ONBI Practical 7: Comparison of techniques

RM Parton 2014

Aims of practical 7:

One of the most common issues confronting people new to microscopy is the confusing array of different techniques available. The aim of this practical is to familiarise yourselves with some of the different imaging techniques and modalities available and to appreciate that different techniques are required for different samples and to address different questions:

This practical will examine:
* Fast dynamic synchronised nuclear divisions in the *Drosophila* early embryo.
* Complex tissue organisation in the *Drosophila* late embryo.
* Two different imaging techniques

You should consider the advantages and disadvantages of each technique to assess these aims.

You should and also consider ways of optimisation though sample preparation, setup of the hardware (imaging parameters) and experimental design.

Organisation:

Practical 7: 2:00 to 5:30 (change over stations at **3:30 with 30 min break**)

Point Scanning Confocal vs the Z1 light sheet (Group A - these systems only)

Group A1	Confocal 2:00 – 3:30; Z1 4:00 – 5:30
Group A2	Z1 2:00 to 3:30; Confocal 4:00 – 5:30

Spinning Disc vs Wide-Field deconvolution (Group B - These systems only)

Group B1	Disc 2:00 – 3:30; Decon 4:00 – 5:30
Group B2	Decon 2:00 to 3:30; Disc 4:00 – 5:30

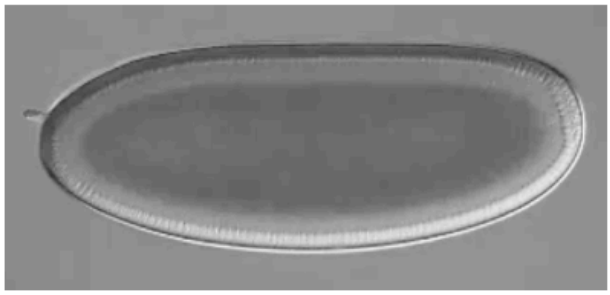
Drosophila embryo prep:

The drosophila embryo prep was described in Practical 1. The embryo is an intact living organism and is widely used to study various aspects of cell and tissue development and differentiation and can be cultured easily from the very early division stages through embryogenesis to the emergence of the first instar larva. The embryo is a relatively large and complex sample and, especially in later stages, can be challenging to microscopy.

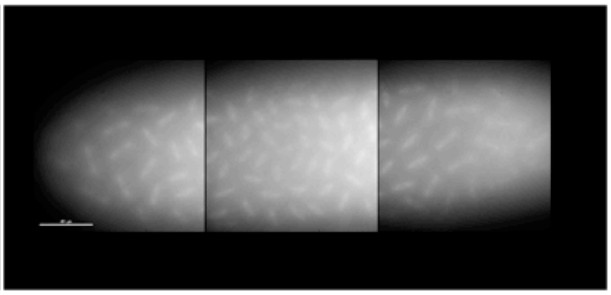
Drosophila embryo development:

http://www.sdbonline.org/sites/fly/aimain/images.htm
http://www.sdbonline.org/sites/fly/aimain/2stages.htm
http://cuttlefish.bio.indiana.edu:7082/allied-data/lk/interactive-fly/aimain/2stages.htm#dafka2
http://www.starklab.org/members/kazmar/2012/04/19/Fluorescence-a-nearly-free-lunch/

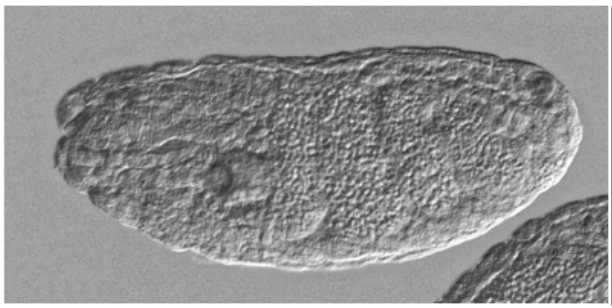
Early development – Syncitial blastoderm



