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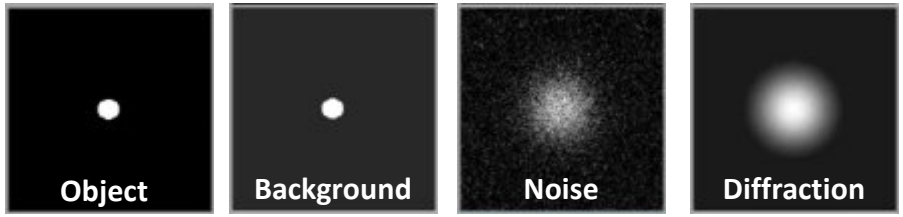
How to acquire reliable and quantifiable image data

Hong Yu, Westmead Imaging Facility 14 Nov 2019

How reliable is my image data?

Image ≠ Real object
(only indirect evidence)



Black box?

Pre-imaging

- Design: Abs, probes, etc
- Sample prep
  fixation
  Ab binding
  labelling
  …

Imaging

- Microscope settings
- Acquisition settings

Post-imaging

- Image processing & restoration
- Analysis methods

Outline

Microscope settings

1. Choose the right objective
   - Cleaning
   - High NA
   - Oil objective
2. Check ups
   - Kholer illumination (BF)
   - Contrast (BF)
   - Fluo channels

Imaging settings

1. BF imaging
2. Z stacking
3. Avoid saturation
4. Minimise bleaching
5. Nyquist sampling

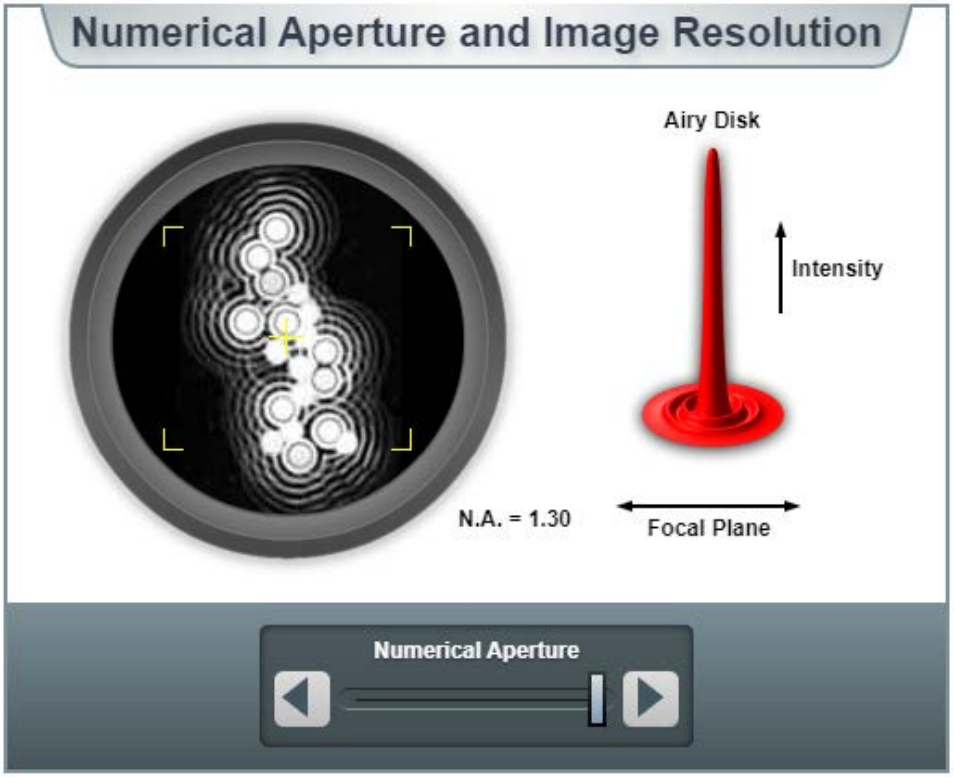
Microscope settings__objective

- **Cleaning? Yes, do it every time before you start observing!**

Microscope settings__objective

- **Choose high NA (numerical aperture) objective**

Resolution (r) = 1.22λ/(NA(obj) + NA(cond))

