CYTEK®
TRANSCEND THE CONVENTIONAL

Cytek® Amnis® ImageStream®X Mk II Flow Cytometer

Visualize The Path To Discovery

Visualize A New Standard

The Cytek Amnis ImageStream^{X} system is a high-throughput, high-resolution imaging flow cytometer that provides hundreds of parameters for making single-cell measurements on millions of cells in a sample. It continues to enable major discoveries published in high-impact journals every year. The system combines the information-rich data from microscope-quality images with the statistical power and throughput of a flow cytometer. Combined with intuitive image analysis tools powered by machine learning and advanced AI algorithms, the ImageStream^{X} system is helping researchers visualize a new standard in scientific discovery.

ACHIEVE **CLARITY** FROM COMPLEXITY

The ImageStream^{X} system rapidly collects microscope-quality images and uses powerful image analysis tools to get the science right

CONFIRM YOUR FINDINGS

The ImageStream^{X} system offers image alignment and spectral compensation, helping researchers identify the location of each probe

GET RESULTS **FAST**

The ImageStream^{X} system collects clear images that make cell morphology meaningful, quantifiable, and translatable for advanced AI tools

Applications

Drug Discovery

Cell Signaling

Co-Localization

Hematology

CAR-T

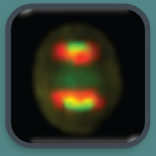
Extracellular Vesicles

Label Free

Sickle Cell

Toxicology

Cell Cycle



Oceanography

Cell Death

Microbiology

Genomics

Immunology

Virology

Powerful Flow Cytometry Meets High-Resolution Imaging

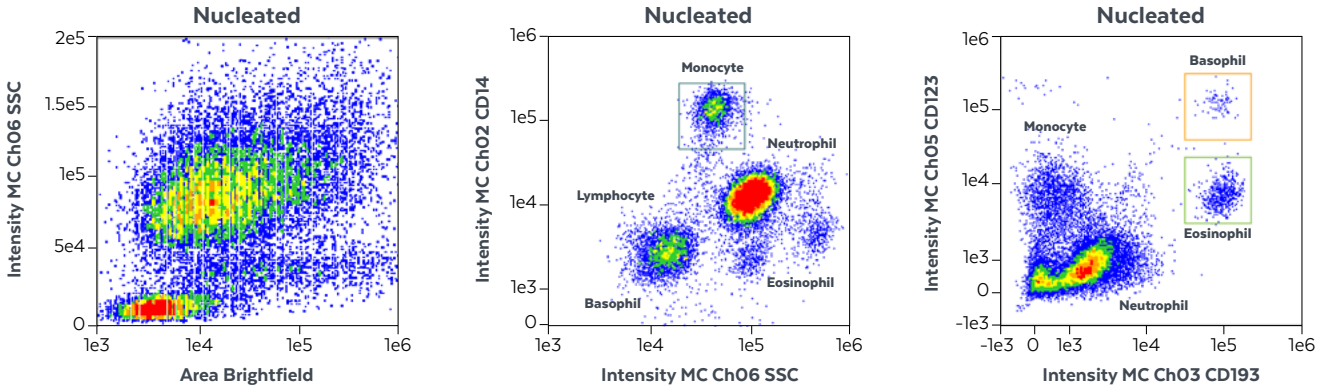
Enhance and expand your research with images acquired on the ImageStream^X system and analyzed using IDEAS® image analysis software. This powerful combination enables visual verification of cells within any region of interest, supports detailed assessment of cell health, and helps uncover unexpected biological insights. Capture up to ten fluorescent markers, along with a side scatter (SSC) image and brightfield images, for every cell. The ImageStream^X instrument can save data files in FCS 3.0 format during or after acquisition, enabling flow data to be analyzed using familiar workflows. Its imaging flow cytometry technology generates dot plots that directly correlate with conventional flow cytometry plots, supporting seamless integration with cell sorting experiments and evaluation of samples collected before or after sorting.

Design features, like a dedicated SSC laser, adjustable laser intensities, and brightfield imaging, allow the system to generate comprehensive data that correlate with flow cytometry data to help you make informed decisions regarding cells of interest, which populations to sort, and the state of those cells.

The ImageStream^X system works seamlessly with cell sorting:

- Before the sort: Verify sample quality and staining specificity
- During the sort: Optimize sort regions using images collected from the ImageStream^x system
- After the sort: Image the sorted population to verify purity, measure translocation, quantify co-localization, assess cell health, or perform other functional assays

Figure 1



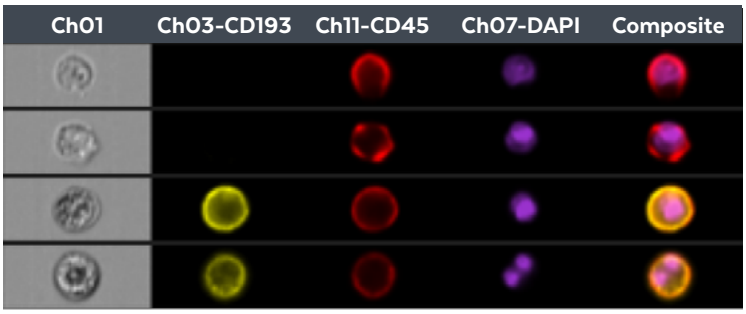


Figure 1: Human whole blood fluorescently labeled with CD45 pan-leukocyte marker, CD14, CD193, CD123, and DAPI (4',6-diamidino-2-phenylindole) DNA stain. Plots show each of the major blood cell types. Images show nuclear morphology in the DAPI image and the staining pattern of surface fluorescence.

Sensitive And Flexible For Any Research Need

Exceptional Fluorescence Sensitivity

The patented architecture of the ImageStream^X system provides high fluorescence sensitivity across the visible spectrum. The four plots below demonstrate the system's ability to discriminate all 8 peaks of the SPHERO™ Rainbow Calibration Particles across the spectrum from FITC to PE-Cy7. High sensitivity is demonstrated by the distinct separation between the negative population and next dimmest peak, combined with low coefficients of variation (CVs).

Figure 2

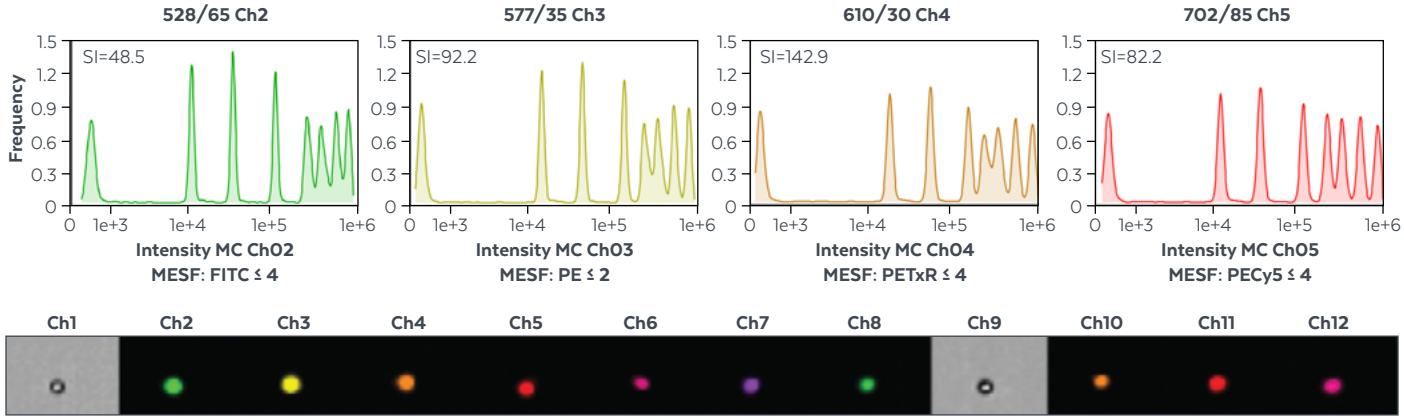


Figure 2: SPHERO Rainbow Calibration Particles (Spherotech, 8 peaks) imaged using a 488 nm laser at 200 mW. These beads are used to measure photonic sensitivity.

5-Part White Blood Cell Differential

With its exceptional sensitivity, the ImageStream^X system excels at resolving mixed subpopulations in heterogeneous samples. As shown in Figure 3, human peripheral blood mononuclear cells (PBMCs) are partitioned into five distinct populations using CD45 expression and SSC intensity. High fluorescence sensitivity and tight CVs resolve monocytes from lymphocytes and facilitate the detection of rare basophils. The dedicated SSC laser clearly resolves eosinophils from neutrophils.

Figure 3

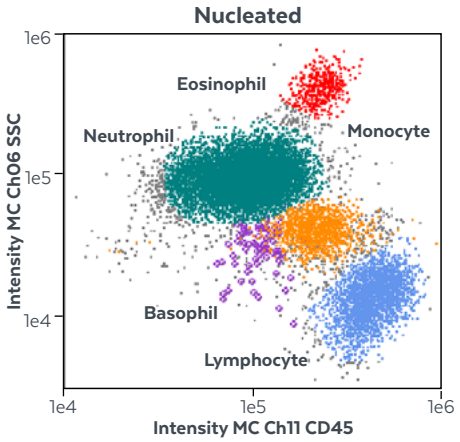


Figure 3: PBMCs stained with CD45 and plotted against SSC can be used to identify five different leukocyte populations.

