

Finding **cures**. Saving **lives**. Giving **hope**.

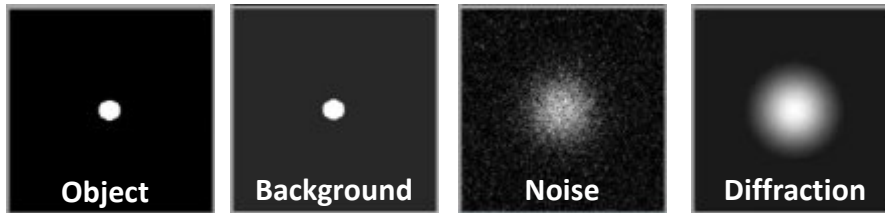


How to acquire reliable and quantifiable image data

Hong Yu, Westmead Imaging Facility 14 Nov 2019

How reliable is my image data?

Image \neq 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
 - Kohler 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

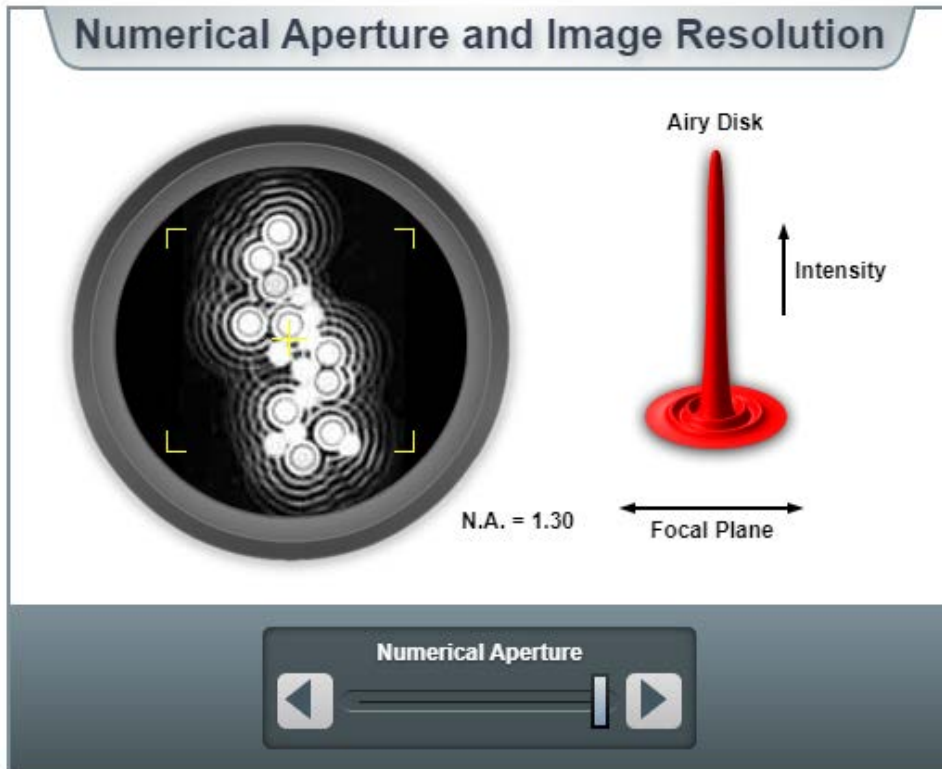
- **Cleaning? Yes, do it every time before you start observing!**



Microscope settings__objective

- Choose high NA (numerical aperture) objective

$$\text{Resolution (r)} = 1.22\lambda / (\text{NA(obj)} + \text{NA(cond)})$$



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

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Microscope settings__objective