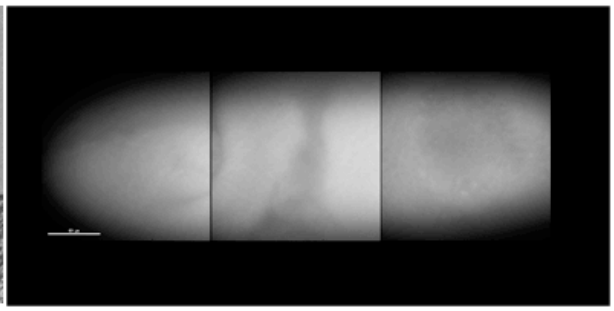
Mitotic Spindles - Jupiter YFP



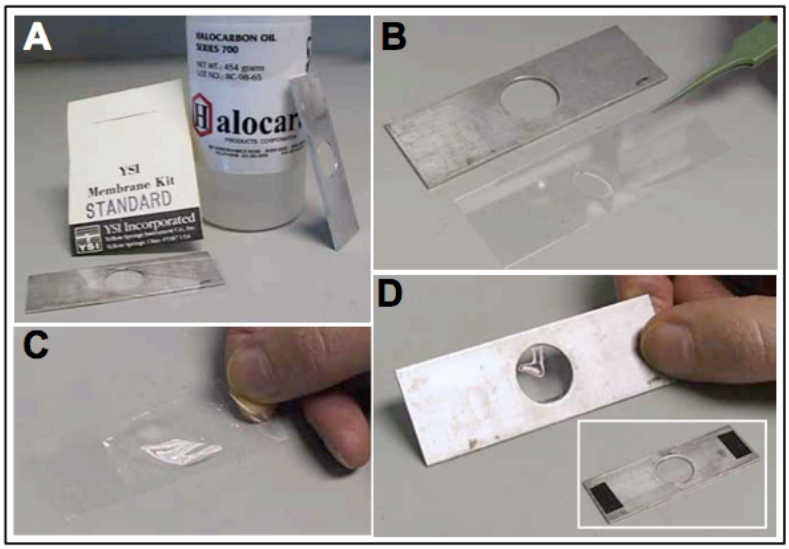
Later development – Gastrulation



Cell distribution and division pattern - Jupiter YFP



Arrangement for mounting *Drosophila* embryos in Halocarbon oil for imaging on either an upright or inverted platform.



Comparison of techniques:

In this practical you will compare two different imaging techniques: Point-scanning confocal microscopy with Digital Light Sheet microscopy (DLSM)

http://microscope.olympus-global.com/en/ga/product/fv1200/

http://www.zeiss.co.uk/microscopy/en_gb/products/imaging-systems/lightsheet-z-1.html

OR

The PerkinElmer spinning disc confocal with the GE Deltavision Wide-field Deconvolution system

http://www.perkinelmer.com/pages/020/cellularimaging/technologies/spinningdiskconfocal.xhtml

http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-us/brands/deltavision/

Experimental Protocol

1. Samples of fluorescently labelled embryos, either fixed or live, will be provided. They must be mounted in a way that is appropriate to the imaging technique used.

2. Set up the instrument you are working on to permit bright-field and fluorescence imaging.

3. Examine the specimens: look at different stages of development, examine shallow and deep imaging. What features can you resolve? What issues are there? Decide what you would like to achieve from your imaging – what biological questions could you investigate?

4. Attempt to improve and optimize you imaging. Remember with live cell imaging you may be causing damage that affects development and compromises with image quality will have to be made. You should aim to collect several data sets to record optimization of the imaging and to address your biological question.

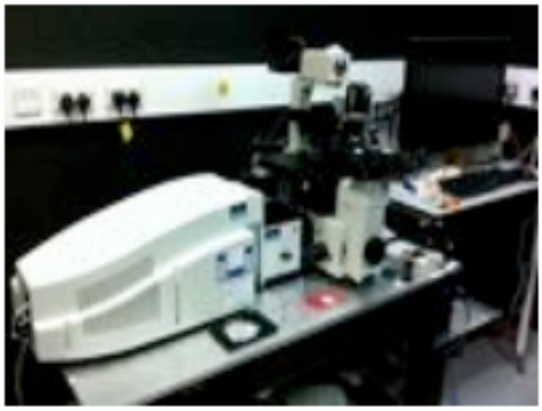
5. Move on to the second imaging and perform the same tasks. What are the differences between the two techniques? Where might one technique have an advantage over the other?

DV-Elite Widefield deconvolution

* Olympus IX 71 inverted stand
* 7-lline solid-state illumination
* Evolve-Delta back thinned EMCCD camera
* 10 position emission filter wheel
* Automated dichroic changer
* Environmental chamber with humidity / CO_2
* Integrated workflow for deconvolution

Fast sensitive live-cell imaging, best with thin or well-contrasted samples. Useful with weakly-labeled, sensitive or very dynamic samples.

Perkin Elmer Ultraview spinning disc confocal



* Olympus IX 81 inverted stand
* 5-llines solid-state laser illumination (405,488,515,568,633)
* Hamamatsu back thinned EMCCD camera
* FRAP module (Photobleaching and Photoactivation)
* Environmental chamber

Relatively fast (compared to point-scanning confocal) live-cell imaging, best with thicker more challenging samples with significant out of focus fluorescence. Optical sectioning not as good as point scanning systems.

Z1 light sheet

Preparation of samples for the Z1 light sheet:

Imaging on the light sheet:

1 Microscope
- Axio Scan.Z1
- Magazines for 12 or 100 slides
- Trays for four 26 mm x 76 mm slides or two 52 mm x 76 mm slides