Images Of Every Cell

The ImageStream^X instrument operates like a conventional flow cytometer but also provides images of every cell. Powerful and intuitive analysis software seamlessly links quantitative data to images. Users can:

- Click on a dot in any plot to view its corresponding image
- Click on a bin in any histogram to view every cell in that bin
- Draw gates on dot plots and view the resulting populations to validate results

With its imaging capabilities, users can investigate outliers and determine whether gates are positioned correctly. When a gate is drawn on a plot, users can click inside or outside the gate to determine the correct placement and confirm cell identity. With visual feedback, gate size, shape, and position can be optimized to improve data quality.

Figure 4A

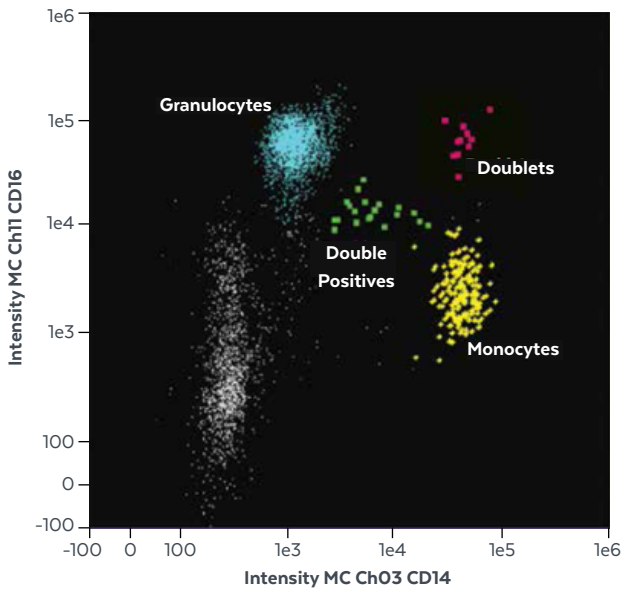


Figure 4B

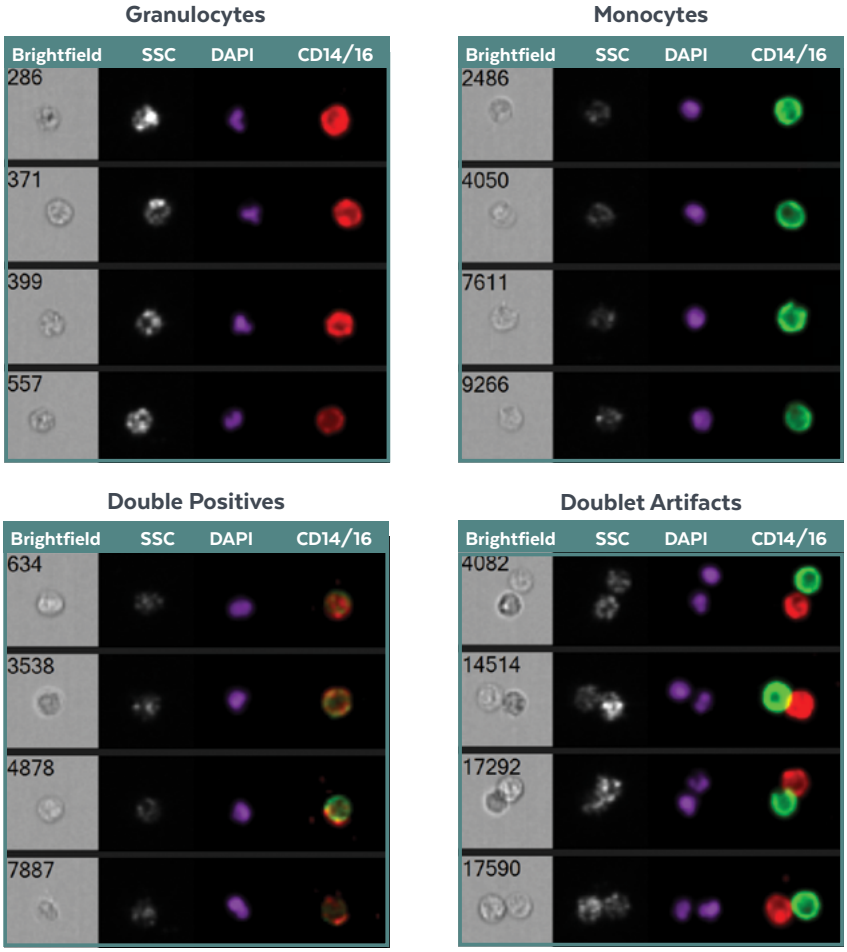


Figure 4: A) Dot plot of PBMCs stained with CD14 and CD16. **B)** Corresponding images enable doublets to be readily distinguished from the double-positive intermediate monocyte population.

Critical Tools For Reliable Results

High Resolution Images And Image Compensation

The ImageStream^X system offers tools to automatically calculate and apply compensation to every pixel in the image dataset. If not properly compensated, fluorescent light from adjacent channels can corrupt image morphology and cause probes to appear where they are not. Subpixel channel alignment and pixel-by-pixel image compensation support high-quality images and are essential for applications such as co-localization, internalization, and translocation.

Figure 5

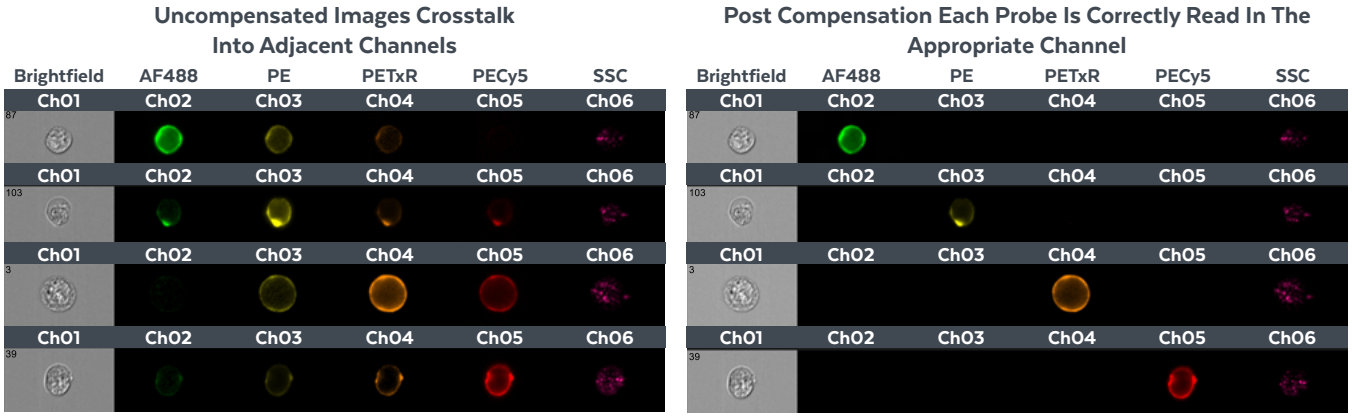


Figure 5: THP-1 cells labeled with an HLA-Biotin primary antibody and a streptavidin secondary antibody conjugated to each individual fluorochrome were used to create single-color compensation controls.

Compliance Solutions For Regulated Settings

Cytek INSPIRE™ software and IDEAS data analysis software work synergistically with the Compliance Manager tool, which was specifically designed for regulated environments. The 21 CFR Part 11-enabled software features allow encrypted sign-on, electronic signatures, and tracking of all related data files throughout data processing workflows.

Figure 6

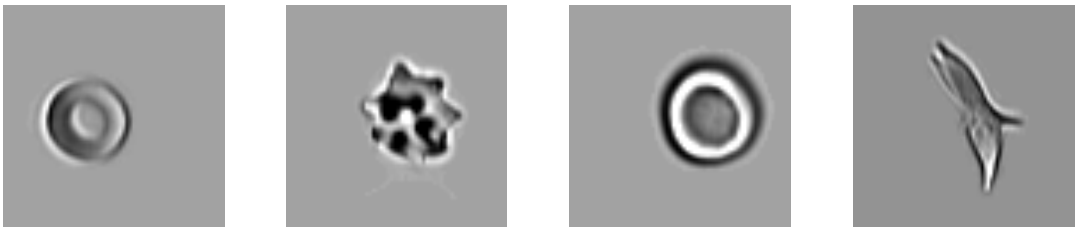


Figure 6: Human red blood cells (RBCs) display normal discocytes, echinocyte, and sickle cell.

Data Acquisition Software

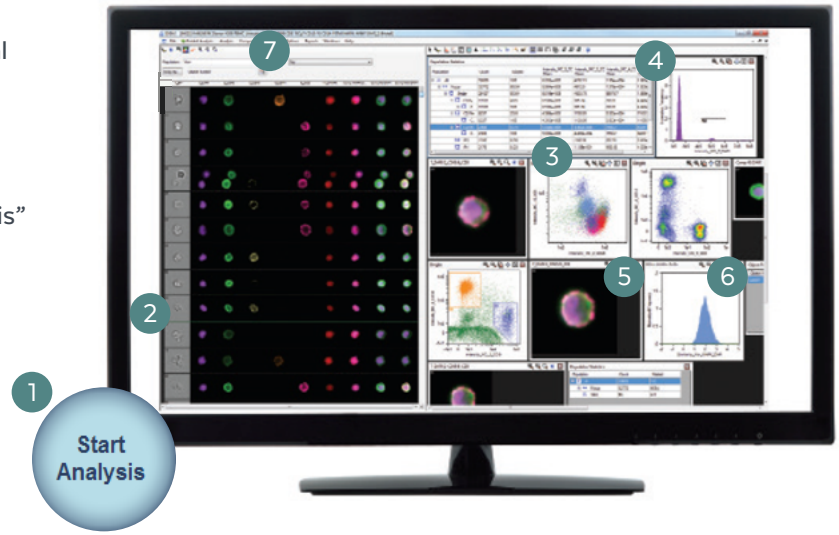
Cytek INSPIRE software offers instrument setting controls, image-based gating, and real-time fluorescence compensation.

