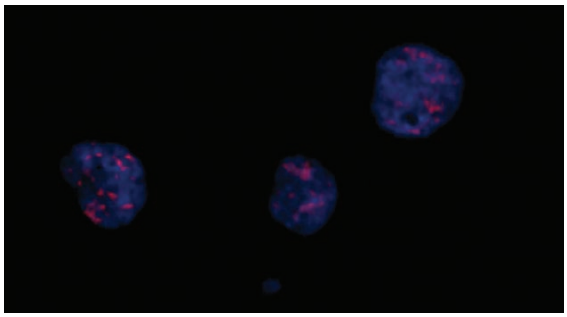


8. γH2AX foci spot counting as a determinant of genotoxicity

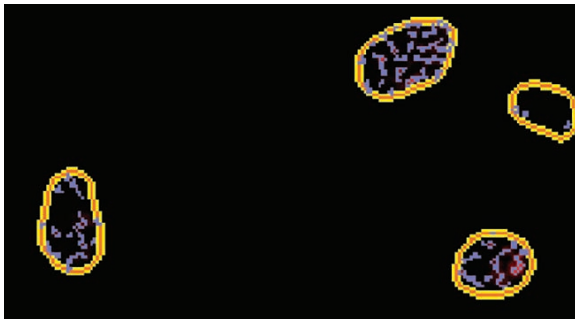
Double stranded DNA breaks represent a critical form of genotoxic effect defined by histone 2AX (H2AX) phosphorylation to γH2AX as part of the DNA repair process. Following immunostaining, automated fluorescence imaging and dual-mask spot counting are performed to quantify labeled foci per nuclei after drug treatment.



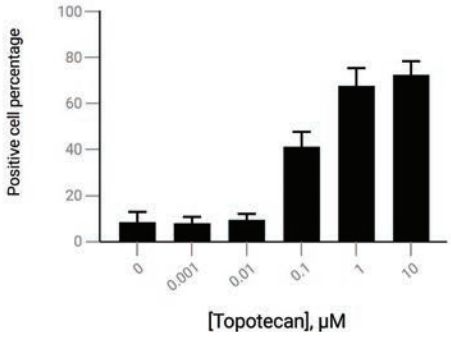
Capture → Images captured of DAPI-stained nuclei and fluorescent antibody-labeled γH2AX signal.



Process → Preprocessing eliminates background signal, revealing actual labeled γH2AX spots.

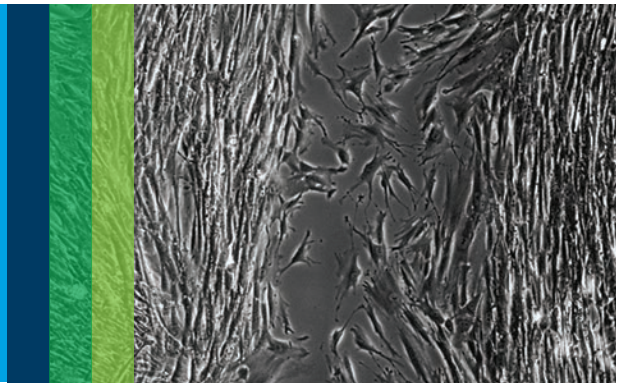


Analyze → Secondary spot counting capability allows quantification of spots per nuclei.



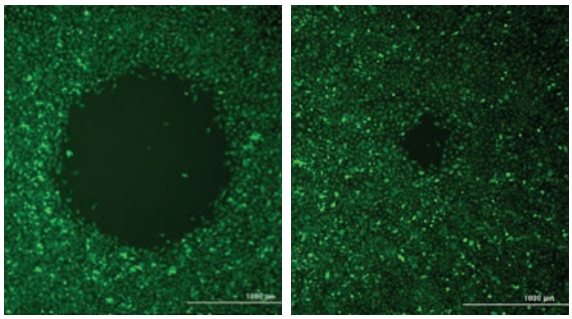
Publish → Statistical determination of minimum spots per nuclei enables calculation of positive γH2AX cell percentage per treatment.

