

Amnis and the ImageStream^X[®]

Frequently Asked Questions



What are the primary applications for the ImageStream^X?

The ImageStream^X is designed for a wide range of applications, including:

- Nuclear translocation studies in mixed populations of cells
- Internalization of ligands, phagocytosis, pinocytosis
- Shape Change (chemokine-induced, etc)
- Cell-Cell interactions, including immune synapse studies
- Apoptosis and autophagy, including LC3 spot counting
- Radiation repair, including γ -H2AX spot counting
- Co-localization of markers within cells or on cell surfaces
- Intracellular trafficking and organelle co-localization
- Cell cycle and mitosis studies in heterogeneous populations
- Sensitive fluorescent in situ hybridization for rare events
- Morphology-based cell classification

How many studies have been published by ImageStream users?

There are over 85 publications in journals that include Nature, Science, Blood, J. Immunology, and numerous others. Please see the Amnis publication list for the most significant recent papers. Reprints are available upon request.

Is ImageStream^X technology unique?

The ImageStream^X is currently the only platform available for the sensitive fluorescence imaging of cells in flow and is covered by over 35 US and international patents. Though imaging in flow is novel, the most important characteristics of the ImageStream^X are its speed, high information content per cell, and high fluorescence sensitivity. High speed allows the imaging and analysis of large populations of cells for statistically robust assay results even with rare sub-populations in heterogeneous samples. The multiple images of each cell help ensure that different cell populations can be distinguished based on their morphology (brightfield imagery), granularity (darkfield imagery), and fluorescence brightness and sub-cellular distribution. The high fluorescence sensitivity ensures detection of even faint markers.

Can the ImageStream^X be used with adherent cells as well as non-adherent cell types?

Most sample preparation protocols for flow cytometry, including those for adherent cells, can be adapted to the ImageStream^X with relatively minor adjustments in cell concentration, sample volume, and probe titration.

How many colors can the ImageStream^X system image?

Each of the ImageStream's two CCD cameras produces six images, for a total of up to 12 images per cell. The 12 images can include darkfield, brightfield, and up to 10 fluorescent images. Each image is used to calculate over 80 parameters that quantitate morphology, fluorescence strength, and fluorescence localization. The ImageStream^X is compatible with a wide variety of fluorescent dyes commonly used in both flow cytometry and microscopy.

How fast is the ImageStream^X?

The system will image approximately 1,000 cells per second, independent of the number of images acquired per cell. The imaging rate is proportional to the sample concentration and assumes two million cells in the

standard sample volume of 50 microliters. The total sample-to-sample cycle time is approximately one minute, including a thorough automated flush between samples.

What is the minimum number of cells per sample?

The ImageStream^X can analyze more than half the standard 50 microliter sample volume, so samples with as few as 10,000 cells will still yield images of over 5,000 cells.

How sensitive is the ImageStream^X?

The ImageStream^X has fluorescence sensitivity equal to or better than modern flow cytometers. The ImageStream^X is significantly more sensitive than flow cytometry for smaller objects like bacteria, yeast, and sub-cellular organelles. Compared to a typical microscope/camera combination, the ImageStream^X will detect fluorescence signals that require exposure times as long as ~1 second on the microscope.

How detailed are the images?

The standard magnification of the system is ~40X. The optical system employs a 0.75 numerical aperture 40X objective lens and is diffraction-limited. The image pixel size is 0.5 x 0.5 microns, so a 10 micron diameter cell covers approximately 300 pixels. The MultiMag option includes additional 60X/0.9 NA and 20X/0.5 NA objectives on a motorized linear slide. The 60X objective provides increased resolution and sensitivity while the 20X objective provides increased speed and a wider field of view for large cells.

How does the ImageStream^X differ from high content imaging systems (HCS) and laser scanning microscopes?

