

FlowCore Users Group Meeting

2nd June 2011

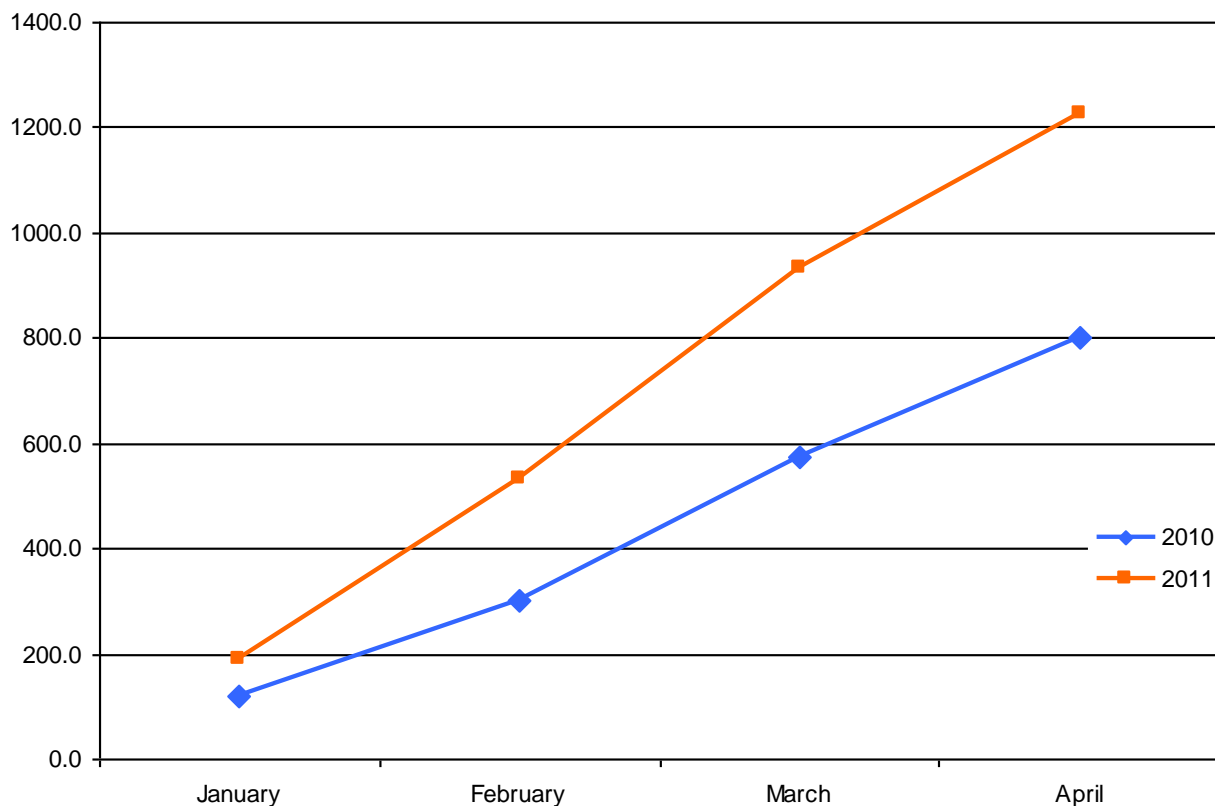


Core Flow Cytometry Facility 



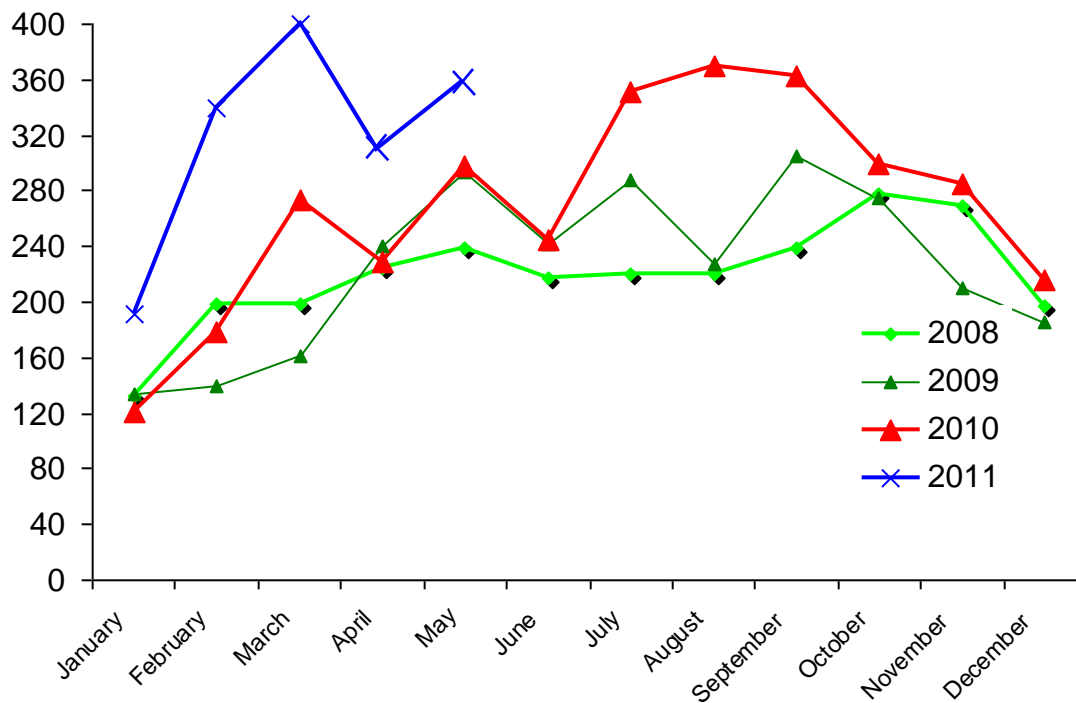
- Welcome to all users
 - www.flowcore.com.au for tips, advice and info
- AF leave: 17 May – 13 June
 - Attending ISAC meeting - International Society for Advanced Cytometry
 - Baltimore, USA
- LifeTechnologies Flow Cytometry Seminar
 - QDots – 30% off list price via MIH quote
 - Useful reagents for assays
 - Slides have been uploaded to flowcore.com.au

Total Sorting Hours 2010 vs 2011



This is a 53% increase in total sorter hours c.f. April 2010
1227.5 hrs vs. 801.5 hrs: Δ of 426 hrs

Total Monthly Sorting Hours





The screenshot shows the Facebook interface for the FlowCore page. The top navigation bar includes the Facebook logo, a search bar, and links for Home, Profile, and Account. The page header for FlowCore is visible, including the name, category (Biotechnology), and an 'Edit page' button. The main content area features a 'Wall' section with a 'Write something...' text box and a dropdown menu for sharing options (Status, Photo, Link, Video, Question). Below this, there are two posts from FlowCore. The first post is a text-based announcement about cancellations and a forum for users, dated May 6th at 10:45. The second post is a celebratory message 'We have liftoff! Welcome to FlowCore!' dated May 5th at 15:54. On the right side, there are sections for 'Admins (1)', 'Use Facebook as FlowCore', 'Notifications', 'Promote with an advert', 'View Insights', 'Suggest to friends', 'Quick tips', and 'Sample Advert: FlowCore'. At the bottom right, there is an 'Advertise Page' button and a 'Sponsored' section with a 'Create an advert' link.

- FlowCore to become 100% Monash at June 30
 - Faculty of Medicine
- Possible changes to emails, phone numbers, branding
- Cost centres

Laser Safety Training Course

- **All users of LSRIIb**
- Must complete ARPANSA approved training course to use CSIRO owned equipment (LSRIIb).
- ½ day course conducted through Monash University.
- Date to be confirmed; likely July.
- All current users conducting or assisting with experiments on LSRIIb are required to attend.
- All non-LSRIIb users will need to undertake the course before gaining access to LSRIIb in the future.
- Laser Safety Training is open to all FlowCore customers.

