The Stream

*Weiguo Feng, FACS user
Of the month*

Special Interest Areas this month:

- Do you sort highly autofluorescent cells?

- Are you gating as accurately as possible for viability?

- Are you trying to quantitate antigen expression levels?

Read about the user of the Month and the Flow Call to find out where to get help.
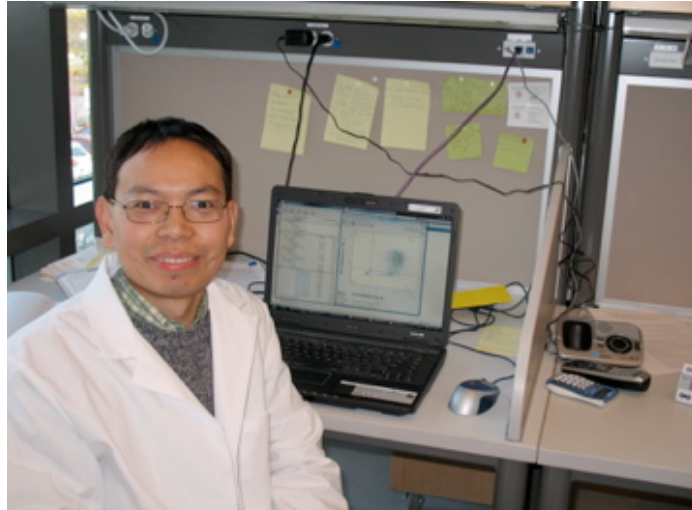
Individual Highlights:

Flow User of the Month: Weiguo Feng Combats Autofluorescence When Sorting Lung Cancer Cells

Hats off to the first FACS user of the month in the new Lorry Lokey Stem Cell Research Building. Weiguo Feng from Max Diehn's lab is this issue's user in the spotlight. The Diehn lab currently focuses on identifying the human lung cancer stem cell. Weiguo is at the forefront of solving some of the unique problems inherent in sorting these cells. He has come up with some excellent ways of dealing with this tissue and his remedies may be helpful to others working with highly autofluorescent tissue.

Last issue we introduced Stephen Willingham's tissue dissociating method as a way of working with tissue that is difficult to disaggregate. Weiguo has another problem. Lung cancer tissue is infiltrated with large numbers of immune cells and highly autofluorescent, making it difficult for him to identify and sort out the rare lung cancer stem cell. Weiguo found that 2 strategies helped him to find these elusive cells. First of all, he changed the fluorophore conjugated

to his marker of interest from FITC to APC. Most cells have less autofluorescence excited by the 633 laser than that excited by the 488. Thus, parameters (such as APC) off the 633 laser will be more easily separated from highly autofluorescent negative cells than those off the 488. Also, Weiguo found that very accurately identifying dead cells was crucial as they can have high autofluorescence. He initially was using a histogram plot to ID live cells. He found that by using a dot plot, he could better distinguish live versus dead. With a new dye and accurate elimination of dead cells using a DAPI vs FSC dot plot, he can easily identify and sort cells with the epithelial marker thought to be one marker on lung cancer stem cells. Other markers further segregating this population are also used. Following the sort of potential lung cancer stem cells, Weiguo injects them into immunodeficient mice and looks for tumor growth, which could indicate that these cells are indeed cancer stem cells. With a few changes to his assay, his research is on it's way to success.

Why use dot plots instead of histograms?

