Cytometry without limits

amnis®
part of EMD Millipore

The ImageStream^{X} Mark II Imaging Flow Cytometer

Get phenotype and functional insights, even with the rarest cells

The new Amnis® ImageStream^{X} Mark II imaging flow cytometer combines the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insights of microscopy. This unique combination enables a broad range of applications that would be impossible using either technique alone.

Like the original ImageStream®, the Mark II produces multiple high resolution images of every cell directly in flow, including brightfield, darkfield (SSC) and up to 10 fluorescent markers with sensitivity exceeding conventional flow cytometers. Compared to the original ImageStream^{X}, the Mark II offers a streamlined workflow, greater flexibility, and optimizations for rare cell applications.

Taken together, the capabilities of the ImageStream^{X} Mark II make it superior for traditional flow applications while greatly expanding the scope of flow cytometry. Applications include the study of cell-cell interactions, phagocytosis, apoptosis and autophagy, the characterization of circulating tumor cells, and many others.

Amnis is now part of EMD Millipore
This union leverages the global presence of Millipore to better reach and serve our customers.

- Faster: Up to 5000 cells/sec with real-time intensity compensation
- Easier: Simple user interface with real-time plotting and graphical gating
- More flexible: Accepts up to 7 lasers and works with sample volumes of 20-200 ul
- More efficient: Up to 95% sample utilization for high yields with rare cell samples
- More affordable: Two-laser configurations starting at $199,000

A Wealth of Applications

Shape Change, Autophagy, Cell Signaling, Trafficking, and more

Quantitative imaging – not just observations

Microscopy offers detailed cellular images and morphologic information, which are useful scientific tools for the study of cell function. However, the interpretation of microscopic imagery 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, flow cytometry lacks any ability to image, so sub-cellular localization and cell function are measured indirectly.

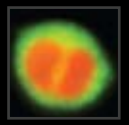
By combining the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insight of microscopy, the ImageStream^X Mark II overcomes the limitations of both techniques and opens the door to an extensive range of novel applications.

Any application you can imagine

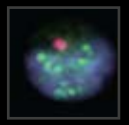
The ImageStream^X Mark II is designed to be a general-purpose platform for cellular studies and is not limited to the applications illustrated in this brochure. The ImageStream^X Mark II utilizes the same dyes and markers employed in microscopy and flow cytometry and can perform virtually any standard flow cytometry assay with the added value of visual confirmation.

Featured applications

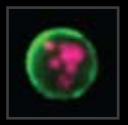
The applications detailed on the following pages demonstrate the types of studies that can be performed using the ImageStream^X Mark II and its powerful companion IDEAS® image analysis software. Over 250 peer-reviewed publications incorporate ImageStream studies.



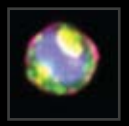
Cell Signaling



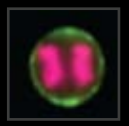
DNA Damage and Repair



Cell Death



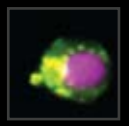
Co-localization



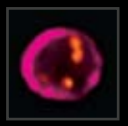
Cell Cycle and Mitosis

Parasitology

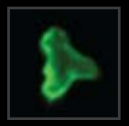
Cell-Cell Interactions



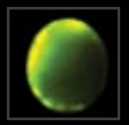
Autophagy



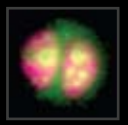
Microbiology



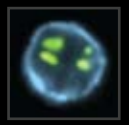
Morphology



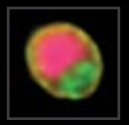
Targeted Immunotherapy



Oncology



Internalization

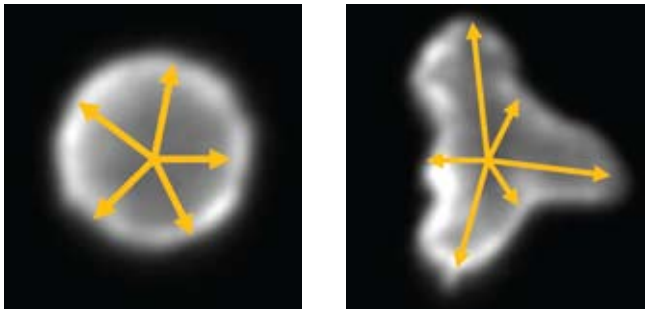


Stem Cell Differentiation

Oceanography

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 Mark II measures cell shape using powerful, pre-defined features in the IDEAS image analysis software. One such feature is the Circularity score:

