Imaging & Microscopy







BioTek

Imaging & Microscopy

Designed for a wide range of applications and budgets.

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

Cytation[™] 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



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.

Five imaging modes



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.

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.

BioTek

CYTATION

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 $0.1 \mu m$ – 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.

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.

Live cell assay support



Temperature control, including the 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 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.



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.



Kinetic image alignment



Movie file creation



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.



Nuclei count and analysis





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.

Original image

Image with mask applied

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





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.

BioTek's AutoScratch[™] Wound Making Tool automates sample preparation for wound healing assays. (see Automation Accessories)

Signal translocation



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

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

Analyze

Subpopulation analysis



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

Kinetic analysis

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

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



Publish

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

Scatter plots & histograms



Total DAPI Fluorescence



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Data analysis



Image & graph annotation



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







Locked raw image

Copy for editing

Raw image retention

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

Select Applications

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

3D NK cell cytotoxicity

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.



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



Process: Each set of z-stacked images is z-projected at each time point for analysis of apoptosis (green fluorescence) or 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% CO₂) and humidity (90%) are maintained during a five day incubation by a BioSpaTM 8 Automated Incubator. Proliferation or drug-induced reduction in proliferation is detected by labelfree 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 percell 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.

Select Applications

Quantifying poly-glutamine aggregates in C. elegans using vivoChip

C. elegans has emerged as a tool for whole organism based high-throughput screening as they model complex human diseases that can not be easily reproduced in vitro. Here, we use a model of Huntington's disease which consists of poly-glutamine aggregation (PolyQ35:YFP). C. elegans were loaded onto the vivoChip™ (Newormics) and imaged in the YFP channel. Outlines of the worms were identified using Gen5, and the secondary mask function was used to count the aggregates per worm.



Capture: : Each vivoChip is automatically imaged at 10x as a 4x8 montage and z-stack in brightfield and YFP channels.

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





Process: Image tiles are stitched together then z-projection and background subtraction are applied.



Publish: Aggregate numbers can be quantified for publication.

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 G1 to G2 phase.



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



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



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



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

Applications

Measuring apoptosis in zebrafish treated with ethanol

Zebrafish embryos are treated with ethanol during the first 24 hours of development and the effects of ethanol treatment on cell death is assessed using acridine orange staining (green emission). Embryos were imaged in 96 well round bottom plates with a 2x objective as z-stacks in the bright field and GFP channels.



Capture: Each well is automatically imaged as a 2x z-stack.



Analyze: The pre-processed images are analyzed, and each individual GFP positive cell is automatically identified and counted.



Process: Images are z-projected then pre-processed in order to better separate individual positive cells.



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

Quantifying cancer biomarker gene expression using RNA FISH

RNA Fluorescence *in situ* hybridization (RNA FISH) is a common method to quantify gene expression, 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.



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



Process: Pre-processing eliminates background signal revealing actual signal from labeled RNA molecules.



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

Select Applications

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 image of a mixed bacterial cluster is shown.



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



Process: Digital phase contrast (DPC) is applied to brightfield images using background flattening and smoothing.



Publish: Imaging and analysis parameters applied to the CF[™] 488A-WGA gram stain method resulted in 99.8% specificity for differentiating bacteria.

Phagocytosis assay

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



Capture: A two channel image at one kinetic timepoint shows black extracellular bioparticles in contrast to the red fluorescence of phagocytized bioparticles (RFP).



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



Process: A time-stamped movie is generated of kinetic images showing an increase in bioparticle phagocytosis over time (orange).



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

Applications

γH2AX foci spot counting as a determinant of genotoxicity

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



 $\begin{array}{l} \textbf{Capture:} \mbox{ Images captured of DAPI} \\ \mbox{stained nuclei and fluorescent} \\ \mbox{antibody labeled } \gamma \mbox{H2AX signal.} \end{array}$



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



Process: Pre-processing eliminates background signal revealing actual labeled γH2AX spots.



Publish: Statistical determination of minimum spots per nuclei enables calculation of induced DNA damage per treatment.

High throughput cell migration assay

Oris[™] Pro is a cell migration assay conducted in a 384- well format. A bio-compatible gel (BCG) 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.



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



Process: : Background flattening is applied to facilitate image analysis.



Publish: Kinetic and endpoint dose responses can quantify potency of migratory inhibitors.

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.



AutoScratch[™] Wound Making Tool

AutoScratch automates the creation of scratch wounds in 24- and 96-well microplates, for cell migration assays.

CO₂/O₂ 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.



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



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

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





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

Imaging & Microscopy Product Comparison

	Lionheart FX	Lionheart LX	Cytation 5	Cytation 1
Imaging Methods				
Fluorescence	٠	•	•	•
High contrast brightfield	٠	٠	•	•
Brightfield	٠		•	
Color brightfield	٠	٠	•	
Phase contrast	٠		•	
Air	1.25x, 2.5x, 4x, 10x, 20x, 40x, 60x			
Phase	4x, 10x, 20x, 40x		4x, 10x, 20x, 40x	
Oil Immersion	60x, 100x			
General				
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	٠			
Joystick controller	•	•	•	
Multi-mode detection capable			•	•
BioStack and BioSpa compatible			•	•

Think Possible with BioTek

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CYTATION 1

View trending applications in imaging & microscopy at **www.biotek.com/IMApps**

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Imaging & Microscopy

About BioTek

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



Think Possible



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