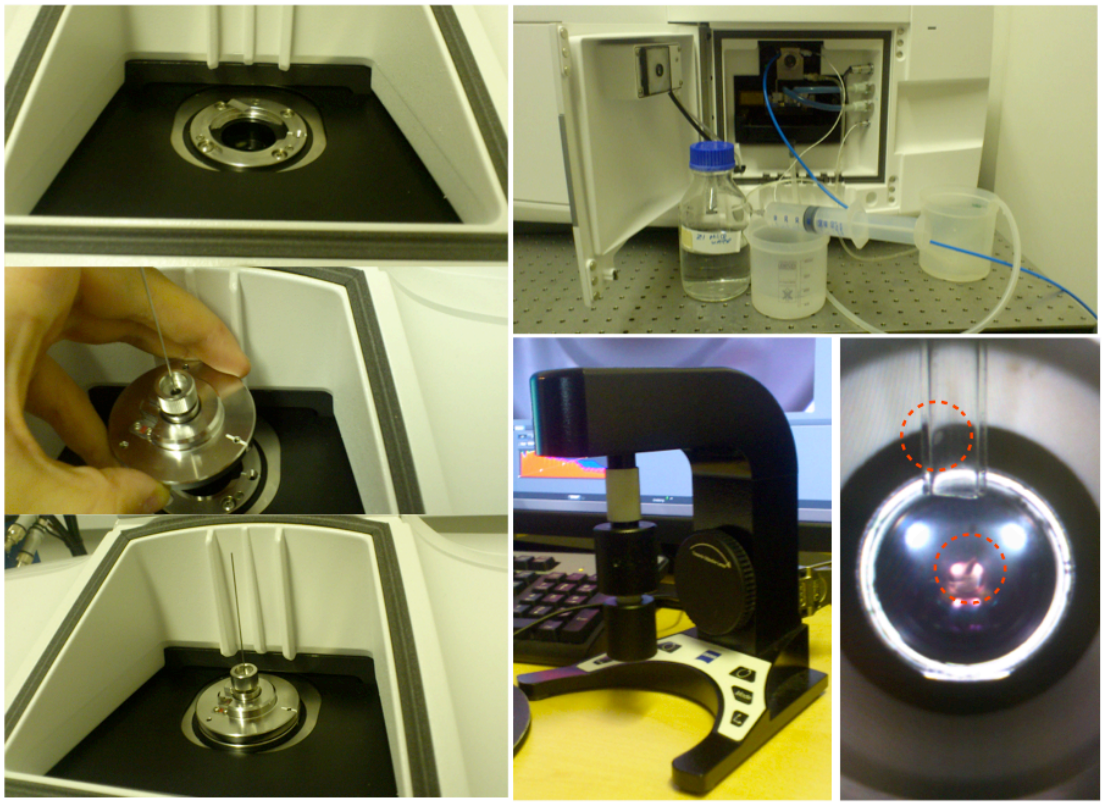
2 Objectives
- N-Achroplan (2.5x, 10x, 20x, 40x)
- Fluar (2.5x)
- Plan-Apochromat (10x, 20x, 40x)

3 Illumination
- Transmitted light: LED (wavelength 400 to 700 nm, maximum at 460 nm)
- Fluorescence: 365 nm, 385 nm, 420 nm, 445 nm, 455 nm, 470 nm, 505 nm, 530 nm, 590 nm, 615 nm, 625 nm or neutral white: 540 - 580 nm, HXP 120 V
- Filter wheels:
  - 10-position ACR for filter cubes or
  - 6-position high-speed excitation
  - 6-position high-speed beamsplitter
  - 6-position high-speed emission

4 Cameras
- Hitachi HV F202
- AxioCam MRm
- Hamamatsu Orca flash 4.0

5 Software
- ZEN slidescan
- ZEN lite
- ZEN browser
- ZEN 2012 (blue edition) image analysis modules

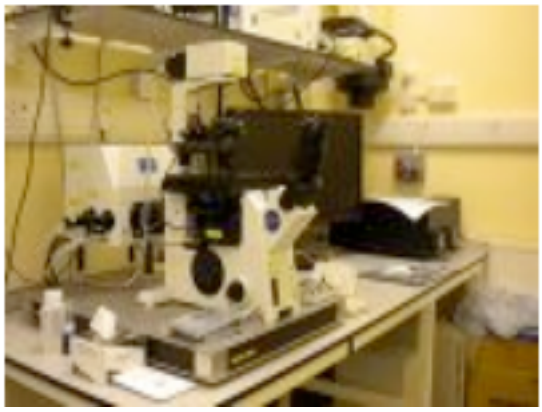
Placing a sample in the Z1:



Very good for long-term (hours to days) live-imaging of large complex 3D samples. Light sheet reduces out-of-focus blur and damage due to illumination.

* Single-side illumination vs dual side illumination.
* Online dual side fusion and post-acquisition fusion.
* Multiview acquisition and processing.

FV1000 / FV1200 Olympus Point scanning Confocal



* Olympus IX 81 inverted stand
* Multiple-llines laser illumination (405,488,515,559,635)
* Multiple PMTS or sensitive GaAsP detectors
* Simultaneous or sequential imaging
* FLIM (Becker and Hickel card) – FV1000

Excellent optical sectioning “out-of-focus” discrimination giving sharp highly contrasted imaging even with thicker highly labeled more challenging samples. Slower and less sensitive that wide-field deconvolution or spinning disc confocal. Improved sensitivity with the use of GaAsP detectors. Very flexible for multichannel imaging.