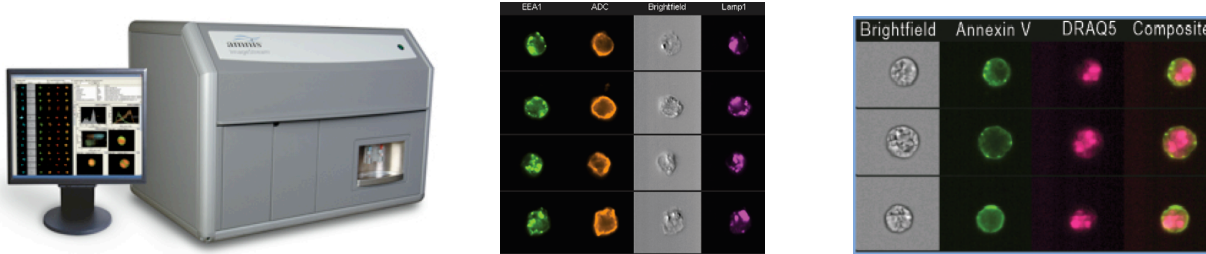


ImageStream Core at Duke University:

The ImageStream System from Amnis Corporation combines high-speed automated image capture and quantitative image analysis tools in a single platform. The ImageStream is currently the only platform available for the sensitive fluorescence imaging of cells in flow. As such, the ImageStream System has advantages over both flow cytometry and over fluorescent microscopy. For example, while flow cytometry allows detection of rare sub-populations of cells within mixed populations and can be highly quantitative due to the ability to acquire large numbers of events, flow cytometry cannot identify the subcellular localization of probes, capture changes in shape or count spots or co-localization events. At the same time, while fluorescence microscopy (confocal or traditional) can assess subcellular localization, changes in morphology, this technique cannot readily assess large numbers of cells for statistical evaluation of the populations. In addition, microscopy cannot capture statistical data on events occurring within minor populations of cells, such as stem cells or circulating tumor cells.



The unique features of the The ImageStream system:

- (1) The ImageStream system collects images of cells that have been hydrodynamically focused in a flow cuvette with sheath as the background. This eliminates the problems inherent to all plate-based microscopy platforms of non-specific cell overlap and the difficulty of identifying precise cellular boundaries that occurs on slides.
- (2) The Amnis ImageStream captures high resolution digital images of cells in flow at rates that can exceed 1000 cells/second. The ImageStream is able to detect four colors of fluorescence along with brightfield and darkfield imagery of each cell.
- (3) The acquired data is analyzed with the Amnis Image Data Exploration and Analysis Software (IDEAS™) package, which operates on a PC platform. The IDEAS™ analysis software quantifies over 500 morphometric and photometric parameters for each cell based on its imagery, including parameters that measure sub-cellular location of probes. This technology combines the quantitative power of large sample sizes common to flow cytometry with the high information content present in microscopic images. The IDEAS software is easy to use and is adaptable, with the ability to easily create new analytical functions using the pre-calculated values (e.g. nuclear to cytoplasmic area ratio). There are existing validated algorithms for measuring translocation, co-localization, internalization, and spot counting (to name a few) and there is continuing platform evolution by Amnis biologists and algorithm scientists.
- (4) The ImageStream is equipped with 488 nm (200 mW variable power), 658 nm (80 mW) and 405 nm (350 mW) lasers. The images obtained at the highest resolution setting have a pixel size of 0.5 microns, with 16 micron depth-of-focus. The system also has an Autosampler for 96 well plates
- (5) Instrument operation is straightforward, featuring automatic sample load, empty, flush, and purge as well as automatic focus and flow speed synchronization. The system auto-calibrates each day prior to use, and the shut-down procedure includes an automated cleaning and sterilization procedure.
- (6) Established applications of the ImageStream technology currently are:
 - 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
 - Co-localization of markers
 - Intracellular trafficking
 - Cell cycle and mitosis studies
 - Morphology-based cell classification
 - Radiation repair, including γ -H2AX spot counting

Logistics: The ImageStream is located at Duke University Medical Center, in the Levine Sciences Research Building (LSRC). Interested investigators should contact Gerry Blobe (gerard.blobe@duke.edu) or Mythreye Kathikeyan (mythreye.k@duke.edu). User fees do apply. For more information about the machine please check out the following web site: www.amnis.com/imagestream.html.