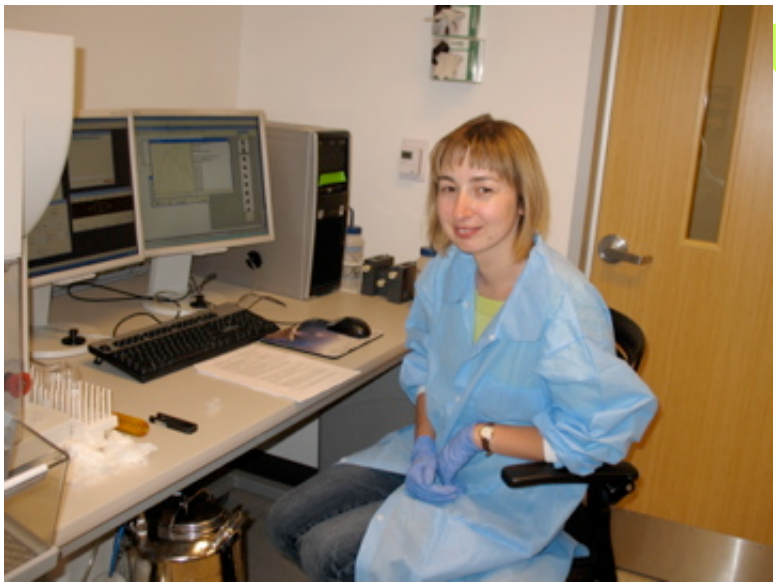
October 2012

Volume 1, Issue 4

The ~~Stream~~ Clog



Flow User of the Month: Is your late-night sorting schedule making you FAT? Maria Costa sorts fat cell progenitors to study the relationship between sleep and metabolism.

Wondering what causes that unwelcome accumulation of fat during bathing suit season? Recent research by this month's FACS User of the Month Maria Costa reveals that those extra pounds might be related to specific genes that affect sleep patterns.

Maria uses flow cytometry to identify and sort progenitors from mouse fat pads that can differentiate into fat cells. Her main research question: Are fat progenitors in a mutant mouse with a circadian rhythm abnormality different quantitatively or qualitatively from those of a normal mouse? And, how does this relate to the mouse's propensity to become obese? (You could conclude it stays up late sorting.)

Maria's project was initially daunting. It involved studying complex details about cell sorting, a skill Maria was just learning. A closer look at her systematic approach using flow cytometry demonstrates that she has developed an efficient method to study any type of progenitor.

Maria's first challenge was determining how to process her sample to tease out the mesynchymal progenitors. Then, she had to identify the progenitor-specific markers for flow sorting and validate them with an assay to prove the existence of the progenitors. Finally, she could begin to study the differences between the progenitors she isolated in normal and mutant mice.

The first steps progressed slowly, but her research is now advancing rapidly. This is the case with many projects in the Stem Cell Institute, and Maria has some advice to those just starting projects – her 3-Step Approach is outlined on page 2.

Following her 3-Step Approach, Maria was able to isolate, identify and sort cells of interest. She is now making progress studying the difference of these cells in the circadian rhythm mutant mice model. Maria's research very well may help scientists determine which circadian rhythm genes affect obesity and, in turn, find solutions.

Special interest areas this month:

- Are you trying to identify a progenitor population?
- Are you just learning to identify your cells by flow cytometry?
- Are clogs slowing down your sorts?

Get helpful tips from the User of the Month and Flow Call.

Individual Highlights:

Maria's Three Step Approach to fat cell progenitor isolation

Step 1: Extract cells from the tissue

Maria retrieves fat progenitors from the fat pads of mice. She minces the tissue and then digests it with collagenase. A centrifugation step causes the mature fat cells to float. The mature cells are discarded, leaving the stromal cells of interest in the pellet. The sorter identifies and sorts the progenitors from these cells.

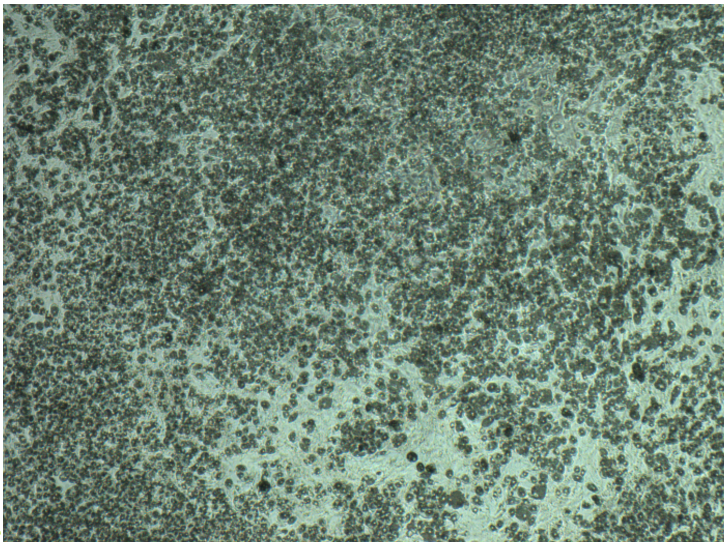
Step 2: Learn to operate FACS sorter

Like Maria, many of you in the Stem Cell Institute are new to using the cell sorter. Practice is required to learn to operate the instrument and optimize it for a particular type of cells. Maria, who had quite a lot of experience analyzing "straightforward" cells by flow cytometry, found she spent a lot of time examining her cells on the easier-to-operate flow analyzer. Learning to identify the location of the rare cells she needed on the analyzer gave her an edge when she moved to the sorter.