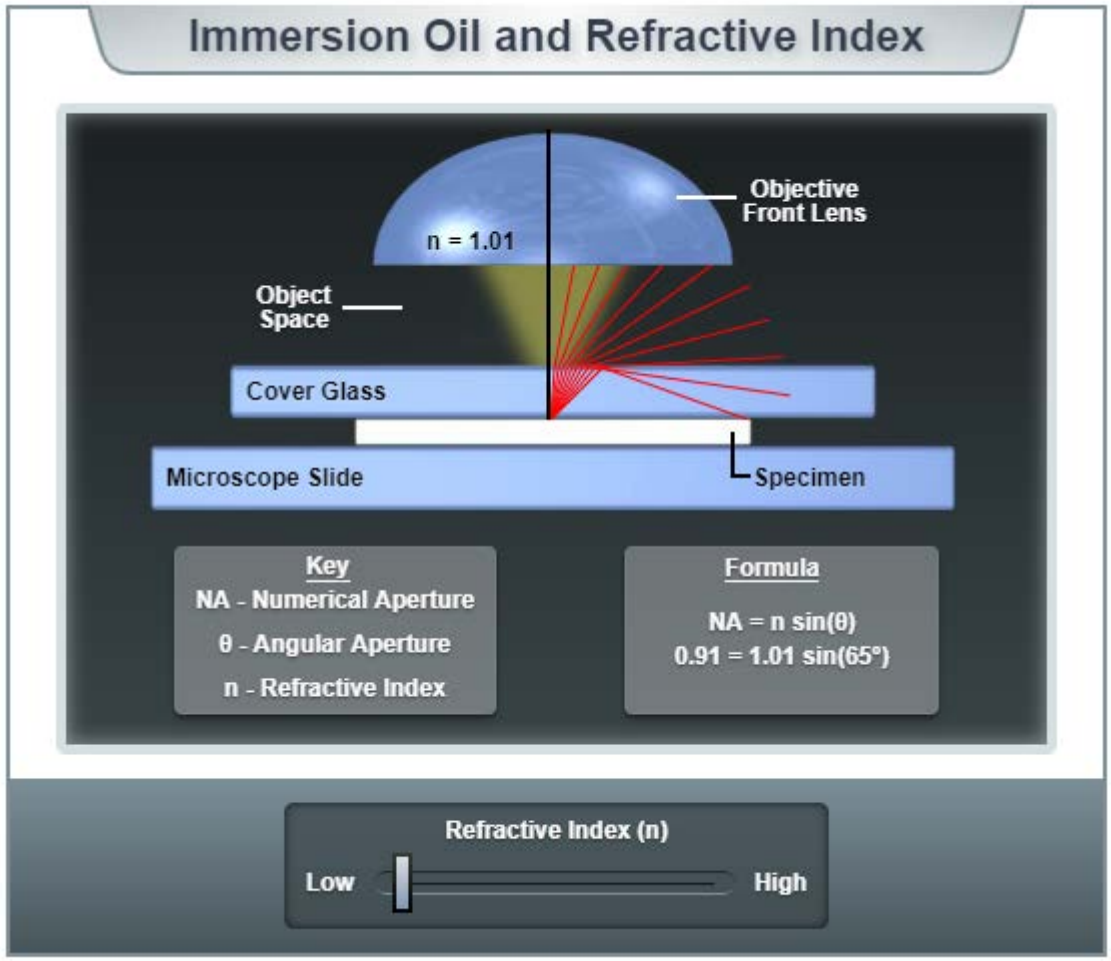


NA

https://www.microscopyu.com/microscopy-basics/resolution

Microscope settings__objective

- **Choose an oil lens if you can**

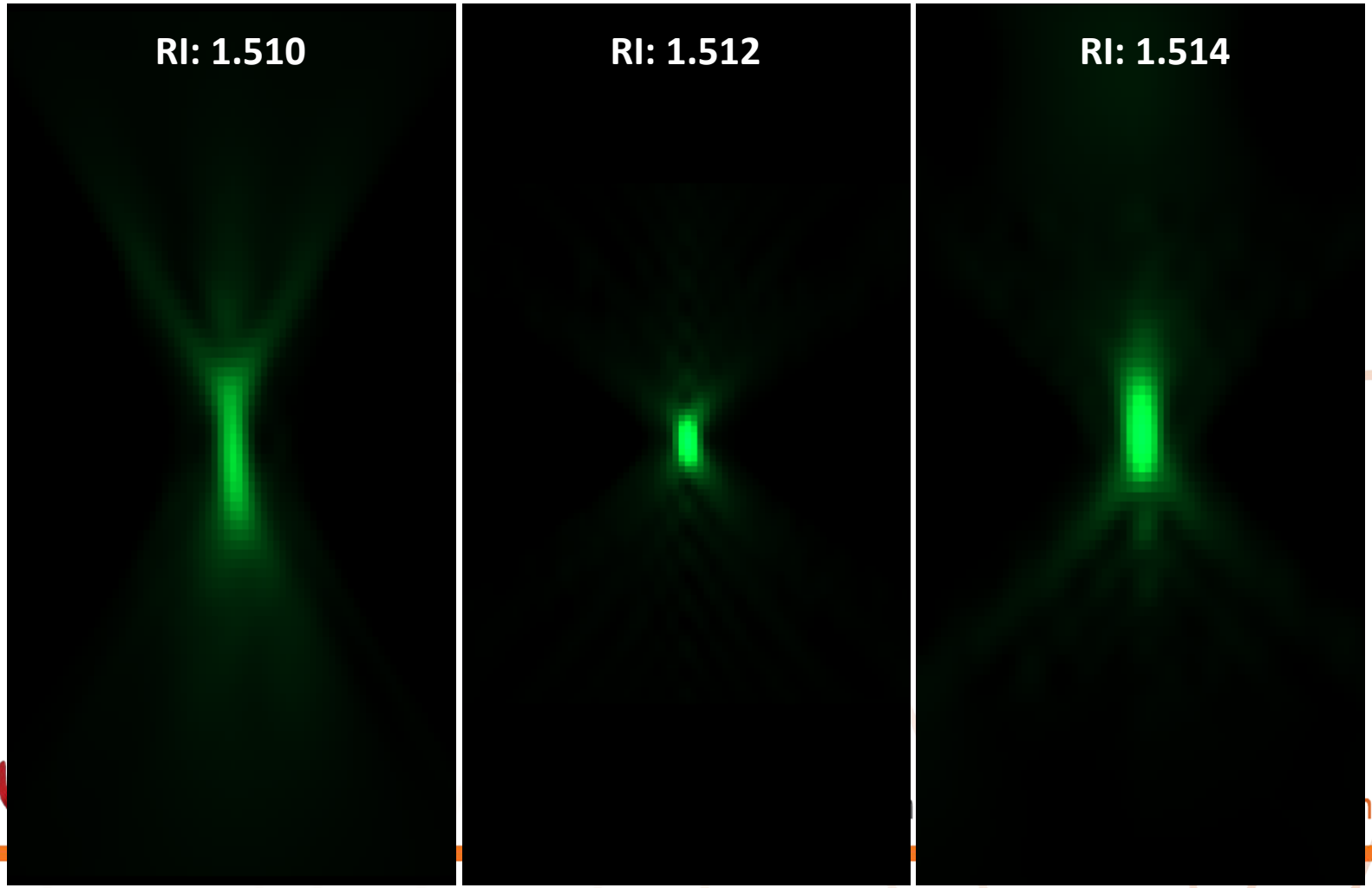


Imaging medium	Refractive Index (RI)
Glass	1.51
Typical oil	1.51x
Silicone oil	1.407
Water	1.33
Air	1.00