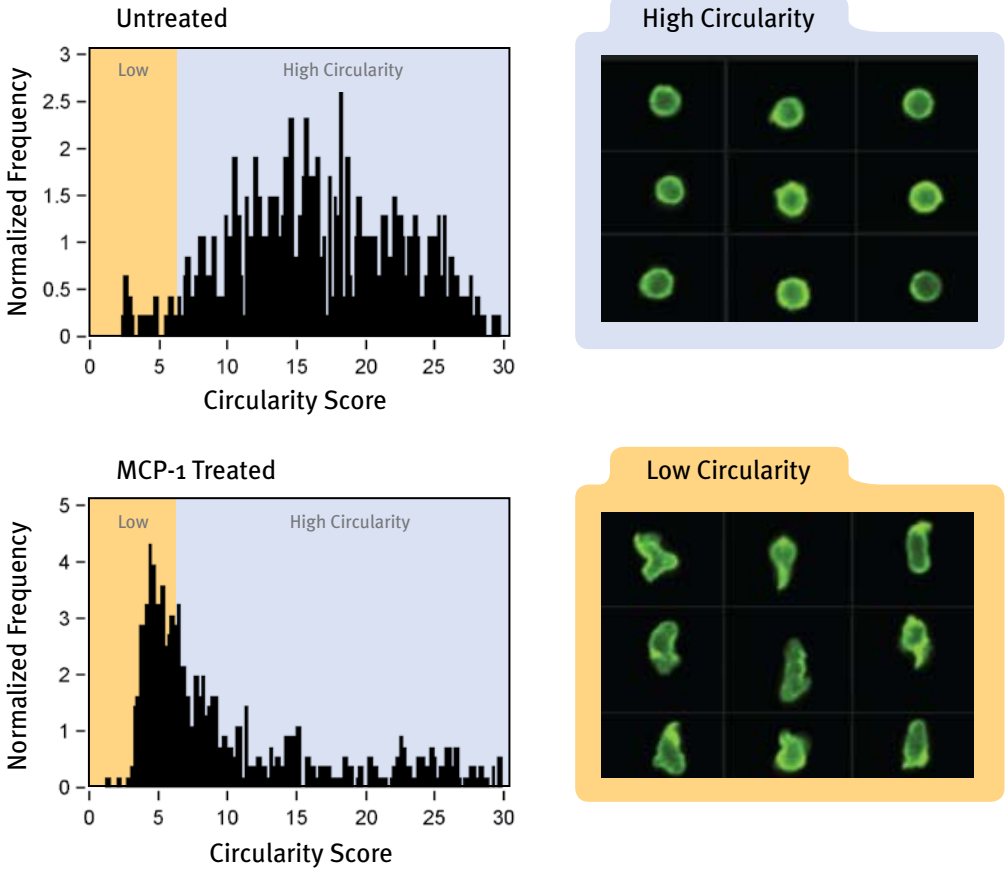


23.8 4.6

Circularity Scores

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.

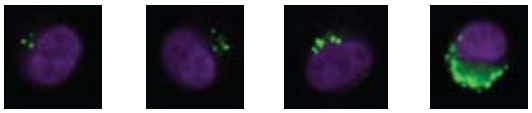
Example: Shape Change in Primary Monocytes



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 inflammatory response – such as drugs for autoimmune disorders – result in an increase in Circularity scores.

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:



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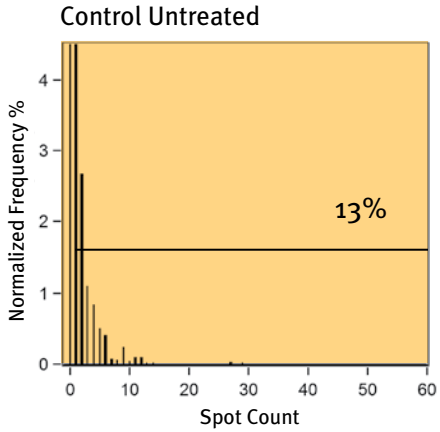
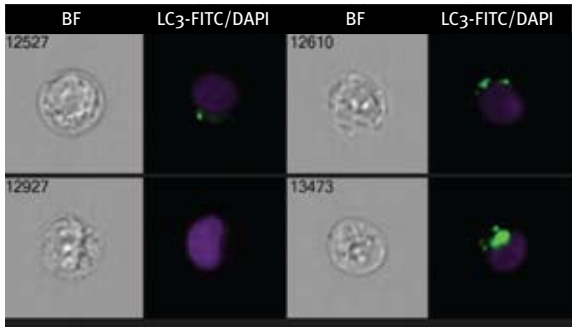
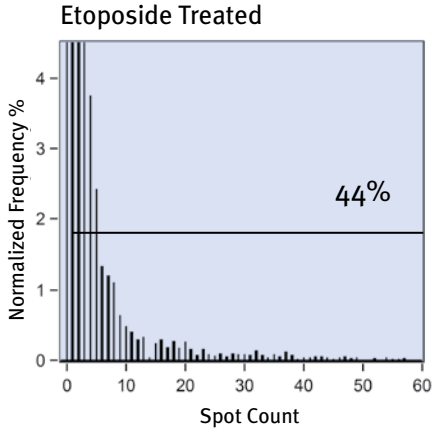
Spot Count Scores

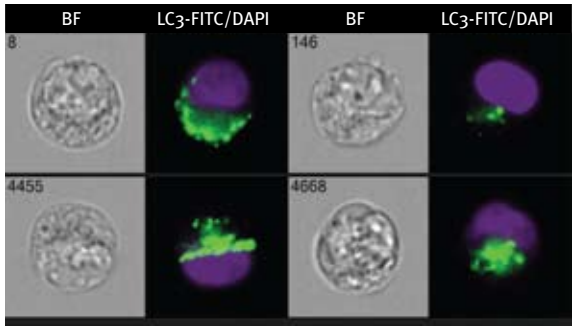
The IDEAS image processing software included with the ImageStream^X Mark II determines the Spot Count of every cell. In this example, cells with varying number of LC3-FITC (green) spots are shown with their corresponding Spot Count. The experiment is described at right.

Example: Autophagy in the Human CML Cell Line K562







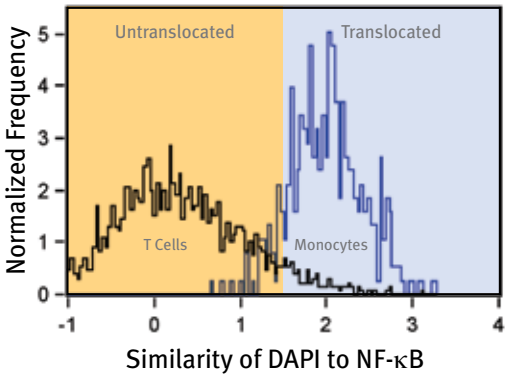


K562 cells were treated with etoposide to induce autophagy. Representative brightfield and merged LC3-FITC (green) and DAPI (purple) images are shown above for control and treated cells. The number of LC3 puncta were quantified for each cell using the Spot Count feature of the IDEAS software and each sample of over 10,000 cells was characterized by a Spot Count histogram. The percentage of cells exhibiting one or more puncta increased from 13% (control) to 44% (treated).