- Choose an oil lens if you can

Immersion Oil and Refractive Index

Key
NA - Numerical Aperture
 θ - Angular Aperture
n - Refractive Index

Formula
 $NA = n \sin(\theta)$
 $0.91 = 1.01 \sin(65^\circ)$

Refractive Index (n)
Low High

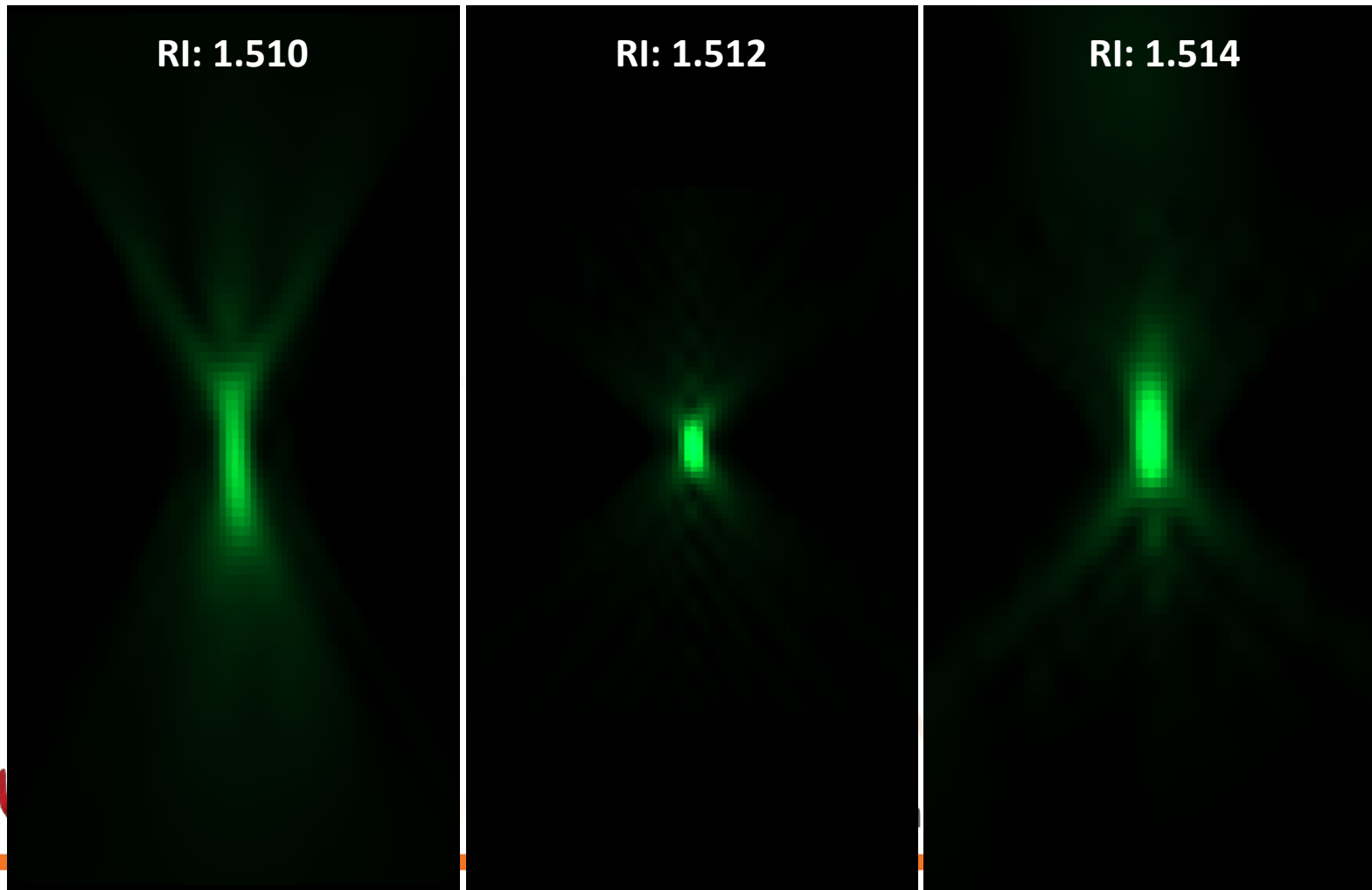
Imaging medium	Refractive Index (RI)
Glass	1.51
Typical oil	1.51x
Silicone oil	1.407
Water	1.33
Air	1.00

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

ding cures. Saving lives. Giving hope.