The most fundamental difference is that the ImageStream^X images cells in suspension rather than on slides or plates, so it is much better suited to blood, cells in other bodily fluids, and non-adherent cell cultures. More significant, however, is that HCS and laser scanning systems are optimized to image only a few hundred cells per sample in a semi-quantitative manner. These systems are typically used in pharmaceutical screening environments with cellular assays that employ homogeneous cell cultures, just a few strong fluorescent markers, and that have very distinct readouts. In contrast, the ImageStream^X is designed to image tens of thousands of cells per sample with high fluorescence sensitivity and up to 12 high resolution and highly quantitative images per cell. These capabilities enable detailed studies of complex and/or subtle biological phenomena in highly heterogeneous cell samples. The ImageStream^X can be employed for HCS with the addition of the AutoSampler option.

Couldn't I just use a cell sorter followed by microscopy to do what the ImageStream^X does?

It is easy to conceive of using a cell sorter to isolate rare cells on a slide for subsequent imaging. In practice, this is very difficult to accomplish because the cells of interest must be phenotypically distinct from other cells in the sample in order to be sorted with high purity. In most cases, a mixture of cells is sorted which cannot be differentiated by the microscope due to its limited sensitivity and fewer fluorescent colors relative to the sorter. Even if a pure population is sorted, the microscope is limited in its ability to quantitate multicolor imagery accurately due to fluorescence crosstalk. Even if this limitation is overcome by using a limited number of spectrally distinct colors, the microscope is constrained in the number of cells it can image in a reasonable period of time, so statistical population information will be unavailable. Variation from cell to cell in small analysis populations can mask subtle differences that the analysis of a larger population would reveal clearly.

What is the EDF option?

EDF stands for Extended Depth of Field and it allows the ImageStream^X to keep the entire cell in focus rather than just a single plane of focus as in conventional microscopy. EDF is extremely useful for assays that rely on the counting of spots, such as fluorescence in situ hybridization, but it is also generally useful. EDF produces imagery similar to a maximum projection of a confocal image stack, but it imposes no speed or sensitivity penalty compared to the standard ImageStream^X optical system. EDF also allows faster operation of the instrument by running a wider fluid core and improves data analysis and consistency by ensuring uniform focus for virtually all the cells in the data file.

How many lasers can be fitted to the ImageStream^X?

The ImageStream employs a 488 nm solid state laser and can be outfitted with up to four additional lasers. These include 405 nm, 561 nm, 592 nm, and 658 nm variable power solid state lasers.

Does the ImageStream^X require a dedicated operator?

No. Amnis recommends that each site have one or two advanced users, but these users can readily train others. The ImageStream^X is highly automated, including one-button calibration at the beginning of the day and one-button sterilization at the end of the day. Instrument operation can be learned in a matter of hours and users can become functional with the IDEAS data analysis software in a day or two. Amnis offer numerous training and support options including telephone and web conferencing, on-site training, and advanced training at our facility.

What's the largest object that can be imaged?

With the standard 40X objective, the imaging field of view is 60 microns wide. With the 60X objective, the field width is 40 microns and with the 20X objective the field width is 120 microns. Flow forces tend to orient long objects vertically, so the main constraint is cell width.

Is clogging a problem?

The narrowest pathway in the system is 250 microns and all fluids are pumped via motor-driven syringe pumps, so clogging problems are rare. To ensure trouble-free operation, Amnis recommends that samples be filtered through a 70 micron nylon mesh before running on the instrument.

Don't the cells tumble in flow?

No. The fluidic system is designed to be extremely laminar, non-pulsatile, and to prevent cell tumbling.

Can the ImageStream^X image live cells or cell conjugates?

Yes. The fluid pressures and shear forces within the ImageStream^X are very low. Please keep in mind that the system has no provision for controlled incubation.

What is the SpeedBead[®] reagent, how is it used, and how much does it cost?

The SpeedBead reagent is a suspension of small microspheres that is run at all times in the system. It is used during the daily ImageStream^X calibration routine (ASSIST) as well as by the real-time velocity detection and autofocus systems. The ASSIST calibration routine is a comprehensive set of automated self-tests and that use SpeedBead imagery to ensure that all the internal systems (illumination, collection, fluidic, electronic, etc.) are functioning within factory specifications. The routine takes about 10 minutes to run and requires no user intervention. The SpeedBead reagent is provided free of charge during the initial year of warranty coverage and is also included as part of Amnis' service contracts.

Is the ImageStream^X compatible with the sheath fluids used by conventional flow cytometers?