https://www.microscopyu.com/tutorials/immersion

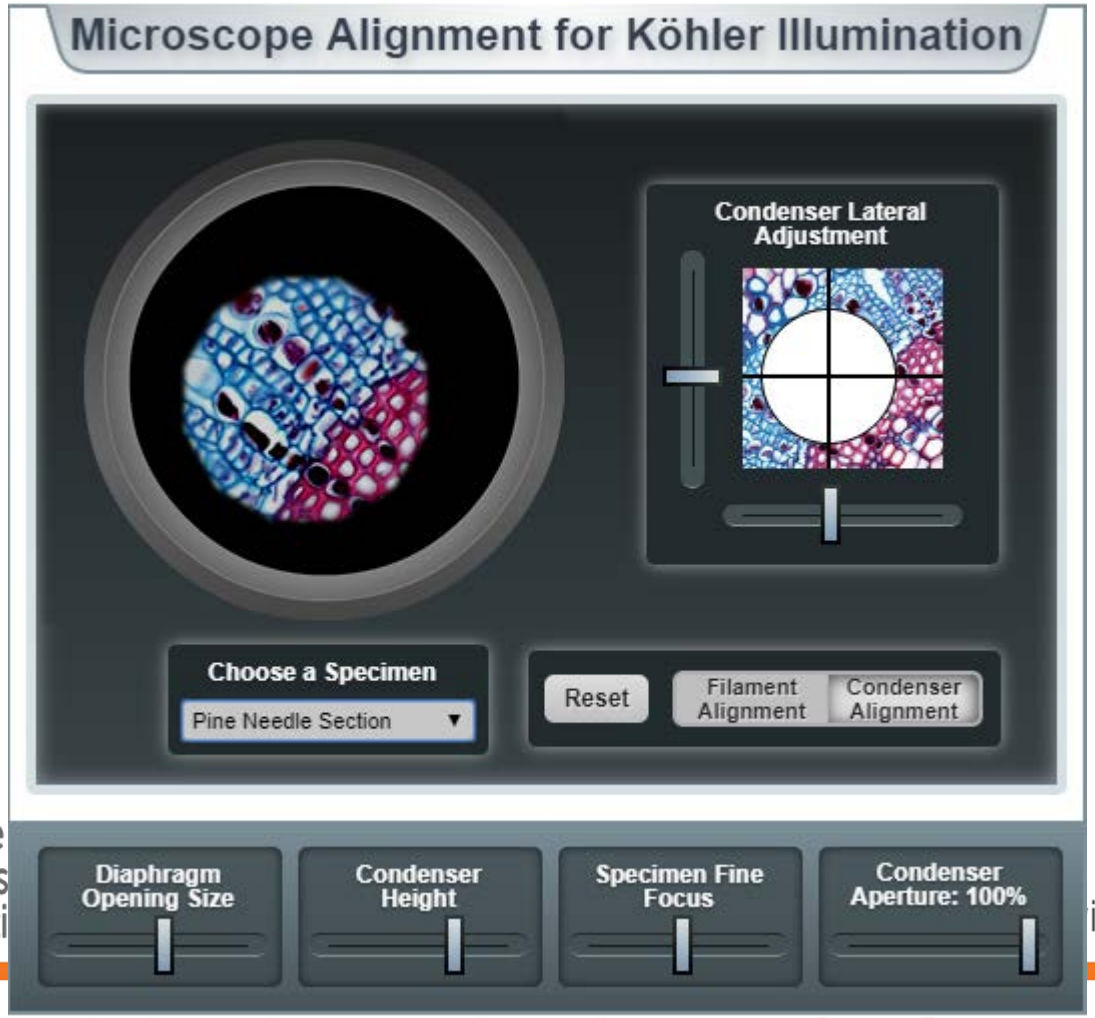
Microscope settings__objective

- **Match with sample medium (refractive Index match)**



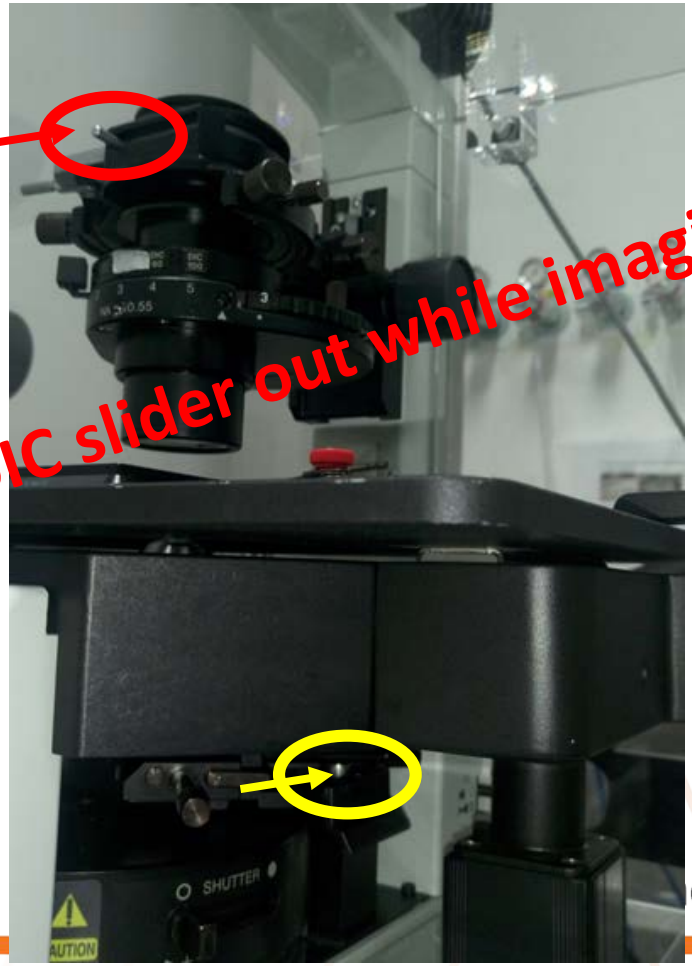
Microscope settings__check ups