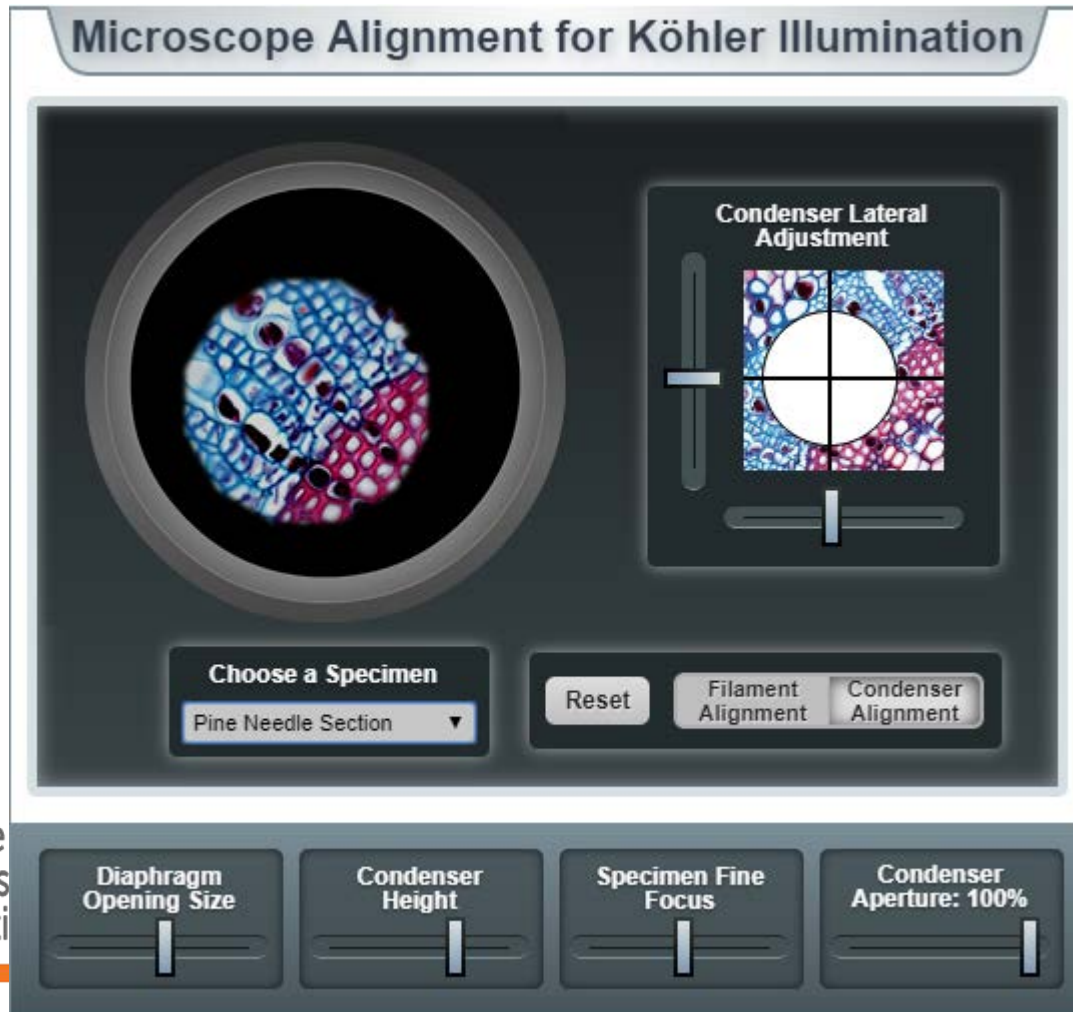
Microscope settings__objective

- Match with sample medium (refractive Index match)