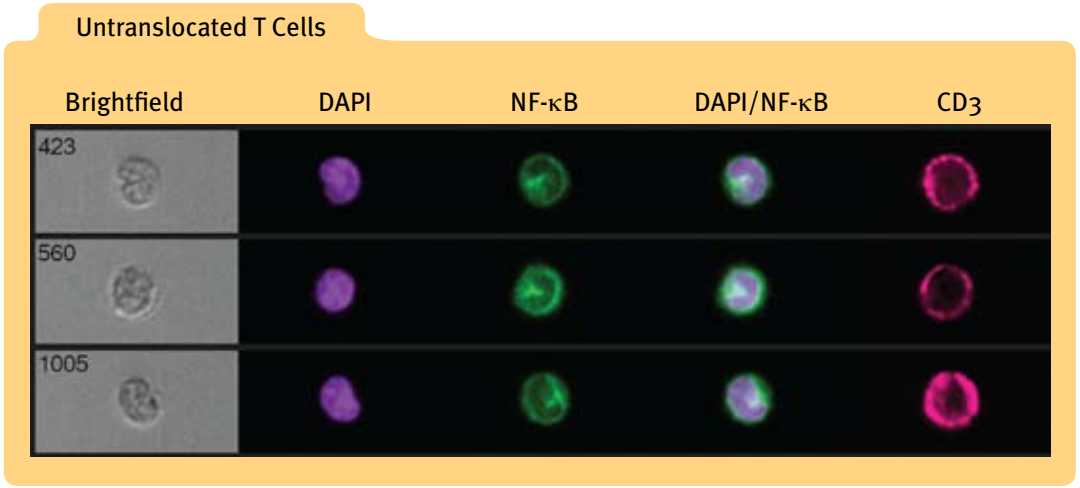
Cell Signaling

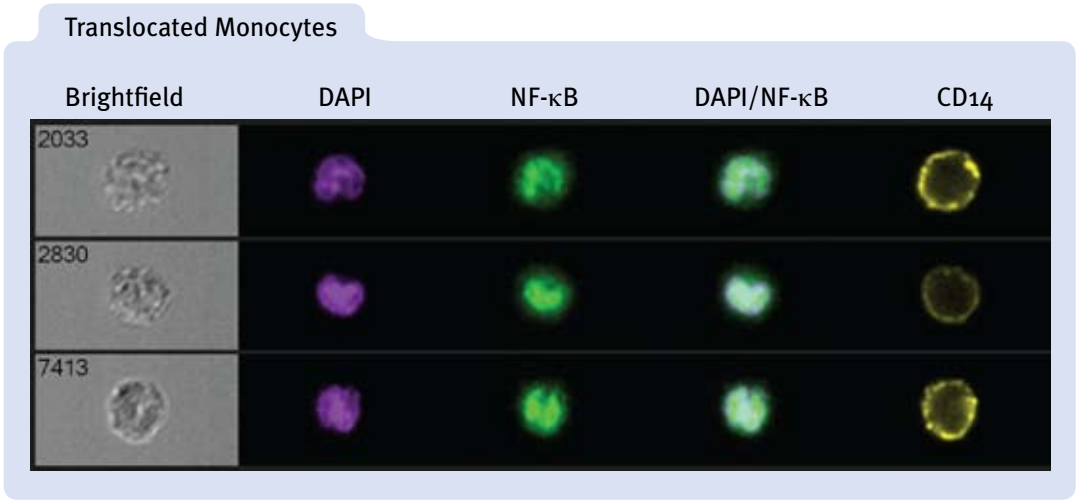
Molecular translocation of transcription factors from the cytoplasm to the nucleus is a pivotal event in many processes critical to cellular activation, differentiation, and host defense. The IDEAS software package quantifies nuclear translocation events by automatically correlating the images of the transcription factor and the nucleus using the Similarity score.

Example: Translocation of NF-κB in Whole Blood Leukocytes



NF-κB translocation is quantified in immunophenotypically-defined whole blood leukocytes imaged at 60X magnification. This example shows that lipopolysaccharide specifically induces NF-κB nuclear translocation in monocytes (blue histogram, images at lower right) but not T cells (black histogram, images at upper right).