- Kohler illumination https://www.microscopyu.com/tutorials/kohler



Microscope settings__check ups

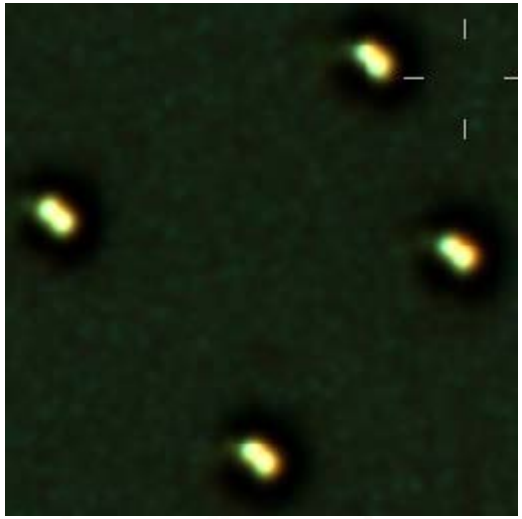
- Contrast: phase or DIC



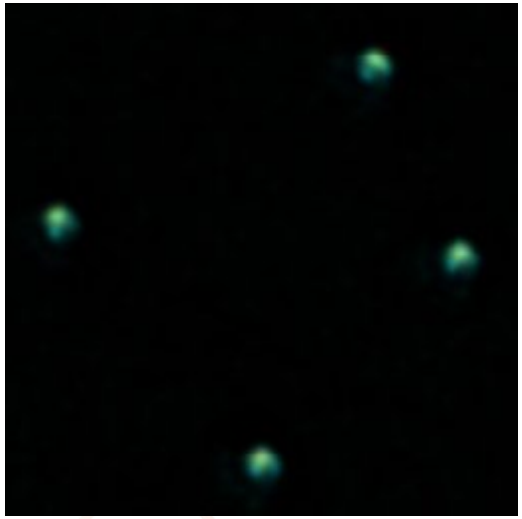
Leave DIC slider out while imaging fluo!

