



# Cytek<sup>®</sup> Amnis<sup>®</sup> Imaging Flow Cytometers

**Microscopy In Flow** 

amnis

CYTEK

# 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, 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<sup>®</sup> Amnis<sup>®</sup> ImageStream<sup>®X</sup> Mk II and Cytek<sup>®</sup> Amnis<sup>®</sup> FlowSight<sup>®</sup> instruments overcome the limitations of both techniques and open the door to an extensive range of novel applications.



### Amnis<sup>®</sup> FlowSight<sup>®</sup>

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<sup>®</sup> ImageStream<sup>®x</sup> 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

### **Powerful Flow Cytometry**

The ImageStream<sup>X</sup> 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<sup>X</sup> Mk II camera operates with a pixel size of 0.1/0.25/1  $\mu$ m<sup>2</sup> 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  $\mu$ m<sup>2</sup> 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.





Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

# 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.



FlowSight® 12 channel imagery of 3-micron diameter Spherotech 8-peak Rainbow beads.

#### 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<sup>X</sup> 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.

#### Granulocytes



Monocytes



#### **Double Positives**

Brightfield	SSC	DAPI	CD14/16
634			
•			
3538			
0			0
4878			
0			. Q
7887			
63			

#### **Doublet Artifacts**



### Data Acquisition Software

#### Fast and easy.

Cytek<sup>®</sup> INSPIRE<sup>™</sup> 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<sup>™</sup> Software

# Software That Turns Data Into Understanding

Cytek<sup>®</sup> IDEAS<sup>®</sup> 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<sup>®</sup> 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<sup>X</sup> Mk II and FlowSight instruments with their powerful companion IDEAS image analysis software.

#### Any application you can imagine

The ImageStream<sup>X</sup> 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.



Cell Signaling



Cell Cycle and Mitosis



Internalization and Co-localization



Oceanography



Cell-cell Interaction



Shape Change and Chemotaxis



Immunological Synapse



Stem Cell Biology



Parasitology



Microbiology



Surface and Intracellular Co-localization



DNA Damage and Repair



Micronucleus Counting

## Quantifying Nuclear Translocation...

#### 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.

BF	NFkB	7AAD	NFkB/7AAD
-	۲		۲
-	۲		۲
Ø.			
= = = 20 µm			

THP-1 Control (no LPS) Mean similarity score = 0.4



THP-1 + 1 μg/mL LPS Mean similarity score = 3.2



### ...with Fluorescent Image Similarity

#### 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<sup>x</sup> 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<sup>x</sup> Mk II system can provide for studies when greater details are needed.



