# CYTEK®
## TRANSCEND THE CONVENTIONAL



# Cytek® Amnis® ImageStreamX® Mk II Flow Cytometers

Microscopy In Flow

# Spanning the Research Disciplines in the Life Sciences

Microscopy offers detailed cellular images and morphologic information, which are useful scientific tools for the study of cell function. However, the interpretation of microscopy images can be subjective, qualitative, and laborious.

Flow cytometry is excellent for quantitative phenotyping and yields statistically robust results by rapidly interrogating large numbers of cells. However, conventional flow cytometry lacks any ability to image, so sub-cellular localization and functional studies are

difficult at best.

By combining the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insight of microscopy, the Cytek® Amnis® ImageStream®X Mk II instrument overcomes the limitations of both techniques and opens the door to an extensive range of novel applications.
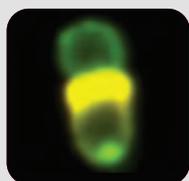
Immunology
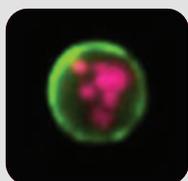
Oncology
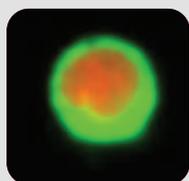
Biochemistry
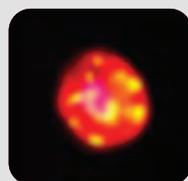
Drug Discovery
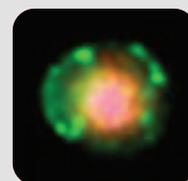
Stem Cell Biology

Hematology
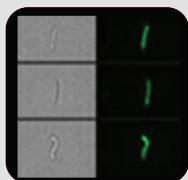
Microbiology
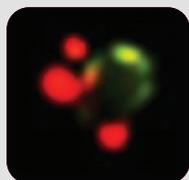
Virology

Nanotechnology

Toxicology
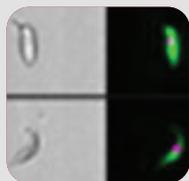
Parasitology

Oceanography

Phycology
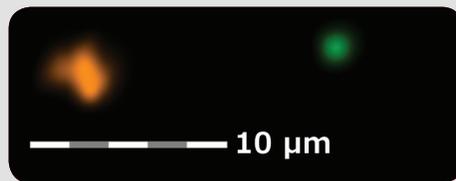
Small Particle Analysis

10 μm

# Powerful Flow Cytometry

The ImageStream$^X$ Mk II system delivers multiple images of every cell in flow, including brightfield, darkfield (SSC), and up to 10 fluorescent markers at high speed. The ImageStream$^X$ Mk II camera operates with a pixel size of 0.1/0.25/1 µm$^2$ with 60X/40X/20X magnification, respectively, allowing visualization of fluorescence location from the membrane, cytoplasm, subcellular organelles, and nucleus at high resolution.

The innovative design of Amnis flow cytometers increases signal and minimizes noise to provide exceptional photonic sensitivity. Design details like a dedicated side scatter laser, adjustable laser intensities, and brightfield imagery for the direct measurement of cell size allow the system to resolve cell populations more effectively than far more expensive cytometers. The ease of use, outstanding performance, and imagery of each cell meet the needs of flow cytometry novices and experts alike.

## Beyond forward and side scatter

Traditional flow cytometers do an admirable job of using low-resolution scattering characteristics to approximate size and intracellular granularity. Amnis imaging flow cytometers produce familiar 'size vs. complexity' scatter plots, but with the power of 20X magnification—or more—can report absolute rather than relative cell size by measuring the actual diameter of objects using the brightfield images.
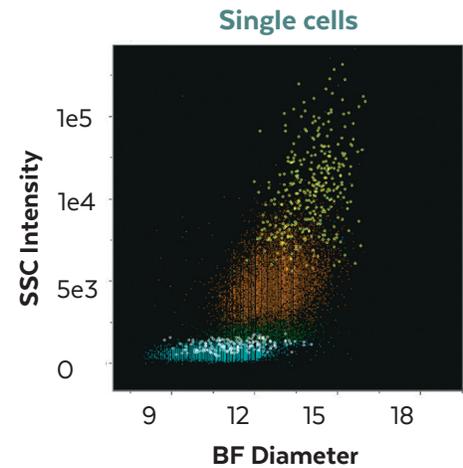
## Multichannel immunophenotyping

Immunophenotyping requires multiple fluorescent channels in addition to dual scatter. Below is a 6-color immunophenotype of human PBMCs using antibodies against CD3, CD4, CD14, CD16, CD45, and CD123, plus DAPI. The arrangement of detection channels, available laser options, and automated compensation wizard allows for the straightforward separation of complex cell populations.

**Single cells**
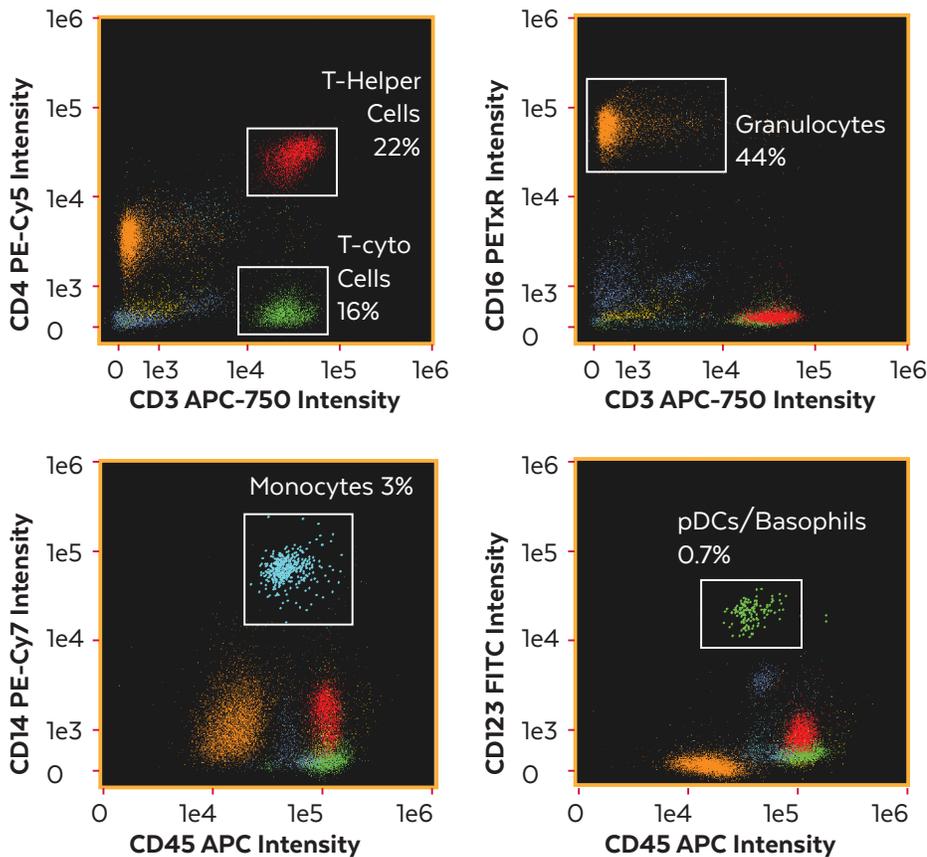
# Sensitive and Flexible for Any Research Need

## Exceptional fluorescence sensitivity

The patented architecture of Amnis imaging flow cytometers provides extraordinary fluorescence sensitivity across the visible spectrum, outperforming other imaging devices. The four plots below demonstrate the ability of the Amnis systems to discriminate all intensities in the Spherotech 8-peak calibration bead set, across the spectrum from FITC to PE-Cy7. Note the distinct peak separation, low coefficients of variance (CVs), and high sensitivity from the FITC to the PE-Cy7 channels.
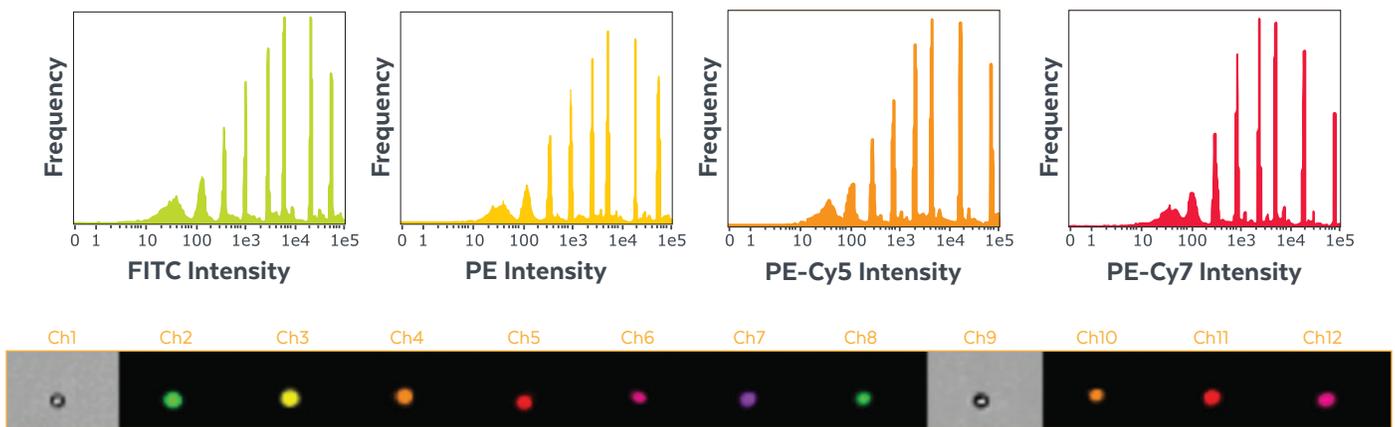


Amnis system 12 channel imagery of 3-micron diameter Spherotech 8-peak Rainbow beads.