Microscope settings__check ups

DIC Slider in



DIC Slider out



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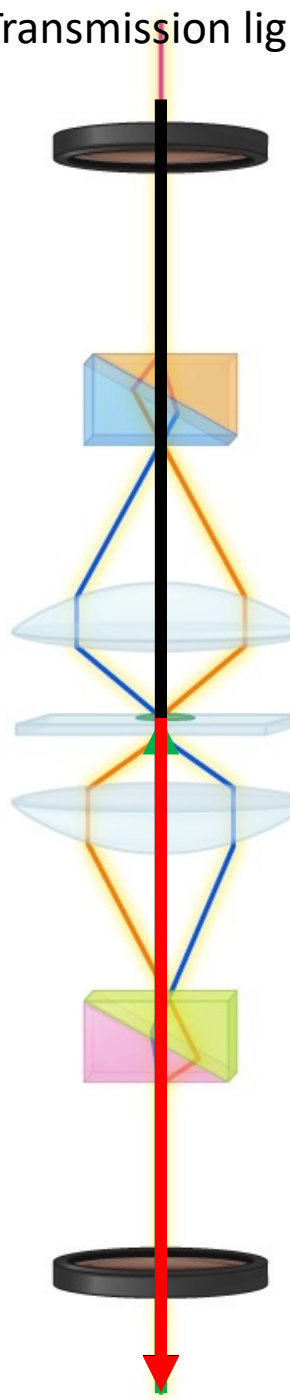
- Fluo channels

DIC Microscope Configuration



1. Polarising filter (“polarizer”, 45°)

2. Normaski Prism (beamsplitter, interpreted in condenser)

Condenser lens

Sample

Objective lens

3. Normaski Prism (beamcombiner, “DIC slider”)

4. Polariser filter (also “analyser”, 135°)

Microscope settings__check ups

Check fluo staining under microscope before taking images on the computer!

- Strong or faint?
- Background?
- Staining pattern?

- Fluo channels

Outline

Microscope settings

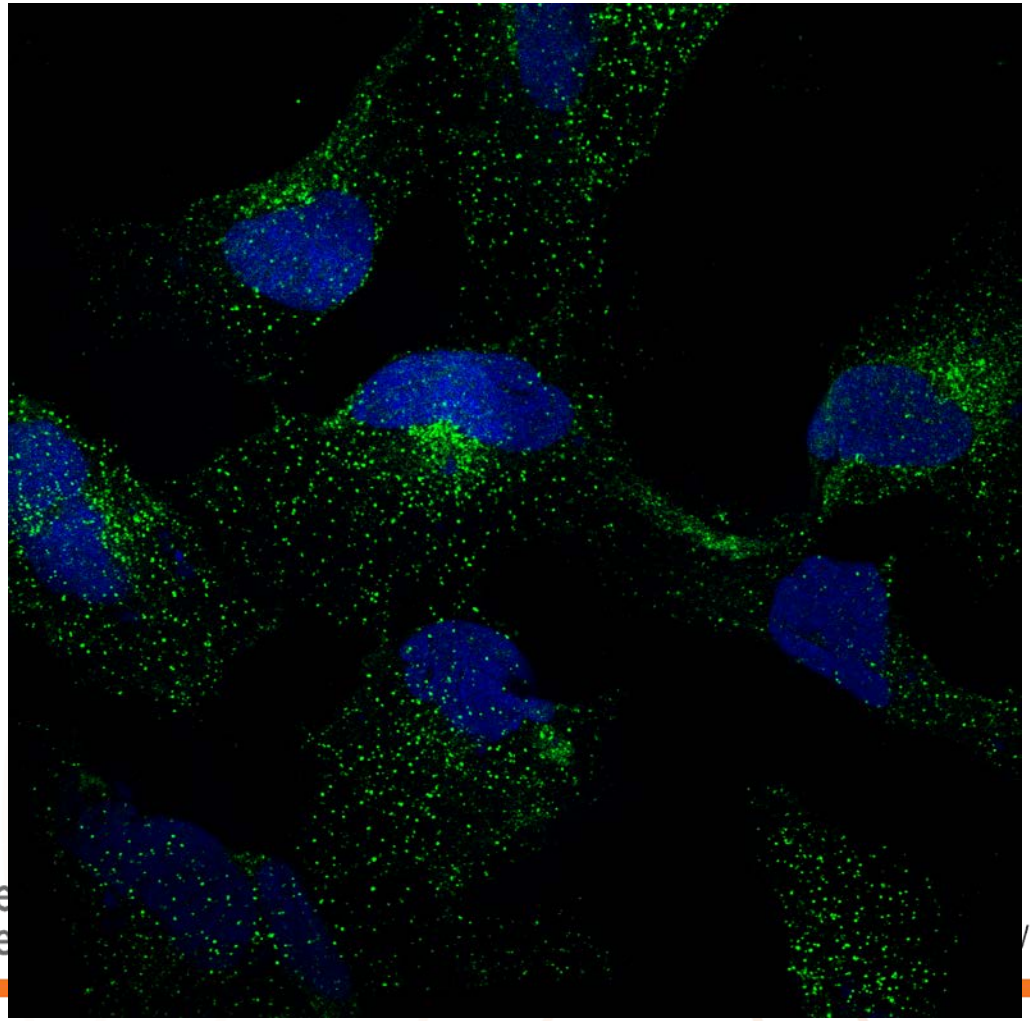
1. Choose the right objective
   - Cleaning
   - High NA
   - Match with sample medium
2. Check ups
   - Kholer illumination (BF)
   - Contrast (BF)
   - Fluo channels

