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, 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 Amnis® ImageStream® X Mk II and Amnis® FlowSight® Instruments overcome the limitations of both techniques and open the door to an extensive range of novel applications.

**Amnis® FlowSight®**
Imaging Flow Cytometer

- **Capable**: Applicable to every research discipline
- **Sensitive**: Camera-based detection dramatically increases resolution over traditional flow cytometry
- **Affordable**: Smaller footprint with configurations for any lab focus and budget
- **Powerful**: Characterizes populations by virtually any visual or fluorescent attribute

**Amnis® ImageStream® X Mk II**
Imaging Flow Cytometer

- **High-throughput**: Analyzes thousands of cells per second at up to 60X magnification
- **Intuitive**: Simple user interface with real time plotting and gating
- **Adaptable**: Can be configured with 1 to 6 lasers
- **Boundless**: Variable magnification images small particles and your largest cells

Spanning the Research Disciplines in the Life Sciences.

- Immunology
- Oncology
- Biochemistry
- Drug Discovery
- Stem Cell Biology
- Hematology
- Microbiology
- Virology
- Nanotechnology
- Toxicology
- Parasitology
- Oceanography
- Phycology
- Small Particle Analysis
Powerful Flow Cytometry.

The ImageStream® Mk II and FlowSight Systems deliver multiple images of every cell in flow, including brightfield, darkfield (SSC), and up to 10 fluorescent markers at high speed. The ImageStream® Mk II camera operates with a pixel size of 0.1/0.25/1 μm² with 60X/40X/20X magnification, respectively, allowing visualization of fluorescence location from the membrane, cytoplasm, subcellular organelles, and nucleus at high resolution. The FlowSight System operates at 20X magnification with a 1 μm² pixel. 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 systems 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 in brightfield images.

Multichannel immunophenotyping

Immunophenotyping requires multiple fluorescence channels in addition to dual scatter. Below is a 6-color immunophenotype of human PBMC 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.

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 FlowSight Instrument 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.

5-Part white blood cell differential

Because of its exceptional sensitivity, the FlowSight System excels at the resolution of mixed sub-populations in heterogeneous samples. Human peripheral blood mononuclear cells (PBMC) 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 FlowSight and ImageStream® Mk II Instruments operate like conventional flow cytometers, but also provide 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.

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 others.

INSPIRE™ Software
Software That Turns Data Into Understanding.

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

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.

IDEAS® Software

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® Mk II and FlowSight Instruments with their powerful companion IDEAS image analysis software.

Any application you can imagine
The ImageStream® Mk II and FlowSight Systems are designed to be general-purpose platforms for cellular studies and are not limited to the applications illustrated in this brochure.

---

**Applications**
- **Parasitology**
- **Stem Cell Biology**
- **Microbiology**
- **Internalization and Co-localization**
- **Surface and Intracellular Co-localization**
- **Cell Signaling**
- **Cell Cycle and Mitosis**
- **DNA Damage and Repair**
- **Cell-cell Interaction**
- **Shape Change and Chemotaxis**
- **Immunological Synapse**
- **Micronucleus Counting**
- **Oceanography**
- **Stem Cell Biology**
- **Parasitology**
- **Microbiology**
20X resolution tells the story

Translocation of NFkB 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. For this data, the 20X objective of the FlowSight System is used to locate NFkB in relation to 7-AAD fluorescence from the nucleus in untreated THP-1 cells and cells stimulated with lipopolysaccharide (LPS). The similarity feature of the IDEAS Software produces a score for every cell, quantifying the co-localization of NFkB and 7-AAD.

A closer look at NFkB signaling with 60X magnification

Here, THP-1 cells stimulated or not with LPS and stained with anti-NFkB and 7-AAD to counterstain the nucleus were collected on the ImageStream® Mk II System using the 60X objective. The IDEAS Software similarity feature demonstrates binning of samples consistent with the FlowSight histogram, and establishes the quality of visual detail that the ImageStream® Mk II System can provide for studies when greater details are needed.
<table>
<thead>
<tr>
<th>Laser</th>
<th>Fluorophore</th>
<th>Ex</th>
<th>Em</th>
<th>Bandpass*</th>
<th>Ch width</th>
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**Notes:**
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### Table of Fluorophores and Corresponding Channels

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Quantitative imaging means Luminex’s imaging flow cytometers have 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.