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



THP-1 Control (no LPS) Mean similarity score = 3.8



## Amnis<sup>®</sup> Spectral Imaging Channels

Laser	Fluorophore		Ex	Em	-ÿ-	Fluorophore	Ex	Em	-;;;-	Fluorophore	Ex	Em	÷	F
		CH 1					CH 2				CH 3			
	Ch1/Ch9 BF *or*					QD525	350-450	525	5	eFluor565 NC	UV - 405	565	2	e
	Alexa Fluor <sup>®</sup> 350		346	442	1					QD565	350-450	565	5	C
375	BV421™		405	421	5					QD585	350-450	585	5	
(with installed 405)	Cascade Blue		377	420	1									
	DAPI		345	461	1									
	Hoechst		352	455	1									
	Pacific Blue		410	455	1									
						Alexa Fluor <sup>®</sup> 488	496	514	3	Cy3	514	566	1	
						BODIPY FI	503 484	512	3	DSRed	557	592	1 5	
						DiO DyLight™ 488	484	501 518	3	PE RFP	496,565 555	578 584	5 2	
						FITC	494	520	3		333	504	2	
						GFP/EGFP	475/488	509						
488						LysoTracker Green	504	511						
						MitoTracker Green	490	516						
						PKH2 & PKH67	490	504						
						Rhodamine 110	496	520						
						SYBR <sup>®</sup> Green	494	521						
						Syto13 (DNA/RNA)	488D/491R	509D/514R						
	BI	RIGHTFIE	ELD			YFP	514	527						
										Alexa Fluor <sup>®</sup> 546	556	573	5	-
										CellMask/Tracker	522	535		
										Dil DSRed	549 557	565 592	3	F.
										DSRed DyLight™550	562	592 576	3 3	
561										Nile Red	515-530	525-605	5	
										PE	496,565	578	5	
										PKH26	551	567		
										Spectrum Orange	559	588		
										Sytox Orange	547	570		
785														
Ch width		435-480					480-560				560-595			
Bandpass*		(457/45)					(528/65)				(577/35)			
		CH 7					СН 8				СН 9			
	Alexa Fluor <sup>®</sup> 350		740	442	1	eFluor525 NC	UV - 405		-					
			346	442	1		00 - 403	525	1					
375	BV421™		405	421	5	QD525	350-450	525 525	1 5					
375 (with 405	BV421™ Cascade Blue		405 377	421 420	5 1	QD525								
	BV421™ Cascade Blue DAPI		405 377 345	421 420 461	5 1 1	QD525								
(with 405	BV421™ Cascade Blue DAPI Hoechst		405 377 345 352	421 420 461 455	5 1 1 1	QD525								
(with 405	BV421™ Cascade Blue DAPI Hoechst Pacific Blue		405 377 345 352 410	421 420 461 455 455	5 1 1 1 1		350-450	525	5					
(with 405	BV421™ Cascade Blue DAPI Hoechst Pacific Blue Alexa Fluor®405		405 377 345 352 410 402	421 420 461 455 455 421	5 1 1 1 1 1	Alexa Fluor® 430	350-450 434	525	5					
(with 405	BV421™ Cascade Blue DAPI Hoechst Pacific Blue Alexa Fluor®405 BV421®		405 377 345 352 410 402 405	421 420 461 455 455 421 421	5 1 1 1 1 1 5	Alexa Fluor® 430 BV510™	<b>350-450</b> 434 405	525 541 510	5					
(with 405	BV421™ Cascade Blue DAPI Hoechst Pacific Blue Alexa Fluor®405		405 377 345 352 410 402 405 377	421 420 461 455 455 421 421	5 1 1 1 1 1 5 1	Alexa Fluor®430 BV510™ Cascade Yellow	<b>350-450</b> 434 405 402	525 541 510 545	5 1 3					6
(with 405	BV421™ Cascade Blue DAPI Hoechst Pacific Blue Alexa Fluor®405 BV421® Cascade Blue		405 377 345 352 410 402 405	421 420 461 455 455 421 421 420	5 1 1 1 1 1 5	Alexa Fluor® 430 BV510™	<b>350-450</b> 434 405	525 541 510	5 1 3 1					(
(with 405 not installed)	BV421™ Cascade Blue DAPI Hoechst Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP		405 377 345 352 410 402 405 377 435	421 420 461 455 425 421 421 420 485	5 1 1 1 1 1 5 1 2	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange	<b>350-450</b> 434 405 402 410	525 541 510 545 551	5 1 3 1 1					e (
(with 405 not installed)	BV421™ Cascade Blue DAPI Hoechst Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI		405 377 345 352 410 402 405 377 435 345	421 420 461 455 421 421 420 485 461 420	5 1 1 1 1 5 1 2 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1					
(with 405 not installed)	BV421™ Cascade Blue DAPI Hoechst Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI		405 377 345 352 410 402 405 377 435 345 400	421 420 461 455 421 421 420 485 461 420	5 1 1 1 1 5 1 2 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	IGHTFIELD			
(with 405 not installed)	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht		405 377 345 352 400 402 405 377 435 345 400 352	421 420 461 455 421 421 420 485 461 420 455	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	IGHTFIELD			e (
(with 405 not installed)	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	RIGHTFIELD			6
(with 405 not installed)	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	RIGHTFIELD			6 ( ) , ,
(with 405 not installed) 405	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	RIGHTFIELD			e ( ( , , , , , , , , , , , , , , , , ,
(with 405 not installed) 405	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	RIGHTFIELD			( ( ( ( ( ( (
(with 405 not installed) 405	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	IGHTFIELD			, ( ( ( ( ( ( ( (
(with 405 not installed) 405	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	IGHTFIELD			, ( ( ( ( ( ( ( ( ( ( ())))))))))))))))
(with 405 not installed) 405 592	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	RIGHTFIELD			
(with 405 not installed) 405	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	RGHTFIELD			
(with 405 not installed) 405 592	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	IGHTFIELD			
(with 405 not installed) 405 592	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	IGHTFIELD			
(with 405 not installed) 405 592	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet		405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	IGHTFIELD			
(with 405 not installed) 405 592 642	BV421™ Cascade Blue DAPI Pacific Blue Alexa Fluor®405 BV421® Cascade Blue CFP DAPI DAPI DyLight™405 Hoescht LIVE/DEAD Violet	435-505 (457/45)	405 377 345 352 410 402 405 377 435 345 400 352 416	421 420 461 455 421 421 420 485 461 420 455 451	5 1 1 1 1 5 1 2 1 1 1 1	Alexa Fluor® 430 BV510™ Cascade Yellow Pacific Orange Pacific Orange	350-450 434 405 402 410 410	525 541 510 545 551 551	5 1 3 1 1 1	BF	ST0-595 (582/25)			