1. Instant Population Viewer
2. Image Gallery
3. Instrument Status At A Glance
4. Real Time Intensity Compensation
5. Gating Without Guesswork
6. Efficient Sample Handling
7. Intuitive Instrument Set Up
8. Dot Plots And Histograms

Software That Turns Data Into Results

Cytek IDEAS software with machine learning and Amnis AI software combine image analysis, statistical rigor, and visual confirmation in an efficient software suite that works seamlessly with your ImageStream^X system datasets.

1. Use Guided Workflows By Pressing “Start Analysis”
2. Images For Every Dot
3. Virtual Cell Sort
4. Comprehensive Population Statistics
5. Flexible Image Display Tools
6. Graph What You See
7. Machine Learning Module



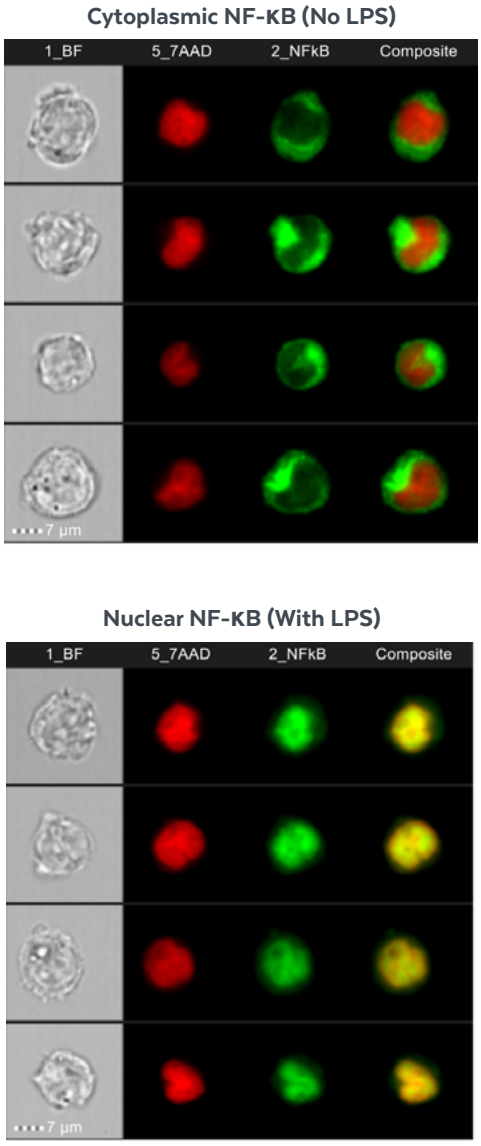
Amnis AI Analysis Software: Powered by deep learning, this standalone software simplifies analysis workflows and reveals subtle cellular heterogeneity with exceptional accuracy, across a range of sample types. Computer-assisted tagging, object map visualization, and advanced analytics enable rapid extraction of insights from ImageStream^X system data.

Applications: Nuclear Translocation

NF-κB Signaling Kit With 60X Magnification

Translocation of NF-κB from the cytoplasm to the nucleus is a key event in the response to many cellular stressors. Imaging flow cytometry enables researchers to quantitatively analyze translocation across thousands of cells. Here, THP-1 cells, unstimulated or stimulated with lipopolysaccharide (LPS), were stained with an anti-NF-κB probe as well as 7-AAD nuclear counterstain and collected on the ImageStream^X system using the 60X objective. The Similarity feature from the IDEAS image analysis software clearly separated translocated events (green histogram) from cells with cytoplasmic NF-κB (orange).

Figure 7



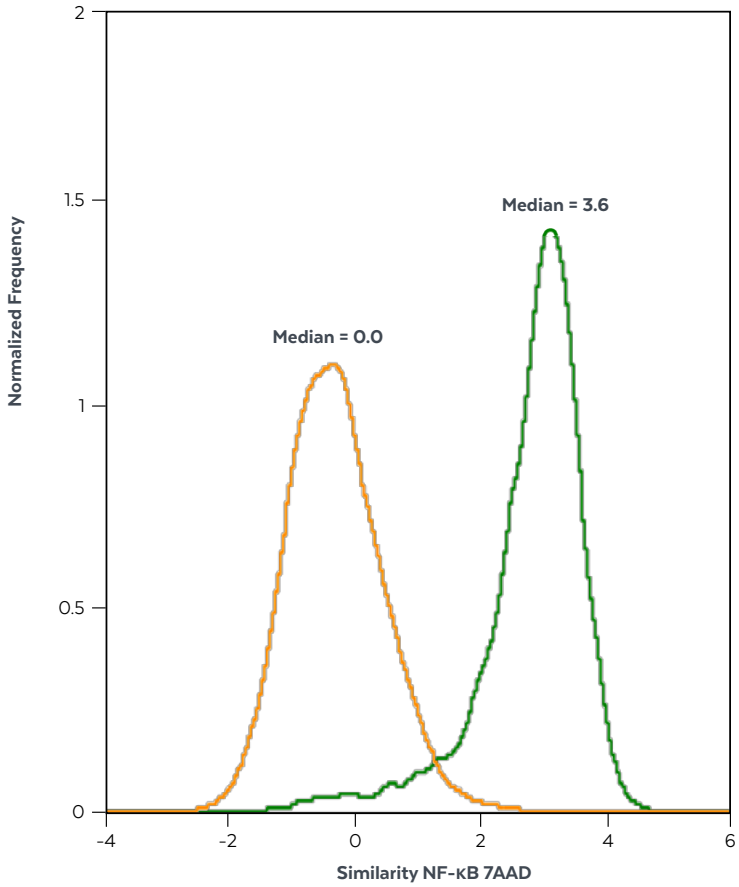
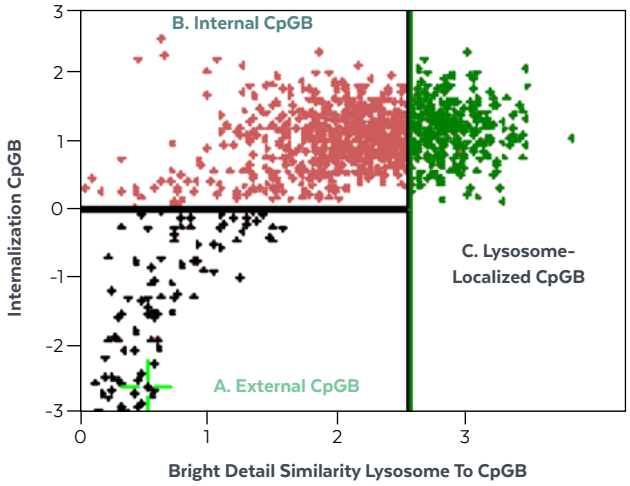


Figure 7: THP-1 cells stained with 7-AAD nuclear dye and anti-NF-κB-AF488. The "No LPS" control shows cytoplasmic NF-κB (orange histogram). "With LPS" shows translocation of NF-κB to the nucleus (green histogram).

Applications: Co-Localization

The ImageStream^X system greatly improves co-localization studies by combining the rapid collection of large numbers of cell images with objective measurement of co-expressed cellular components.

Figure 8



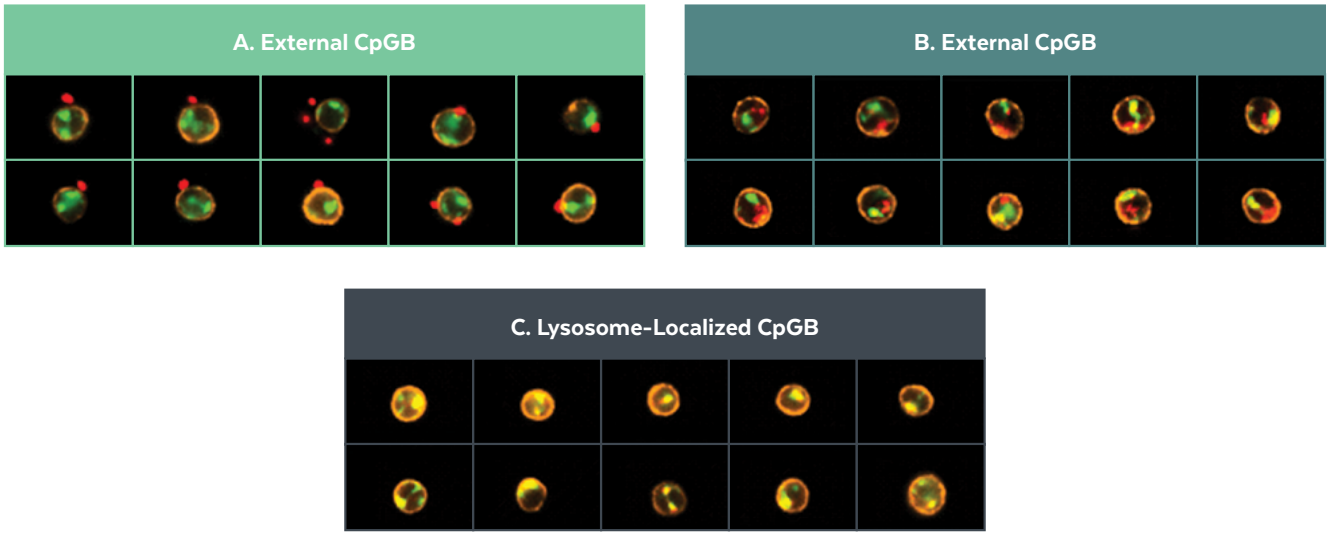


Figure 8: Human PBMCs were incubated at 37ºC for 30 minutes and labeled with blood dendritic cell antigen 2 (BDCA2) to identify primary plasmacytoid dendritic cells (pDCs) and CD107 to mark the lysosomes, then treated with CpGB tetramer particles to measure internalization and co-localization. Representative merged images in the tables above show lysosomal trafficking of CpGB within pDCs. Trafficking is quantified using the Internalization (x-axis) and the Bright Detail Similarity (y-axis) scores. The image gallery shows pDCs (orange), CpGB (red), and lysosomes (green) at 40X magnification. Cells within the lower left region of the dot plot have surface-bound CpGB. As CpGB molecules enter pDCs, the Internalization score increases (upper left region). Once CpGB traffics to lysosomes, the Similarity between the CpGB and lysosome image pair increases (upper right region).