Amnis recommends the use of phosphate-buffered saline as sheath fluid. The ImageStream system's fluidics are conceptually similar to those of a flow cytometer but the actual design is quite different and will not function properly with commercially available sheath fluids intended for flow cytometers. The ImageStream^X uses standard cleanser, debubbler, and sterilizer solutions which can be purchased from a variety of sources at nominal cost.

What is Time Delay Integration (TDI)?

Instead of using photomultiplier tubes, the ImageStream employs a custom CCD camera to image the cells. TDI is a method of CCD camera operation that greatly increases sensitivity, even at very high imaging rates. Essentially, TDI is a means of electronically panning the camera to track the cells in the flow stream. As the cells move down the field of view, the photoelectrons on the CCD chip are shifted down the chip. This process is synchronized by measuring the velocity of the cells and constantly adjusting the shift rate of the photoelectrons to match. The end result is that light from each cell can be collected ~1000X longer than standard imaging techniques without blurring. With TDI operation, all objects in the flow core are imaged without the need for a triggering system.

Can IDEAS[®] export data for analysis by other flow cytometry software packages?

The IDEAS data analysis package can export calculated feature values in plain text format to a file or to the Windows clipboard. Feature values can also be exported to an FCS file

Can IDEAS export raw image data for analysis by other image analysis packages?

IDEAS can export the raw image data for user-specified cell populations in 16-bit TIFF format.

Can IDEAS be customized to suit my own needs?

The parameters calculated by IDEAS can be user-customized to a large extent. For instance, if a cell is counterstained with a fluorescent DNA binding dye, the nucleus will be imaged along with a brightfield image of the cell as a whole. The cytoplasmic area can then be calculated by subtracting the nuclear image area from the cellular image area. The nuclear to cytoplasmic area ratio could also be calculated by dividing the nuclear area by the cytoplasmic area calculated previously. Once defined, these user parameters are automatically calculated for all cells and can be plotted on histograms and dot plots just like any other standard parameter.

Does IDEAS require special computer hardware?

IDEAS will run on most modern PC's running Windows XP or Vista. Four gigabytes of RAM are recommended to facilitate the analysis of larger data files (more than 10,000 cells) and/or the analysis of multiple data files at the same time. IDEAS can also take advantage of higher video resolution settings to display more graphs and images at a time. The best way to transfer data is via gigabit ethernet.

How big are the data files?

A typical 10,000 cell file requires roughly 500 MB for both raw and processed data file storage.

What do you recommend for data storage and transfer?

The ImageStream^X is available with a dedicated high speed workstation that incorporates a 1 terabyte RAID and can be connected to the instrument via a gigabit ethernet link. The workstation's RAID is typically sufficient for at least the first year of data storage. Data can be backed up to DVD, external hard drives, or a storage server via a network.

Is IDEAS Apple Macintosh compatible?

IDEAS is a Windows application but a number of our users run it on Intel-based Apple hardware using Parallels, VMware, and other Windows environments. IDEAS is not officially supported by Amnis on Apple hardware.

How big is the instrument?

The ImageStream^X is approximately 90cm wide x 60cm deep x 65cm tall (36" x 24" x 26") and weighs about 130Kg (300lb). The instrument incorporates its own suspension system and can be placed on any sturdy table or bench big enough for the instrument itself as well as the computer that runs it. The ImageStream^X consumes ~500W of electrical power and requires no external connections or tanks.

What is included with the instrument?

The ImageStream^X includes a Windows-based computer for instrument operation and a copy of the IDEAS data analysis software package. The instrument and computer are covered by a comprehensive one year parts and labor warranty with free telephone and web support. Also included is installation, one week of training for two users at Amnis, and one year's worth of the SpeedBead calibration reagent.

What is the history of Amnis?

Amnis has been in business for over 10 years. It is privately held and has been funded by sales revenues, venture capital, and grants. Product revenues have grown steadily year-over-year since the ImageStream was introduced in 2005. 2008 was a record year for sales, despite the worldwide recession. We entered 2009 with well-controlled costs, significant capital reserves, an excellent reputation for customer support, and a large installed base of ImageStream systems in 15 countries world-wide.