Kylie Price 23-3-13

# OVERVIEW: Optimising a Multicolour Experiment

**Have a well-defined scientific question, with a gating strategy prepared**

1. Make a wish-list of antigens you are interested in and put them in order of importance

2. Assign your antigens into categories: primary, secondary and tertiary antigens

3. Purchase the most reagents for primary antigens, fewer for secondary antigens and only one or two for tertiary antigens (using the brightest fluorophores e.g. PE and APC)

4. Titrate all antibodies

5. Screen all antibodies in single colour experiments to determine the best detector for each antigen

6. Make your grid and begin to block off detectors starting with either the tertiary antigens or the lowest expression

7. Evaluate candidate antibodies for interference. Start with antibodies identifying major subsets (eg. CD3, CD4, CD8) and add remaining antibodies one at a time in the order you will be gating

8. Troubleshoot problem antibodies (e.g. check compensation, perhaps leave problem antibodies out or try a different fluorophore)

9. Check your spreading error. Analyse individual antibody-conjugates (single stained) on the x-axis versus all the detectors being used on the y-axis. Check for spreading errors in other detectors. Troubleshoot if you have spreading error (dilute antibody further, check compensation or change the fluorophore)

10. Where possible use CompBeads for your compensation

11. Where possible include many controls, Biological, Specificity and Gating controls (FMOs only needed when it is difficult to determine the positive/negative boundary)

12. Check compensation using Biexponential Plots

13. Always include an overview of your gating strategy (as supplementary information)