Acquisition settings

1. **BF imaging**
2. **Z stacking**
3. **Avoid saturation**
4. **Sampling according to Nyquist**
5. **Minimise bleaching**

Acquisition settings: BF imaging

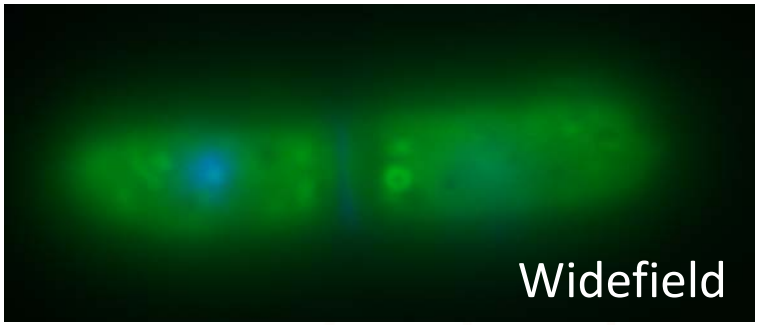
1. BF imaging: always good to include BF imaging data!!!

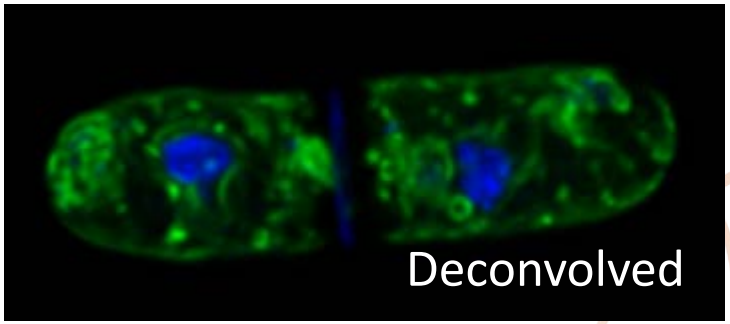


Acqusition settings: Z stacking

2. Z stacking: always good to run Z stacking!

Allowing post-acquisition processing: deconvolution, 3D rendering etc



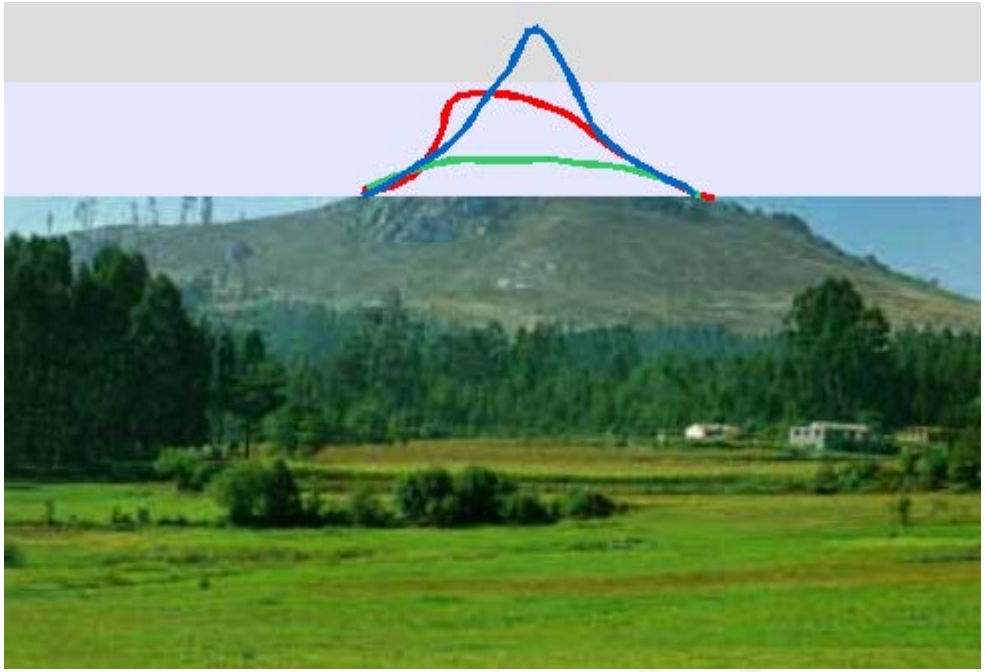


Courtesy images: GE Health

Acquisition settings: avoid saturation

- Never ever saturate signals (no clipping)!