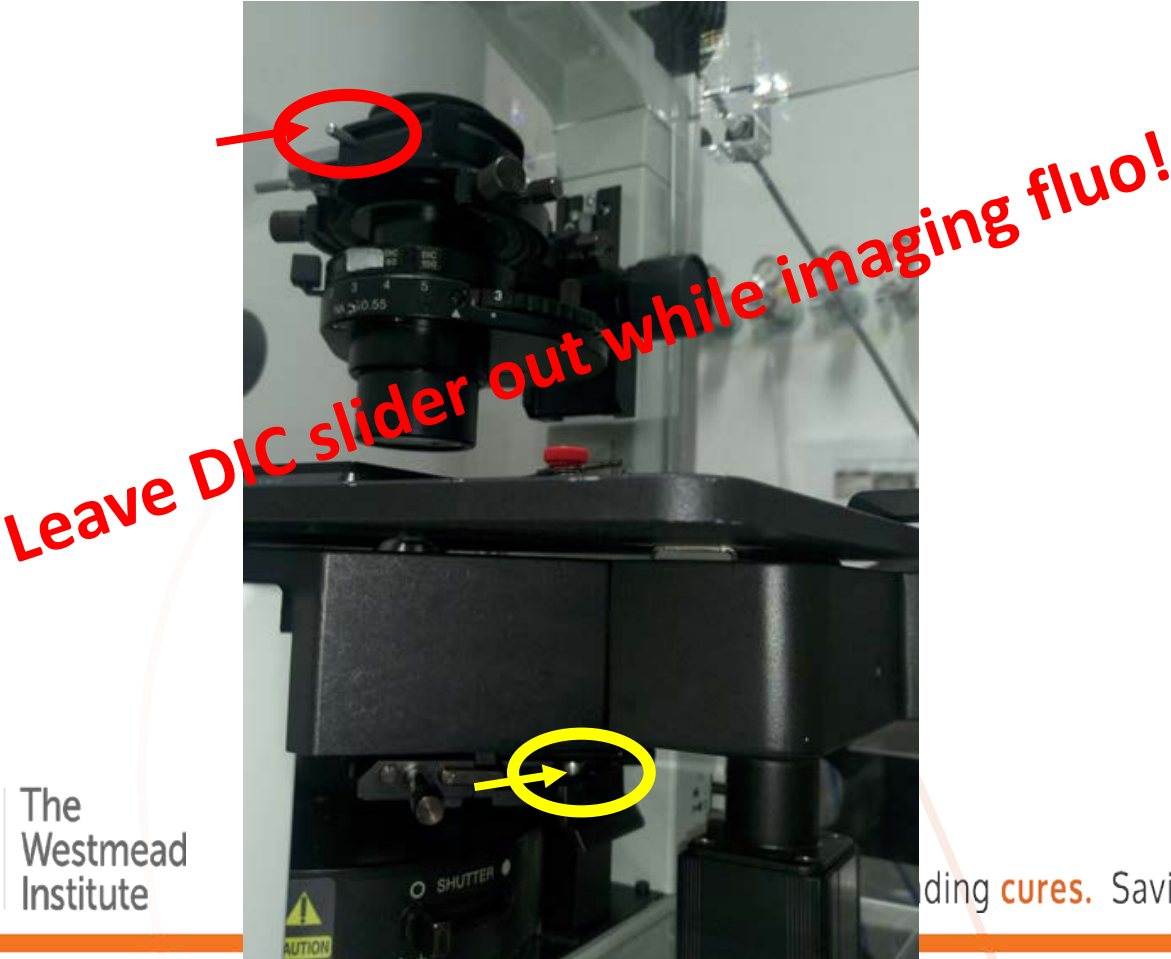
Microscope settings__check ups

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



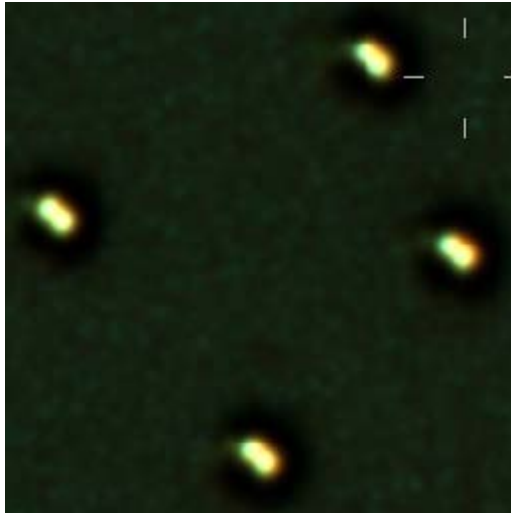
Microscope settings__check ups

- Contrast: phase or DIC

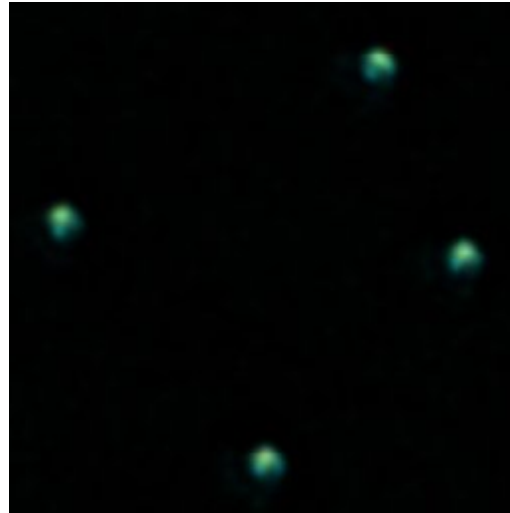


Microscope settings__check ups

DIC Slider in

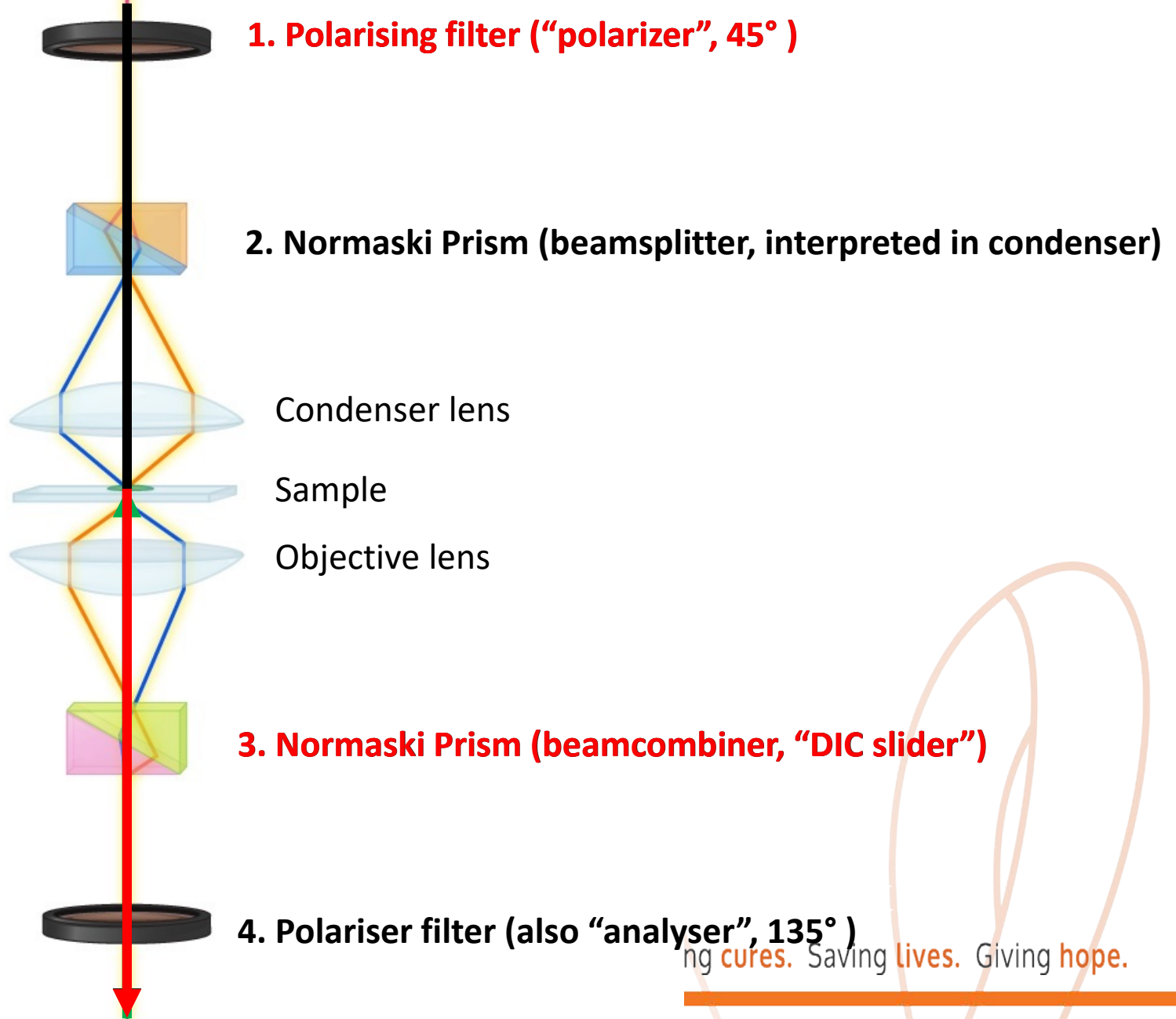