Select applications

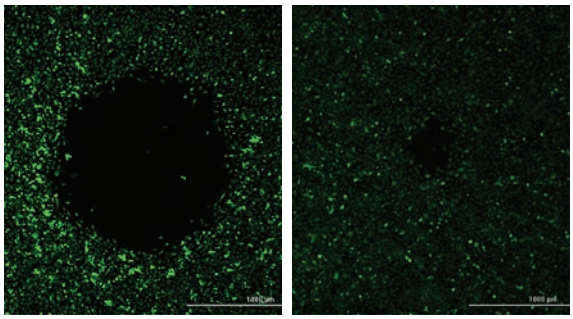


9. High-throughput cell migration assay

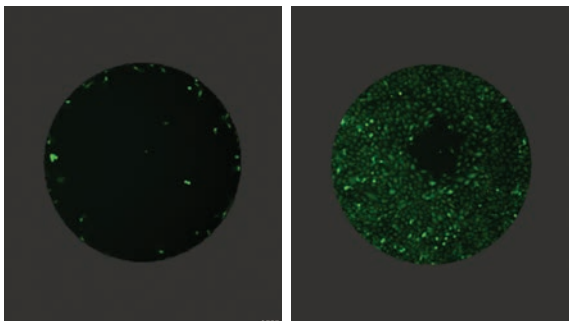
Oris Pro is a cell migration assay conducted in a 384-well format. A biocompatible gel is used to create a cell-free zone following media/cell addition. Image analysis of percent confluence is used to quantify the effect of migratory inhibitors.



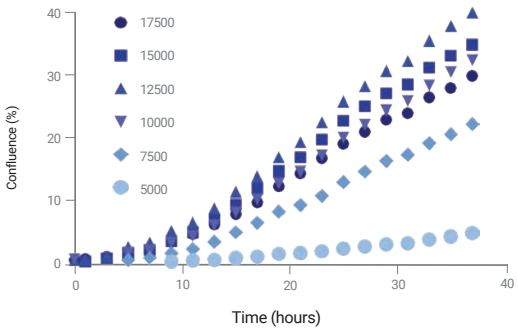
Capture → Cell migration into the detection zone is monitored kinetically.



Process → Background flattening is applied to facilitate image analysis.



Analyze → A disk-shaped "plug" is applied to determine percent confluence within the cell-free zone.

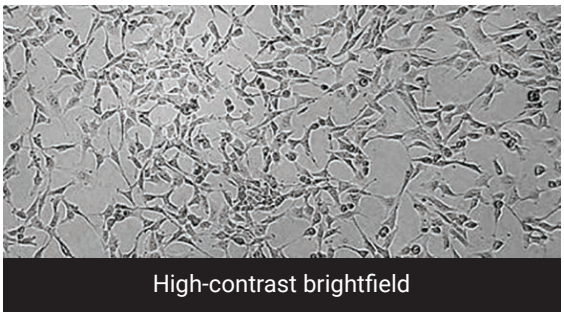


Publish → Kinetic and end-point dose responses can quantify potency of migratory inhibitors.

Select applications

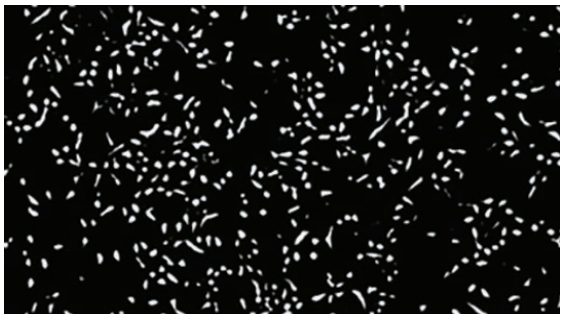
10. Label-free cell proliferation

Cells are seeded into 96-well microplates at 2,000 cells per well. Environmental conditions, including temperature (37 °C), gas (5% CO_2), and humidity (90%), are maintained during a five-day incubation by the Agilent BioTek BioSpa 8 automated incubator. Proliferation or drug-induced reduction in proliferation is detected by label-free cell counting using high-contrast brightfield.

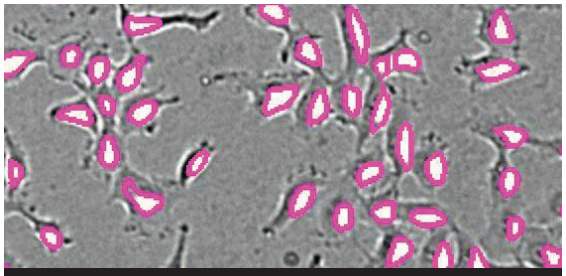


High-contrast brightfield

Capture → Each well is kinetically monitored every two hours using high-contrast brightfield.

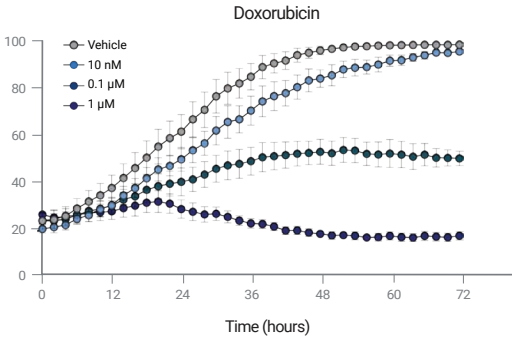


Process → All images are processed to maximize contrast of cells over background.