Multicolour Flow Cytometry

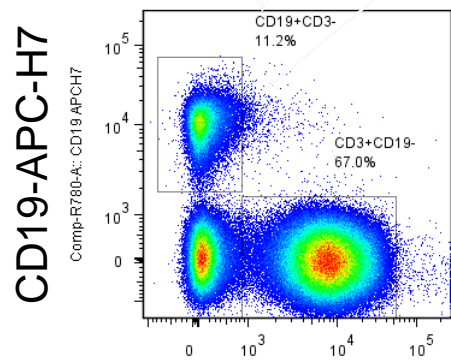
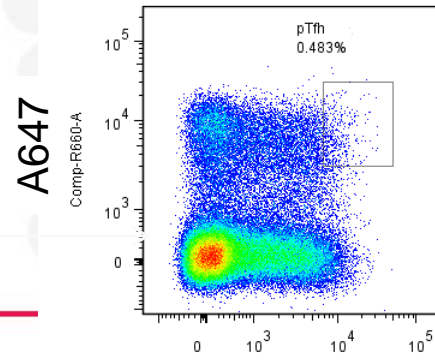
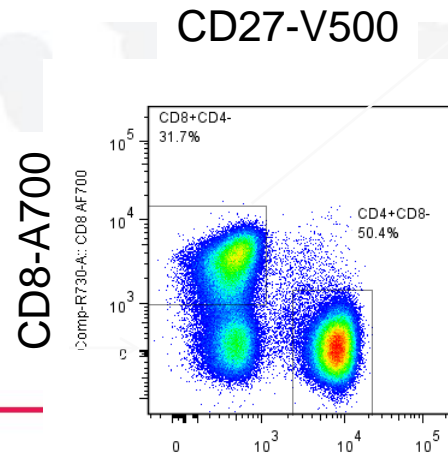
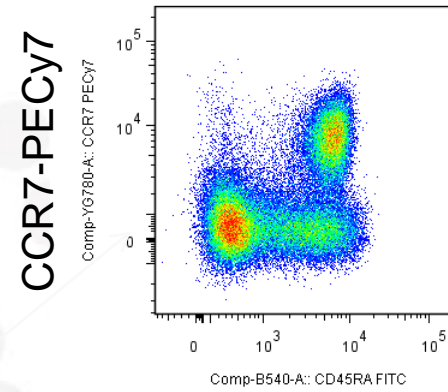
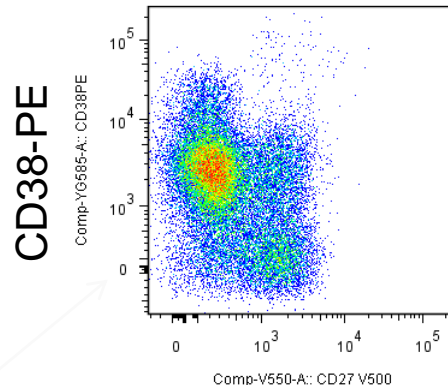
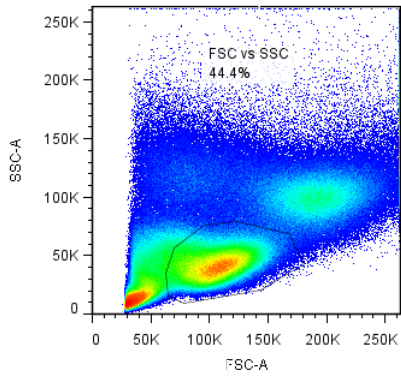
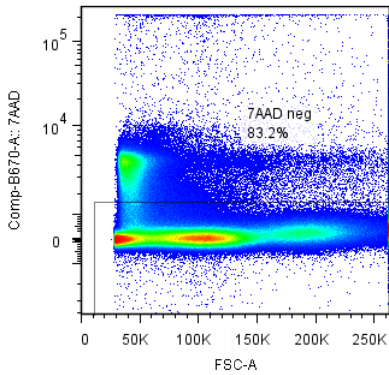
Courtesy of Di Yu