### Mean Similarity Score vs. ng/mL TNFa

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<th>Count Singles</th>
<th>Count Positive</th>
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### Internalization Identifies Trogocytosis

**20X objective for a wider field of view**

The FlowSight Instrument is optimized for imaging large objects such as epithelial cells, macrophages, neutrophils, fibroblasts, and even large eukaryotic parasites. Here, *Entamoeba histolytica* demonstrates amoebic trogocytosis of immune cells. Following attachment to Jurkat cells, the FlowSight Instrument measures every *E. histolytica* expressing Jurkat markers internalized or on their surface.
Co-localization and Trafficking.

The ImageStream® 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.

Lysosomal trafficking of CpGB within pDC 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).

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 FlowSight 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 are shrunken and fragmented.

Lysosomal trafficking of CpGB within pDC 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.
**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.

The IDEAS image processing software included with the ImageStream 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.

**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 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.

**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.

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).
Microalgae.

**Mixed cultures of microalgae**
The images below demonstrate microalgae identification in mixed cultures using morphological parameters and the ImageStream® Mk II Instrument at 40X magnification.

**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® Mk II System at 60X magnification.

Quintessential Cell Interactions at the Immunological Synapse.

**FlowSight 20X images**

**SEB Dose Response Curve**

Raji B cells were exposed to SEB (0-20 μg/mL) and incubated with human primary T cells.
Take the Analysis Even Further with Higher Resolution.

- 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
- NFκB translocation is measured in the T cells specifically

Additional excitation lasers
The 488 nm blue laser comes standard with the FlowSight and 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. Twelve channels are standard on the FlowSight Instrument.

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
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.

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.
## FlowSight Instrument specifications

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</tr>
<tr>
<td>Imaging rate</td>
<td>4,000 cells/sec</td>
</tr>
</tbody>
</table>

### 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
- 400W, 100-240 VAC, 50/60 Hz
- No external air or water necessary

### Physical characteristics
- 18” W x 18” H x 25” D in (457 mm x 465 mm x 635 mm)
- 135 lbs. (61 kg)

### Illumination
- **Excitation**: Standard: 488 nm; Optional: 405 nm, 561 nm, and 642 nm
- **Side scatter**: 785 nm standard
- **Brightfield**: Multi-channel

## ImageStream^X Mk II Instrument specifications

<table>
<thead>
<tr>
<th>Performance Characteristics</th>
<th>Magnification 40X</th>
<th>60X</th>
<th>20X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numeric aperture</td>
<td>0.75</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Pixel size</td>
<td>0.5 x 0.5 µm</td>
<td>0.3 x 0.3 µm</td>
<td>1.0 x 1.0 µm</td>
</tr>
<tr>
<td>Field of view</td>
<td>60 x 128 µm</td>
<td>40 x 170 µm</td>
<td>120 x 256 µm</td>
</tr>
<tr>
<td>Imaging rate</td>
<td>2,000 cells/sec</td>
<td>1,200 cells/sec</td>
<td>5,000 cells/sec</td>
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</table>

### 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.

Ordering Information

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Instruments</strong></td>
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</tr>
<tr>
<td>Amnis® FlowSight® Flow Cytometer</td>
<td>100370</td>
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<tr>
<td>Amnis® ImageStream® Mk II Flow Cytometer</td>
<td>100220</td>
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<td><strong>Reagents</strong></td>
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<tr>
<td>Amnis® SpeedBead® Kit</td>
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<td>FlowSight® Calibration Beads</td>
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<td><strong>Kits</strong></td>
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<td>Amnis® NFkB Translocation Kit</td>
<td>AC50000</td>
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<td>Amnis® Protein Aggregate and Silicone Oil Detection Kit</td>
<td>APH00001</td>
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<td>Amnis® Intracellular Staining Kit</td>
<td>AC50002</td>
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<td><strong>Service Plans</strong></td>
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<td>Amnis® FlowSight® IQ/OQ</td>
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<td><strong>Training Options</strong></td>
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<tr>
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<tr>
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<td>Onsite ImageStream® Mk II or FlowSight® training - FAS 5 consecutive days; Up to 5 people</td>
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<td><strong>Software</strong></td>
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<td>Amnis® IDEAS® Image Analysis Software - 21CFR enabled</td>
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*AF: Auto Focus **SSC: Side Scatter Laser, 785 nm
For more information, please visit luminexcorp.com/flowsight-imaging and luminexcorp.com/imagestreamx-mark-ii.

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