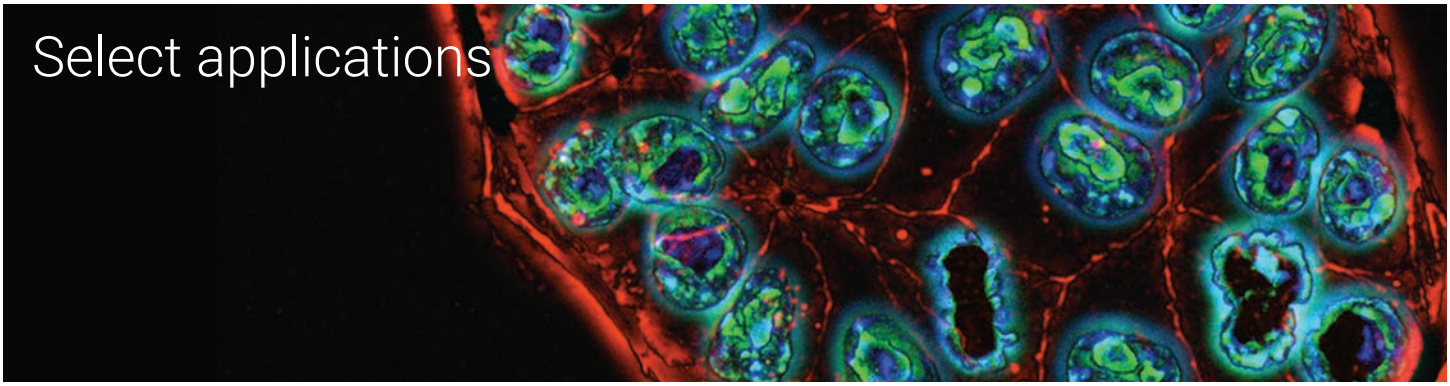
Overlay with object masks

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



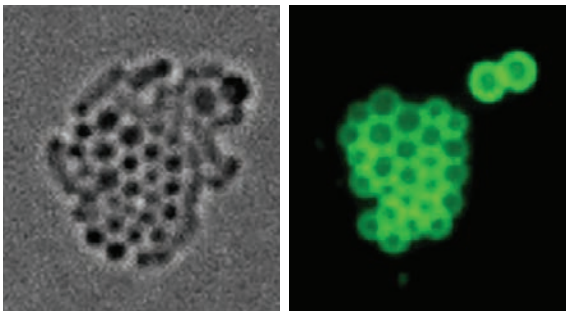
Publish → Antiproliferative agent pharmacology can be published.

Select applications

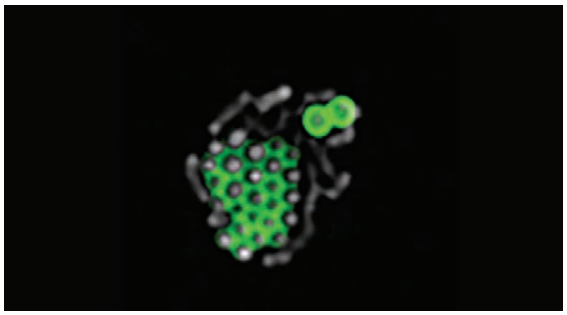


11. Gram stain imaging

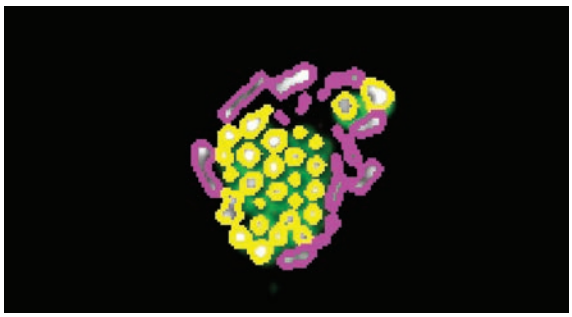
Gram staining classifies bacterial strains based on differences in their cell wall. Green fluorescence results when CF 488A-wheat germ agglutinin (WGA) binds to N-acetyl glucosamine in bacterial peptidoglycan. Gram-positive bacteria appear bright green as they have a thick, exposed peptidoglycan layer. An outer membrane and thin peptidoglycan layer restrict the signal in gram-negative bacteria.