## 5-Part white blood cell differential

Because of its exceptional sensitivity, the ImageStream[X] system excels at the resolution of mixed sub-populations in heterogeneous samples. Human peripheral blood mononuclear cells (PBMCs) are partitioned into 5 distinct populations using CD45 expression and side scatter intensity. High fluorescence sensitivity and tight coefficients of variance (CVs) resolve monocytes (green) from lymphocytes (blue) and facilitate the detection of rare basophils (white). The dedicated side scatter laser clearly resolves eosinophils (yellow) from neutrophils (orange).
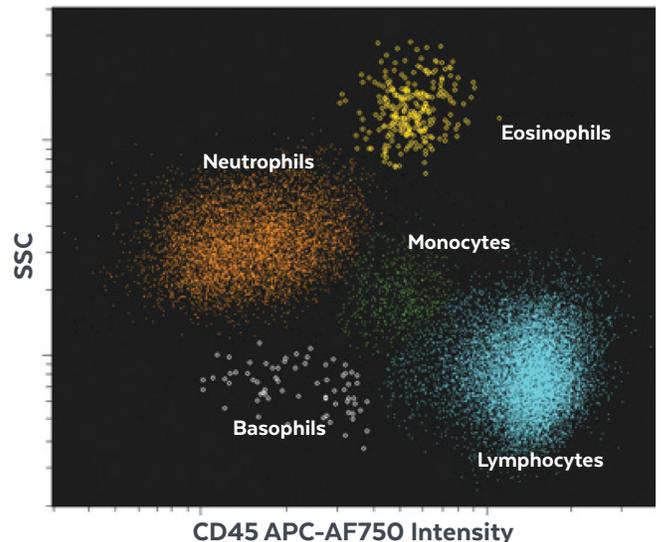
# Sensitive and Flexible for Any Research Need

## Images of every cell

The ImageStream[X] Mk II instrument operates like a conventional flow cytometer, but also provides imagery of every cell. Powerful and intuitive analysis software seamlessly links quantitative data to images:

- Click on a dot in any plot to see 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
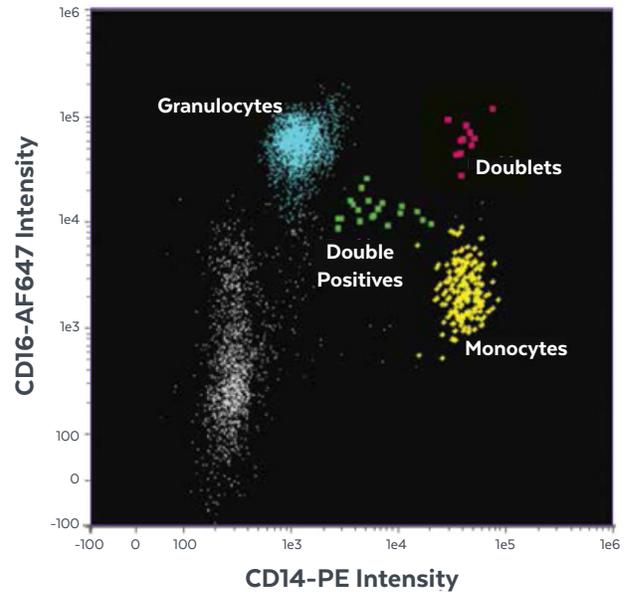


With imaging capabilities, you'll never wonder about outliers or whether your gates are in the right place, as shown in the example above. Once you've drawn a gate on a plot, you can click inside and out to determine if it's in the right place, as shown in the example to the right. With visual feedback, you can optimize gate size, shape, and position for better data quality.
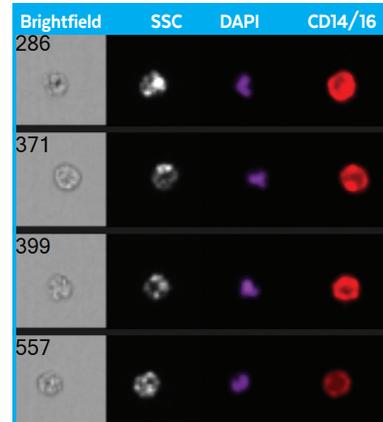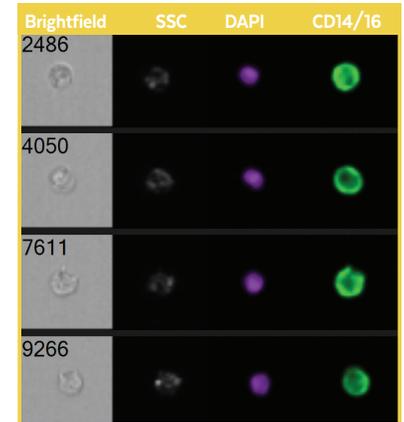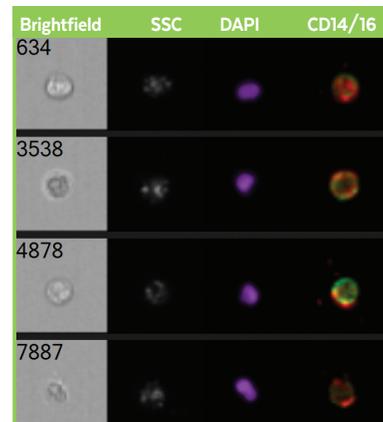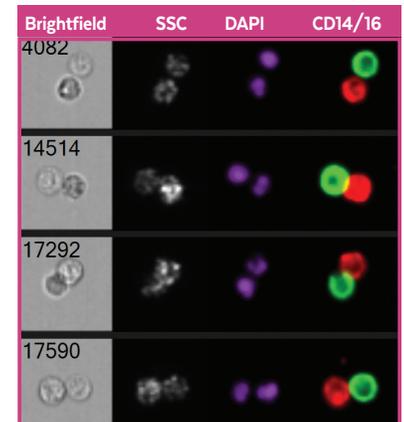
# Data Acquisition Software

## Fast and easy.

Cytek® INSPIRE™ software offers powerful, image-based gating and real time fluorescence compensation.

1.  **Instant Population Viewer**: Every population is added to a pull-down list as soon as you draw a gate. Simply select a population of interest from the list to view the corresponding cells during data acquisition.

2.  **Image Gallery**: Imagery of cells of interest appear in the gallery as they are acquired, allowing you to inspect morphology, assess staining patterns, and optimize laser power settings.

3.  **Instrument Status at a Glance**: Convenient gauges, indicators, and text alerts provide continuously updated instrument operational status.

4.  **Real Time Intensity Compensation**: An easy to use compensation wizard quickly guides you through the setup of multi-color compensation matrices.

5.  **Gating Without Guesswork**: Gates are easily drawn using graphical tools, and verified for accuracy by visual inspection of gated cells.

6.  **Efficient Sample Handling**: Up to 95% of the sample volume is utilized, facilitating the analysis of rare cells. Unused sample can be recovered for further analysis.

7.  **Intuitive Acquisition**: A simple and intuitive user interface provides complete control of sample acquisition settings and data storage criteria.

8.  **Familiar Dot Plots and Histograms**: Data plots are updated in real time, just as with conventional flow cytometers. Unlike conventional cytometers, you can also plot morphologic parameters such as area, cell width, cell height, aspect ratio, and more.