Applications:
Protein Therapies And Cell Health

Protein Aggregation Detection Kit

Protein injectables are tested to ensure minimal aggregation and silicone oil contamination, especially following shipment and storage. The ImageStream^X system offers a reliable method for measuring particle size and composition and can be multiplexed to include measurements of protein-silicone interactions and bacterial contamination. The ImageStream^X system provides enhanced detection of subvisible protein aggregates ranging from <1 µm to >20 µm in a single sample.

Figure 9

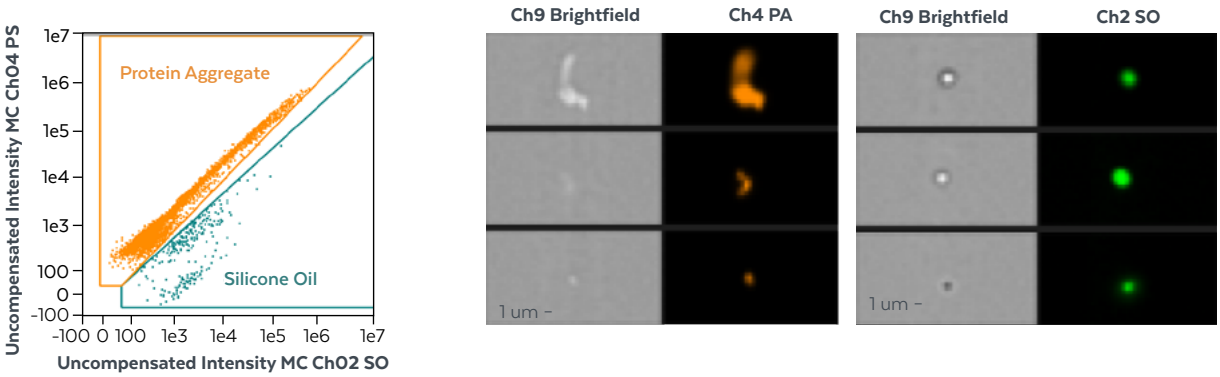


Figure 9: Aggregated IgG stained with PROTEOSTAT® Aggresome Detection reagent (orange) to identify protein aggregates and PMPBF2 (green) to label silicone oil droplets.

Detection Of Apoptosis By Nuclear Fragmentation

Conventional flow cytometers can use membrane-impermeant dyes to identify dead or dying cells that have lost membrane integrity. However, distinguishing whether cell death occurs through apoptosis or necrosis can be challenging. The ImageStream^X system simplifies this analysis by revealing the nuclear morphology of every cell. As shown in this sample of cells labeled with DAPI, viable cells exhibit normal nuclear morphology, whereas apoptotic cells display shrunken and fragmented nuclei.

Figure 10

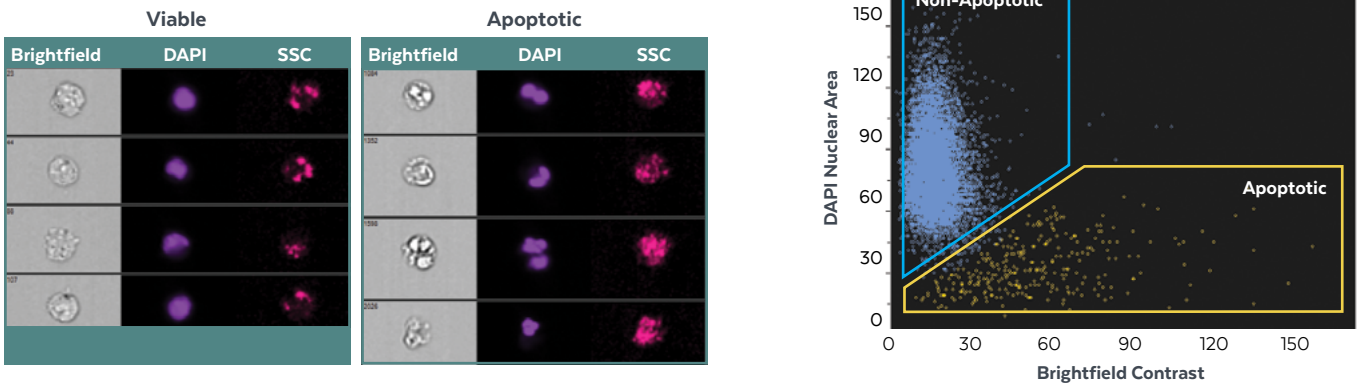
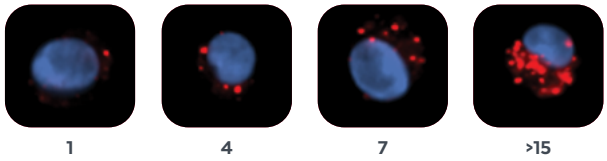


Figure 10: Ramos cells treated with camptothecin to induce apoptosis, then fixed and stained with DAPI to label the nuclei.

Applications:
Autophagy Dependent Cell Death

During autophagy, cytoplasmic LC3 is processed and recruited to the outer membrane of autophagosomes. Autophagic cells can be identified by visualizing LC3 puncta and enumerating the spots within each cell.



Spot Count Scores

The Spot Count feature of the IDEAS image processing software package determines the spot count of every cell. In this example, cells with varying numbers of LC3-RFP (red) spots are shown with their corresponding spot count.

Autophagy In The Human Osteosarcoma Cell Line U2OS

After serum starvation, U2OS cells develop autophagosomes that can be enumerated using the IDEAS software Spot Count feature. The Spot Count value is low in healthy cells and high in cells undergoing autophagy.

Figure 11

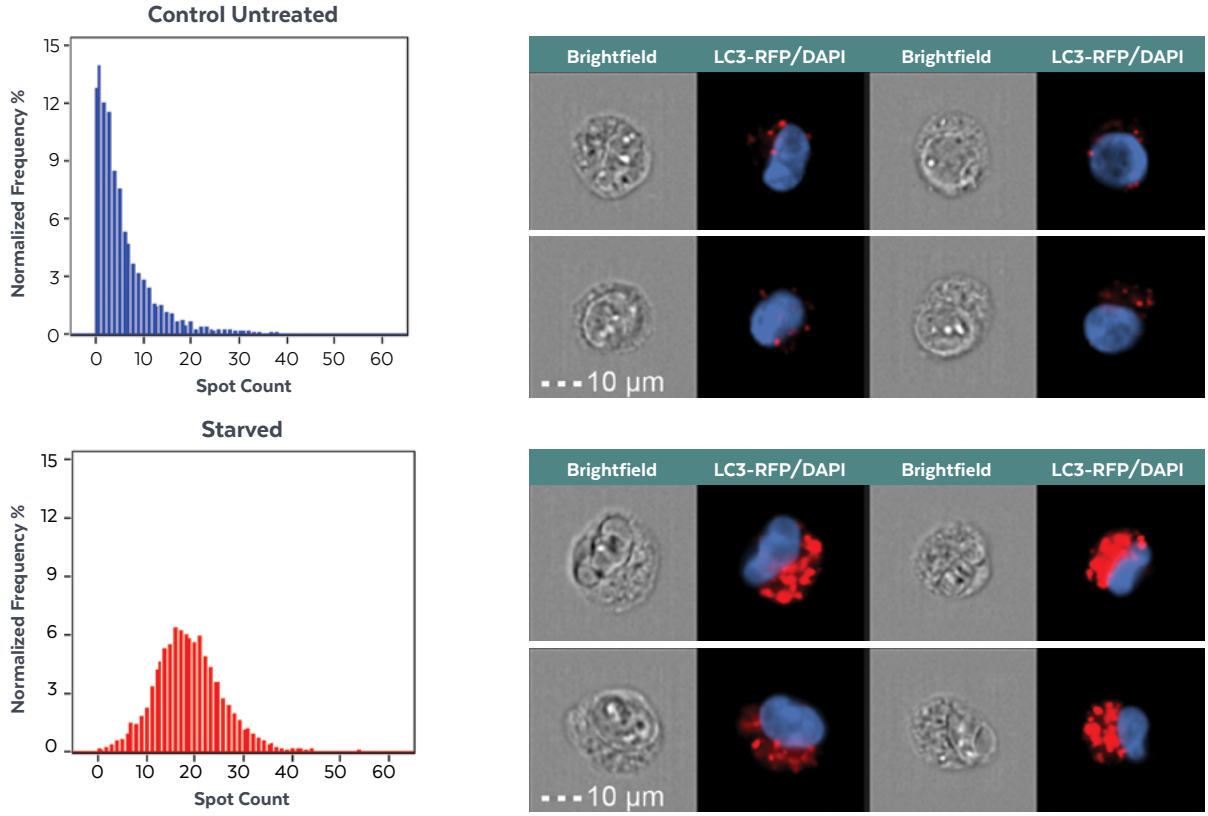
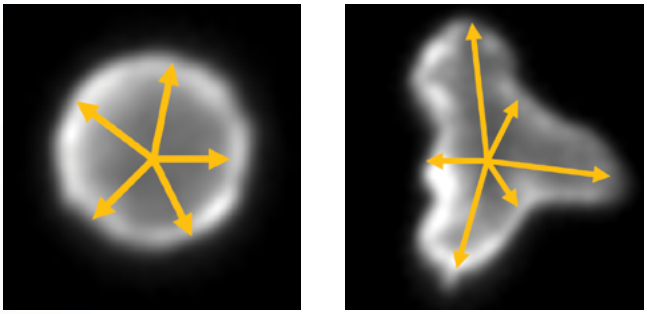


Figure 11: U2OS cell line expressing LC3-RFP (red) and stained with DAPI (blue). Untreated samples show minimal autophagosome formation. Serum starvation for four hours at 37°C stimulates autophagosome clustering.