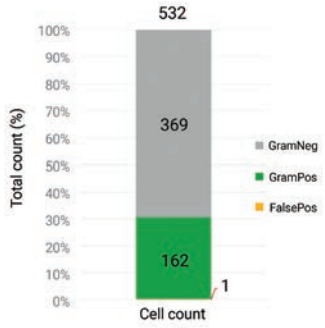
Capture → Raw image acquisition is done in brightfield and GFP using 60x oil immersion. A zoomed-in image of a mixed bacterial cluster is shown.



Process → Digital phase contrast is applied to brightfield images using background flattening and smoothing.

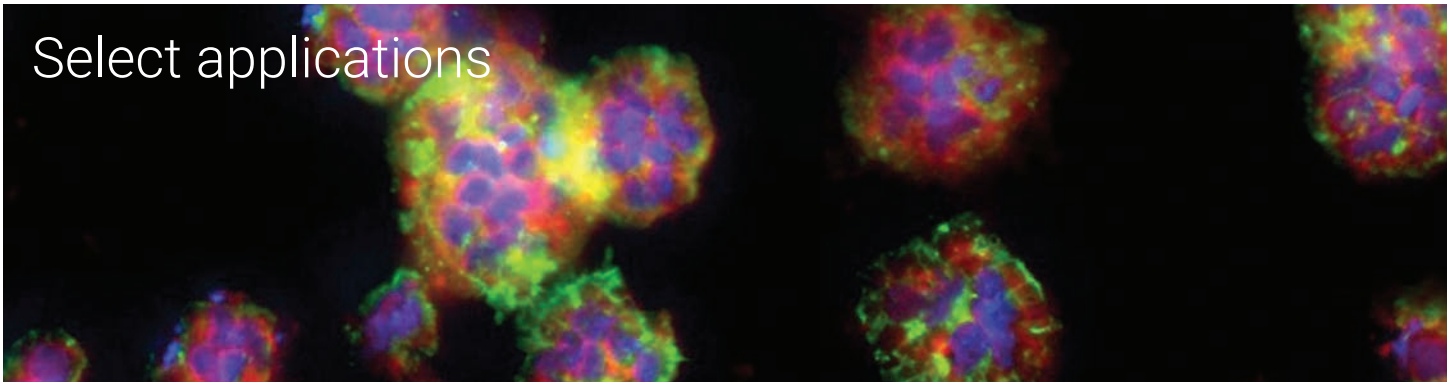


Analyze → Gram-positive (yellow) and gram-negative (pink) cells are distinguished using subpopulation criteria.



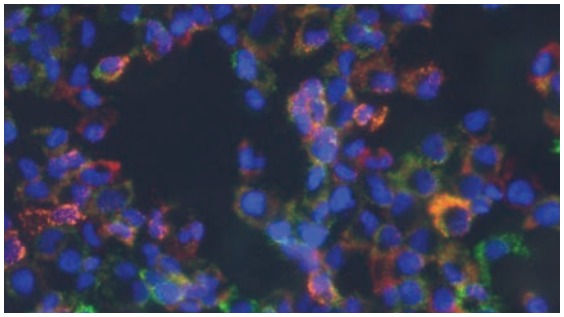
Publish → Imaging and analysis parameters applied to the CF 488A-WGA gram staining method resulted in 99.8% specificity for differentiating bacteria.

Select applications

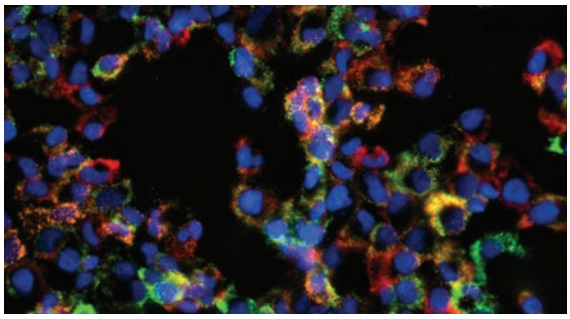


12. Quantifying cancer biomarker gene expression using RNA FISH

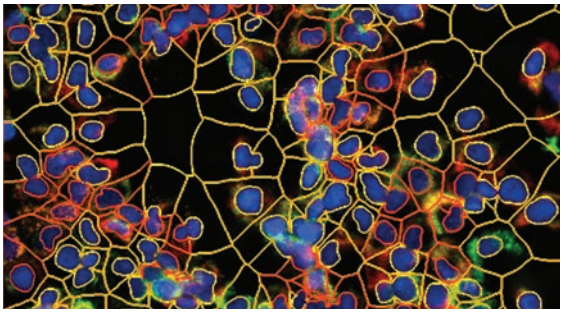
RNA fluorescence in situ hybridization (RNA FISH) is a common method for quantifying gene expression, and is often used in cancer research. Highly specific probes and amplification systems allow image-based quantification of relative RNA expression, while counterstaining with a nuclear stain allows for normalization of expression to cell number.