## INSPIRE™ Software

# Software That Turns Data Into Understanding

Cytek® IDEAS® with Machine Learning and Amnis® AI software combines image analysis, statistical rigor, and visual confirmation in an easy to use software suite that works seamlessly with your ImageStream data sets.

1. **Inspect Your Populations**: The Image Gallery allows you to see every image of every cell or perform a "virtual cell sort" to inspect and validate the cells within a specific population.

2. **Images for Every Dot**: Every dot in every scatter plot is linked to the corresponding cell imagery. Simply click on a dot to see the associated cell images or vice-versa.

3. **Graphical Population Definitions**: Define populations using familiar graphical tools and combine them with logical functions.

4. **Comprehensive Population Statistics**: Characterize your cell populations with a wide range of statistical metrics to reveal differences in cell morphology, phenotype, and function.

5. **Flexible Image Display Tools**: Create composite images, pseudo-color representations, and a host of other image transformations for reporting and publication.

6. **Graph What You See**: Virtually anything you see in the imagery can be plotted as a histogram or dot plot. Hundreds of parameters are calculated for every cell, including fluorescence intensity, fluorescence location, cell shape, cell texture, and numerous other morphologic and photometric features.

7. **Machine Learning Module**: Adds the power of computer learning and the ability to create experiment specific super-features tailored to maximize separation of your critical populations using the thousands of features in your IDEAS analysis.

**Amnis® AI analysis software:** This stand alone software package takes advantage of deep learning using convolutional neural networks to greatly simplify analysis and accurately identify even the most subtle of cell differences. With computer aided tagging, object map plots for identifying unique cell types, and accuracy analytics, using artificial intelligence with your ImageStream data has never been easier.

## IDEAS® Software with Machine Learning

# A Wealth of Applications

## Any application you can imagine.

### Featured applications

The applications detailed on the following pages demonstrate the types of studies that can be performed using the ImageStream^X Mk II instrument with the powerful companion IDEAS® with Machine Learning image analysis software.

### Any application you can imagine

The ImageStream^X Mk II system is designed to be a general-purpose platform for cellular studies and is not limited to the applications illustrated in this brochure.
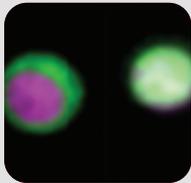


Cell Signaling
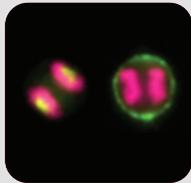
Cell Cycle and Mitosis
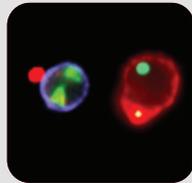
Internalization and Co-localization
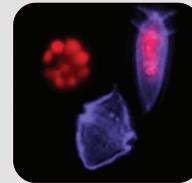
Oceanography
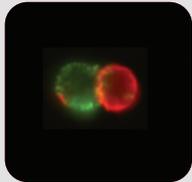
Cell-cell Interaction
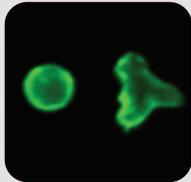
Shape Change and Chemotaxis
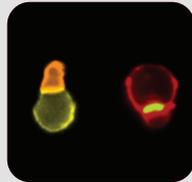
Immunological Synapse
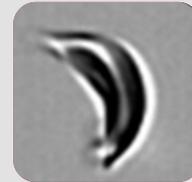
RBC Morphology
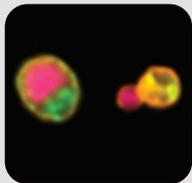
Stem Cell Biology

Parasitology
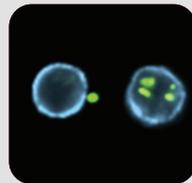
Microbiology
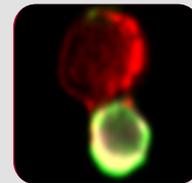
CAR-T CRISPR/ Cas9
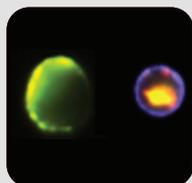
Surface and Intracellular Co-localization
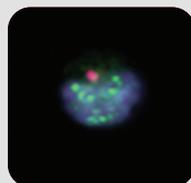
DNA Damage and Repair

Micronucleus Counting
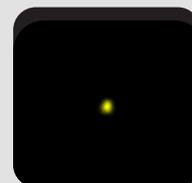
EVs Nanoparticles

# Quantify Nuclear Translocation with Fluorescent Image Similarity

## A closer look at NF–κB signaling with 60X magnification

Translocation of NF–κB from the cytoplasm to the nucleus of the cell is a key event in the response to the presence of cell stressors. Only imaging flow cytometers can analyze translocation quantitatively, in thousands of cells. Here, THP-1 cells unstimulated or stimulated with LPS are stained with anti-NF–κB and 7-AAD to counterstain the nucleus, were collected on the ImageStream^X Mk II system using the 60X objective. The IDEAS software similarity feature is clearly able to separate translocated events (blue histogram) from cells with cytoplasmic NF–κB (Yellow)
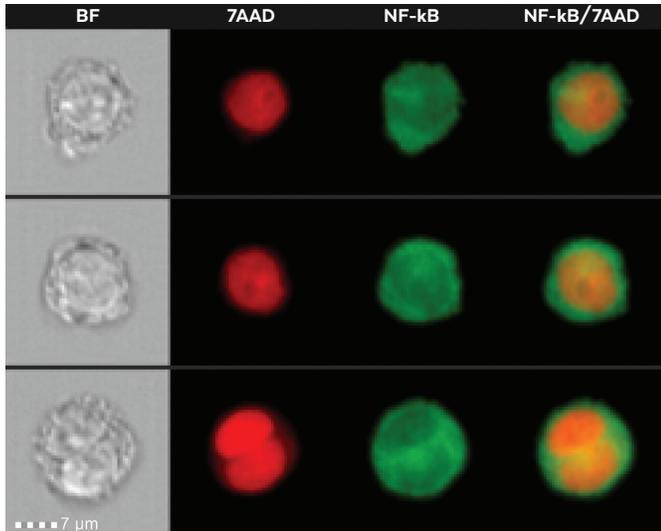


**THP-1 Control (no LPS)**
**Mean similarity score = 0.2**
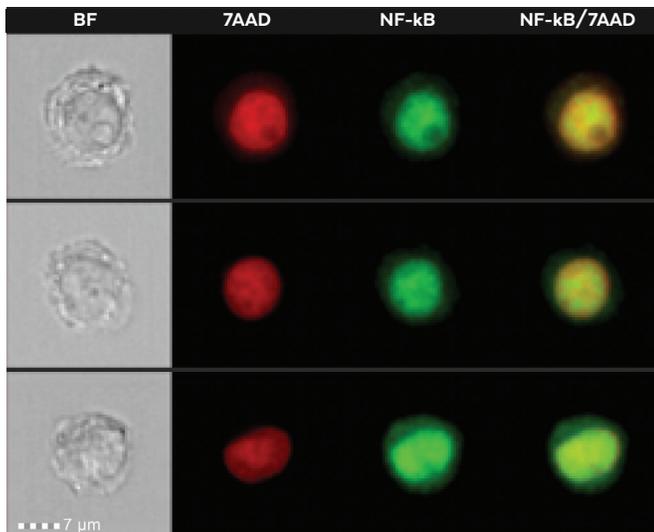


**THP-1 Control (no LPS)**
**Mean similarity score = 3.8**
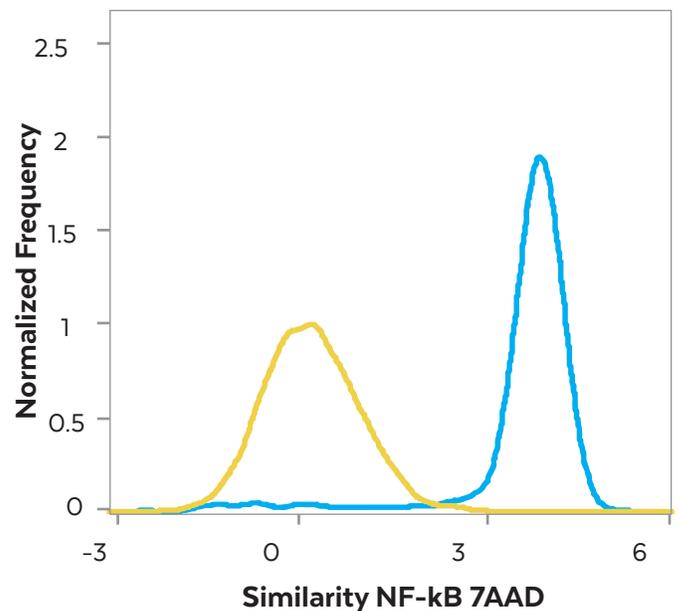
# Quantitative Imaging and Robust Population Statistics

Quantitative imaging means Cytek's ImageStream$^X$ Mk II has a powerful and intuitive image processing package with thousands of analysis parameters and optimized analysis wizards for many common image-based applications, including nuclear translocation, shape change, internalization, and apoptosis.