DIC Slider out



DIC Microscope Configuration

Transmission light



Microscope settings__check ups

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

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

Outline

Microscope settings

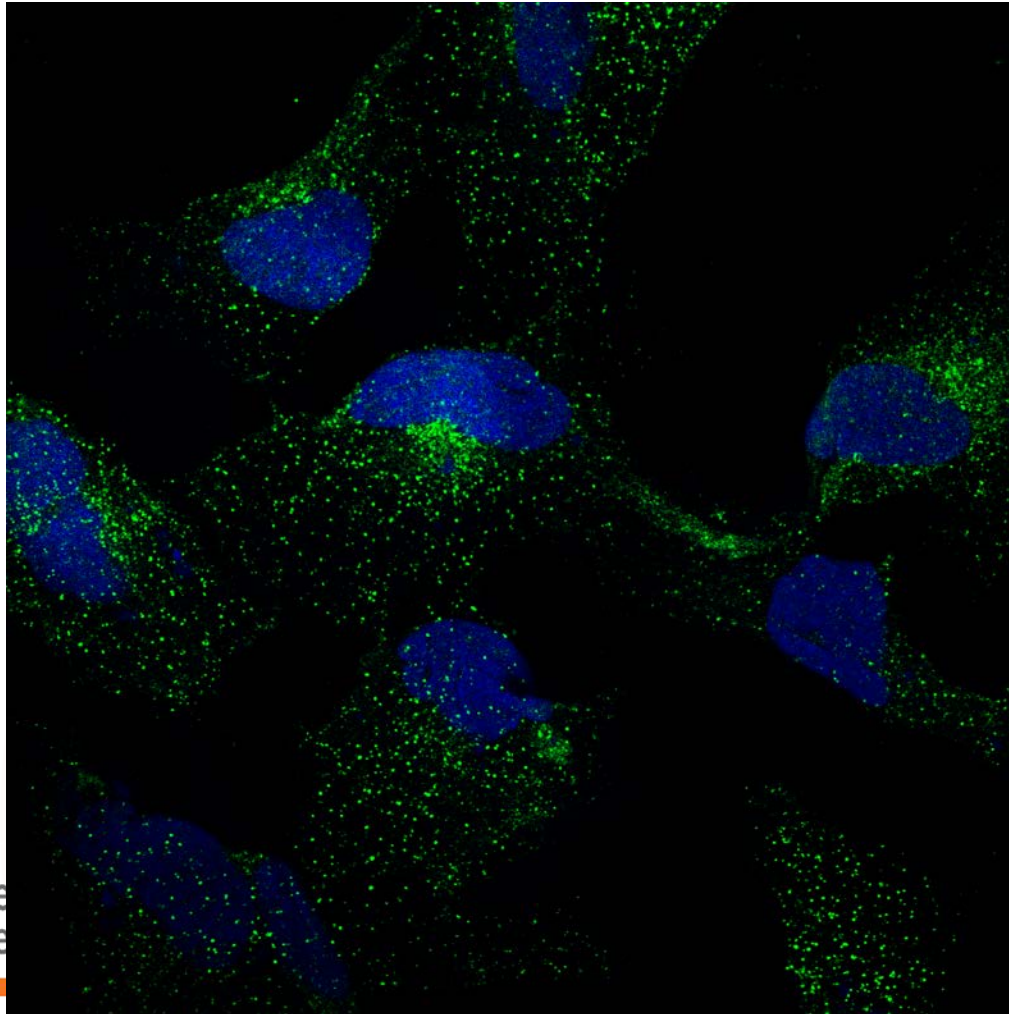
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Acquisition settings