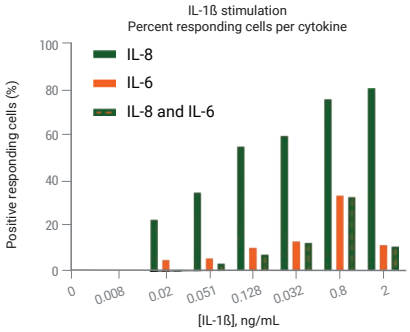
Capture → Images captured of DAPI-stained nuclei in addition to hybridized, amplified, and fluorescently labeled RNA targets.



Process → Preprocessing eliminates background signal, revealing actual signal from labeled RNA molecules.

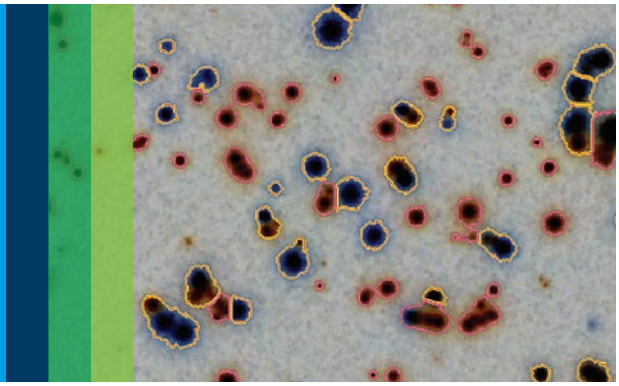


Analyze → Secondary masks quantify mean fluorescence signal from labeled targets. Subpopulation analysis identifies cells responding to treatment.



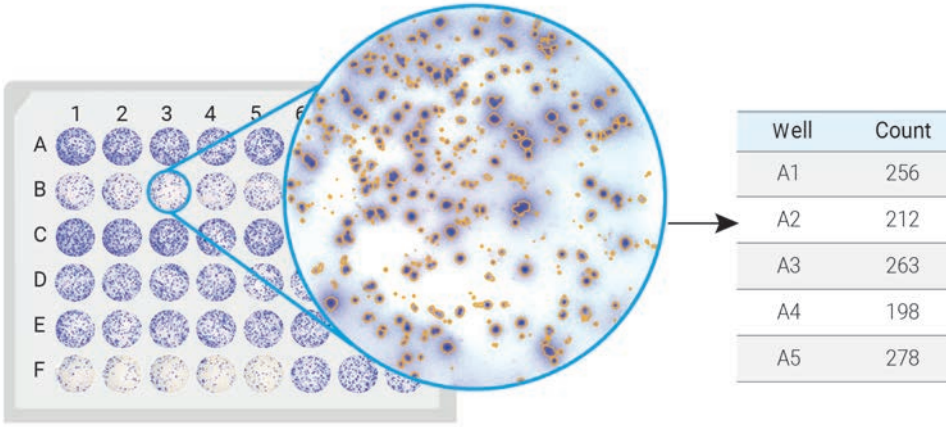
Publish → Normalization of responding cells to total cell count enables calculation of percent response via RNA expression per treatment.

Select applications



13. ELISpot imaging

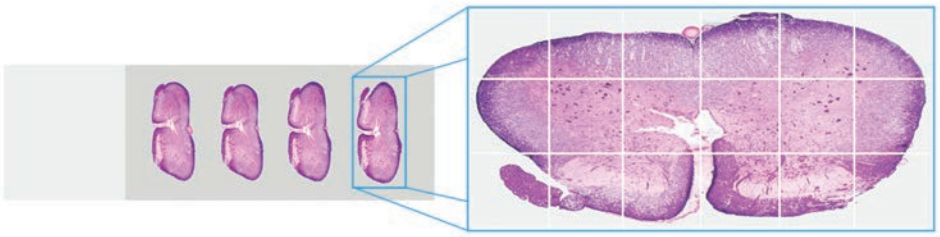
Cytation's upright imaging module can be used to automate assays such as ELISpot, in which cell secretions are rendered visible through the use of a colorimetric reaction. Cytation fully automates image acquisition, processing, image analysis, and object count.



Well	Count
A1	256
A2	212
A3	263
A4	198
A5	278

14. 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.



