Discussion Session: Instrument Quality Control

Gareth Howell, Facility Manager

Bio-imaging Facility

- LSM510 confocals
- Delta Vision
- Eppendof Microinjection

Currently seeking funding for two additional confocals and a live cell imaging station

Flow Cytometry and Cell Sorting Facility

- BD LSRFortessa analyser
- BD FACSAriaII cell sorter

Core service to University researchers
Funding obtained through external equipment calls and internal infrastructure sources

Why perform instrument QC?

Image quality

Reproducibility

Quantitative accuracy

QC in flow cytometry

Generally accepted FCM performance needs to be closely monitored

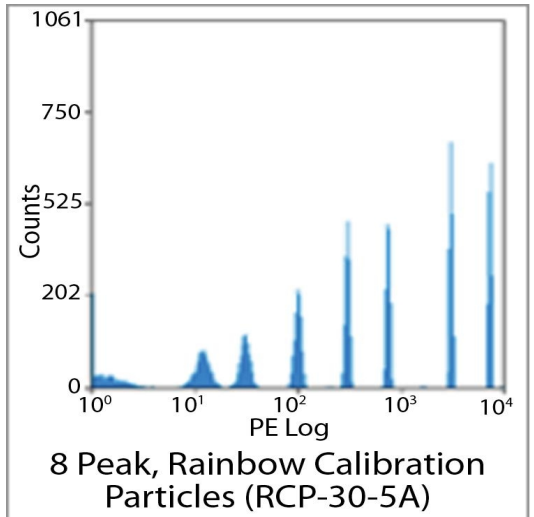
Range of reagents available (fluorescent beads)

Sampling at 1000's of events per second enables large data sets to be acquired - quantitative

Quantitative anlaysis (MFI, CV, %) enables daily assessment of performance

Assess detector response, linearity, etc

Microscopy QC - qualitative

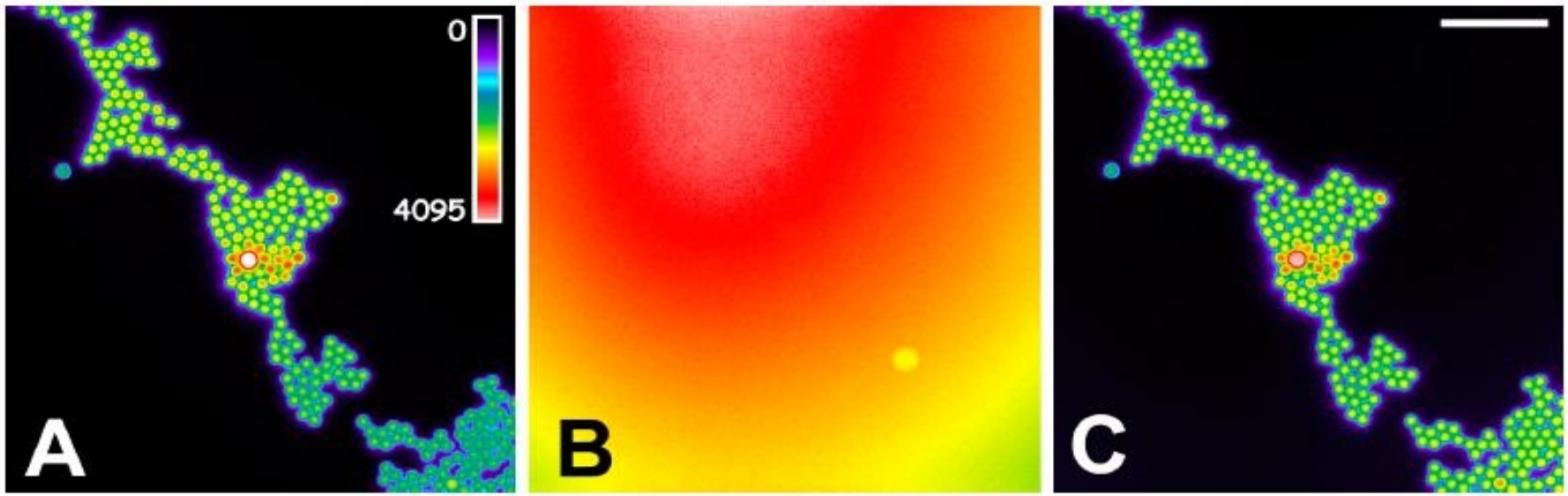


8 Peak, Rainbow Calibration Particles (RCP-30-5A)

What aspects can we check on microscope?

