Amnis Imagestream cytometry

In this edition of the newsletter we will be covering the upgrades which were made to the Amnis® imaging flow cytometer and a **new facility initiative to ease use of this instrument.** The new Amnis® ImageStreamX mkII combines the speed and sensitivity of flow cytometry with the detailed imagery and functional insights of microscopy. This unique combination enables a broad range of applications that would be impossible using either technique alone.

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**Features of the upgraded Imagestream cytometer**

Over the summer the facility ImageStream was upgraded to an ImageStreamX mkII. The new instrument has superior fluidics and software which have made this a significantly more user-friendly instrument. In addition, several hardware upgrades have enhanced both image acquisition and fluorescence detection.

- The red laser was changed from 658nm to 642nm to better excite common fluorophores like APC and AlexaFluor647.
- A 758nm laser has been added to allow the collection of side scatter in either channel 6 or channel 12. This allows for the simultaneous collection of side scatter and PE-Cy7 which was previously impossible.
- A rotating nosepiece with three objectives; 20x, 40x and 60x has been installed, replacing the single 40x objective.

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**A broad range of applications are possible.**
Intuitive image analysis that incorporates traditional flow cytometry gating

The IDEAS software package incorporates traditional flow cytometry analysis with detailed image analysis. Imaged cells can be gated as events through scatterplots or histograms and the gated images further processed to quantify fluorescence within segmented portions of cells. Every dot on a scatterplot is linked to the image of that cell in the image gallery, which helps to build confidence that your analytic approach is accurately reflecting trends. Once the ideal analysis strategy is selected, a template can be created and files can be batch analyzed.

For example, with a DNA binding dye, the nucleus can be masked within the image and fluorescence within or exterior to the nucleus compared, thereby measuring the extent of nuclear translocation of your protein of interest. Below is an example of such a study.

Data graciously provided by Matthew Staron, Kaech lab.

Staff support for data acquisition and analysis

To help you incorporate this technique into your research, we are now providing more staff support during the early stages of your study as you gain experience. Experimental design for this instrument requires finesse as compensation is different from that of a typical flow cytometer. Please contact Christine Cote to request training for the Amnis. She is available for experimental design consultation and can suggest fluorophore combinations. If it would be helpful, Christine can also assist you during initial acquisitions. She can also help you design an image analysis strategy to quantify the biological trends you are interrogating.

The Yale FACS Facility website has the Amnis Sample Preparation guide to direct your experimental design. The Amnis website has a wealth of information available for users with the creation of a free account. Through the website, they also make IDEAS available to everyone free of cost.