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

Acquisition settings: BF imaging

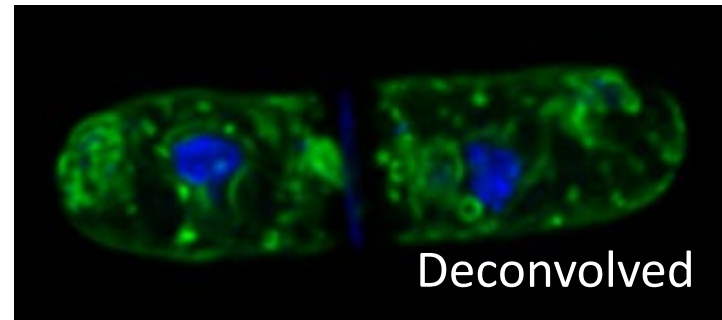
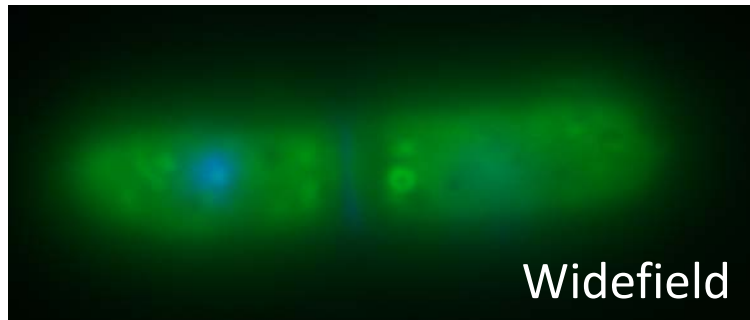
1. BF imaging: always good to include BF imaging data!!!



Acquisition settings: Z stacking

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

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

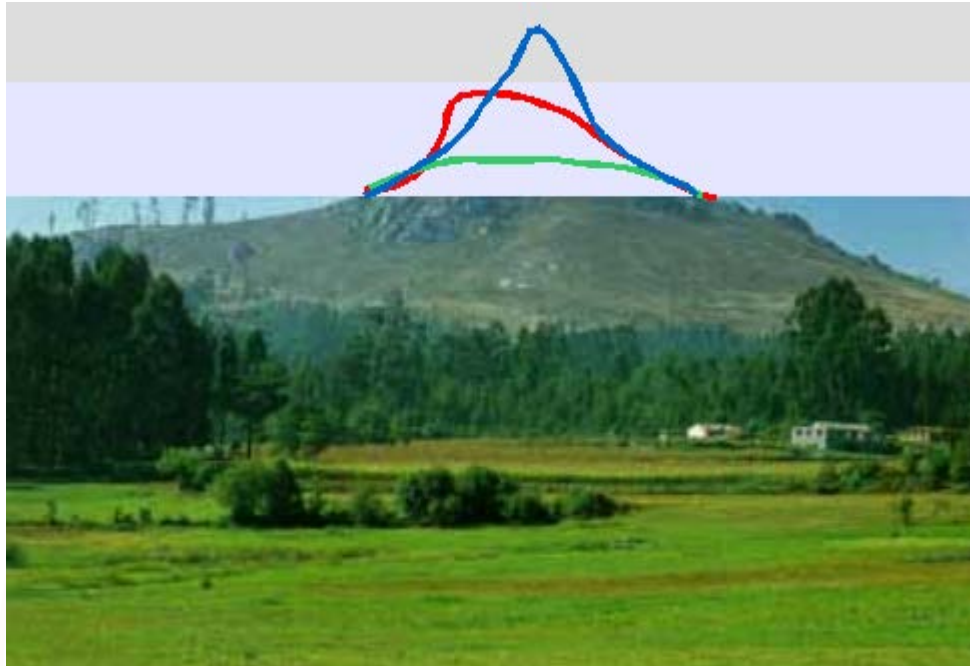


Courtesy images: GE Health

Acquisition settings: avoid saturation

- Never ever saturate signals (no clipping)!

