**Day / Date / Time:**
Tuesday  
14 August 2012  
12:00pm – 1:00pm

**Venue:**
CeLS Auditorium  
@ Centre for Life Sciences,  
Level 1, 28 Medical Drive  
Singapore 117456

**Convener:**
A/Prof Paul MacAry

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**Adventures in Polychromatic Flow Cytometry - A Multicolour Kaleidoscope or a Technicolor Yawn?**

**Dr Paul Edward Hutchinson**  
Flow Cytometry Laboratory  
Immunology Programme  
Centre for Life Sciences  
National University of Singapore

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**Abstract**

Advances in flow cytometry technology and the increase in the range of available fluorochromes have expanded the number of antibody combinations researchers can use, such that it is now common to see experiments where cells are labeled with eight or more different antibodies. This use of more fluorescent colours has been termed ‘polychromatic cytometry’, and researchers are utilising it to enable advances in fields ranging from immunology to stem cell biology. However the addition of more antibodies to your experiment also increases the complexity of both data acquisition and analysis, so in order to fully utilise the power of polychromatic cytometry you need to be aware of the pitfalls and problems involved. For data acquisition it is essential that the correct single colour controls are used so that there is accurate compensation of the overlap between the multitude of the different fluorochromes being measured. For data analysis it is important that the right control samples are included in the experiment antibody staining panel, particularly fluorescence minus one controls which will give you the most accurate negative threshold for staining once the compensation has been factored in. Also when you consider that even a 6 antibody staining protocol will have up to $2^6$ (64!) possible staining combinations, it is becoming clear that the standard gate and analyse procedures using one and two parameter histograms is limiting the ability to fully analyse the acquired data. These issues will be discussed with examples from real data and I will give some tips that will help to make your polychromatic cytometry experiments successful. I will also talk about the latest cytometry technologies such as the CyTOF Mass Spec Cytometer and Sony Spectral Analyser Flow Cytometer, which are offering the possibility to use 30 or more different antibody combinations at the same time; and also new reagent technology such as the Brilliant Violet dyes and Upconversion Nanoparticles which are expanding the number of available fluorochromes that can be measured on your flow cytometer.