Applications: Morphology

Change in cell shape are correlated with changes in function, particularly during monocyte activation, stem cell differentiation, and cellular response to drugs. The ImageStream^X system measures cell shape using powerful, pre-defined features in the IDEAS image analysis software. The Circularity Score is one such feature.

The Circularity Score measures variation in the radius of the cell. Round cells (left) have high Circularity Scores while irregularly shaped cells (right) rate low Circularity Scores.



23.8 4.6

Circularity Scores

Shape Change In Primary Monocytes

Monocyte chemoattractant protein-1 (MCP-1) induces monocyte shape change and migration to sites of inflammation, as evidenced by the significant decrease in the Circularity Score of the MCP-1 treated sample relative to the untreated control. In contrast, treatments that reduce the inflammatory response, such as drugs used for autoimmune disorders, increase Circularity Scores.

Figure 12

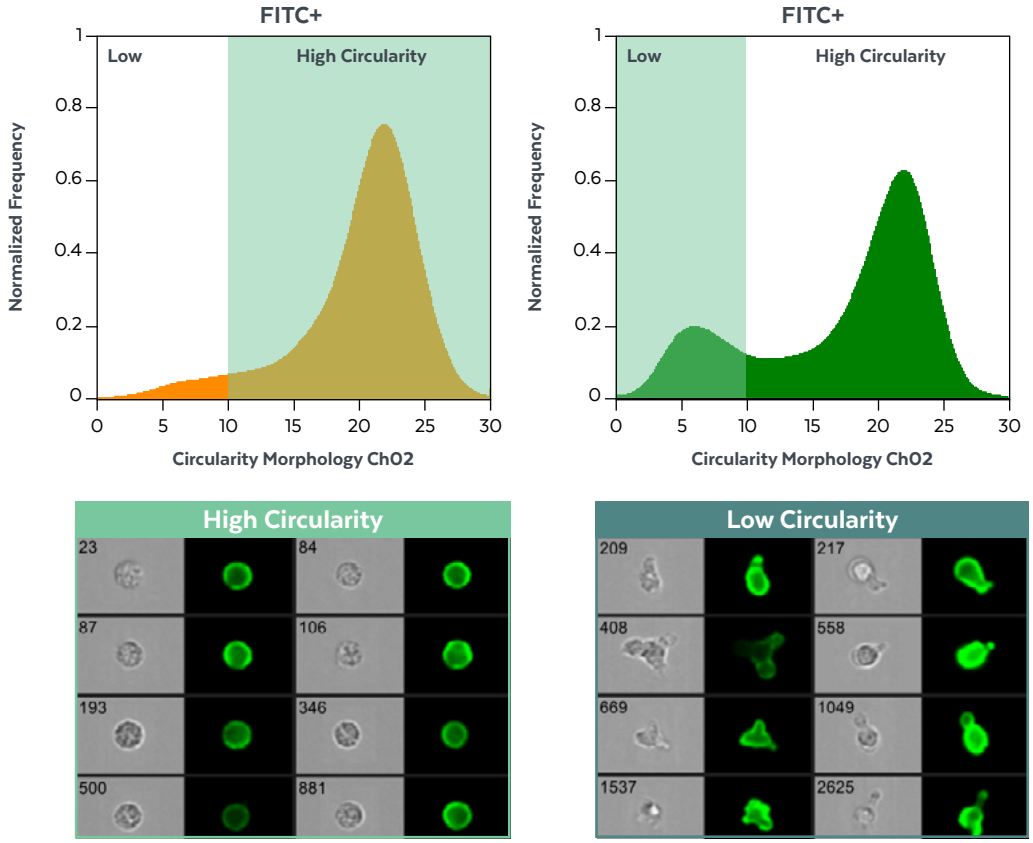


Figure 12: THP-1 human monocytes stained with CD14 FITC. More cells in the MCP-1 treated sample (green) display an elongated morphology with lower Circularity Scores compared to the untreated sample (orange).

Applications: Oceanography

Microalgae Quality Control

The images below demonstrate the detection of bacterial contamination, cellular debris, and clusters in mixed microalgae cultures. A mixed culture of *T. pseudonana* and *C. cryptica* contaminated with bacteria was analyzed on the ImageStream^X system at 60X magnification.

Figure 13

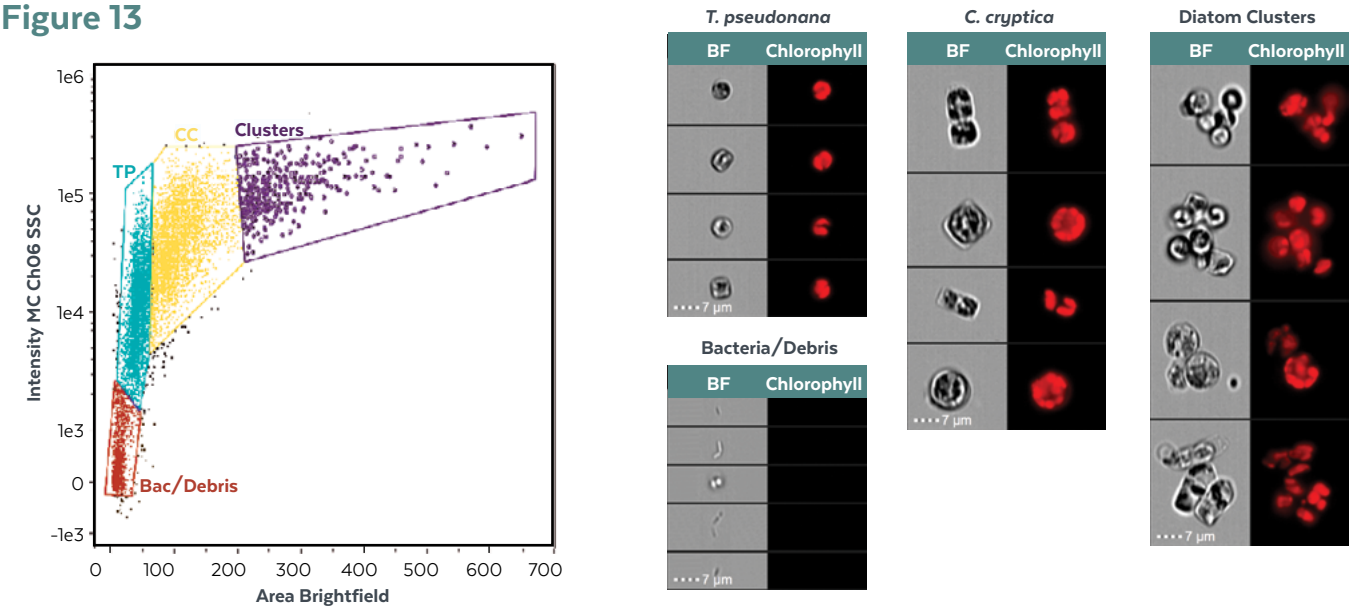


Figure 13: Mixed culture of microalgae *T. pseudonana* and *C. cryptica* with contaminating bacteria.

Wharf Tows

Wharf tows are a useful method for assessing the nearshore health of local aquatic ecosystems. Here, a plankton net with 10 µm mesh was used to collect samples. Samples were analyzed on the ImageStream^X system, and each species of phytoplankton was characterized using algorithms that measure morphology.

The ImageStream^X system and the environment:

- Streamline: Automate acquisition and complex image analysis
- Comprehensive: Discover the diversity within each sample
- Adaptable: Measure microalgae, pollen grains, air particulates, and more

Figure 14

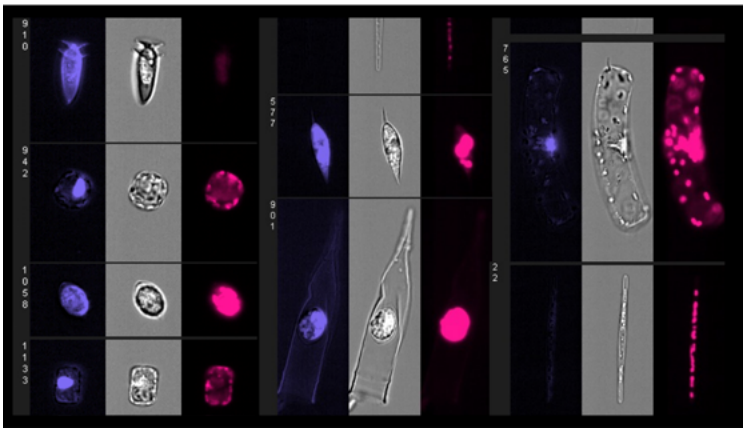
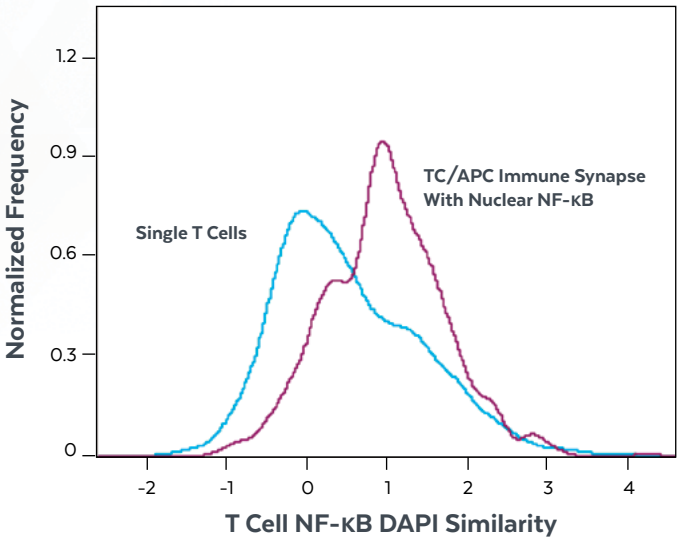


Figure 14: Ocean water filtered with 10 µm mesh.