Zucker and Price (1999) Methods 18:447-458; Waters (2009) JCB 185:1135-1148

Field illumination -- influence on quantitative microscopy



Fluorescent dyes -- check slides ---beads of consistent intensity

Condition of objectives – dirt or contamination?

What aspects can we check on microscope?

Zucker and Price (1999) Methods 18:447-458; Waters (2009) JCB 185:1135-1148

Image resolution

Alignment

sub diffraction fluorescent beads

lateral (xy) axis

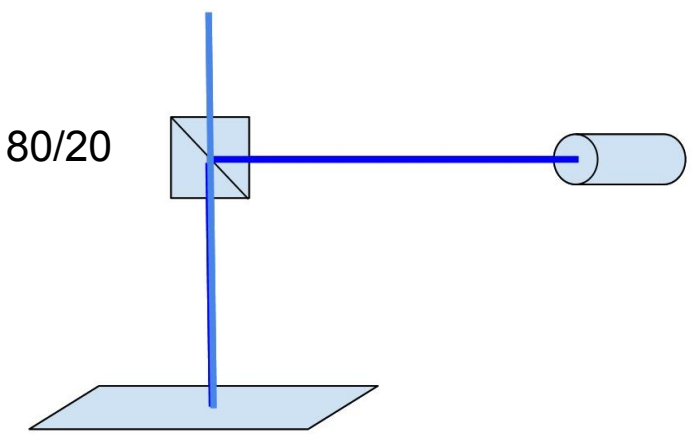
axial (z) axis FWHM

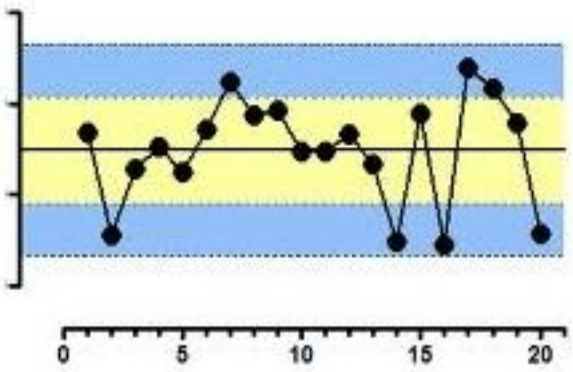
Detector / PMT quality

Signal:noise assessment

image fluorescent beads - measure signal and ROI in background

Laser performance test





<u>Procedure</u>
Glass slide
80/20 dichroic
LP emission filter >laser line
Set PMT to collect signal at 100 units
Set offset to 0