### And Corresponding Fluorophores

						1						ght®	ImageStream <sup>x®</sup> Camera 1	ImageStream <sup>x®</sup> Camera 2
Fluorophore	Ex	Em	-ờ	Fluorophore	Ex	Em	÷	Fluorophore	Ex	Em	÷;-	FlowSight®	ageS	ageS
	CH 4				CH 5				CH 6			Ē	ٽ≟	ڻ <u>۲</u>
eFluor625 NC QD625	UV - 405 350-450	625 625	5	eFluor700 NC QD705	UV - 405 350-450	700 705	1 5	QD800	350-450	800	5			
AldeRed	488	615		7-AAD	546	647	3	PE-Alexa Fluor <sup>®</sup> 750	496,565	775	3			
Aldered PE-Alexa Fluor <sup>®</sup> 610	488 496,565	630	3	DRAQ5	546 646	647	3	PE-Alexa Fluor 750 PE-Cy7	496,565	775	5 4			
PE-Texas Red <sup>®</sup> (ECD)	496,565	613	2	FuraRed-lo	472	657		/.	,					
RFP	555	584	2	LDS751	543	712								
				PE-Alexa Fluor <sup>®</sup> 647	496,565	669	5							
				PE-Cy5	496,565	670	4							
				PE-Cy5.5	496,565	690	3					•	•	
				PerCP	482	675	2							
				PerCP-Cy5.5 Pl	482	690 617	3							
				ы	535	617								
Aloue Et®Ecco	570	<b>CO7</b>	-	7.440	540	C 47	-	DE Alexa Etra-Paco	400 505	775				
Alexa Fluor <sup>®</sup> 568 DyLight™594	578 593	603 618	3	7-AAD DRAQ5	546 646	647 697	5	PE-Alexa Fluor <sup>®</sup> 750 PE-Cy7	496,565 496,565	775 774	4			
PE-Texas Red <sup>®</sup> (ECD)	496,565	613	2	LDS751	543	712		PE-Cy/	490,505	//4	4			
PE-Alexa Fluor <sup>®</sup> 610	496,565	628	5	PE-Alexa Fluor <sup>®</sup> 647	496,565	669	5							
RFP	555	584	4	PE-Cy5	496,565	670	4							
mCherry*	587	610	4									•		
									SSC					
	595-642				642-745				745-780			C	h widt	٦
	(610/30)				(702/85)				(762/35)			Ba	andpas	s*
	СН 10				СН 11				CH 12					
eFluor625 NC	UV- 405	625	5	eFluor650 NC	UV- 405	650	5	QD800	350-450	800	5			
QD625	350-450	625	5	QD705	350-450	705	5							
												•		
	590	617	2											
Alexa Fluor <sup>®</sup> 594		618												
Alexa Fluor <sup>®</sup> 594 DyLight™594	593													
	593 587	610	1											
DyLight™594		610 603	1 5											
DyLight™594 mCherry	587			Alexa Fluor <sup>®</sup> 647	650	668	5	APC-Alexa Fluor <sup>®</sup> 750	650	774	2			
DyLight™594 mCherry	587			Alexa Fluor <sup>®</sup> 660	663	690	2	APC-Cy7	650	774	2			
DyLight™594 mCherry	587			Alexa Fluor <sup>®</sup> 660 APC	663 645	690 660	2 5	APC-Cy7 APC-eFluor750	650 633	774 750				
DyLight™594 mCherry	587			Alexa Fluor <sup>®</sup> 660 APC Cy5	663 645 650	690 660 670	2	APC-Cy7 APC-eFluor750 APC-H7	650 633 652	774 750 785	2 4			•
DyLight™594 mCherry	587			Alexa Fluor <sup>®</sup> 660 APC Cy5 DiD	663 645 650 644	690 660 670 665	2 5	APC-Cy7 APC-eFluor750 APC-H7 Cy7	650 633 652 743	774 750 785 767	2 4 3	•		
DyLight™594 mCherry	587			Alexa Fluor <sup>®</sup> 660 APC Cy5 DiD DRAQ5	663 645 650 644 646	690 660 670 665 697	2 5	APC-Cy7 APC-eFluor750 APC-H7 Cy7 eFluor780	650 633 652 743 753	774 750 785 767 785	2 4 3 2	•		•
DyLight™594 mCherry	587			Alexa Fluor <sup>®</sup> 660 APC Cy5 DiD	663 645 650 644	690 660 670 665	2 5	APC-Cy7 APC-eFluor750 APC-H7 Cy7	650 633 652 743	774 750 785 767	2 4 3	•		
DyLight™594 mCherry	587			Alexa Fluor <sup>®</sup> 660 APC Cy5 DiD DRAQ5	663 645 650 644 646	690 660 670 665 697	2 5	APC-Cy7 APC-eFluor750 APC-H7 Cy7 eFluor780	650 633 652 743 753 496,565	774 750 785 767 785	2 4 3 2		h widtl	