Applications: Cell Signaling

In this experiment, T cell binding to antigen-presenting cells (APCs) was analyzed using morphology to measure both immune synapse formation and NF-κB translocation within T cells.

Figure 15 shows CD3 T cells binding to CD19 APCs and forming an immune synapse, which is visible when actin (red) migrates to the cell conjugate interface. Following APC binding, T cells induce immune synapse formation and translocate NF-κB to the nucleus during activation, as measured by the Similarity feature.



The ImageStream^X system and cell-cell interactions:

- Unique: Enables analysis that is very difficult to perform with other methods
- Multiplexed: Measures NF-κB translocation induced by APC binding and immune synapse formation
- Robust: Works with many cell types, including CAR T, CAR NK, and others

Figure 15

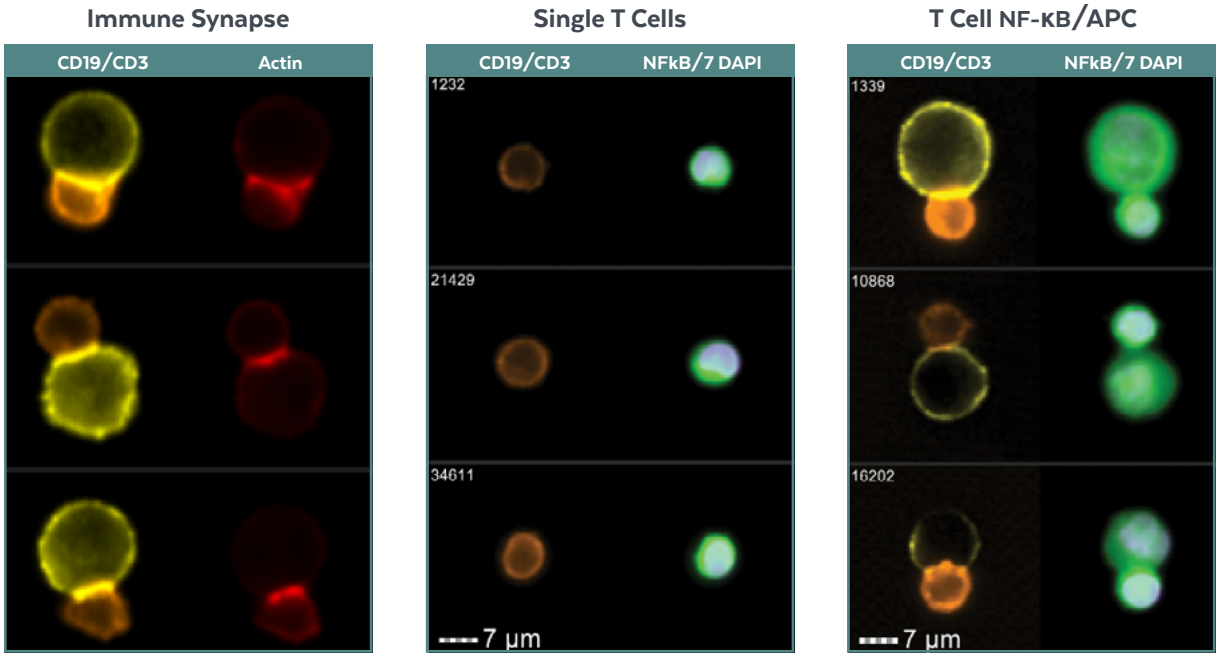


Figure 15: Human T cells mixed with APC (Raji) cells and stained with CD3+ (orange), CD19+ (yellow), Actin (red), NF-κB (green), and DAPI nuclear stain (purple). Immune synapse formation is measured by the presence of an actin band at the conjugate interface followed by NF-κB migration to the nucleus of the T cell, resulting in an increased Similarity Score in the histogram above.

Applications: Extracellular Vesicles Research

High precision is essential for detecting the subtle biological signals generated by viruses, small particles, and the shedding of extracellular vesicles (EVs). The high photonic sensitivity of the ImageStream^X system makes it well suited for studying these small particles. By minimizing background and amplifying signal, the High Gain Mode on the ImageStream^X system can detect particles as small as 27 nm under optimized conditions. Whether studying EV production, measuring single-EV concentrations, or quantifying the uptake of small particles by other cells, the ImageStream^X system provides a versatile platform for a wide range of applications.

Figure 16A

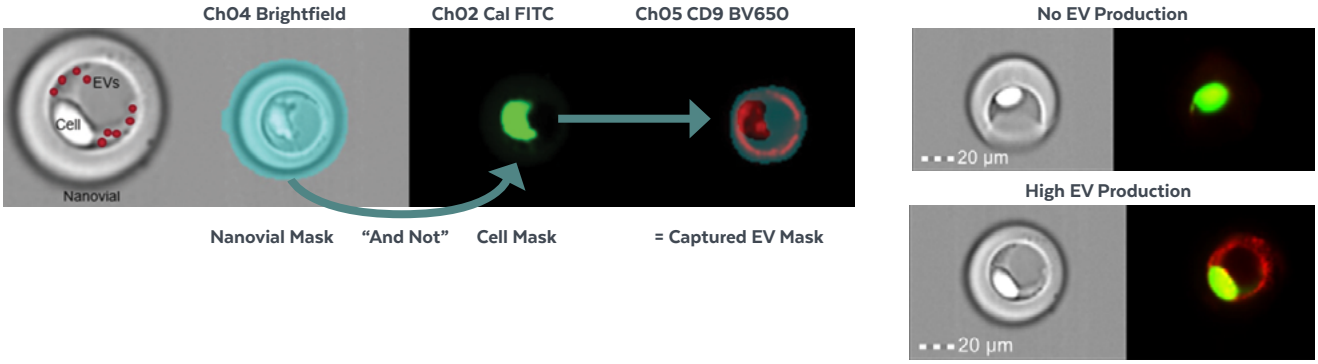


Figure 16B

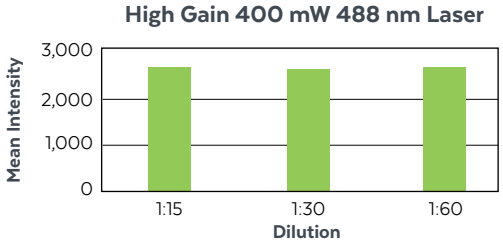


Figure 16C

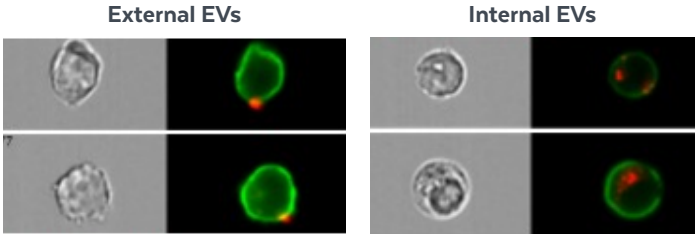


Figure 16: A) EV production and capture in nanovials: EV production is measured on a per-cell basis by identifying signals outside the cell captured by the encapsulating hydrogel (nanovial). Mesenchymal stem cells (MSCs) shedding EVs are captured in nanovials and stained with calcein and CD9. **B)** Viral particles in circulation: Serial dilution of viral particles demonstrates that mean intensity remains constant while concentration decreases, indicating that single particles (rather than swarms) are detected. Total sfGFP viral particle intensity was measured at different concentrations. **C)** EV binding and internalization: EV uptake can be measured using the Internalization feature, while the number of EVs inside the cells can be determined using the Spot Count feature. Representative images of Jurkat cells stained with CD45-FITC (green) and EVs stained with CD63-PE (red).

The ImageStream^X system and small particles:

- Sensitive: High photonic sensitivity provides accurate and reliable results
- Versatile: Measures EV production, circulation, and uptake with a single system
- Flexible: Uses SSC and/or fluorescence for detection

Cell Painting And Machine Learning

Multiplexed Cell Painting

Cell painting is a method for staining critical cellular organelles to establish a standard phenotypic profile for each cell type and is used to identify normal cells from those responding to drug treatment. The advantage of this approach is that a standard cell painting protocol can be applied without the need to develop novel assays for each new compound being tested. In this example, K562 cells were treated with varying doses of the drug phenformin, and changes in cell morphology were tracked using the machine learning module in IDEAS software.