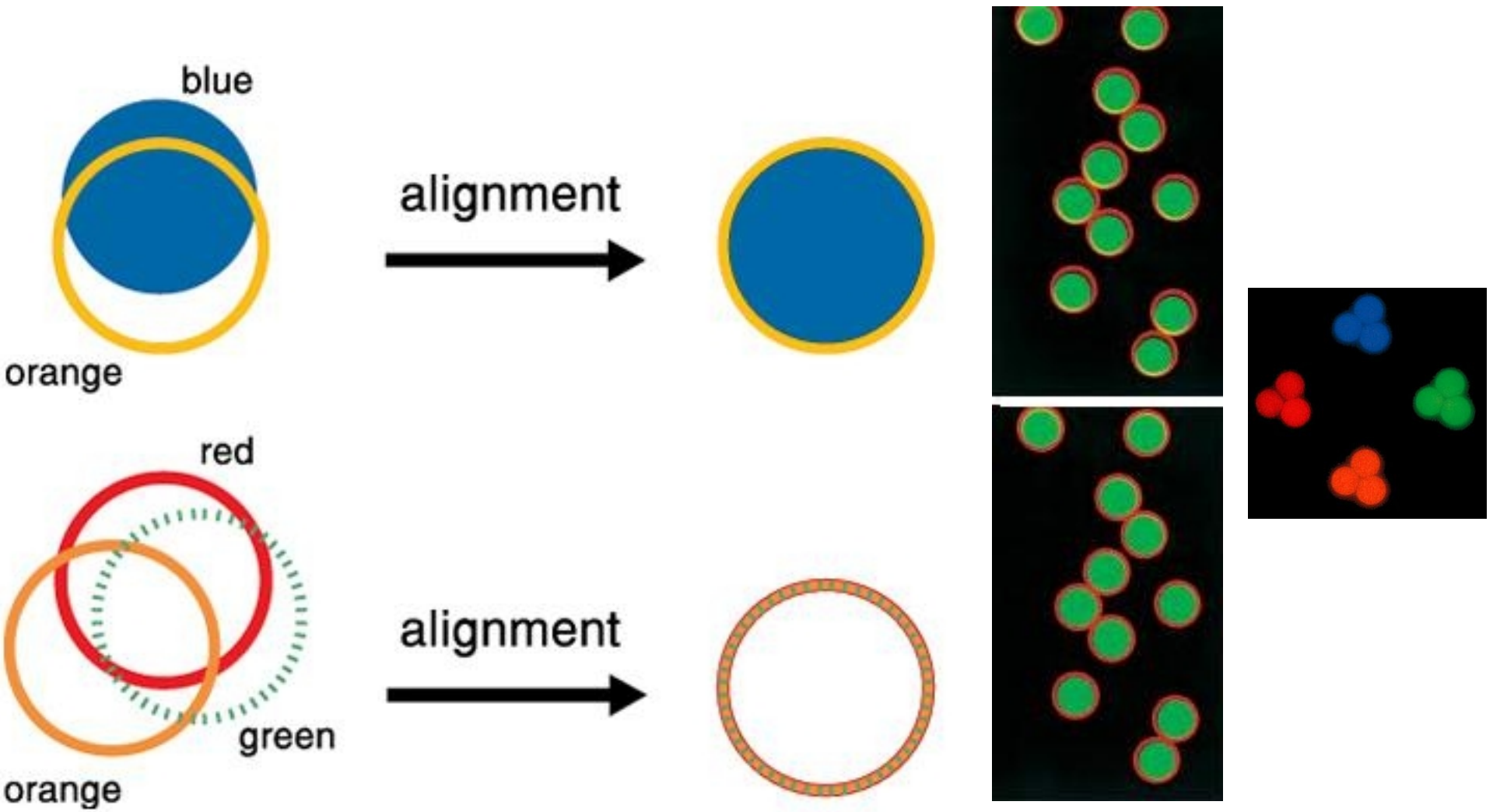
Record PMT gain value

Log over time +/- 2 SD on LJ plot

Baseline settings

Registration

Focal check beads (Invitrogen)
Zeiss LSM510 align pinholes to correct mis-registration



Epi-fluorescence? What checks to perform?

Alignment as per confocal checks

- Check beads
- Field illumination
- Registration

What checks on camera? What do you use in your lab?

Conclusion - Routine tasks

Clean objectives (1 / 2 days, users)
- oil on 'dry' objectives!
- isopropanol on lens tissues / cotton buds (ethanol)
- 'The Clean Microscope' Zeiss guide

Laser checks (weekly / fortnightly)

Field illumination (weekly / fortnightly)

Detector checks (signal:noise checks) Monthly/ quaterly?

Axial checks - when required