### Quantitative Imaging and Robust Population Statistics

Quantitative imaging means Cytek'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.



### 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.



Data courtesy of Dr. Katherine Ralston, UC Davis.





### **Co-localization and Trafficking**

The ImageStream<sup>×</sup> 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.



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

		External CpGB		
٢	٨	÷Ò	۲	-
1	6	٥	•	۲
		Internal CpGB		Ter la companya de la
0	<b>S</b>	٢	4	۲
۲	٢	۲	6	9
		Lysosome-Localized CpGB		
٩	•	0	٩	Ŷ
Ø	1	0	۲	۲

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 Iysosomes (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 Iysosomes, the similarity between the CpGB and Iysosome 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 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.



### 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<sup>X</sup> 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.



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<sup>X</sup> 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.



**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.









### Microalgae

#### Mixed cultures of microalgae

The images below demonstrate microalgae identification in mixed cultures using morphological parameters and the ImageStream<sup>x</sup> 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<sup>x</sup> Mk II system at 60X magnification.



### Quintessential Cell Interactions at the Immunological Synapse



#### FlowSight 20X images

Cell doublets consisting of one T cell with one antigen presenting cell are isolated and interrogated at the point of cell-cell contact.



Raji B cells were exposed to SEB (0-20  $\mu$ g/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
- NFkB translocation is measured in the T cells specifically



#### ImageStream<sup>×</sup> Mk II 60X images

Single T cells

T cell: APC



### Modular Options for the FlowSight and ImageStream<sup>×</sup> Mk II Instruments





The 488 nm blue laser comes standard with the FlowSight and ImageStream<sup>X</sup> 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<sup>x</sup> 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<sup>X</sup> 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.

