



# Flow cytometry with vision

### Introducing FlowSight

CAPABLE: Sensitive and flexible for every need INTUITIVE: Easy-to-use, with imagery for every cell AFFORDABLE: Designed and priced for every lab



#### SENSITIVE AND FLEXIBLE FOR EVERY NEED

The FlowSight offers high performance in a small package. Its innovative design increases signal and minimizes noise to provide unmatched fluorescence sensitivity. Twelve standard detection channels simultaneously produce brightfield, darkfield and up to ten channels of fluorescence imagery of every cell. With these unique capabilities, the FlowSight enables a broad range of applications.

Ch1	Ch2	Ch3	Ch4	Ch5	Ch6	Ch7	Ch8	Ch9	Ch10	Ch11	Ch12
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Twelve channel imagery of 3 micron diameter Spherotech 8-peak Rainbow beads

#### EASY-TO-USE, WITH IMAGERY FOR EVERY CELL

The FlowSight operates like a conventional flow cytometer but also provides imagery of every cell. Powerful and intuitive analysis software seamlessly links quantitative data to imagery:

- Click on a dot in any plot to see the corresponding cell imagery.
- Click on a bin in any histogram to view all the cells in that bin.
- Draw gates on dot plots and view the resulting populations to validate results.

#### **DESIGNED AND PRICED FOR EVERY LAB**

The FlowSight is powerful enough for the core lab but sized and priced for any lab. The system can be factory configured or field upgraded with up to four excitation lasers (405, 488, 561, 642 nm), a 96-well plate AutoSampler, and a powerful quantitative image processing option. Whether in a base configuration or fully optioned, the FlowSight sets a new standard of value.







### **Powerful Flow Cytometry**

### Expand your application range with high performance and flexibility

With 12 detection channels and up to four excitation lasers, the FlowSight can measure up to 10 fluorescent probes simultaneously with unrivalled sensitivity. Numerous thoughtful design details like a dedicated 785 nm scatter laser, adjustable laser intensities, and brightfield imagery for the direct measurement of cell size allow the FlowSight to resolve cell populations more effectively than far more expensive cytometers. The ease of use, outstanding performance, and imagery of each cell allow the FlowSight to meet the needs of beginners and experts alike.

#### UNPARALLELED FLUORESCENCE SENSITIVITY

The patented architecture of the FlowSight provides unparalleled fluorescence sensitivity in all fluorescence detection channels. The four plots to the right demonstrate the ability of the FlowSight to discriminate all intensities in the Spherotech 8-peak calibration bead set across the spectrum from FITC to Cy7. The FITC and PE channels exhibit excellent peak separation using less than 5% of the available laser power. The red (PE-Cy5) and far-red (PE-Cy7) channels exhibit peak separations exceeding the best flow cytometers.



#### FIVE-PART WHITE BLOOD CELL DIFFERENTIAL

The FlowSight excels at the resolution of mixed sub-populations in heterogeneous samples. In this example, human peripheral blood mononuclear cells (PBMC) were partitioned into five distinct populations using CD45 and side scatter. High fluorescence sensitivity and tight CVs resolve monocytes (green) from lymphocytes (blue) and facilitate the detection of rare basophils (white). The dedicated scatter laser clearly resolves eosinophils (yellow) from neutrophils (orange) and monocytes (green).

#### **EIGHT-COLOR IMMUNOPHENOTYPING**

The FlowSight is the only compact flow cytometer with twelve channels of detection. Shown below is an eight-color immunophenotype of human PBMC using antibodies against CD45, CD14, CD16, CD19, CD3, CD4 and CD123, plus DAPI. The arrangement of detection channels, available laser options, and automated compensation wizard allow the straightforward separation of complex cell populations.





### **Intuitive Visual Verification**

### Better flow cytometry through imaging

The FlowSight stands apart from other flow cytometers by producing up to 12 images of each and every cell. The unique image collection system simultaneously produces a side scatter (darkfield) image, one or two transmitted light (brightfield) images, and up to 10 fluorescence images. The FlowSight operates with a pixel size of 1 micron (~20X magnification) allowing visualization of fluorescence from the membrane, cytoplasm, or nucleus. Identifying cell conjugates or distinguishing single cells from doublets and debris is effortless. The data acquisition and analysis software allows you to click on a dot in any plot, select a bin in any histogram, or draw a gate on any dot plot to see the corresponding cell imagery. Flow cytometry has never been so intuitive.

#### **GATING WITHOUT GUESSWORK**

With the imaging capabilities of the FlowSight, you'll never wonder about those outliers or whether your gates are in the right place. 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 single cell identification example at right. With visual feedback, you can optimize your gate size, shape, and position for better data quality.





#### **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 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.



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## **Quantitative Imaging**

#### Accelerate discovery with quantitative image analysis

A Quantitative Imaging (QI) upgrade includes optical, computer, and software enhancements that increase the imaging and analysis capabilities of the FlowSight even further. The QI option incorporates a powerful but 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.