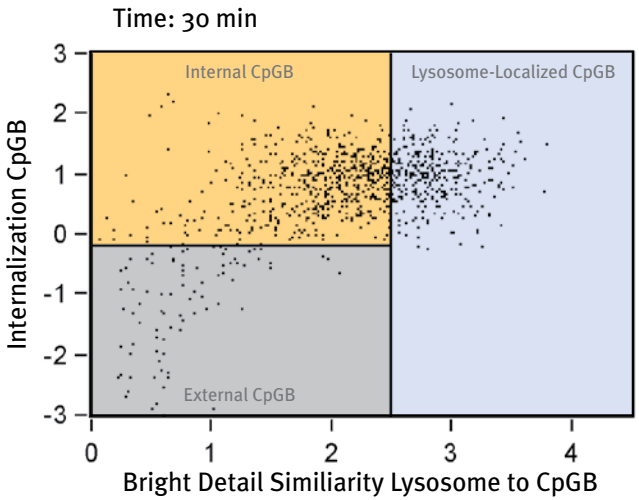


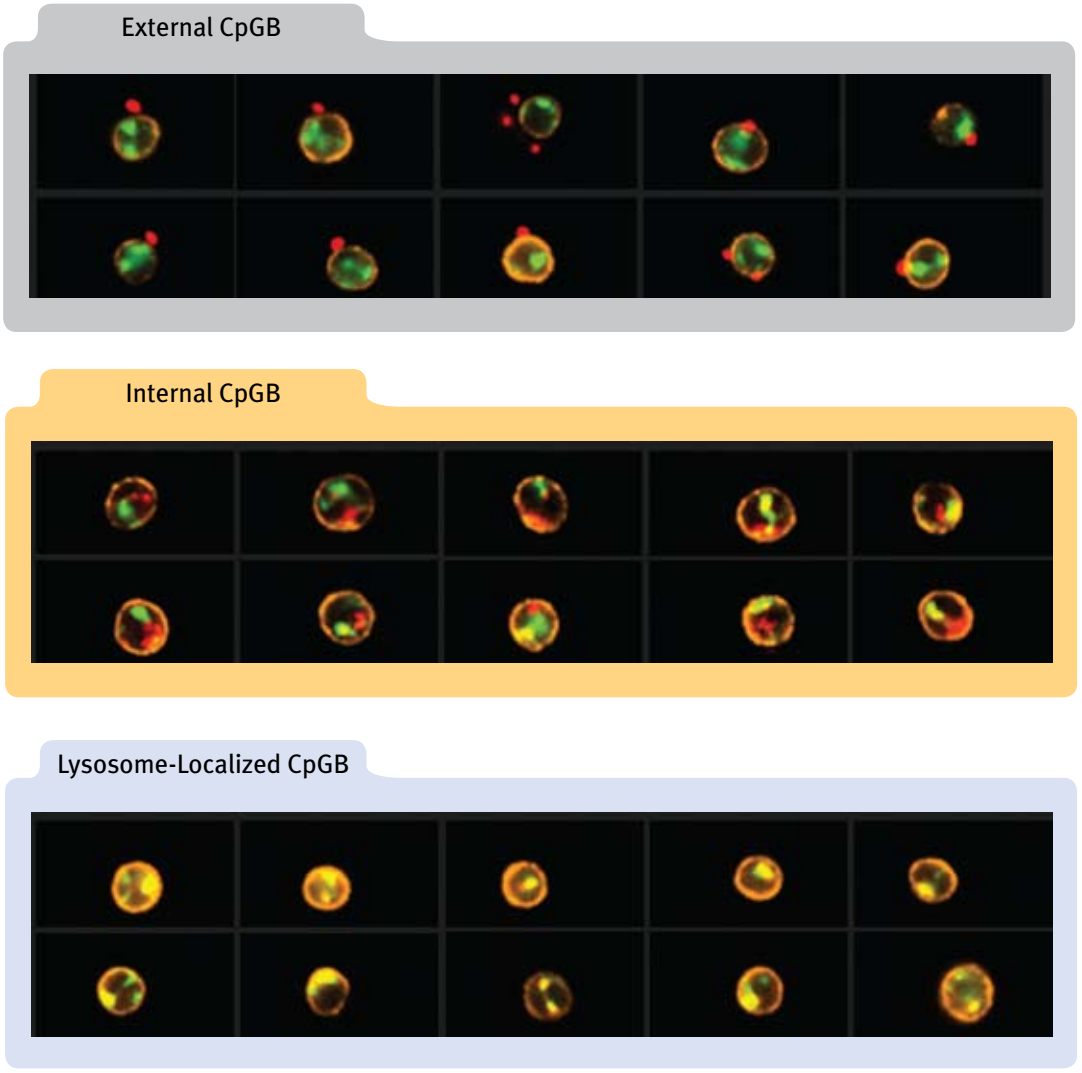


Co-localization and Trafficking

The ImageStream^X Mark II 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.

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





Lysosomal trafficking of CpGB within pDC is quantified using the Internalization (Y-axis) and the Bright Detail Similarity (X-axis) scores, and representative merged images of pDC (orange), CpGB (red), and lysosomes (green) are shown at right. Cells within the lower left region of the plot 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.