Objective, quantitative image analysis on large numbers of cells is backed by a large set of statistical parameters for data reporting.

Select the wizard to use for analysis:

- Open File
- Display Properties
- Begin Analysis
- Feature Finder
- Apoptosis
- Cell Cycle - Mitosis
- Co-localization
- Internalization
- Nuclear Localization
- Shape Change
- Spot
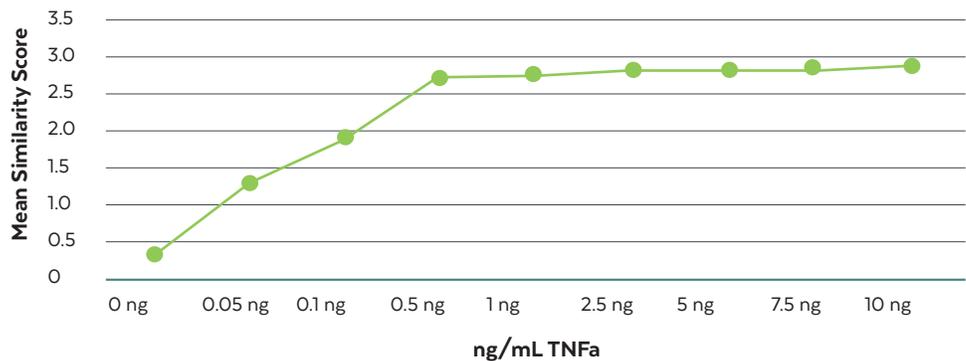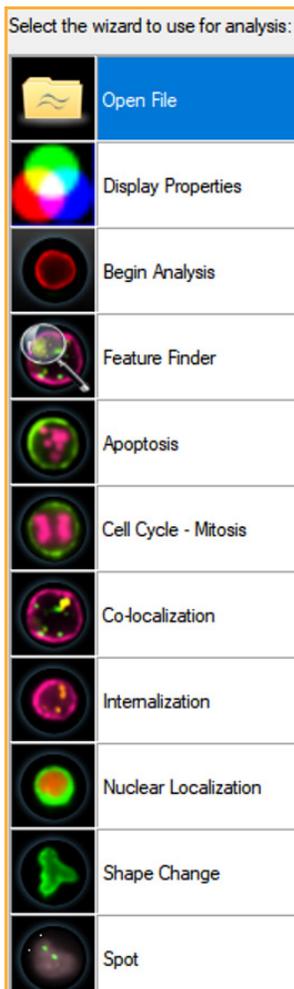
| File | Count All | Count Focus | Count Singles | Count Positive | Mean Similarity | Std Dev Similarity |
|------|-----------|-------------|---------------|----------------|-----------------|--------------------|
| TNFa_0ng_2_2016.daf | 10000 | 4903 | 4265 | 3740 | 0.34 | 0.71 |
| TNFa_0-05ng_3_2016.daf | 10000 | 4621 | 4060 | 3635 | 1.28 | 0.81 |
| TNFa_0-1ng_4_2016.daf | 10000 | 4280 | 3739 | 3365 | 1.90 | 0.82 |
| TNFa_0-5ng_5_2016.daf | 10000 | 4861 | 4167 | 3516 | 2.68 | 0.66 |
| TNFa_1ng_6_2016.daf | 10000 | 3811 | 3311 | 2910 | 2.72 | 0.63 |
| TNFa_2-5ng_7_2016.daf | 10000 | 3893 | 3425 | 3070 | 2.80 | 0.58 |
| TNFa_5ng_8_2016.daf | 10000 | 4162 | 3685 | 3180 | 2.80 | 0.52 |
| TNFa_7-5ng_9_2016.daf | 10000 | 4361 | 3782 | 3387 | 2.82 | 0.58 |
| TNFa_10ng_10_2016.daf | 10000 | 4005 | 3456 | 2988 | 2.90 | 0.55 |

# Co-localization and Trafficking

The ImageStream$^X$ Mk II instrument greatly improves co-localization studies by combining the rapid collection of large numbers of cell images with objective measurement of the similarity of bright image details.

**Time**: 30 min



**Example:** Internalization and Trafficking of CpGB in Primary Plasmacytoid Dendritic Cells (pDC)
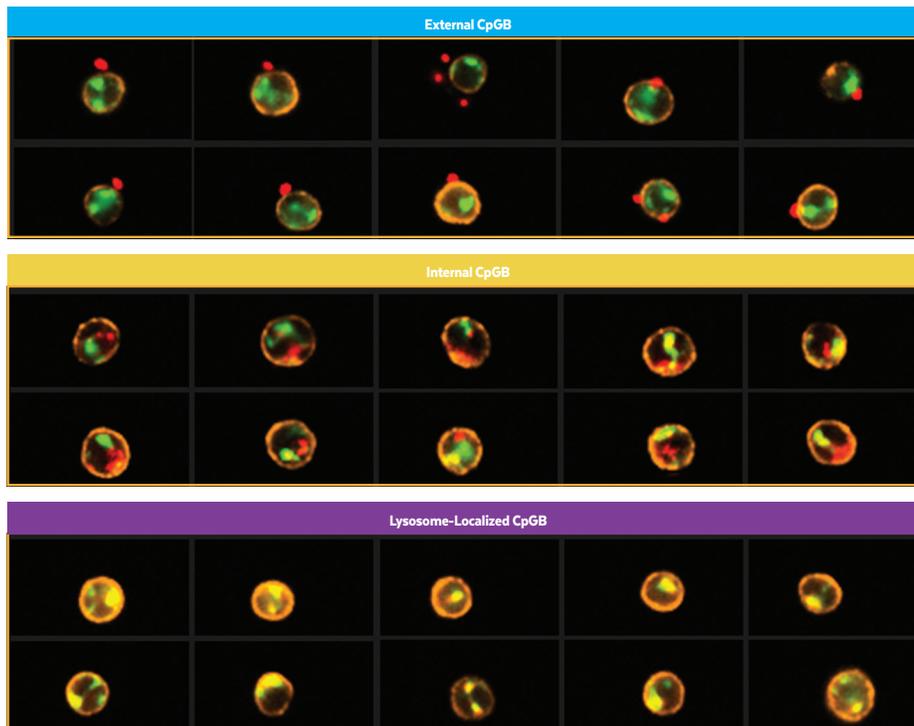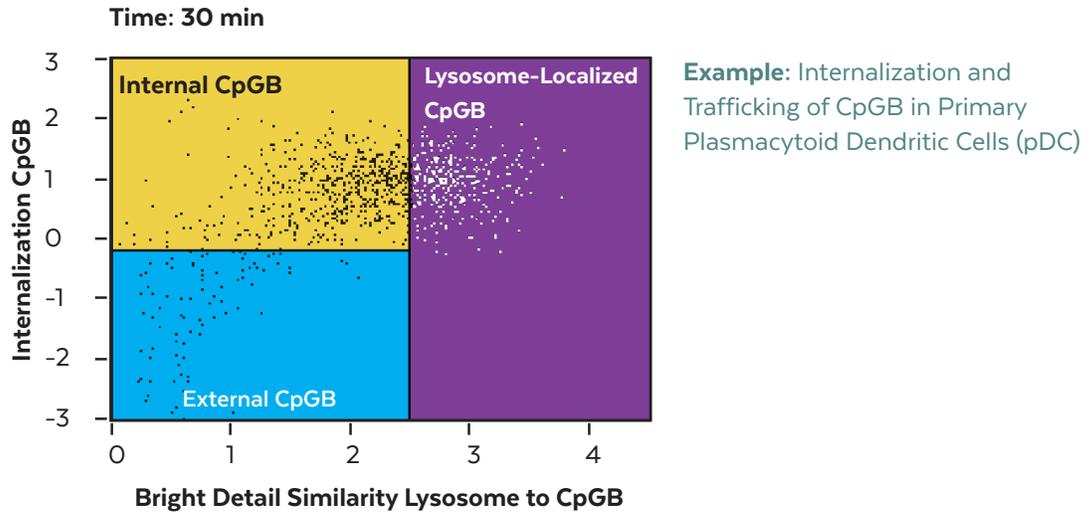


Lysosomal trafficking of CpGB within pDCs is quantified using the Internalization (Y-axis) and the Bright Detail Similarity (X-axis) scores. Representative merged images of pDC (orange), CpGB (red), and lysosomes (green) are shown at 40X magnification. Cells within the lower left region of the plot and have surface-bound CpGB. As CpGB molecules enter the pDC, the Internalization score increases (upper left region). Once the CpGB traffics to the lysosomes, the similarity between the CpGB and lysosome image pair increases (upper right region).