Figure 17

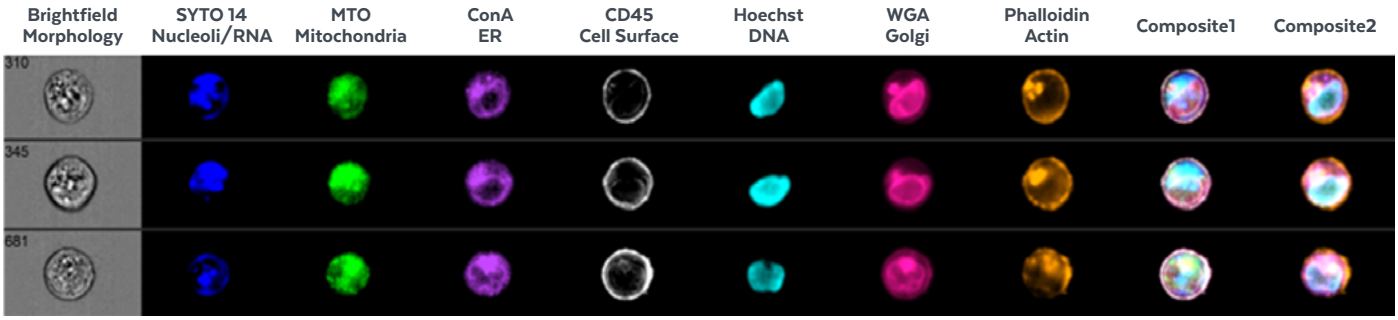


Figure 17: K562 cells stained with Syto14, MTO, ConA, CD45, Hoechst, WGA, and phalloidin. Cells were treated with varying doses of phenformin to induce changes in morphology.

Machine Learning Module

The IDEAS software machine learning module creates custom features to track changes in each fluorescently labeled cellular component and identifies regions where cell responds to drugs. These changes may reveal dose-dependent responses and help uncover the mechanism of action by identifying how cells respond to treatment.

The ImageStream^X system and IDEAS software with machine learning:

- Faster: Analyze more objects and more images per cell than with microscopy alone
- Comprehensive: Stain multiple organelles and measure responses to multiple treatments in a single assay
- Enlightening: Discover critical cellular changes induced by drug treatments

Figure 18

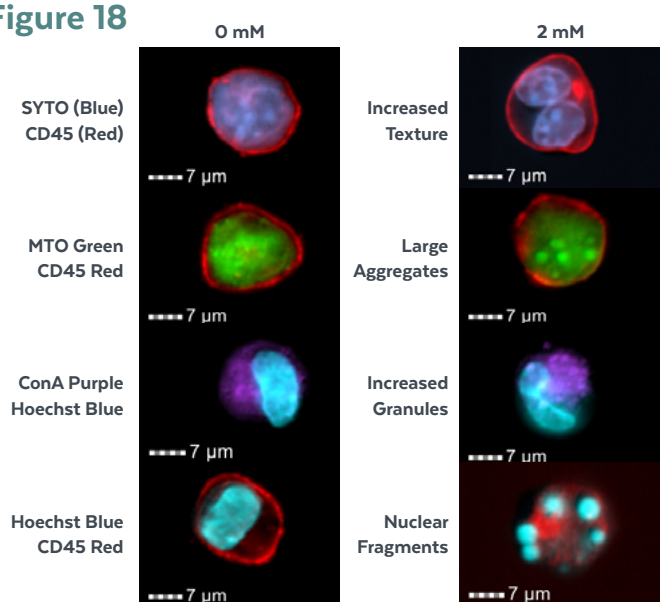


Figure 18: The images above highlight the organelles affected by drug treatment and show cells before (0 mM) and after (2 mM) phenformin treatment.

Micronucleus Detection With Amnis AI Image Analysis Software

The Amnis AI image analysis software seamlessly integrates the power of artificial intelligence for data analysis on the ImageStream^X system. Multiple classifiers, including deep learning with convolutional neural networks (CNNs), allow users to train custom models for advanced image analysis. Unsupervised population clustering combined with a prediction algorithm and accuracy analytics brings this powerful capability to every laboratory.

In this example, Amnis AI software was used to classify nine unique populations of nuclear morphologies, including the identification of micronuclei (MN). MN form when chromosome fragments lag during cell division, indicating toxic exposure. This approach helps automate the otherwise labor-intensive task of counting MN using microscopy.

Figure 19

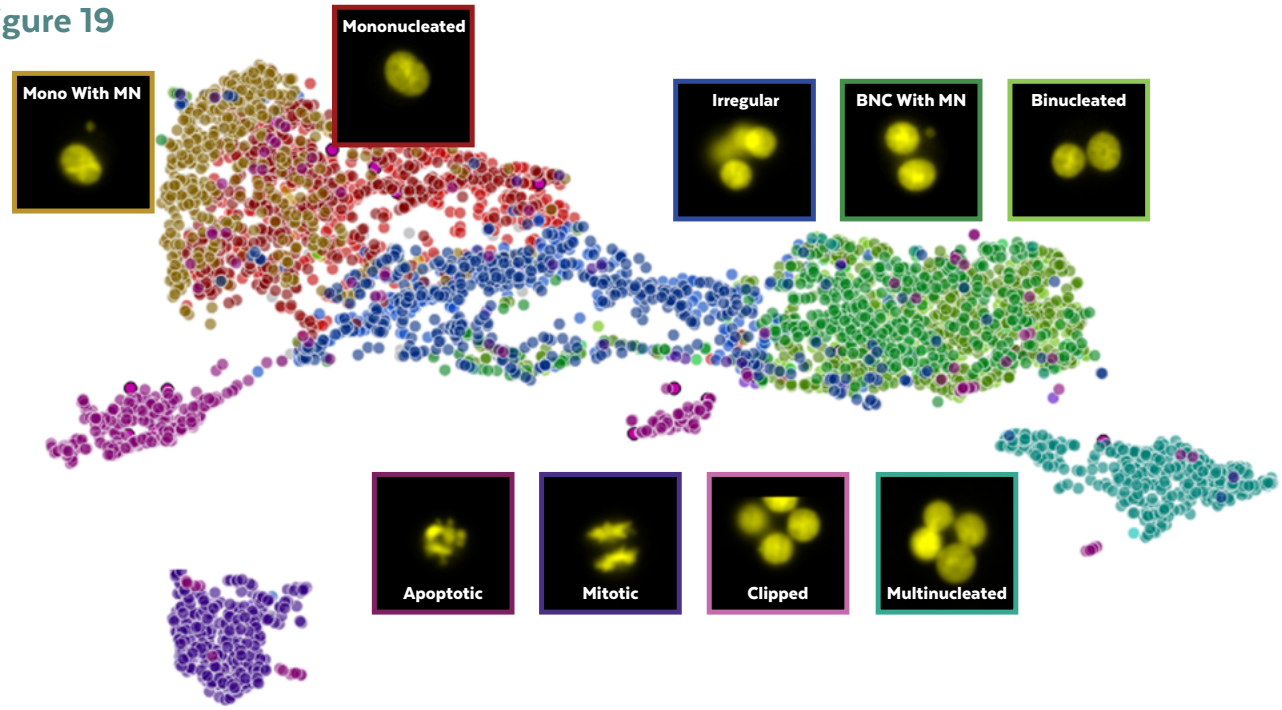
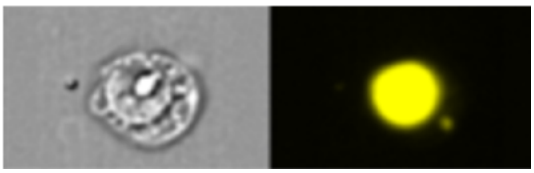


Figure 19: UMAP plot generated using Amnis AI software and trained on multiple cell lines, including TK6, CHO-K1, and L5178Y cells. Samples were treated with various test chemicals to induce MN formation and stained with Hoechst DNA dye.

The ImageStream^X system and micronucleus detection:

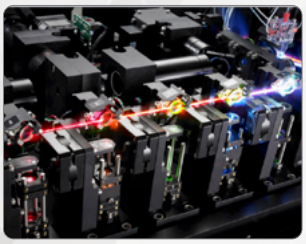
- Screening: Significantly faster than manual microscope-based counting
- Advanced: The Amnis AI model classifies distinct nuclear morphologies, providing reproducible and robust results
- Adaptable: Tests a variety of cell types and compounds

Mononucleated Cell With MN



Binucleated Cell With MN

Customizable Modular System

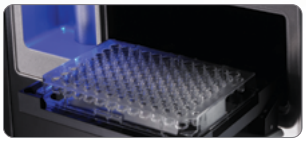


Additional Excitation Lasers

The standard 488 nm blue laser can be upgraded to a high-powered 488 nm configuration and further expanded with optional 375 nm ultraviolet, 405 nm violet, 561 nm yellow-green, 592 nm orange, and 642 nm red lasers. This flexible laser architecture supports a broader range of fluorophores and enables greater experimental versatility for complex multicolor applications.

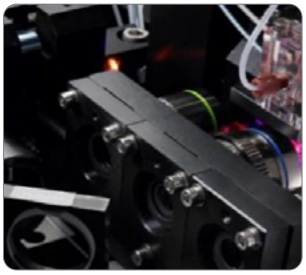
12 Channels Of Detection

Up to 12 high-resolution image channels are available with the addition of a second camera and its associated optics.



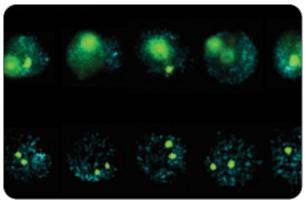
Multi-Well Plate Autosampler

The Autosampler enhances productivity with unattended sample loading from 96-well plates. Its fully integrated design streamlines dose-response and time-course studies.