Image curtesy: https://svi.nl/ClippedImages

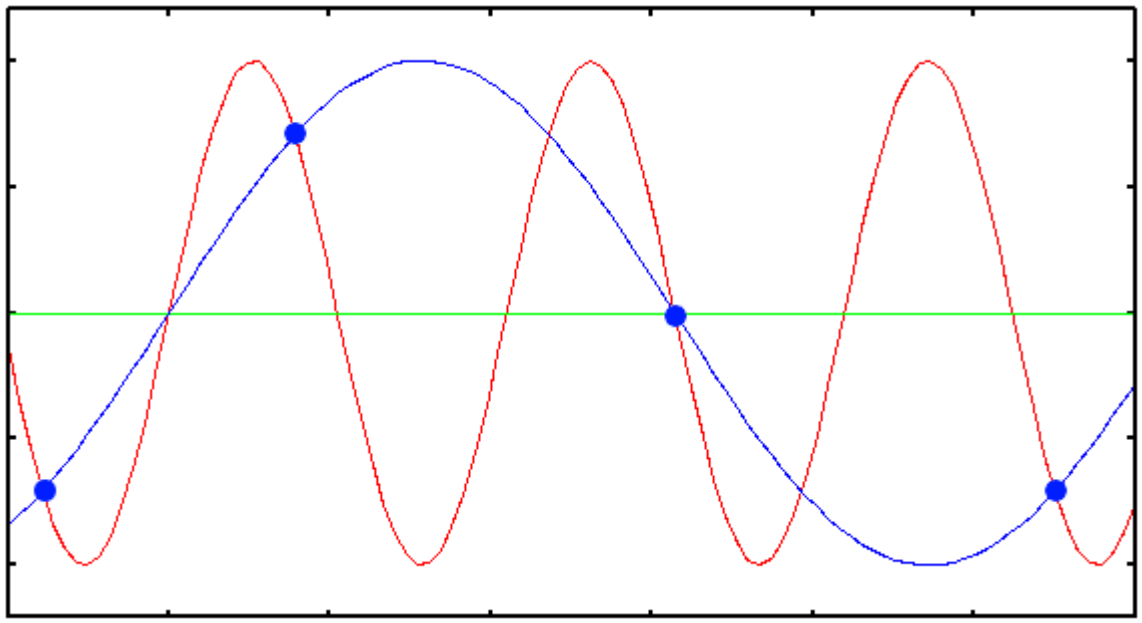


Acquisition settings: minimise bleaching

- Illumination strength and acquisition time
- Scanning speed: slow down?
- Averaging: be cautious with low signal

Acquisition settings: Nyquist sampling

- Sample according to Nyquist Rate



Nyquist rate and PSF calculator

Confocal

1.3

488 nm

520 nm

1

Oil 1.515

Calculate a Point Spread Function

Calculate

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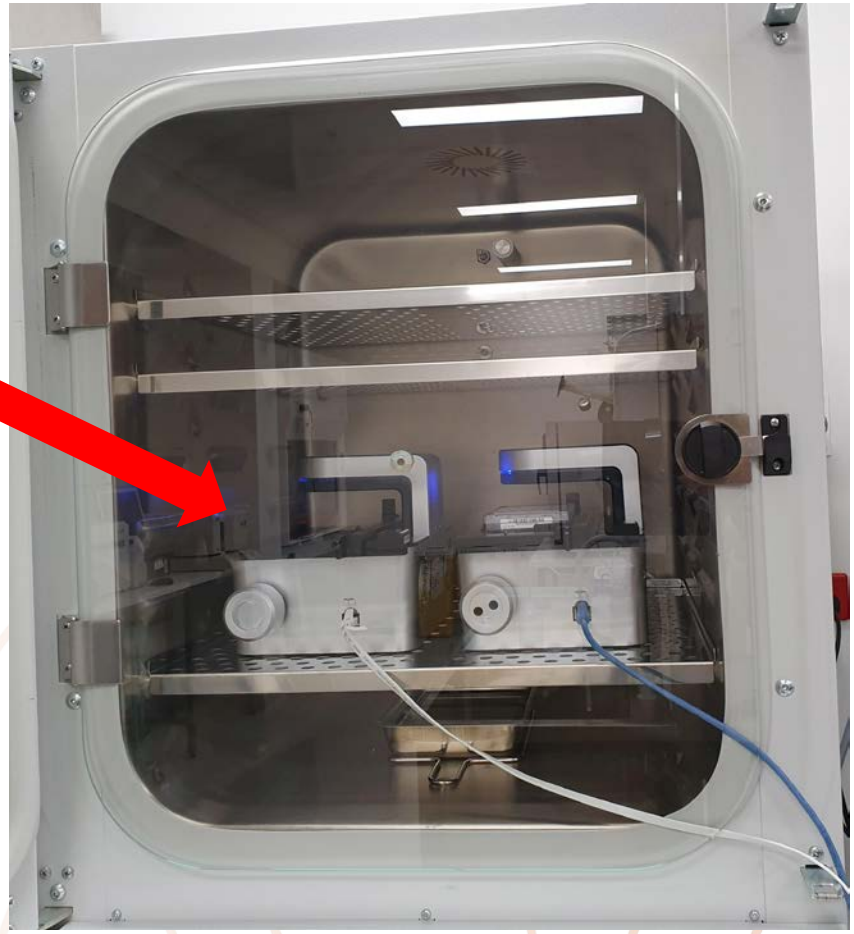
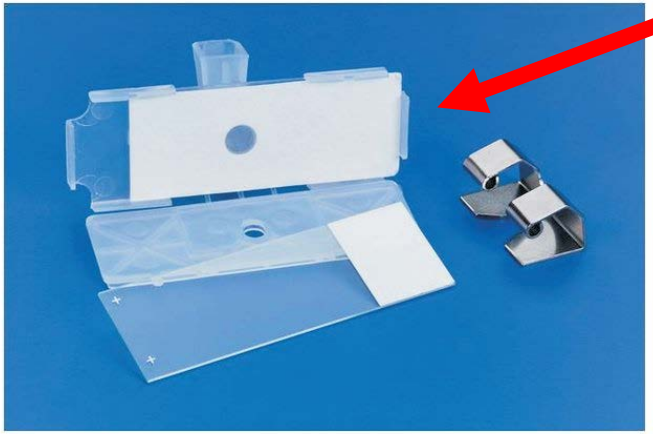
New/old instrument: Juli BR live cell analyser