*Data courtesy of Dr. Patricia Fitzgerald-Bocarsly, University of Medicine and Dentistry, New Jersey.*

# Apoptosis and Necrosis

## Apoptosis and necrosis detection by image analysis

The apoptosis wizard analyzes the nuclear morphology and brightfield image contrast of each cell to detect apoptosis in any sample containing a nuclear stain.
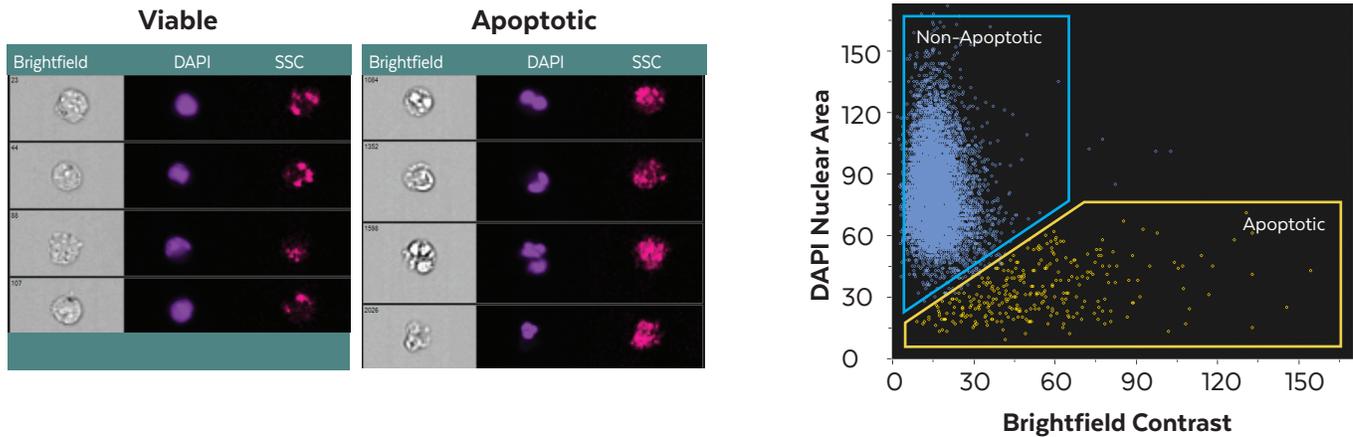


## Necrosis versus apoptosis

Conventional flow cytometers can use membrane-impermeant dyes to identify dead or dying cells that have lost membrane integrity. However, it can be difficult to determine if cell death is via apoptosis or necrosis. The ImageStream[X] Mk II system simplifies this determination by revealing the nuclear morphology of every cell. As shown in this sample of THP-1 cells labeled with propidium iodide, the nuclei of necrotic cells have normal nuclear morphology, while the nuclei of apoptotic cells appear shrunken and fragmented.
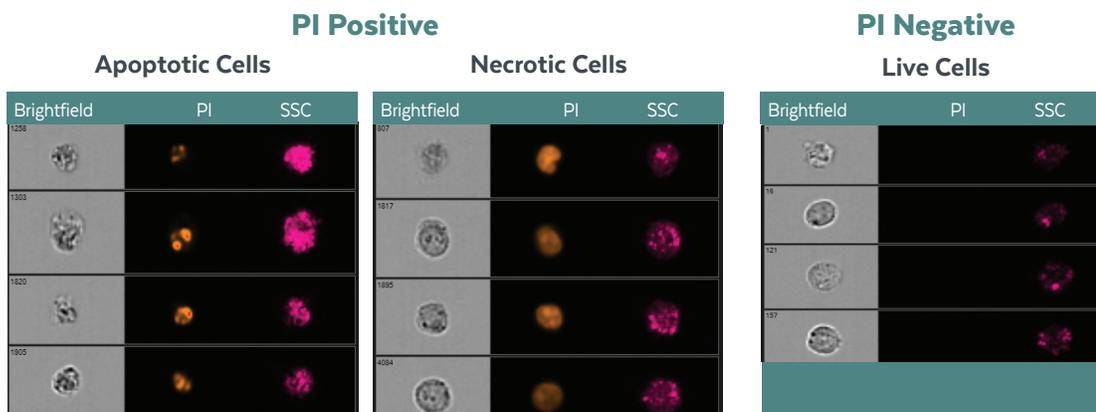
# Autophagy

During autophagy, cytoplasmic LC3 is processed and recruited to the outer membrane of autophagosomes. Cells undergoing autophagy can be identified by visualizing LC3 puncta and enumerating the spots within each cell using the Spot Count feature of the IDEAS software package.



**1**  **4**  **7**  **>15**

**Spot Count Scores**
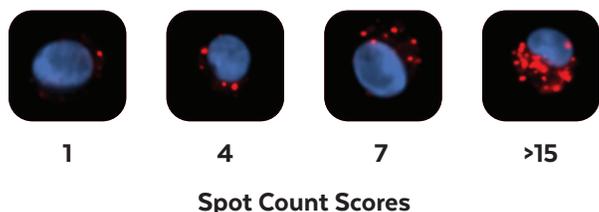
The IDEAS image processing software included with the ImageStream$^X$ Mk II system determines the Spot Count of every cell. In this example, cells with varying number of LC3-RFP (red) spots are shown with their corresponding Spot Count.

## Example: Autophagy in the human osteosarcoma cell line U2OS

A serum starvation step can induce autophagy in U2OS cells. This data demonstrates how the IDEAS feature Spot Count can be used to quantify the textural variation between healthy cells and those undergoing autophagy.



**Control Untreated**
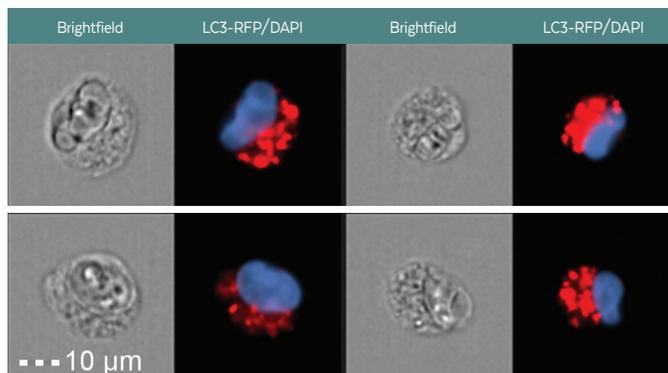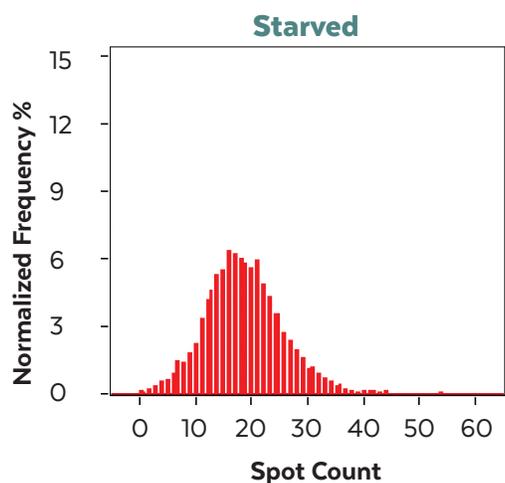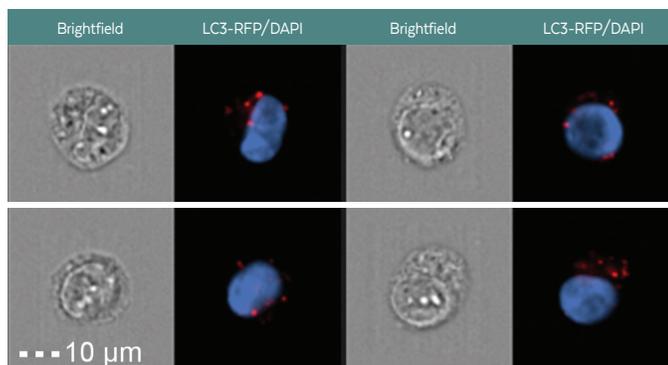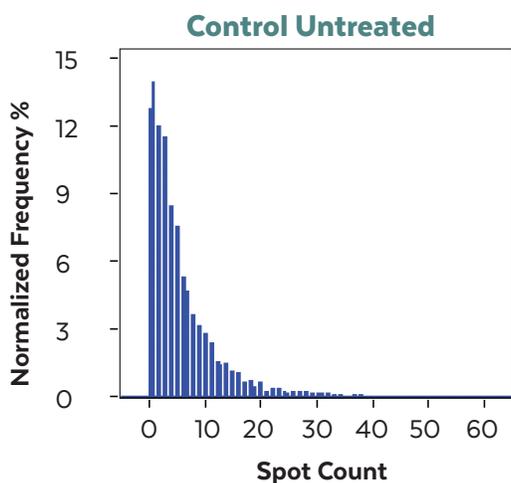


**Starved**

U2OS RFP-LC3 human osteosarcoma reporter cell line was starved for 4 hours at 37°C. Both the control and starved samples were supplemented with a degradation inhibitor. FlowCellect™ RFP-LC3 Reporter Autophagy Kit (Catalog No. FCCH100183).