Violet laser: V405, V500, Q605

Blue laser: FITC, 7AAD

Yellow-green laser: PE, PEcy5.5, PEcy7

Red laser: A647, A700, APC-H7



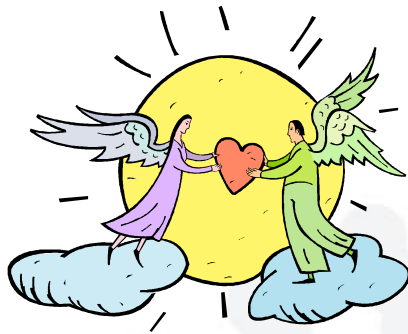
CD3-Q605

CD4-V405

PD1-PEcy5.5



Biexponential Transformation



Good vs. Evil



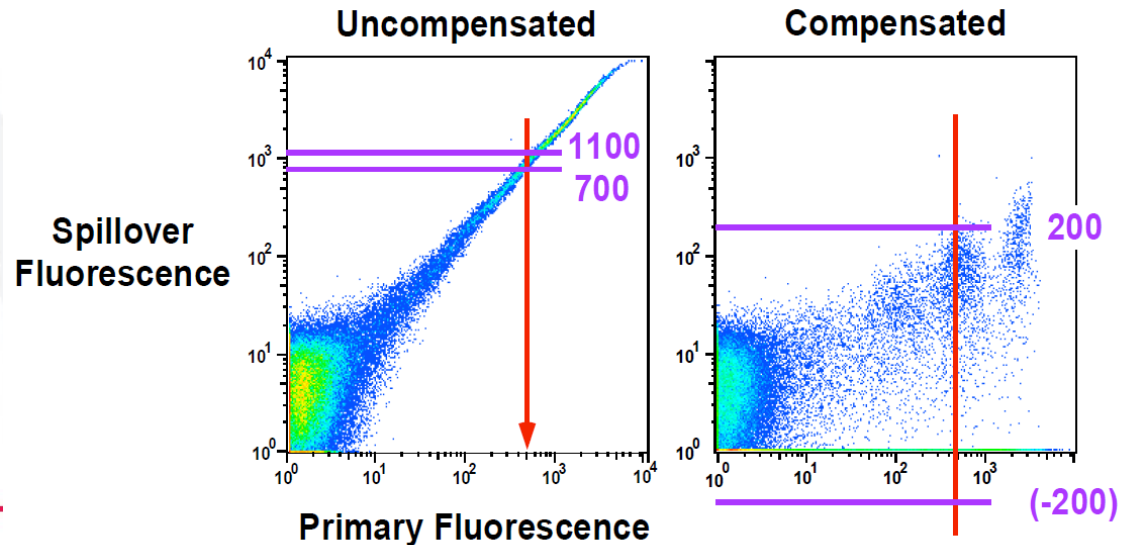
Definition:

A biexponential transformation is a data transformation that, for each (one dimensional) real number input x , outputs an approximation (found, e.g. with the Newton's method) to a solution y of the equation $B(y)-x=0$, where B denotes a b transformation.

Q: Why do we need it anyway?

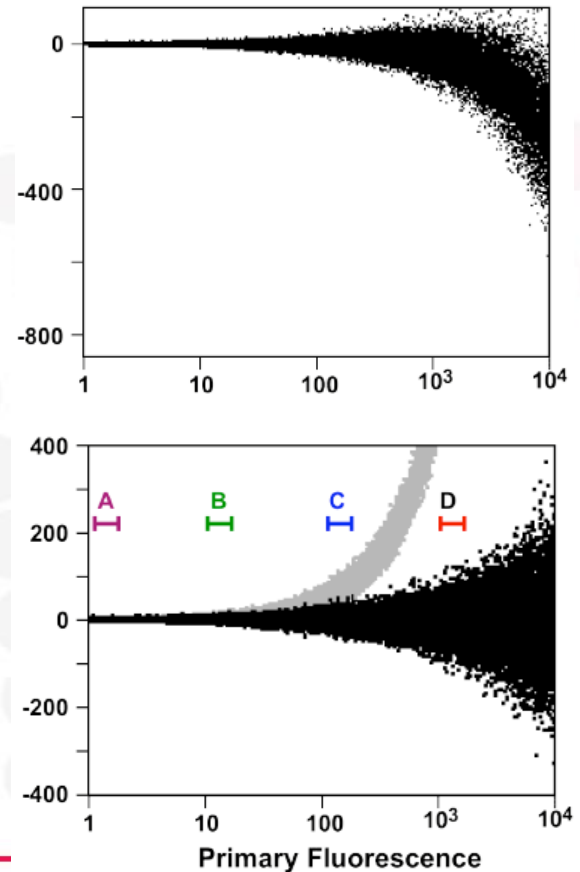
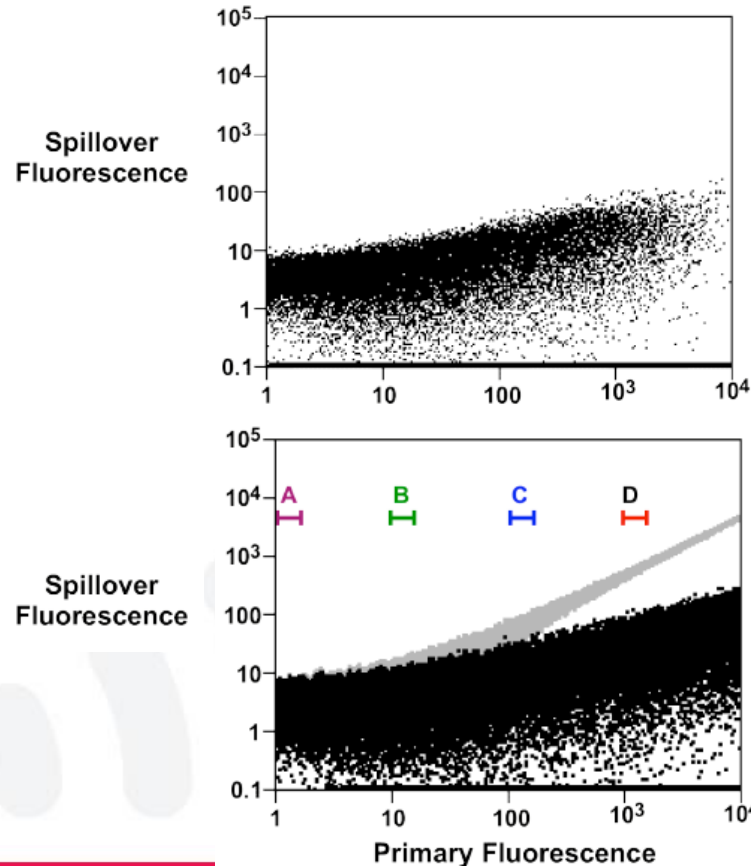