Image courtesy: <https://svi.nl/ClippedImages>



Acquisition settings: minimise bleaching

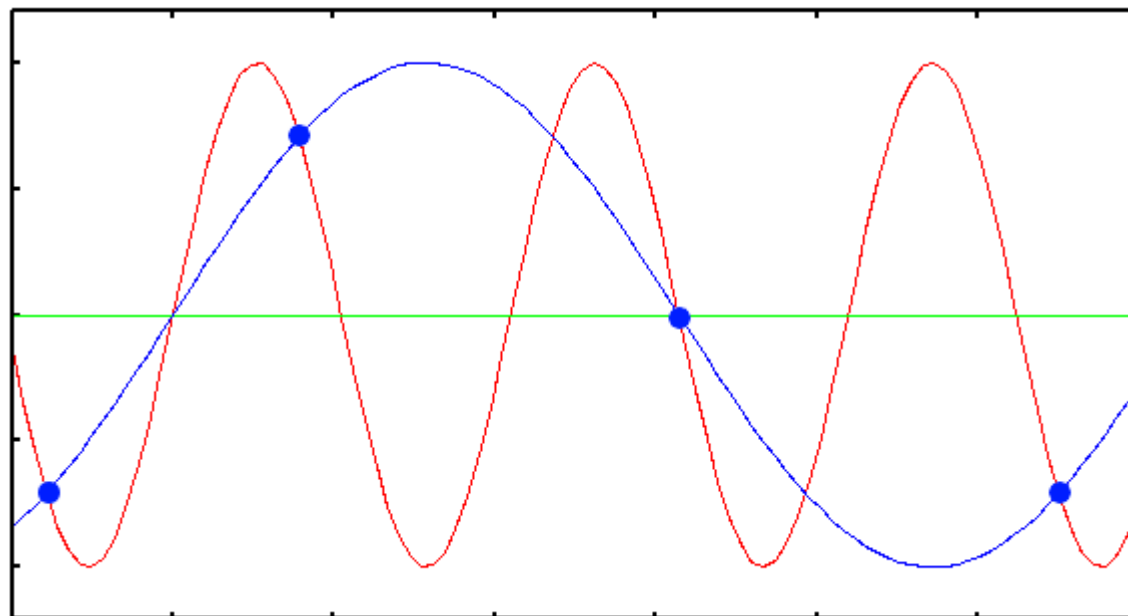
- Illumination strength and acquisition time
- Scanning speed: slow down?
- Averaging: be cautious with low signal