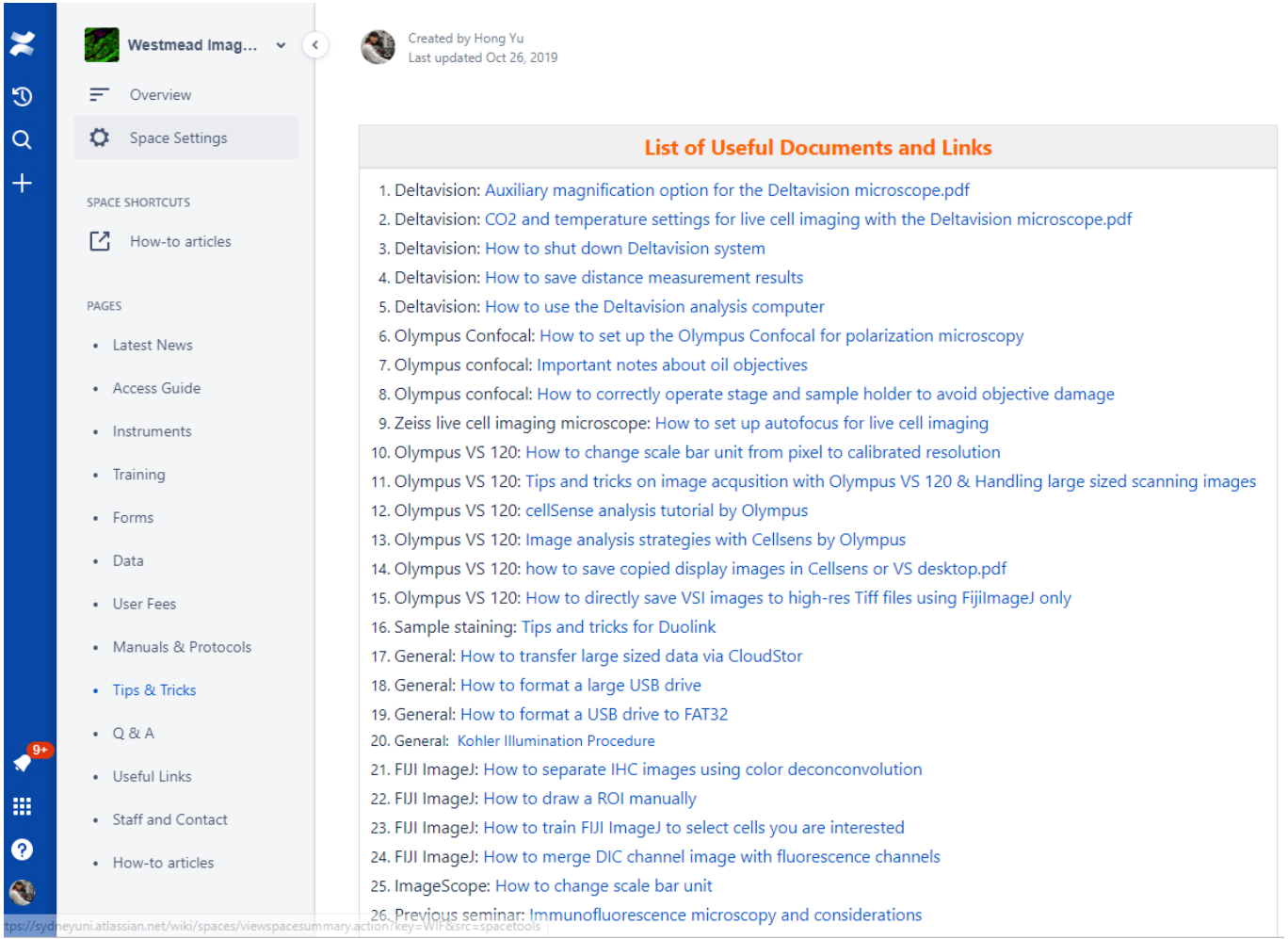
Cytofuge2: cytocentrifuge for sample concentration

- 4 slices in one go
- Concentration recommendations: 100-300 ul, 500-1500 cells/ul
- We provide
  training
  disposable filter concentrators
- Fees: starting 1 Jan 2020



Our website…

https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/765397549/Tips+Tricks



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Thank you!