A: To accommodate data "spread" resulting from compensation of fluorescence spillover measured on a log scale.

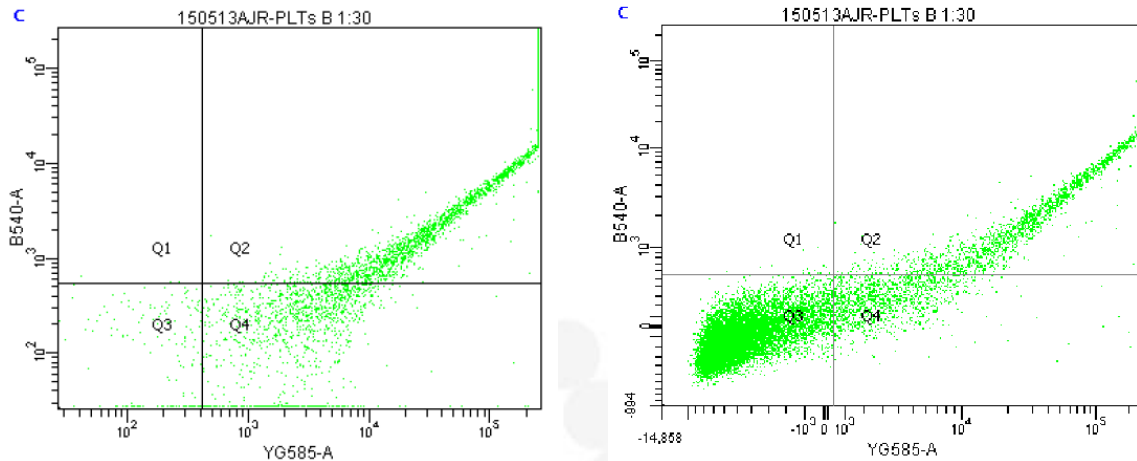


- Adjusting spectral compensation based on visual feedback is what we refer to as “manual” compensation.
- Taken together data spread and compensation will often drive a significant portion of the data into the zero channel. This phenomena results in a “distortion” of the visual feedback we are relying upon to properly set our compensation.
- Biexponential transformation allows us to manually set the compensation more accurately.

- We invoke biexponential transformation to create artificial negative space on our histogram that allows us to better visualize the actual spread of the compensated data.



- Adjusting voltage settings to position negative controls with biexponential transformation on can be very deceiving.



Experiment Name: LBAR110513
 Specimen Name: 150513AJR
 Tube Name: PLTs B 1:30
 Record Date: May 13, 2011 11:45:46 AM
 \$OP: AsccSNilsson

Population	#Events	%Parent	YG585-A Mean	B540-A Mean
<input checked="" type="checkbox"/> P2	17,051	85.3	39,314	30,296
<input checked="" type="checkbox"/> Q1	13	0.1	-1,351	646
<input checked="" type="checkbox"/> Q2	4,013	23.5	180,368	129,049
<input checked="" type="checkbox"/> Q3	11,732	68.8	-5,043	-136
<input checked="" type="checkbox"/> Q4	1,293	7.6	4,422	233

Step 1 - Turn off biexponential transformation on the histogram we are working with.

Step 2 – Run negative control and adjust voltage settings to position the negative population in the lower left quadrant.

Step 3 – Run single color control and turn on biexponential transformation to adjust compensation.

Step 4 – Once compensation is set biexponential transformation can be turned off.