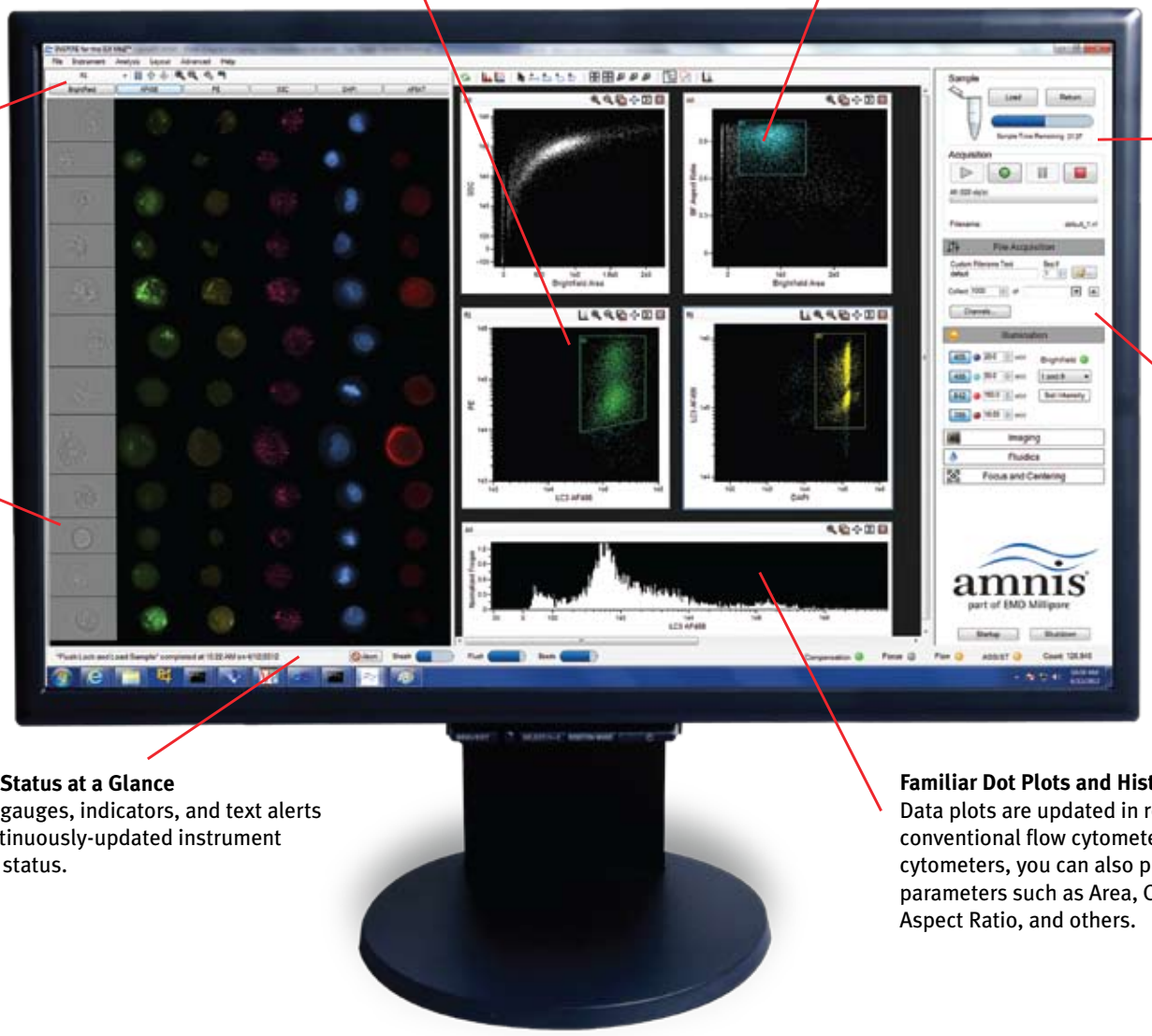
Fast and Easy Data Acquisition Software

INSPIRE® offers powerful image-based gating and real-time fluorescence compensation

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

Gating without Guesswork
Gates are easily drawn using graphical tools and verified for accuracy by visual inspection of gated cells

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.



Efficient Sample Handling
The Mark II utilizes up to 95% of the sample volume, facilitating the analysis of rare cells. Unused sample can be recovered for further analysis.

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

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.

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

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 others.

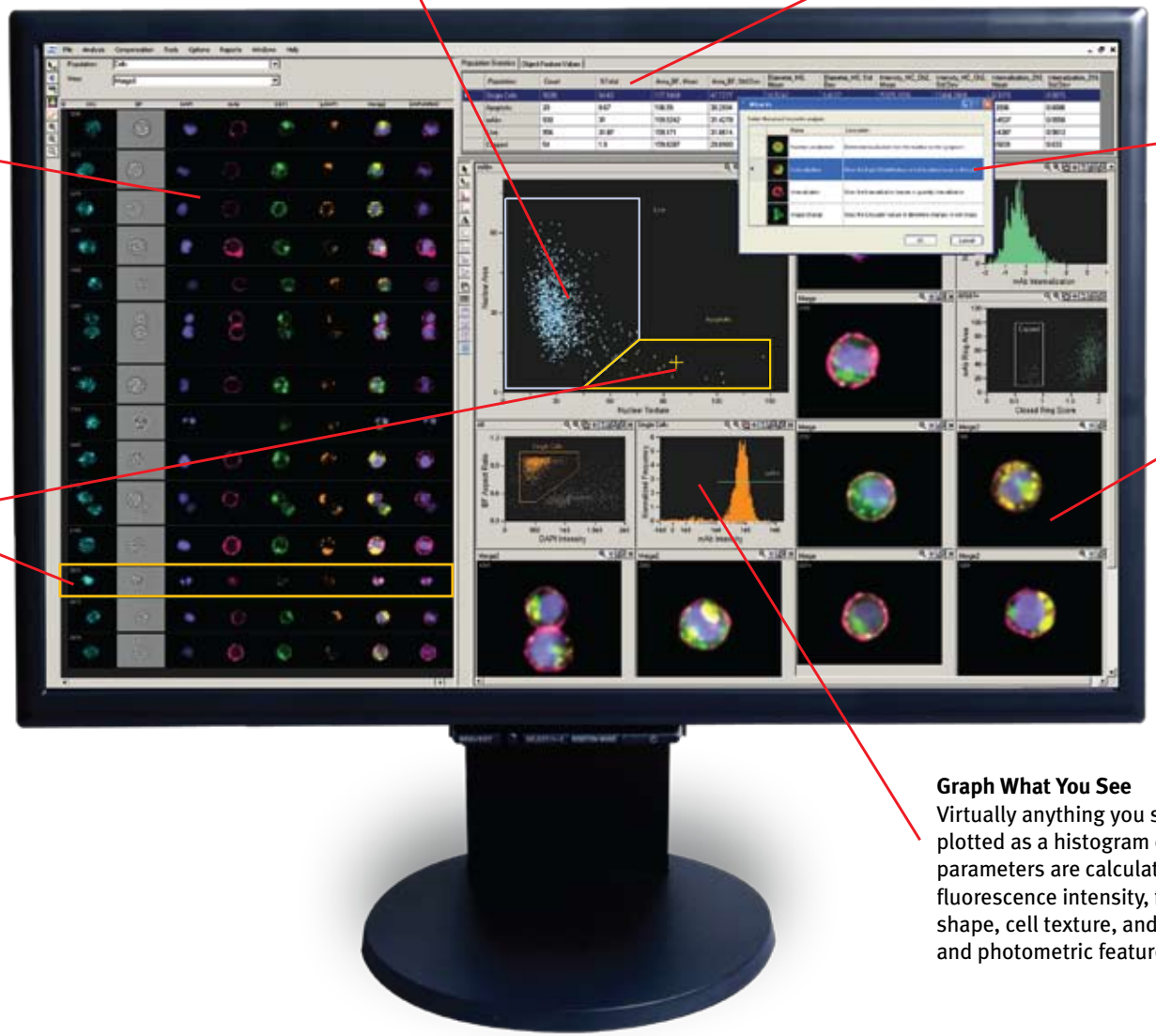
Software that Turns Data into Understanding

IDEAS combines image analysis, statistical rigor, and visual confirmation in an easy-to-use package

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

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

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.