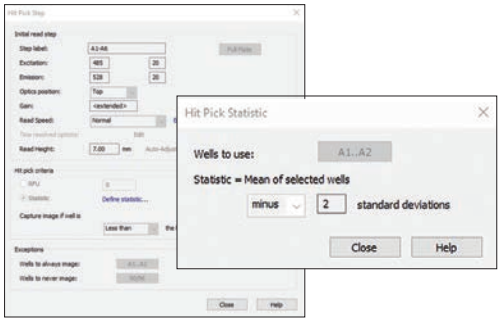
A Low-magnification scan and ROI identification.

B High-magnification imaging.

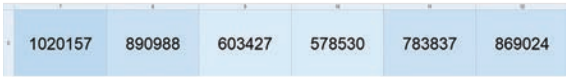
Select applications

15. Hit picking

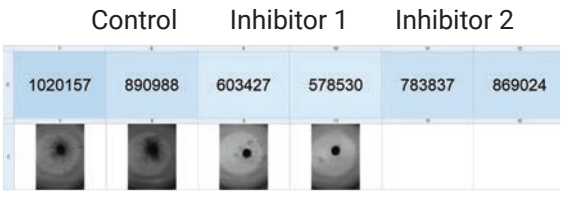
Hit picking combines the joint reading and imaging-based detection capabilities of Cytation cell imaging readers. Control and test wells are read using rapidly performed PMT-based detection. Automatic imaging then immediately commences on test wells that meet predetermined, statistically derived “hit pick” PMT-based signal criteria. The combined process reduces total imaging time and space required to store captured images.



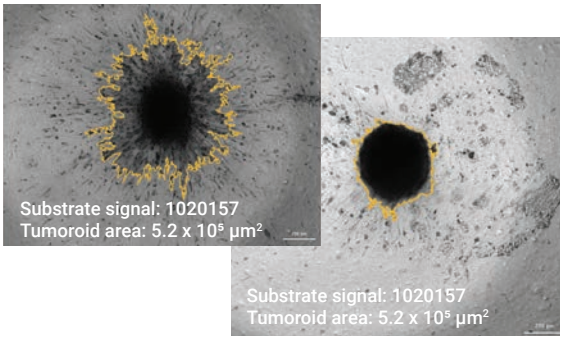
Set criteria → Hit pick criteria are defined, which will trigger test-well imaging.



Read → All control and test wells are read using PMT-based detection.

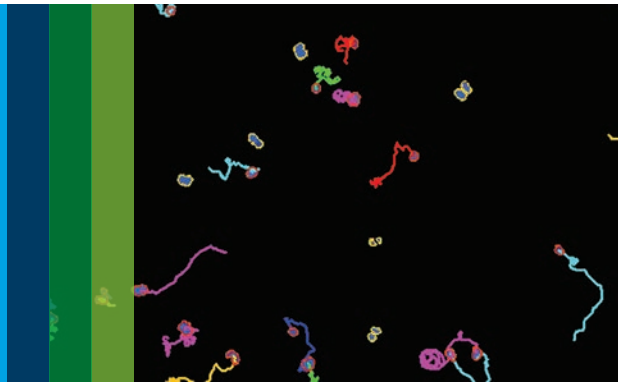


Targeted imaging → Only test wells that meet hit pick PMT-based detection cut-off criteria are imaged.



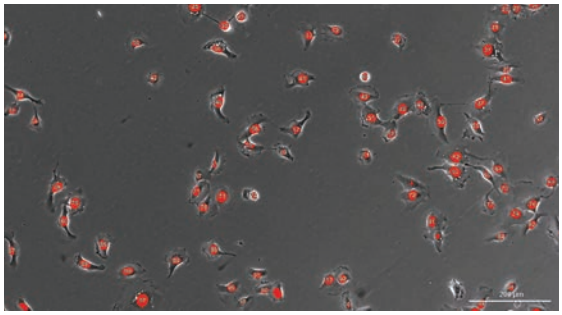
Analysis → Cell analysis is performed to confirm that image-based results from "hit" wells matches PMT-based results.