#### **APOPTOSIS DETECTION BY IMAGE ANALYSIS**

The QI upgrade includes a wizard for the automated identification of apoptotic cells. The wizard analyzes nuclear morphology and the cell's brightfield image contrast to identify apoptotic cells in any sample containing a nuclear stain, as shown at right.

![](_page_7_Picture_5.jpeg)

![](_page_7_Picture_6.jpeg)

SSC

#### Cell signaling via nuclear translocation

The QI upgrade includes a wizard to determine the degree of translocation between the cytoplasm and the nucleus of any labeled signaling molecule. The example at right illustrates the translocation of transcription factor NF- $\kappa$ B (green) from the cytoplasm to the nucleus (magenta). The cells of the control (left gallery) exhibit primarily cytoplasmic-localized NF- $\kappa$ B and negative nuclear translocation scores. The cells of the stimulated sample (right gallery) exhibit primarily nuclear localized NF- $\kappa$ B and positive nuclear translocation scores.

![](_page_8_Figure_2.jpeg)

(a,b)

![](_page_8_Picture_3.jpeg)

![](_page_8_Figure_4.jpeg)

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### **Modular Options**

Expand the range of performance

#### **EXCITATION LASERS**

The standard 488 nm blue laser of the FlowSight system may be augmented with up to three additional lasers at 405 nm (violet), 561 nm (green), and 642 nm (red) wavelengths. Adding excitation lasers increases experimental flexibility by permitting a broader palette of fluorescent markers. All lasers are intensity adjustable to ease protocol development.

#### 96 WELL AUTOSAMPLER

The AutoSampler option for the FlowSight enhances productivity with unattended sample loading from 96 well plates. The fully integrated AutoSampler option greatly facilitates dose-response and time-course studies.

#### QUANTITATIVE IMAGING UPGRADE

The Quantitative Imaging upgrade includes optical, computer, and software enhancements that increase the performance of the FlowSight. Improved optics yield better image quality, better sensitivity and more brightfield options. The post-acquisition image processing software allows highly quantitative determinations of the location and strength of fluorescence signals for applications such as nuclear translocation, shape change, internalization, and apoptosis.

![](_page_9_Picture_8.jpeg)

![](_page_9_Picture_9.jpeg)

![](_page_9_Figure_10.jpeg)

# **FlowSight Specifications**

Innovation and advanced engineering create exceptional performance

#### **PERFORMANCE CHARACTERISTICS**

**Detection:** 12 channels standard – two brightfield images, one darkfield (SSC) image and up to 10 fluorescence images

#### Illumination:

Excitation - 488 nm standard; 405, 561, and 642 nm optional

Side scatter – 785 nm standard

Brightfield – fixed channel 1/9 standard, multi-channel optional

Collection: 20X magnification at 0.6NA with a 1.0 micron pixel size

Event Rate: Up to 2,000 cells per second

#### **AUTOMATED INSTRUMENT OPERATIONS**

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

#### **OPERATIONAL REQUIREMENTS**

400W, 90-240 VAC, 50-60 Hz No external air or water necessary

#### **PHYSICAL CHARACTERISTICS**

17.7 W x 18.3 H x 24.7 D inches (450mm x 465mm x 635mm) 135 lbs. (61 kg)

#### SPECTRAL IMAGING BANDS AND APPLICABLE DYES

CHANNEL 1	CHANNEL 2	CHANNEL 3	CHANNEL 4	CHANNEL 5	CHANNEL 6	CHANNEL 7	CHANNEL 8	CHANNEL 9	CHANNEL 10	CHANNEL 11	CHANNEL 12
430-480 nm	505-560 nm	560-595 nm	595-642 nm	642-740 nm	740-800 nm	430-505 nm	505-560 nm	560-595 nm	595-642 nm	642-740 nm	740-800 nm
Brightfield	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 Dil 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 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)	DAPI Hoechst 33258 CFP Alexa Fluor 405 Marina Blue Pacific Blue Cascade Blue LIVE/DEAD Violet DyLight 405 eFluor 450 Spectrum Aqua	Alexa Fluor 430 Pacific Orange Cascade Yellow Lucifer Yellow Qdot 525 Qdot 545	Qdot 565 Qdot 585 Brightfield	Qdot 605 Qdot 625 eFluor 605	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-H7 APC-eFluor780 DyLight 750

Excitation Lasers:	405 nm	488 nm	561 nm	642 nm	Darkfield (SSC) Laser:	785 nm	
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![](_page_11_Figure_0.jpeg)

#### **AMNIS CORPORATE AND INTERNATIONAL DISTRIBUTION OFFICES**

#### Amnis United States Patents

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![](_page_11_Picture_4.jpeg)

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![](_page_11_Picture_9.jpeg)

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