Wizards Simplify Analysis
Pre-configured and optimized analysis wizards are provided for many common applications.

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

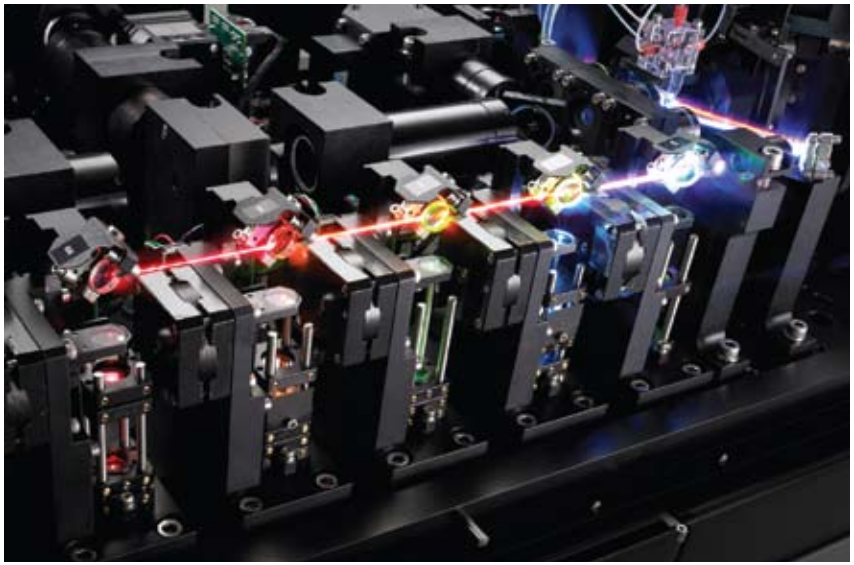
Images for Every Dot
Every dot in every scatter plot is linked to the corresponding cell imagery. Simply click on a dot to see the associated cell images or vice-versa.

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.

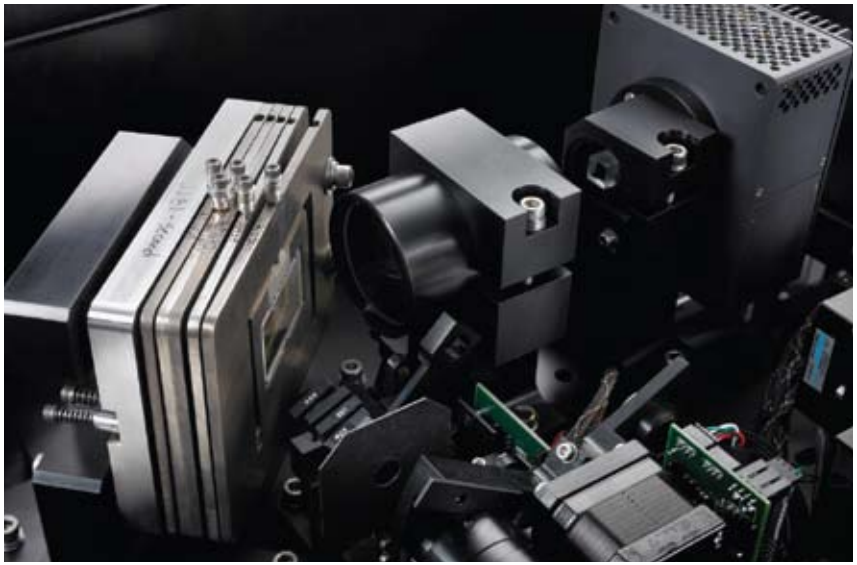
Modular Options

The ImageStream^X Mark II has numerous options to serve a wide range of needs and budgets

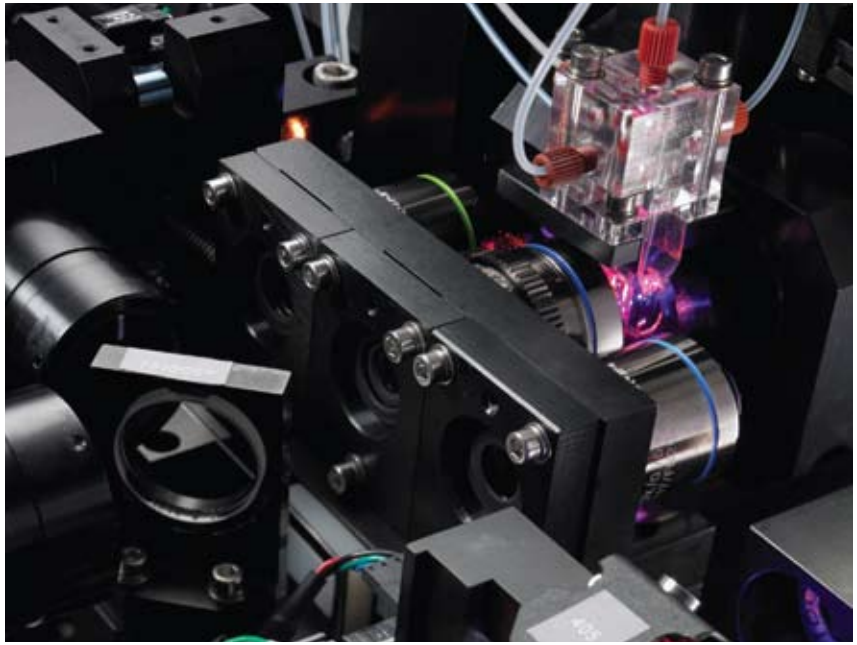
Seven Lasers: The standard 488 nm laser of the ImageStream^X Mark II may be augmented with up to six additional lasers, including 375, 405, 561, 592, and 642 nm. A high power 488 nm laser upgrade is also available for even higher sensitivity.