Select applications

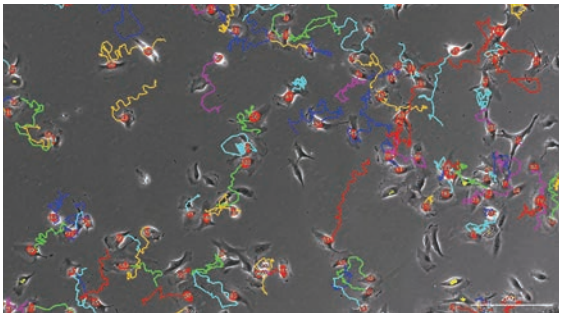


16. Single-cell object tracking

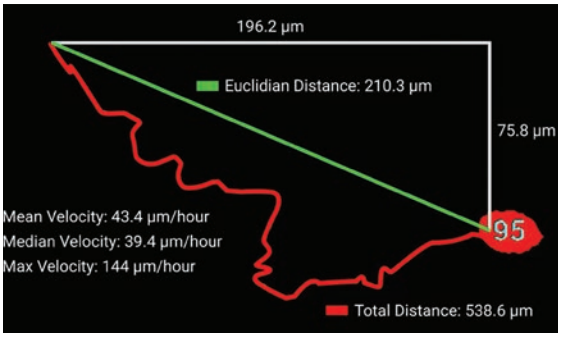
The Gen5 object tracking module automates the ability to track single objects over time. Objects are identified using primary cell analysis metrics. Single objects or populations are selected, and kinetic tracking then occurs. Multiple metrics can be calculated, including total distance moved, Euclidean distance, and mean, median, and maximum movement rates.



Identify → Objects within the image are masked then selected for tracking.



Track → Objects are tracked over the lifetime of the kinetic experiment.



Quantify → Tracking metrics are automatically generated and returned on a per-cell or population level.

Related instruments and accessories

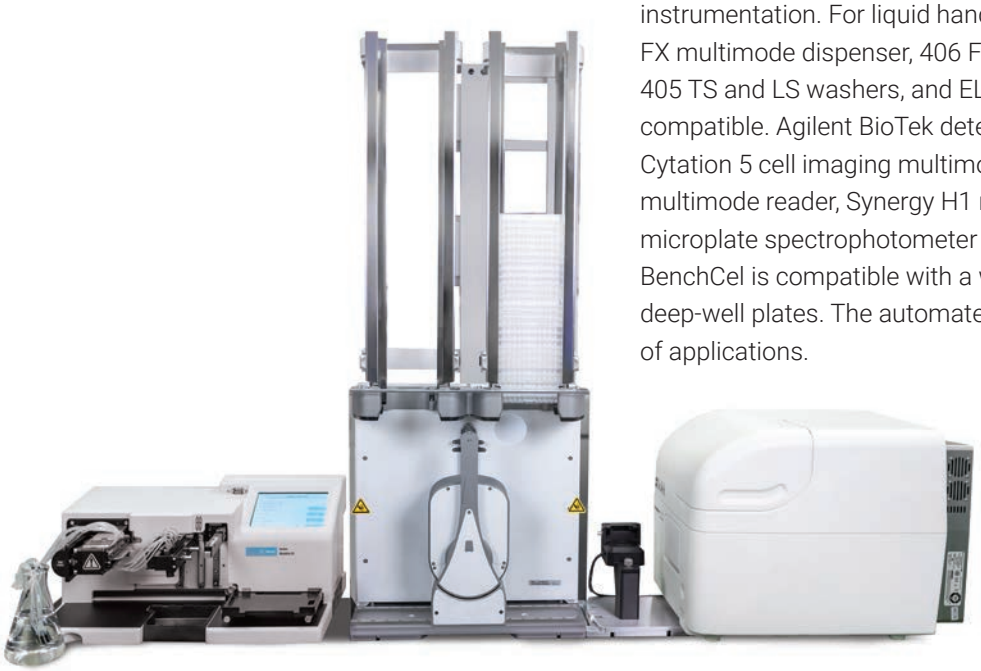
For higher-volume processing or long-term workflows, Cytation cell imaging readers integrate with Agilent automation solutions.

Cytation C10 integrated with BioStack

The Agilent BioTek BioStack microplate stacker manages up to 50 microplates for automated imaging or multimode operations, including de- and relidding of microplates used with cell-based assays.