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

Outline

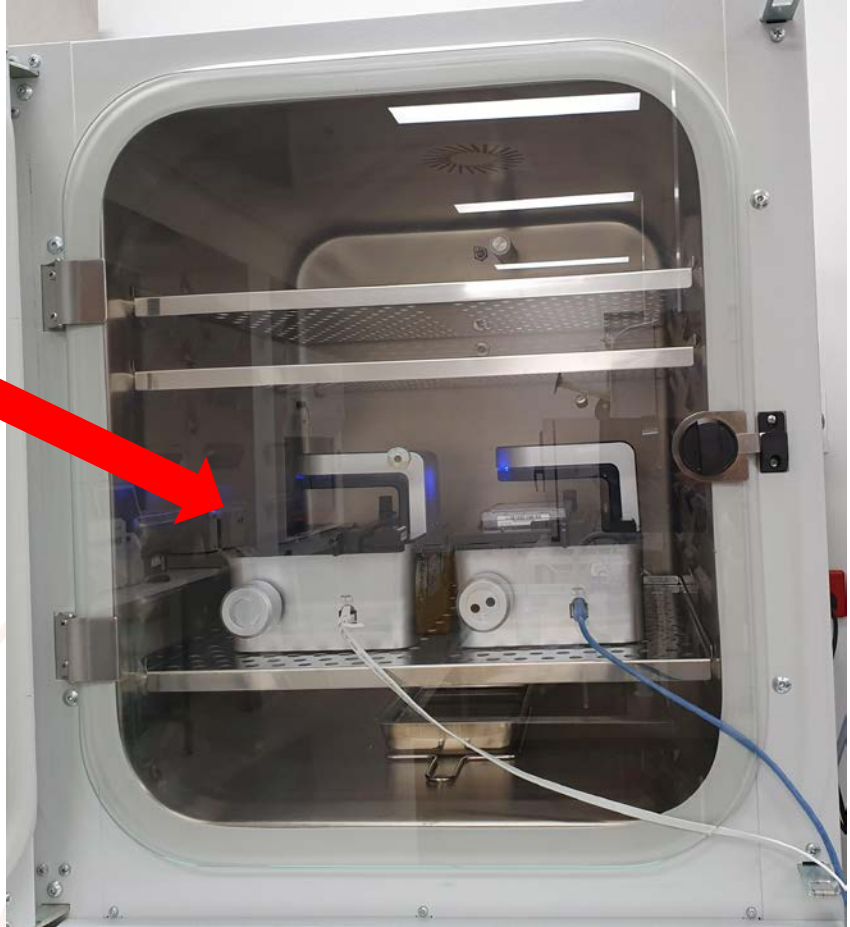
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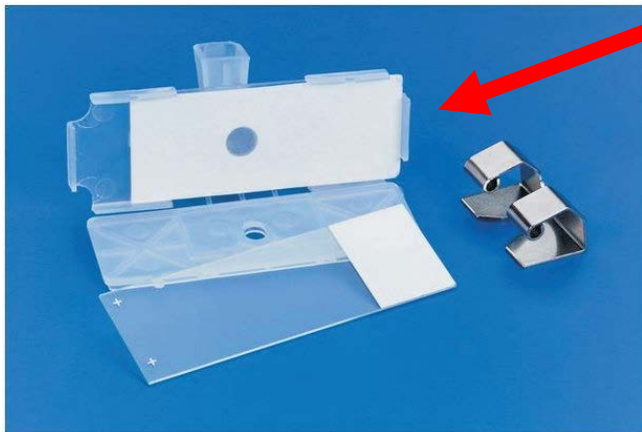
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New/old instrument: Juli BR live cell analyser



Cytofuge2: cytocentrifuge for sample concentration

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



Our website...

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

The screenshot shows a Confluence page interface. On the left is a navigation sidebar with a blue header and various icons. The main content area is titled 'List of Useful Documents and Links' and contains a numbered list of 26 items. The page is created by Hong Yu and last updated on Oct 26, 2019.

Westmead Imag... Created by Hong Yu
Last updated Oct 26, 2019

Overview
Space Settings

SPACE SHORTCUTS

How-to articles

PAGES

- Latest News
- Access Guide
- Instruments
- Training
- Forms
- Data
- User Fees
- Manuals & Protocols
- Tips & Tricks
- Q & A
- Useful Links
- Staff and Contact
- How-to articles

List of Useful Documents and Links

1. Deltavision: Auxiliary magnification option for the Deltavision microscope.pdf
2. Deltavision: CO2 and temperature settings for live cell imaging with the Deltavision microscope.pdf
3. Deltavision: How to shut down Deltavision system
4. Deltavision: How to save distance measurement results
5. Deltavision: How to use the Deltavision analysis computer
6. Olympus Confocal: How to set up the Olympus Confocal for polarization microscopy
7. Olympus confocal: Important notes about oil objectives
8. Olympus confocal: How to correctly operate stage and sample holder to avoid objective damage
9. Zeiss live cell imaging microscope: How to set up autofocus for live cell imaging
10. Olympus VS 120: How to change scale bar unit from pixel to calibrated resolution
11. Olympus VS 120: Tips and tricks on image acquisition with Olympus VS 120 & Handling large sized scanning images
12. Olympus VS 120: cellSense analysis tutorial by Olympus
13. Olympus VS 120: Image analysis strategies with Cellsens by Olympus
14. Olympus VS 120: how to save copied display images in Cellsens or VS desktop.pdf
15. Olympus VS 120: How to directly save VSI images to high-res Tiff files using Fiji/ImageJ only
16. Sample staining: Tips and tricks for Duolink
17. General: How to transfer large sized data via CloudStor
18. General: How to format a large USB drive
19. General: How to format a USB drive to FAT32
20. General: Kohler Illumination Procedure
21. Fiji ImageJ: How to separate IHC images using color deconvolution
22. Fiji ImageJ: How to draw a ROI manually
23. Fiji ImageJ: How to train Fiji ImageJ to select cells you are interested
24. Fiji ImageJ: How to merge DIC channel image with fluorescence channels
25. ImageScope: How to change scale bar unit
26. Previous seminar: Immunofluorescence microscopy and considerations

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Thank you!