# Morphology

Change in cell shape is correlated with change in function, particularly in the case of macrophage activation, stem cell differentiation, and cellular response to drugs. The ImageStream[X] Mk II instrument measures cell shape using powerful, pre-defined features in the IDEAS image analysis software. One such feature is the Circularity score.

The Circularity score is a measure of how much the cell radius varies. Round cells (left) have high Circularity scores while irregularly shaped cells (right) have low Circularity scores.



**23.8**        **4.6**

**Circularity Scores**
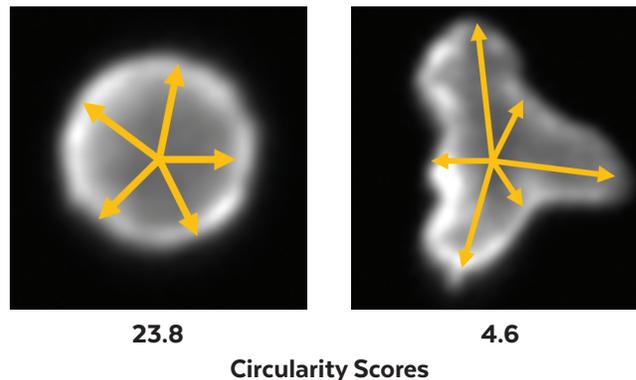
## Example: Shape change in primary monocytes

Chemoattractant 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 for autoimmune disorders – result in an increase in Circularity scores.



**Untreated**



High Circularity



**MCP-1 Treated**



Low Circularity
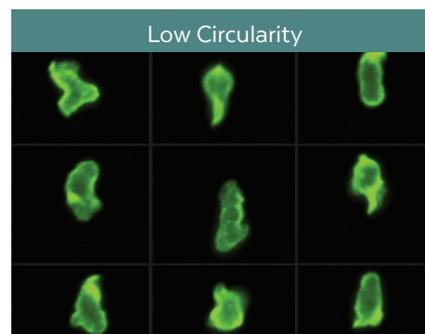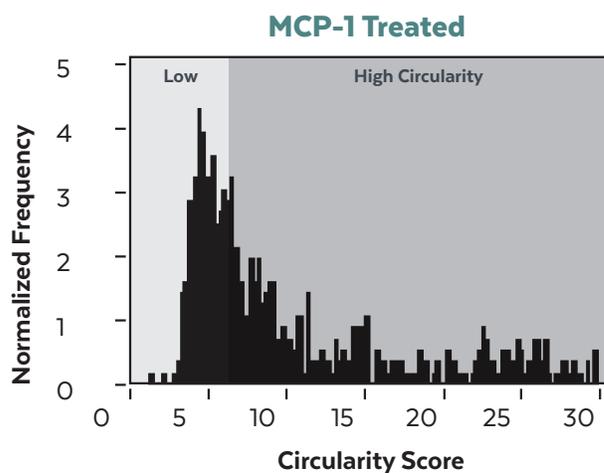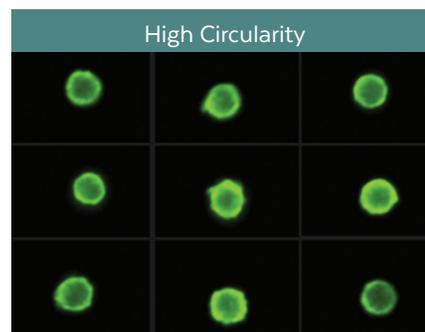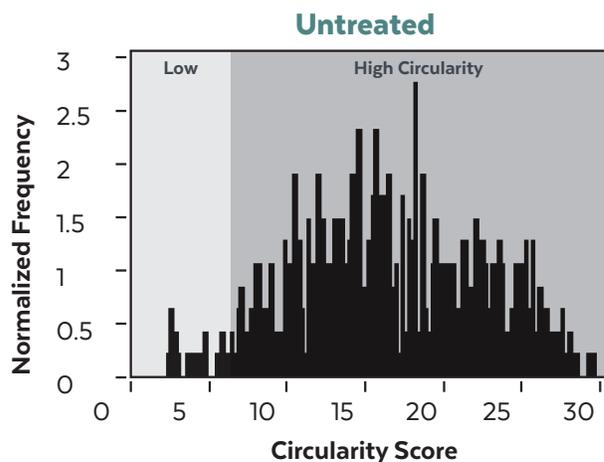
# Microalgae

## Mixed cultures of microalgae

The images below demonstrate microalgae identification in mixed cultures using morphological parameters with the ImageStream$^X$ Mk II instrument at 40X magnification.