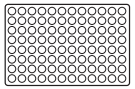
12 Image Channels: Up to 12 channels of detection are available with the addition of an optional second camera and associated optics.



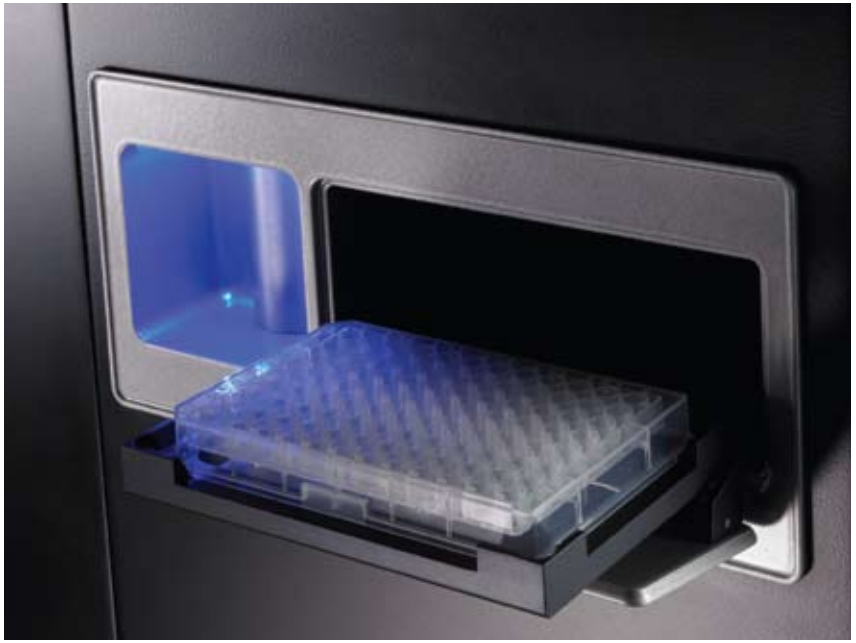
MultiMag: The MultiMag option provides 60X and 20X objectives on a motorized stage, in addition to the standard 40X objective. The 60X objective offers greater resolution for the morphologic analysis of cells as small as yeast and bacteria, while the 20X objective offers a 120 micron wide field of view for very large cells.

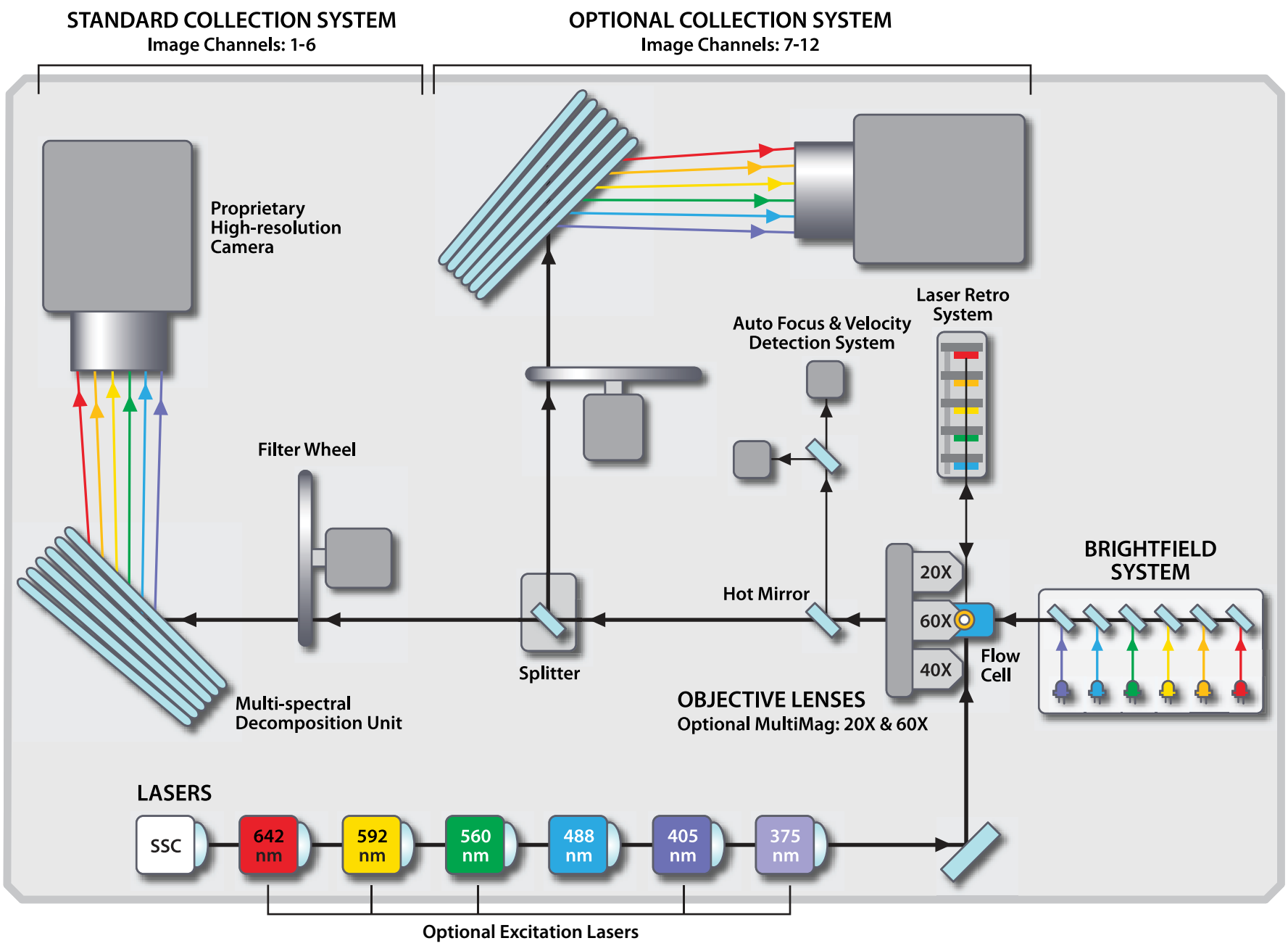


Extended Depth of Field: The EDF™ option incorporates Wavefront Coding™ technology from CDM Optics, which is a combination of specialized optics and unique image processing algorithms, to project all structures within the cell into one crisp plane of focus.