BenchCel microplate handler

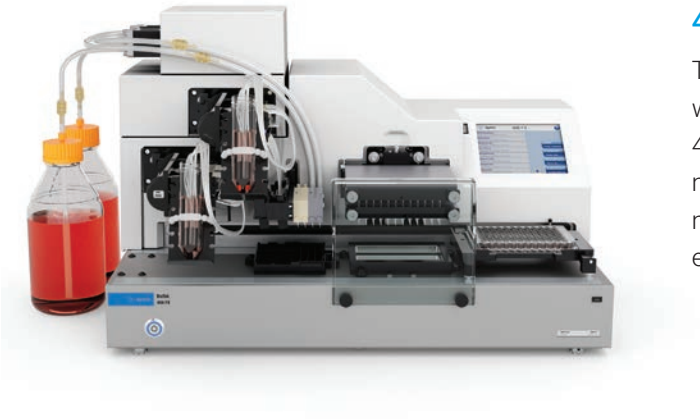
The Agilent BenchCel microplate handler is a compact, automated system that can be integrated with a variety of Agilent BioTek instrumentation. For liquid handling, the Agilent BioTek MultiFlo FX multimode dispenser, 406 FX and EL406 washer dispensers, 405 TS and LS washers, and ELx405 Select deep well washer are compatible. Agilent BioTek detection instruments, including the Cytation 5 cell imaging multimode reader, Synergy Neo2 hybrid multimode reader, Synergy H1 multimode reader, and Epoch 2 microplate spectrophotometer can also be added. In addition, the BenchCel is compatible with a wide range of microplates, including deep-well plates. The automated workflows enable a wide variety of applications.



Related accessories and technologies

BioSpa 8 automated incubator

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



406 FX washer dispenser

The 406 FX washer dispenser automates multiple plate washing and reagent dispensing steps in one instrument. The 406 FX is ideal for cell-based, ELISA, multiplex assays, and many other common protocols. The Agilent BioTek Dual-Action manifold, and proprietary Agilent BioTek Ultrasonic Advantage ensure excellent washing performance and easy maintenance.

AutoScratch wound making tool

The AutoScratch wound making tool automatically creates reproducible scratch wounds in cell monolayers grown in 24- or 96-well microplates for cell migration and invasion studies.

Related accessories and technologies

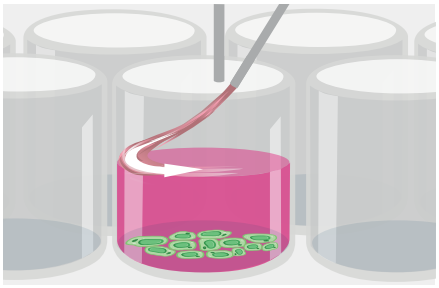
CO_2/O_2 control and reagent injectors

The compact gas controller maintains control of CO_2 and O_2 levels for live cell assays. The gas controller is for use with Lionheart FX and Cytation systems.

Confocal hardware

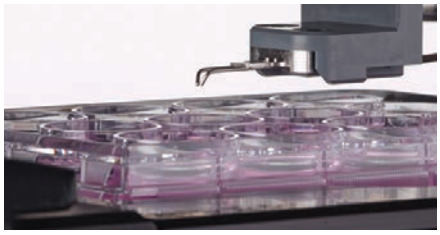
The Cytation C10's confocal microscope uses a Hamamatsu sCMOS camera, along with 60 and 40 µm Nipkow spinning disks. A deep-sectioning disk (DSD) allows a deeper look into thick sample biologies such as tissues and spheroids.

Angled injector tips



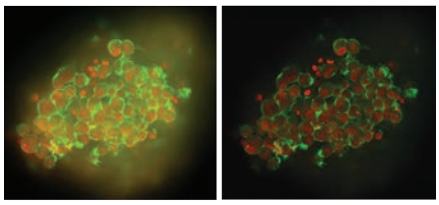
Angled injector tips protect cell monolayers from shear stress during injection.

Dual-reagent injector module



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

Deep-sectioning disk



The deep-sectioning spinning disk (DSD) diminishes crosstalk in thick samples, allowing a clear view into thick tissue samples and spheroids. Gather more detailed data from deep within challenging sample types. Spheroid Z-slice captured with standard 60 µm disk (left) and 60 µm DSD (right).

High-quality objectives

Agilent BioTek uses high-quality, optical components, including Olympus objectives. Air objectives are available for all Cytation and Lionheart imagers. Oil immersion objectives are compatible with Lionheart and water immersion objectives are available for Cytation C10.

Imaging cubes

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

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

Labware adapters

From microscope slides, cell culture dishes, and chamber slides to microplates, T75 flasks, and hemocytometers, the Agilent BioTek range of labware adapters supports many imaging workflows.

Humidity control

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

Agilent BioTek imaging and microscopy

Lionheart FX
automated microscope

Lionheart LX
automated microscope

Cytation C10
confocal imaging reader

Cytation 7
cell imaging multimode reader

Cytation 5
cell imaging multimode reader

Cytation 1
cell imaging multimode reader

Agilent BioTek imaging and microscopy

Instrument 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 and 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 8 Compatible			•	•	•	•
BenchCel Compatible				•	•	•
Multimode 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					
Magnifications Available–Water	40x, 60x					
Inverted Microscope	•	•	•	•	•	•
Upright Microscope				•		

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