Step 3: Identify your cells in flow

Maria began working with these fat cell progenitors at a time when little was published about how to identify them. Searching the literature, she found clues about which markers may be present on her cells, and she performed detailed preliminary work sorting different populations to isolate specific progenitors. Luckily, she could easily identify which of her isolated populations were indeed the progenitors – she had a functional assay. She seeded the sorted cells into plates and stimulated them with factors known to change progenitors into fat cells. She imaged the cells four days after stimulation, looking for characteristic lipid droplets in the cytoplasm. Those that turned into fat cells after stimulation confirmed that she had correctly identified the progenitors.

A confluent culture of preadipocytes differentiating into adipocytes. The dark cells that have clear droplets are the adipocytes.



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 seem to experience a lot of clogs during my sorts. How come this happens, and how do I prevent it from happening in the future? It really slows me down.

-Anonymous clogger

A Dear Clogger,

Note that this issue of The Stream has been renamed "The Clog!" Flow Call has noticed lately an increase in clogs causing problems on the cytometers. Clogs not only slow you down, but if they are so bad as to clog the whole sample line, then they also delay everyone after you. This can lead to a costly sample line replacement.

Additionally, clogging that occurs during sorting can misalign stream deflection, causing the stream to hit a surface inside the chamber. This can increase aerosol production and hazard potential. We should all take extra precautions to prevent clogs from occurring during our sorts.

Here are some factors that can affect aggregate formation and clogging:

1. Nozzle size

It is important to pick an appropriate nozzle size to match your cells. Cells should not be more than one fifth the size of the nozzle.

2. Aggregation Formation

Poorly prepared samples can contain aggregated cells that can lead to clogs. There are several methods to prevent/remove aggregation.

Always filter (strain) your cells before loading onto a cytometer. BD sells 5ml polystyrene tubes with nylon mesh strainer caps (cat #352235), and individual 100, 70, and 40-micron cell strainers (cat #352360, 352350, 352340). Partec also sells filter units with mesh diameters ranging from 10-150 microns.

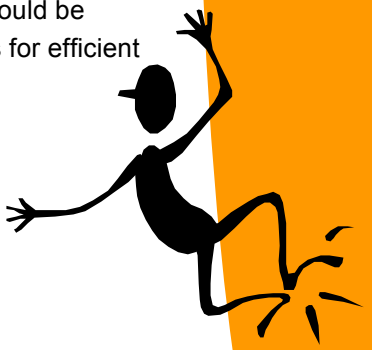
A cheaper option is to purchase nylon mesh (sold by the yard by Elko Filtering Company www.elkofiltering.com). Cut the mesh into squares, autoclave it, place it on top of tubes, and pipette cell suspension through the mesh. Remember that prewetting strainers can minimize cell loss during straining.

Dead cells found in thawed frozen samples or samples subjected to long dissociation processes can stimulate aggregation formation as the DNA they release sticks to live cells. These aggregates can form after filtration, so filtration will not necessarily help in this case. Eliminate cells aggregating in this situation by adding 20 μg/ml DNAase for 10 minutes at 37°C.

Highly concentrated cell suspensions can also cause clumping. Cells should be diluted to a density that allows for efficient sorting with minimal clogging.

Let's all work t
stop clogs
before they
happen

-Flow Call



New Users Section

We have had many new users join since the last newsletter. Following is a list of some new users who completed their training recently. More will follow in the next issue. Don't hesitate to ask them if they need help or advice.

Sharareh Gholamen sorts cells and looks for tumor initiating cell surface markers in Glioblastoma and Medulloblastoma.

Jonathan Davila and **Henrik Ahlenius** (Wernig lab) will be sorting induced neurons.

Members of the Wu Lab have recently begun using the sorters: **Francie Barron** will be sorting cardiomyocytes based on GFP or tdTomato expression.

Members of the Wysocka Lab have also recently begun using the sorters. **Eliezer Calo-Velazquez** sorts haploid cell lines by size. **Christa Buecker** performs similar sorts and also sorts cells based on expression of GFP, RFP, and BFP. **Edward Grow** sorts cells expressing active transposon reporters.

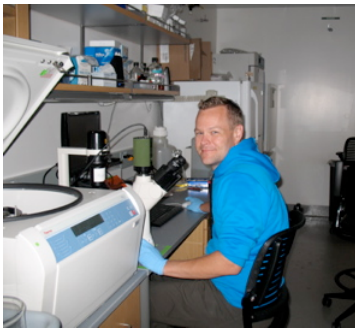
New User Photos

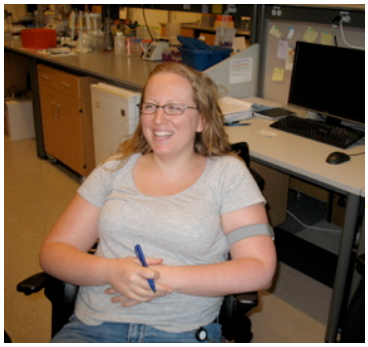
Sharareh Gholamen

Jonathan Davila

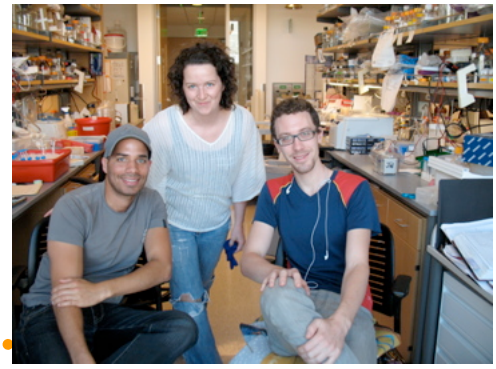
"If you see these new users at the Aria, check in to see if they need some extra help."

Henrik Ahlenius





Francie Barron



Wysocka Lab members: Eliezer Calo-Velazquez, Christa Buecker, and Edward Grow