Kylie Price, 22 March 2013

QUESTIONS TO ASK WHEN YOU ARE CRITIQUING FCM DATA:

1. Were the unstained cells treated the same as the stained cells (e.g. stimulated)?

2. What were the controls (**FMO**, biological controls, experimental controls)?

3. Did they use CompBeads?

4. Has an antibody titration been done? Ask to see results, expressed as a Stain Index.

5. Is the data compensated properly? Look for "diagonals" and over-compensated data (events piled on axis). Ask to see their compensation matrix and keep an eye out for values >50%

6. What plot type was selected and it is meaningful? If they're using contour plots, if so what level of contour did they select?

7. If the authors are using antigen density information: Are they using saturating reagents? Did they carefully control their staining protocol? How did they calculate the antigen density (florescence): mean, median, geometric mean? Is the data unimodal or bimodal?

8. If they used a "lymphocyte" gate, is it appropriate for the experiment? I.e. are activated cells being excluded because they are too large?

9. Are they using rectangular gates on populations that cannot be purely identified by rectangular gates?

10. When they are doing progressive gating (hierarchical gating), have they shown you examples of how they set their gates (show ancestry or backgating)

11. Are they excluding dead cells?

12. Can you see evidence of non-specific staining/binding (diagonals!)?

13. For lymphocyte analysis did they properly exclude monocytes? (Look for diagonals!)

Please ensure all your staff or students perform Antibody Titrations and use CompBeads on your digital cytometers

- Anti-mouse Ig κ beads typically bind human antibodies (as they are most commonly raised in mice)
- The anti-rat Ig κ, anti-hamster Ig κ beads should bind most mouse antibodies

If people need to know how to use FlowJo direct them to:
http://flowjo.com/home/tutorial.html

They can download the Basic and Advanced Tutorials