T. pseudonana
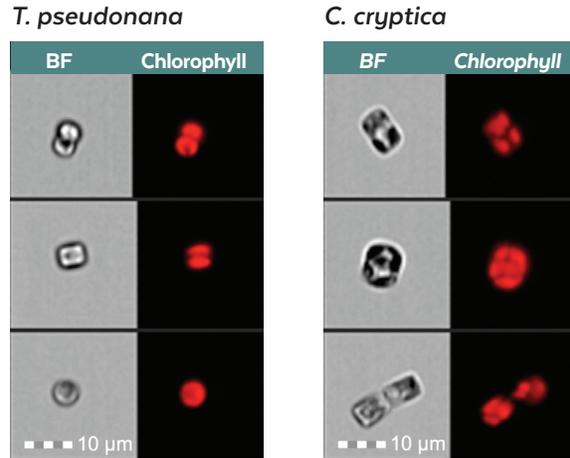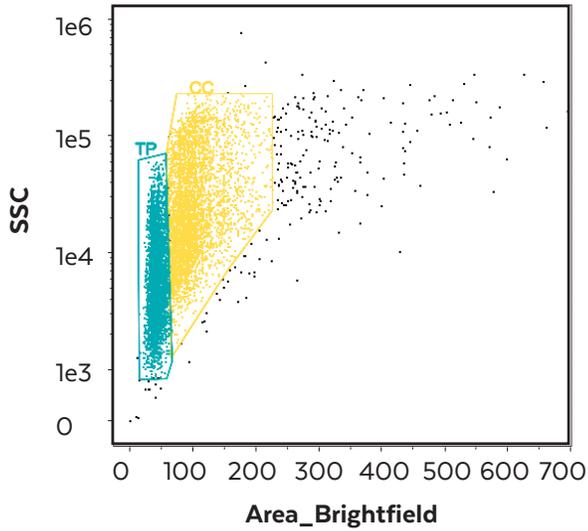
C. cryptica



## Microalgae quality control

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



T. pseudonana

C. cryptica

Diatom Clusters

Bacteria/Debris
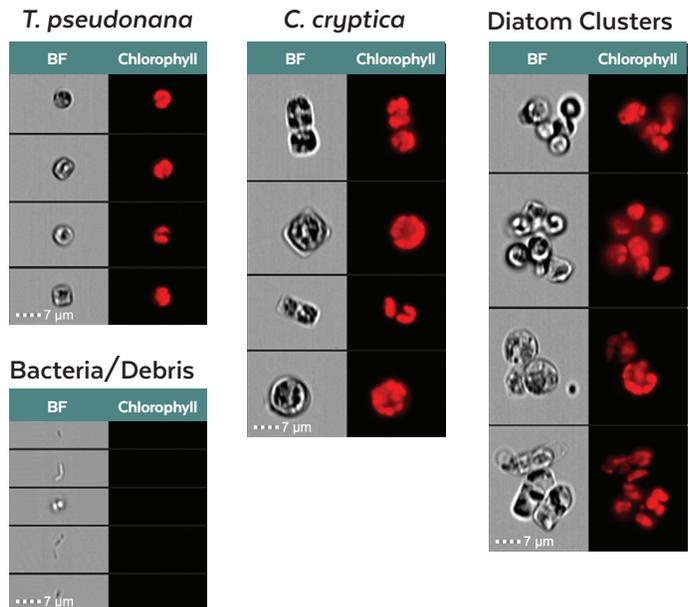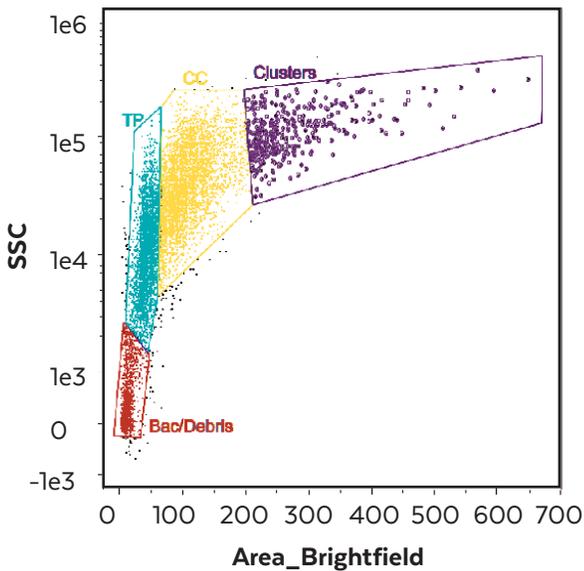
# Quintessential Cell Interactions at the Immunological Synapse

- T:APC conjugates are easily identified using morphological features

- The point of cell–cell contact is identified using a mask (cyan overlay)

- Actin accumulation within the mask confirms formation of an immunological synapse

- All T cells are then identified either in conjugates or not

- NF–kB translocation is measured in the T cells specifically

- Many types of cell-cell interactions can be measured including the binding of CAR-T cells to target cells
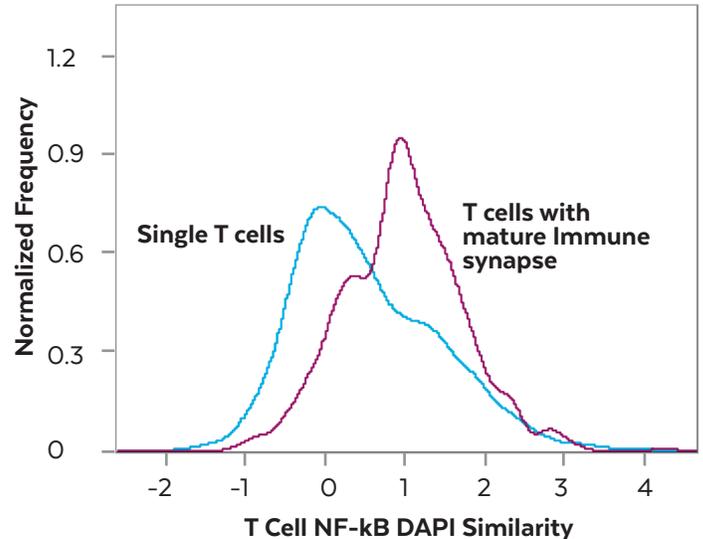


## ImageStream$^X$ Mk II 60X images



Mask Overlay (Cyan) Showing Synapse Identification

Single T Cells

T Cell: APC
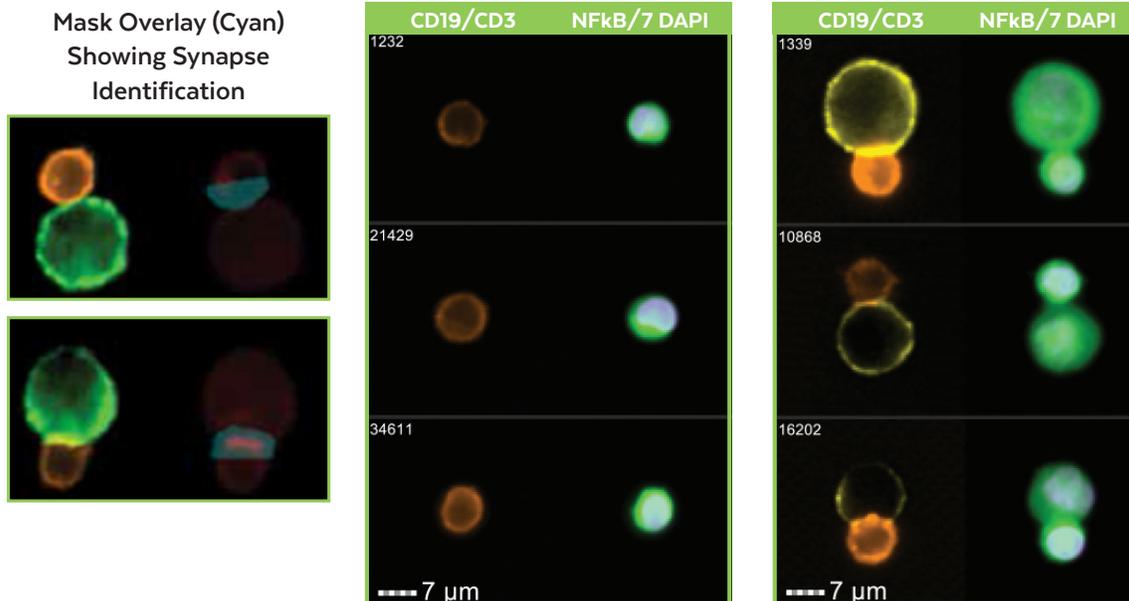
# Modular Options for the ImageStream<sup>X</sup> Mk II Instruments
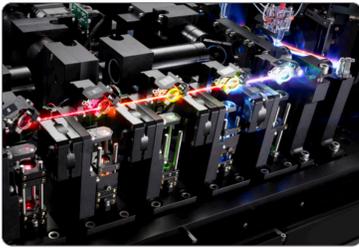
## Additional excitation lasers

The 488 nm blue laser comes standard with the ImageStream$^X$ Mk II instruments. Adding excitation lasers increase experimental flexibility by permitting a broader palette of fluorescent markers. All lasers are intensity adjustable to ease protocol development.
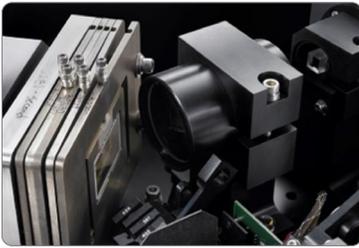
## 12 channels of detection

Up to 12 high resolution image channels are available with the addition of an optional second camera and associated optics for the ImageStream$^X$ Mk II system.

## Multi-well plate AutoSampler

The AutoSampler option enhances productivity with unattended sample loading from 96-well plates. The fully integrated AutoSampler option greatly facilitates dose response and time course studies.
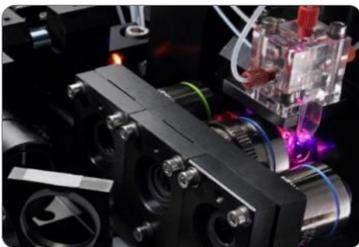
## MultiMag/High Gain Mode

The MultiMag option for the ImageStream$^X$ Mk II system provides 60X and 20X objectives on a motorized stage, in addition to the standard 40X objective. The 60X objective offers greater resolution for the morphologic analysis of cells as small as yeast and bacteria, while the 20X objective offers a 120 micron wide field of view for very large cells. MultiMag also comes with High Gain mode effectively doubling the photonic sensitivity of the camera.
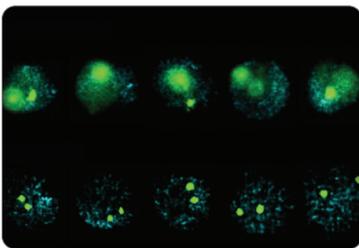
## EDF: Extended depth of field

The EDF option incorporates Wavefront Coding technology from OmniVision CDM Optics, which is a combination of specialized optics and unique image processing algorithms, to project all structures within the cell into one crisp plane of focus. Ideal for automated FISH spot counting.

| Option | ImageStream®X Mk II |
|---|---|
| **Additional Excitation Lasers** | Standard 488<br>Option High Power 488, 375, 405, 561, 592, and 642 |
| **12 Channels of Detection** | 6 Standard High Resolution<br>12 Channel Option |
| **Multi-well Plate Autosampler** | 96-Well Plate |
| **MultiMag** | 40X Standard; 20X and 60X Option |
| **EDF: Extended Depth of Field** | Available |