Option		ImageStream <sup>®x</sup> Mk II
Additional Excitation Lasers	Standard 488 Option 405, 561, 642	Standard 488 Option High Power 488, 375, 405, 561, 592, and 642
12 Channels of Detection	Standard	6 Standard High Resolution 12 Channel Option
Multi-well Plate Autosampler	96-Well Plate	96-Well Plate
MultiMag	Not Available	40X Standard; 20X and 60X Option
EDF: Extended Depth of Field	Not Available	Available

## Progressive Engineering...

### FlowSight instrument specifications

Performance Characteristics	Magnification 20X
Numeric aperture	0.6
Pixel size	1.0 x 1.0 µm
Field of view	60 x 256 µm
Imaging rate	4,000 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 selftest

#### **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



## ...Advances Performance

### ImageStream<sup>X</sup> Mk II instrument specifications

	Magnification					
Performance Characteristics	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 selftest

#### **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

#### **Operational requirements**

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



Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

## The Path to Scientific Enlightenment...

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



#### FlowSight<sup>®</sup> Imaging Flow Cytometer Optical Layout



# Ordering Information

Product Name	Part Number
Instruments	
Cytek® Amnis® FlowSight® Flow Cytometer	100370
Cytek <sup>®</sup> Amnis <sup>®</sup> ImageStream <sup>®x</sup> Mk II Flow Cytometer	100220
Reagents	
Amnis® SpeedBead® Kit	400041
FlowSight <sup>®</sup> Calibration Beads	400300
Kits	
Amnis® NFkB Translocation Kit	ACS10000
Amnis® Protein Aggregate and Silicone Oil Detection Kit	APH10001
Amnis® Intracellular Staining Kit	ACS10002
Service Plans	
Cytek® ImageStream® Mk II Intermediate Maintenance and Service Agreement	SLA-ISXMKII- Intermediate
Cytek® ImageStream®x Mk II Complete Maintenance and Service Agreement	SLA-ISXMkII-COMPLETE
Cytek <sup>®</sup> ImageStream <sup>®x</sup> Mk II Basic Maintenance and Service Agreement	SLA-ISXMkII-BASIC
Cytek® FlowSight® Intermediate Maintenance and Service Agreement	SLA-FS-Intermediate
Cytek® FlowSight® Complete Maintenance and Service Agreement	SLA-FS-COMPLETE
Cytek® FlowSight® Basic Maintenance and Service Agreement	SLA-FS-BASIC
Cytek® Amnis® ImageStream®X Mk II System IQ/OQ	603222
Cytek® Amnis® FlowSight® IQ/OQ	603224
Training Options	
Cytek® ImageStream® Mk II training at Luminex, 3 days - per person	500200
Cytek® Onsite ImageStream® Mk II or FlowSight® training - FAS 1 day; Up to 5 people	500200-1
Cytek® Onsite ImageStream® Mk II or FlowSight® training - FAS 2 consecutive days; Up to 5 people	500200-2
Cytek® Onsite ImageStream® <sup>x</sup> Mk II or FlowSight® training - FAS 3 consecutive days; Up to 5 people	500200-3
Cytek® Onsite ImageStream® Mk II or FlowSight® training - FAS 4 consecutive days; Up to 5 people	500200-4
Cytek® Onsite ImageStream® Mk II or FlowSight® training - FAS 5 consecutive days; Up to 5 people	500200-5
Software	
Cytek® Amnis® IDEAS® Image Analysis Software - 21CFR- enabled	IFC300202
Cytek® Amnis® INSPIRE <sup>™</sup> Software & Amnis® IDEAS® Software - 21CFR-enabled, Amnis® FlowSight®	IFC300203
Cytek® Amnis® INSPIRE <sup>™</sup> Software & Amnis® IDEAS® Software - 21CFR-enabled, Amnis® ImageStream®X Mk II	IFC300204
Cytek® Amnis® IDEAS® 6.3 Image Analysis Software - Single seat license	CN-SW69-01
Cytek® Amnis® IDEAS® 6.3 Image Analysis Software - 12 seat group license	CN-SW69-12
Cytek <sup>®</sup> Amnis <sup>®</sup> IDEAS <sup>®</sup> 6.3 Image Analysis Software - Institutional license	CN-SW69-20
Cytek <sup>®</sup> Machine Learning Module	CN-SW45-01
Cytek® Amnis® AI: Computer-aided Image Analysis Software	CN-SW70-01

For Research Use Only. Not for use in diagnostic procedures. ©2023 Cytek Biosciences, Inc. All rights reserved. Cytek, Amnis, FlowSight, ImageStream, FlowCellect, IDEAS, INSPIRE and SpeedBead are trademarks of Cytek Biosciences, Inc. All other trademarks are the property of their respective owners.



BR243222 April 2023