AutoSampler: The AutoSampler option enhances productivity with unattended sample loading from 96 well plates.




STANDARD COLLECTION SYSTEM
Image Channels: 1-6
OPTIONAL COLLECTION SYSTEM
Image Channels: 7-12
Proprietary
High-resolution
Camera
Laser Retro
System
Auto Focus & Velocity
Detection System
Filter Wheel
BRIGHTFIELD
SYSTEM
20X
60X
40X
Hot Mirror
Flow
Cell
Splitter
Multi-spectral
Decomposition Unit
OBJECTIVE LENSES
Optional MultiMag: 20X & 60X
LASERS
SSC
642
nm
592
nm
560
nm
488
nm
405
nm
375
nm
Optional Excitation Lasers

ImageStream^X Mark II Specifications

Advanced engineering creates exceptional performance

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	4,000 cells/sec

Sample characteristics

Volume: 20-200 µl

Utilization Efficiency: up to 95% of sample

Throughput: 1 sample/min nominal

Automated instrument operations

Start up and shut down

Sample load and acquisition

Laser alignment, focus adjustment, calibration and self test

Operational requirements

350 W, 90-240 VAC, 50-60 Hz

100 Mbps ethernet, minimum

No external air or water necessary

Physical characteristics

36" W x 26" H x 24" D (91 cm x 66 cm x 61 cm)

350 lbs (159 Kg)

Spectral imaging bands and applicable dyes

CHANNEL 1 420-480 nm	CHANNEL 2 480-560 nm	CHANNEL 3 560-595 nm	CHANNEL 4 595-642 nm	CHANNEL 5 642-745 nm	CHANNEL 6 745-800 nm	CHANNEL 7 430-505 nm	CHANNEL 8 505-570 nm	CHANNEL 9 570-595 nm	CHANNEL 10 595-642 nm	CHANNEL 11 642-745 nm	CHANNEL 12 745-800 nm
Brightfield Default	FITC GFP YFP Acridine Orange Alexa Fluor 488 Alexa Fluor 500 Alexa Fluor 514 SYTO Spectrum Green LysoTracker Green DyeCycle Green Calcium Green-1 MitoTracker Green DyLight 488	DsRed DiI Cy3 R-phycoerythrin OFP Alexa Fluor 546 Alexa Fluor 555 DyLight 549 Calcium Orange	7-AAD PE-Texas Red (ECD) PE-Alexa Fluor 610 Propidium Iodide Spectrum Orange MitoTracker Red LysoTracker Red RFP mCherry Alexa Fluor 568 Alexa Fluor 594 Alex Fluor 610 DyLight 594 Texas Red	PerCP PerCP-Cy5.5 PE-Alexa Fluor 647 PE-Alexa Fluor 680 PE-Cy5 PE-Cy5.5 DRAQ5 Nile Blue	PE-Cy7 PE-Alexa Fluor 750 Darkfield (SSC)	CFP DAPI Hoechst 33258 Alexa Fluor 405 Marina Blue Pacific Blue Cascade Blue LIVE/DEAD Violet DyLight 405 eFluor 450 Spectrum Aqua DyeCycle Violet	Alexa Fluor 430 Pacific Orange Cascade Yellow Lucifer Yellow Qdot 525 Qdot 545	Qdot 565 Qdot 585 Brightfield Default	Qdot 605 Qdot 625 eFluor 605 mCherry Alexa Fluor 568 Alexa Fluor 594 Alexa Fluor 610 DyLight 594 Texas Red Spectrum Red Calcium Crimson	Qdot 705 eFluor 650 Nile Blue APC APC-Cy5.5 DyLight 649 MitoTracker Deep Red Alexa Fluor 647 Alexa Fluor 660 Alexa Fluor 680 DRAQ5 Cy5 Cy5.5	Qdot 800 APC-Cy7 APC-Alexa Fluor 750 APC-eFluor780 DyLight 750 Darkfield (SSC)

Excitation Lasers: 375 nm laser | 405 nm laser | 488 nm laser | 561 nm laser | 592 nm laser | 642 nm laser

Darkfield (SSC) Laser: 785 nm laser

Note: Twelve image channel configuration shown. Multiple lasers can be used in six image channel configuration. Other laser lines are available. Please contact Amnis for more information.

Amnis is part of the EMD Millipore family of cell analysis solutions

Amnis ImageStream^{X} Mark II

Amnis FlowSight®

Muse™

Guava® easyCyte™

amnis®
part of EMD Millipore

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Amnis United States Patents

6211955, 6249341, 6256096, 6473176, 6507391, 6532061, 6563583, 6580504, 6583865, 6608680, 6608682, 6618140, 6671044, 6707551, 6763149, 6778263, 6875973, 6906792, 6934408, 6947128, 6947136, 6975400, 7006710, 7009651, 7057732, 7079708, 7087877, 7190832, 7221457, 7286719, 7315357, 7450229, 7522758, 7567695, 7610942, 7634125, 7634126, 7719598, 7889263, 7925069,8005314, 8009189, 8103080, 8131053

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