ImageStream: Filtering samples

In the event that you experience significant sample clumping, follow these steps to filter the sample through 80 um mesh (Small Parts, Inc; cat # CMN-0074) before loading onto the ImageStream:

Method A: Filter before last wash
This method involves filtering a large volume of sample fluid in wash buffer through the mesh prior to the final sample concentration step and transfer into microcentrifuge tubes. The advantage is that it is technically easier; disadvantage is that further clumping may occur after the filtration as a result of the final sample concentration step.

After the final staining step:
1. Add wash buffer
2. Pipet entire volume up from tube
3. Place mesh square over tube opening
4. Pipet the sample volume through the mesh
5. Pellet cells
6. Resuspend in 50 uL of volume and transfer to 0.6 mL microcentrifuge tubes
7. Run on the ImageStream

Method B: Filter after last wash
This method involves filtering a small volume of concentrated sample through the mesh immediately prior to running on the ImageStream. Although technically more difficult, it avoids the possibility of further clumping that can occur with subsequent washing (method A).

Assume a starting sample volume of 50 uL:
1. Place mesh square over 0.6 mL microcentrifuge tube opening
2. pipet 50 uL running buffer into the flipped back microcentrifuge cap
3. Pipet approximately 10 uL of running buffer from the cap onto the mesh at the inside edge of one portion of the tube wall to create a liquid seal and channel for fluid to flow through by capillary action
4. Pipet the sample onto the wet portion of the mesh; the sample should flow through the mesh along the inside of the tube wall. It is important to avoid having the sample fluid seal all the way around the tube, as the sample will then not flow through.
5. Chase any sample on the mesh with 10-20 uL of running buffer (take from the tube cap)
6. Wrist-flick the tube to get the sample to the bottom
7. Measure the volume and add running buffer to Q up to 50 uL
8. Run on the ImageStream

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