# Progressive Engineering Advances Performance

## ImageStream$^X$ Mk II instrument specifications

| Performance Characteristics | Magnification | | |
| --- | --- | --- | --- |
| | 40X | 60X | 20X |
| Numeric aperture | 0.75 | 0.9 | 0.5 |
| Pixel size | 0.5 x 0.5 µm | 0.3 x 0.3 µm | 1.0 x 1.0 µm |
| Field of view | 60 x 128 µm | 40 x 170 µm | 120 x 256 µm |
| Imaging rate | 2,000 cells/sec | 1,200 cells/sec | 5,000 cells/sec |

### Sample characteristics

- **Volume** – 20-200 µL
- **Utilization Efficiency** – Up to 95% of sample

### Automated instrument operations

- Start up and shut down
- Sample load and acquisition
- Laser alignment, focus adjustment, calibration, and self-test

### Operational requirements

- 450W, 100-240 VAC, 50/60 Hz
- No external air or water necessary

### Physical characteristics

- 35" W x 26" H x 25" D in (889 mm x 660 mm x 635 mm)
- 400 lbs. (182 kg)

### Illumination

- **Excitation** – Standard: 488 nm; Optional: High Power 488, 375 nm, 405 nm, 561 nm, 592 nm, and 642 nm
- **Side scatter** – 785 nm standard
- **Brightfield** – Multi-channel

# The Path to Scientific Enlightenment...

...passes through the Amnis® multi-spectral decomposition element, which enables simultaneous collection of brightfield, laser scatter, and multiple fluorescent images per cell.



ImageStream®X Mk II Imaging Flow Cytometer Optical Layout
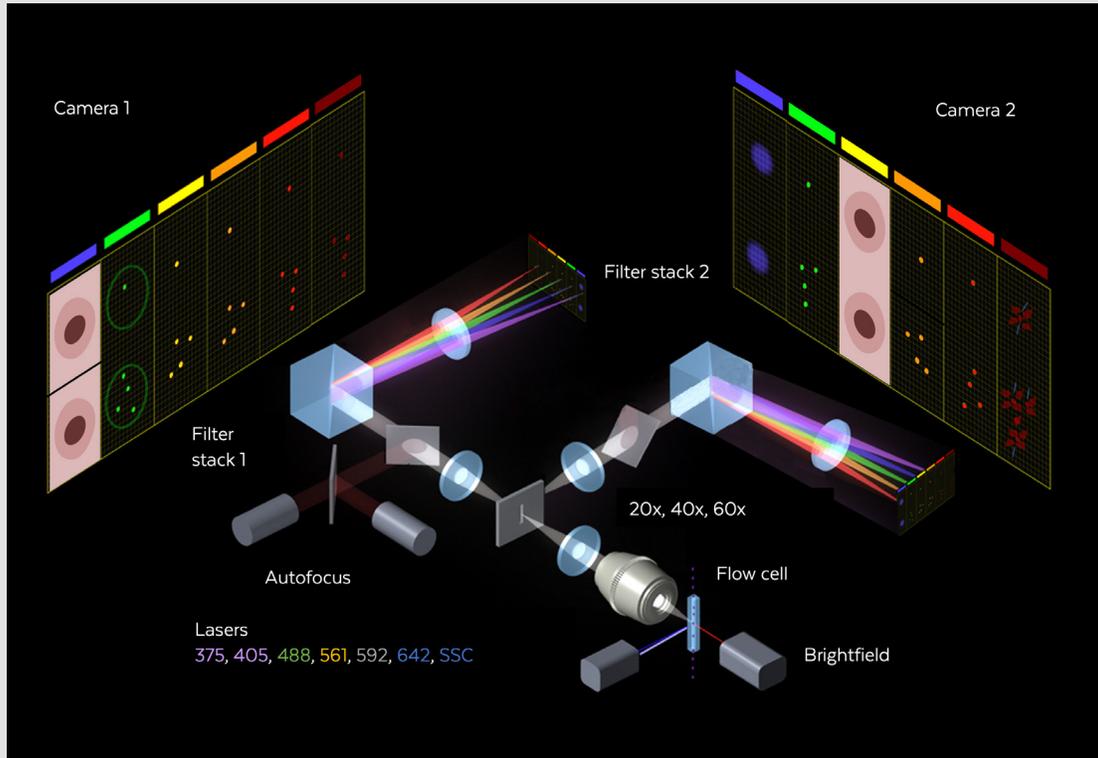
## Amnis® Spectral Imaging Channels and Corresponding Fluorophores

| Camera 1 | | | | | | Camera 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Channel 1 435-480nm | Channel 2 480-560nm | Channel 3 560-595nm | Channel 4 595-642nm | Channel 5 642-745nm | Channel 6 745-785nm | Channel 7 435-505nm | Channel 8 505-570nm | Channel 9 570-595nm | Channel 10 595-642nm | Channel 11 642-745nm | Channel 12 745-785nm |
| Brightfield<br><br>AF350+<br>BV421+<br>AF405+<br>PacificBlue™+<br>DAPI+<br>Hoechst+ | cFluor®B515<br>cFluor®B520<br>FITC<br>AF488<br>BB515<br>GFP<br>SYTO13<br>MT Green | cFluor®BYG575*<br>PE*<br>DSRed<br>AF546<br>Cy3<br>PKH26 | cFluor®BYG610*<br>PE-TexasRed*<br>PE-AF610*<br>ECD*<br>RFP<br>mCherry | cFluor®BYG710*<br>cFluor®B690<br>BB700<br>PerCP<br>PerCP-Cy5.5<br>Draq5*<br>PE-Cy5*<br>7AAD*<br>PI* | cFluor®BYG781*<br>PE-Cy7*<br>PE-AF750*<br>Ch6 SSC | cFluor®V420*<br>BV421*<br>AF405*<br>PacificBlue™<br>DAPI*<br>Hoechst*<br>L/D Violet*<br>D/C Violet*<br>AF350 | cFluor®V547*<br>BV510*<br>PacOrange™<br>AF430 | Brightfield | cFluor®V610<br>BV605<br>eFluor™ 625<br>AF594<br>CF594<br>SpectrumRed | cFluor®R668<br>cFluor®R659<br>AF647<br>CF647<br>APC*<br>DRAQ5™*<br>BV711<br>eFluor™ 700 | cFluor®R780<br>APC-Cy7*<br>APC-750*<br>BV786<br>Ch12 SSC |

*This is not a complete list. Fluorophores of equivalent excitation and emission spectra can replace any of the ones listed here.*
*This chart displays the 12-channel ImageStream®, 6-channel systems will have these fluorophores in the matching 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 Ch1 in systems with a 405 laser, otherwise its aligned to Ch7. Brightfield can be collected in any channel to accommodate fluorescence.

# Ordering Information

| Product Name | Part Number |
|---|---|
| **Instruments** | |
| Cytek® Amnis® ImageStream®X Mk II Flow Cytometer | 100220 |
| **Reagents** | |
| Cytek® Amnis® SpeedBead® Kit | 400041 |
| **Kits** | |
| Cytek® Amnis® NF-kB Translocation Kit | ACS10000 |
| Cytek® Amnis® Protein Aggregate and Silicone Oil Detection Kit | APH10001 |
| **Service Plans** | |
| Cytek® ImageStream®X Mk II Intermediate Maintenance and Service Agreement | SLA-ISXMKII-Intermediate |
| Cytek® ImageStream®X Mk II Complete Maintenance and Service Agreement | SLA-ISXMkII-Complete |
| Cytek® ImageStream®X Mk II Basic Maintenance and Service Agreement | SLA-ISXMkII-Basic |
| Cytek® Amnis® ImageStream®X Mk II System IQ/OQ | 603222 |
| **Training Options** | |
| Cytek® Onsite ImageStream®X Mk II training - TAS 1 day; Up to 5 people | 500200-1 |
| Cytek® Onsite ImageStream®X Mk II training - TAS 2 consecutive days; Up to 5 people | 500200-2 |
| Cytek® Onsite ImageStream®X Mk II training - TAS 3 consecutive days; Up to 5 people | 500200-3 |
| Cytek® Onsite ImageStream®X Mk II training - TAS 4 consecutive days; Up to 5 people | 500200-4 |
| Cytek® Onsite ImageStream®X Mk II training - TAS 5 consecutive days; Up to 5 people | 500200-5 |
| **Software** | |
| 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® 6.3 Image Analysis Software – Single seat license | CN-SW69-01 |
| Cytek® Amnis® IDEAS® 6.3 Image Analysis Software – 20 seat group license | CN-SW69-12 |
| Cytek® Amnis® IDEAS® 6.3 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 |

**CYTEK** ®
TRANSCEND THE CONVENTIONAL