Multiple Magnification And High Gain Mode

Expand the standard 40X configuration by adding 20X and 60X objectives with the Multiple Magnification (MultiMag) option. Objectives are mounted on a motorized stage, allowing for easy transitions between magnifications and autofocus tracking. The 60X objective is critical for small particle detection, while the 20X objective offers a 120 µm wide field of view for very large cells. MultiMag includes High Gain Mode, effectively doubling the photonic sensitivity of the camera.



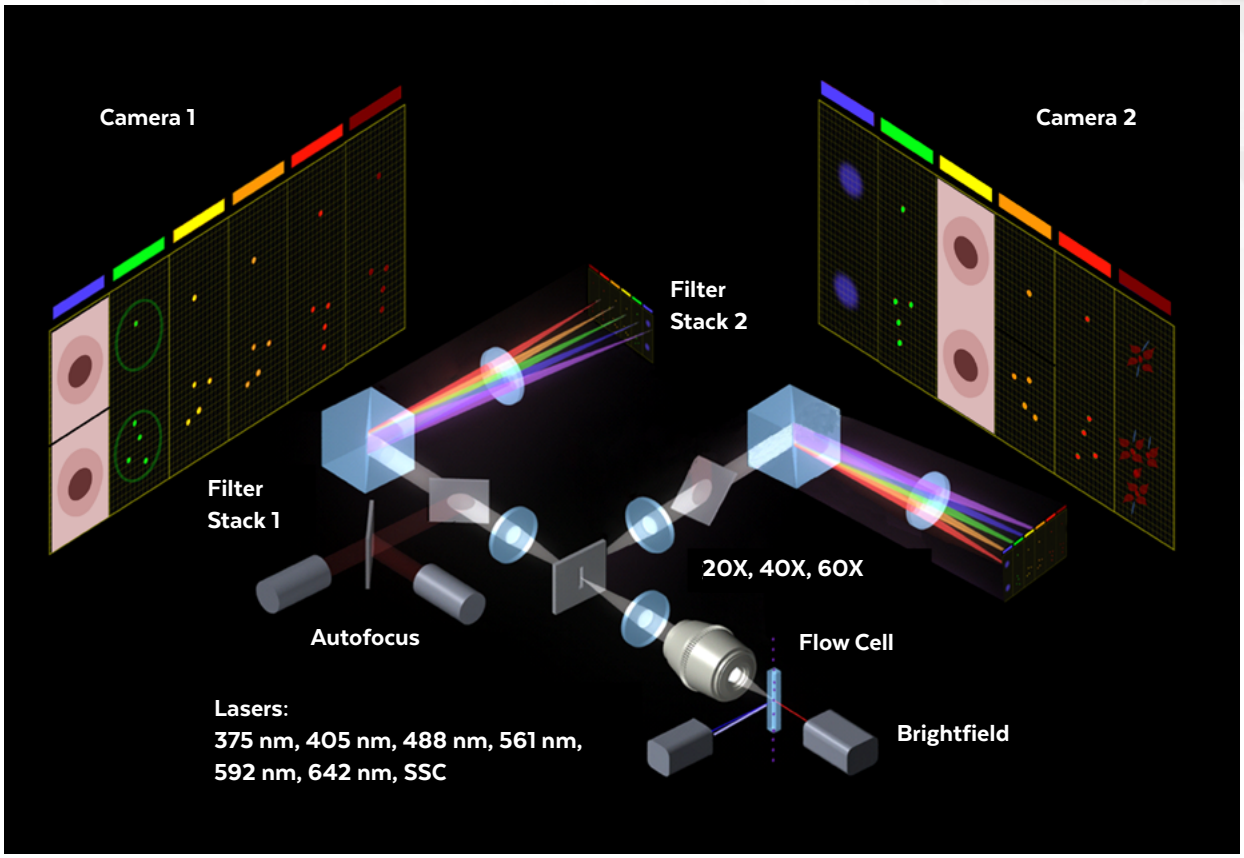
Extended Depth Of Field

The Extended Depth of Field (EDF) element incorporates Wavefront Coding™ technology from OMNIVISION® CDM Optics, Inc., combining specialized optics with advanced image processing algorithms to project all cellular structures into a single, crisp plane of focus. This option is ideal for automated fluorescence *in situ* hybridization (FISH) spot counting.

Upgrades And Options	ImageStream^X System
Additional Excitation Lasers	Upgrade the standard 488 nm laser to a high-powered 488 nm laser, and add optional 375 nm, 405 nm, 561 nm, 592 nm, and 642 nm lasers
12 Channels Of Detection	Upgrade from one camera with six channels to two cameras with 12 channels
Multi-Well Plate Autosampler	96-well plate autoloader and single tube loader
MultiMag	Adds 60X and 20X magnification to a standard 40X system
Extended Depth Of Field (EDF)	Maintains focus through the cell

The Path To Scientific Enlightenment

ImageStream^X Imaging Flow Cytometer Optical Layout



Amnis Spectral Imaging Channels And Corresponding Fluorophores

Camera 1						Camera 2					
Channel 1 435 nm - 480 nm	Channel 2 480 nm - 560 nm	Channel 3 560 nm - 595 nm	Channel 4 595 nm - 642 nm	Channel 5 642 nm - 745 nm	Channel 6 745 nm - 785 nm	Channel 7 435 nm - 505 nm	Channel 8 505 nm - 570 nm	Channel 9 570 nm - 595 nm	Channel 10 595 nm - 642 nm	Channel 11 642 nm - 745 nm	Channel 12 745 nm - 785 nm
Brightfield AF350+ BV421+ AF405+ PacificBlue+ DAPI+ Hoechst+	cFluor® B515 cFluor® B520 FITC AF488 BB515 GFP SYTO13 MT Green	cFluor® B515* PE* DSRed AF546 Cy3 PKH26	cFluor® BYG610* PE-TexasRed* PE-AF610* ECD* RFP mCherry	cFluor® BYG610* cFluor®B690 BB700 PerCP PerCP-Cy5.5 Draq5* PE-Cy5* 7AAD* PI*	cFluor® BYG781* PE-Cy7* PE-AF750* Ch6 SSC	cFluor® V435* BV421* AF405* Pacific Blue DAPI* Hoechst* L/D Violet* D/C Violet* AF350	cFluor® V547* BV510* PacOrange AF430	Brightfield	cFluor® V610 BV605 eFluor625 AF594 CF594 SpectrumRed	cFluor® R668 cFluor® R659 AF647 CF647 APC* Draq5* BV711 eFluor700	cFluor® R780 APC-Cy7* APC-750* BV786 Ch12 SSC

This is not a complete list. Fluorophores of equivalent excitation and emission spectra can replace those listed here as applicable.

This chart displays the 12-channel ImageStream^X system, 6-channel systems will coincide with the fluorophores listed in the chart channels 1-6.

Excitation Lasers	375 nm Laser*	405 nm Laser*	488 nm Laser*	561 nm Laser*	592 nm Laser*	642 nm Laser*

**Some fluorophores will excite with multiple lasers.*

+375 laser is aligned to Channel 1 in systems with a 405 laser, otherwise its aligned to Channel 7. Brightfield can be collected in any channel to accommodate fluorescence.

Ordering Information

Product Name	Part Number
Cytek® Amnis® ImageStream®X Mk II Flow Cytometer	100220
Cytek® Amnis® SpeedBead® Kit	CN-0440-01
Cytek® Amnis® NF-kB Translocation Kit	ACS10000
Cytek® Amnis® Protein Aggregate And Silicone Oil Detection Kit	APH10001
Cytek® ImageStream®X Mk II Gold Maintenance And Service Agreement	SLA-ISXMkII-Gold
Cytek® ImageStream®X Mk II Silver Maintenance And Service Agreement	SLA-ISXMkII-Silver
Cytek® ImageStream®X Mk II Bronze Maintenance And Service Agreement	SLA-ISXMkII-Bronze
Cytek® Amnis® ImageStream®X Mk II System IQ/OQ	603222
Cytek® On-Site ImageStream®X Mk II Training - TAS 1 Day; Up To 5 people	500200-1
Cytek® On-Site ImageStream®X Mk II Training - TAS 2 Consecutive Days; Up To 5 People	500200-2
Cytek® On-Site ImageStream®X Mk II Training - TAS 3 Consecutive Days; Up To 5 People	500200-3
Cytek® On-Site ImageStream®X Mk II Training - TAS 4 Consecutive Days; Up To 5 People	500200-4
Cytek® On-Site ImageStream®X Mk II Training - TAS 5 Consecutive Days; Up To 5 People	500200-5
Cytek® Amnis® IDEAS® Image Analysis Software – 21 CFR Part 11 Enabled	IFC300202
Cytek® Amnis® INSPIRE™ And IDEAS® Software 21 CFR Part 11 Enabled, Software For ImageStream®X Mk II System	IFC300204
Cytek® Amnis® IDEAS® Image Analysis Software – Single Seat License	CN-SW69-01
Cytek® Amnis® IDEAS® Image Analysis Software – 12 Seat Group License	CN-SW69-12
Cytek® Amnis® IDEAS® Image Analysis Software – Institutional License	CN-SW69-20
Cytek® Machine Learning Module	CN-SW45-01
Cytek® Amnis® AI: Computer-Aided Image Analysis Software	CN-SW70-01

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cFluor® BYG610 and cFluor® BYG781 are tandem dyes made with R-PE. cFluor® B690 is a tandem dye made with PerCP. cFluor® R780 is a tandem dye made with APC. Caution: Tandem dyes may show changes in their emission spectra with prolonged exposure to light or fixatives.

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