Introducing ImageStream™
See what you’ve been missing
Seeing is Believing, but ImageStream® is Proof

If you rely on flow cytometry or microscopy, you need the power of the new ImageStream®

A BREAKTHROUGH INTERSECTION OF TECHNOLOGIES

Amnis proudly introduces the new ImageStream® imaging flow cytometer, a tremendous leap forward in cell analysis. Amnis has dedicated 10 years to combining the most advanced technologies in optics, sensors, and image processing to achieve what has never been possible before – a fusion of the analytical power of flow cytometry with the visual detail of imaging.

The ImageStream® quantifies both the intensity and the location of fluorescent probes and can image at incredibly high rates of speed, allowing you to analyze rare sub-populations and highly heterogeneous samples with statistically robust and objective results. By combining the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insights of microscopy, the ImageStream® will rapidly advance your research.

With the ImageStream® you can:

- Image cells directly in suspension with the resolution of a 60X microscope and the fluorescence sensitivity of the best flow cytometers
- Analyze highly heterogeneous samples and rare cell sub-populations at speeds exceeding 1,000 cells per second
- Perform phenotypic and functional studies at the same time using up to five lasers and 12 images per cell
- Quantitate virtually anything you can see using the IDEAS® software package's numerous pre-defined fluorescence and morphologic parameters
A Wealth of Applications
Cell Signaling, Co-localization, Shape Change, Internalization, 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™ overcomes the limitations of both techniques and opens the door to an extensive range of novel applications.

Any Application You Can Imagine
The ImageStream™ is designed to be a general-purpose platform for cellular studies and is not limited to the applications illustrated in this brochure. The ImageStream™ 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™ and its powerful companion IDEAS image analysis software.
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® measures cell shape using powerful, pre-defined features in the IDEAS image analysis software. One such feature is the Circularity score:

Example: Shape Change in Primary Monocytes

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.

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.
Internalization

Measurement of the cellular uptake of specific ligands is important in the study of drug metabolism, host-pathogen interactions, and antigen processing and presentation. The IDEAS software objectively measures localization of internalized probes using a variety of parameters, including the Internalization score:

Example: Phagocytosis by Murine Macrophages

Phagocytosis of FITC-labeled Zymosan particles (green) by RAW cells (orange), a murine macrophage line, incubated at 4°C (top) and at 37°C (bottom). The Zymosan Internalization score is plotted for each sample at left and representative images of cells with surface-bound (top) or phagocytosed Zymosan (bottom) are shown at right.

Internalization Scores

The Internalization score measures the relative amount of signal inside versus outside the cell. In this example, cells with Zymosan (green) bound to the membrane (orange) have negative Internalization scores, while cells that have internalized Zymosan have positive Internalization scores.
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® 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)

Lyosomal 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.
The ImageStream$^X$ Instrument

Think of the possibilities

The ImageStream$^X$ is designed to gather more information from your cells than you ever thought possible. This breakthrough instrument is capable of imaging 1,000 cells per second with the fluorescence sensitivity of conventional flow cytometry, so you can perform image-based studies of dim markers on rare cells, even in heterogeneous samples. The ImageStream$^X$ can accommodate up to five excitation lasers and simultaneously acquires up to 12 images per cell, so you can combine functional studies with detailed phenotypes.

If you think all of this power comes at the expense of image quality, think again. The ImageStream$^X$ produces imagery comparable to the best fluorescence microscopes and operates at 60X, 40X, or 20X magnification, so you can study the fine details of objects as small as bacteria and as large as epithelial cells. Only the ImageStream$^X$ combines the speed, sensitivity, and quantitation of flow cytometry with the visual detail of microscopy in a single platform.
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.

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.

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

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\textsuperscript{X} has numerous options to serve a wide range of needs and budgets.

**Five Lasers:** The standard 488 nm laser of the ImageStream\textsuperscript{X} may be augmented with up to four additional lasers at 405, 561, 592, and 658 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.
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.

MultiMag: The new MultiMag option provides 20X and 60X objectives lenses in addition to the standard 40X lens for greater flexibility and improved resolution. The 60X objective increases magnification for small objects such as yeast and bacteria and offers greater detail with mammalian and plant cells.

Full Color Brightfield: The Full Color Brightfield option provides a full spectrum brightfield light source that allows the ImageStream™ to replicate the RGB brightfield imagery of a microscope.

AutoSampler: The new AutoSampler option enhances productivity with unattended sample loading from multiwell plates.
Optional Excitation Lasers

AF: Autofocus laser, 830 nm
SSC: Side Scatter Laser, 785 nm

STANDARD COLLECTION SYSTEM
Image Channels: 1-6

OPTIONAL COLLECTION SYSTEM
Image Channels: 7-12

LASERS
SSC 658 nm
592 nm
560 nm
488 nm
405 nm

Optional Excitation Lasers
ImageStreamX Specifications
Advanced engineering creates exceptional performance

**Performance Characteristics**

<table>
<thead>
<tr>
<th>Magnification</th>
<th>40X</th>
<th>60X</th>
<th>20X</th>
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<tbody>
<tr>
<td>Numeric Aperture</td>
<td>0.75</td>
<td>0.9</td>
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<tr>
<td>Pixel Size</td>
<td>0.5 x 0.5 µm</td>
<td>0.3 x 0.3 µm</td>
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<tr>
<td>Field of View</td>
<td>60 x 128 µm</td>
<td>40 x 170 µm</td>
<td>120 x 256 µm</td>
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<tr>
<td>Imaging Rate</td>
<td>1,000 cells/sec</td>
<td>600 cells/sec</td>
<td>2,000 cells/sec</td>
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**Sample Characteristics**

- Volume: 50 µl
- Utilization Efficiency: > 50% of sample
- Throughput: 1 sample/min nominal

**Spectral Imaging Bands and Applicable Dyes**

<table>
<thead>
<tr>
<th>CHANNEL 1 (420-480 nm)</th>
<th>CHANNEL 2 (480-560 nm)</th>
<th>CHANNEL 3 (560-595 nm)</th>
<th>CHANNEL 4 (595-660 nm)</th>
<th>CHANNEL 5 (660-740 nm)</th>
<th>CHANNEL 6 (740-800 nm)</th>
<th>CHANNEL 7 (420-505 nm)</th>
<th>CHANNEL 8 (505-570 nm)</th>
<th>CHANNEL 9 (570-595 nm)</th>
<th>CHANNEL 10 (595-660 nm)</th>
<th>CHANNEL 11 (660-740 nm)</th>
<th>CHANNEL 12 (740-800 nm)</th>
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<tr>
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<td>FITC</td>
<td>GFP</td>
<td>YFP</td>
<td>Acidine Orange</td>
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<td>Alexa Fluor 500</td>
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**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)

**Excitation Lasers:**

- 405 nm diode laser
- 488 nm solid state laser
- 561 nm solid state laser
- 592 nm solid state laser
- 658 nm diode 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.