It can be a habit to use histograms to gate populations based on one parameter, but often using a 2 parameter dot plot will help to reveal subpopulations not apparent on a histogram, thus allowing for more selective gating. Can you see how Weiguo is able to draw a more intricate gate around his viable/lineage negative cells using a dot plot than he can do by gating the two separately using histograms? Try using a dot plot instead of a histogram next time (you can use FSC or SSC as one of the parameters) and see if you can better define your populations.
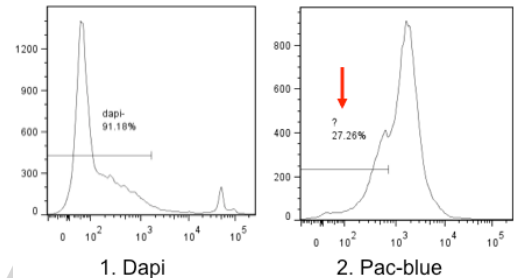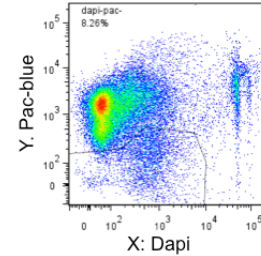
Flow Call- Advice for Flowers

Flow call is the advice column for "The Stream." Email your flow questions to lovelace@stanford .edu

Q Dear Flow Call,

I am interested in quantifying the amount of CD47 expressed on human tumor cells. I have a PE conjugated anti-CD47 antibody. Can I use the PE MFI as a measure of CD47 expression? Is there a better way to quantify antigen expression?

S Willingham

A Dear S,

While MFI can be used to compare expression levels of samples within one run, it can be more difficult to compare expression levels on samples run on different days or different instruments, since instrument performance and settings affect MFI. In order to compare MFI, one would have to verify that the instrument is set up to perform exactly the same way each time samples are run. This can be done by using something called "Application Settings" linked to a daily CST performance check, but the best way to quantitate antigen expression level is to use antigen quantitation beads. BD QuantiBRITE beads are one example of these beads. Each QuantiBRITE PE tube contains a lyophilized pellet of four different bead populations. Each population is conjugated with a pre-established known mean number of PE molecules attached per bead. Analyzing QuantiBRITE PE tubes at the same instrument settings and conditions as the assays allows the conversion of the PE-associated fluorescence values into the number of PE molecules bound per cell. In your case, median absolute CD47 antibody binding for each sample can be determined from a calibration curve constructed from the QuantiBRITE bead data using the FlowJo Data Analysis software calibration tool. Importantly, these beads are designed to work with PE conjugated antibodies conjugated at a 1:1 ratio. Anyone trying to quantitate antigen expression level is encouraged to investigate this method.

New Users Section

Introducing the newest Aria users to the group. The following are just some of the new users that completed their training since we moved to the new building. Don't hesitate to ask if they need help or advice from more experienced users:

Brian Jonas in the Majeti lab will be isolating mouse hematopoietic stem/precurser cells and mouse leukemia stem cells.

Ryan Corces-Zimmerman (Majeti lab) will analyze the efficacy of integration of engineered transposable elements in leukemia and hematopoietic stem cells.

Josh Checketts (Porteus lab) will sort cells according to cell cycle stage to determine the effect of cell cycle on gene targeting.

Richard Voit (Porteus lab) will be sorting GFP positive cells after targeted gene additions to look at the integration site.
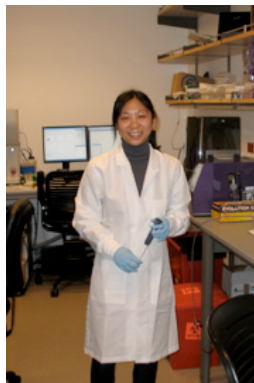
Mike Kareta (Sage lab) will be looking at the role of cancer genes reprogramming regular cells into stem cells..

New User Photos
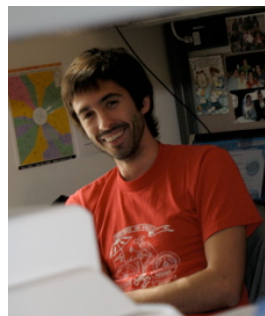
New Majeti lab flow users

Ryan Corces-Zimmerman

Brian Jonas

Emily Piccione

Wan-Jen Hong

"If you see these new users at the Aria, check in to see if they need some extra help."

Porteus lab flow users: Shaina Porter, Josh Checketts, Eric Kildebeck, and Richard Voit

Sage lab flow user: Mike Kareta