Immuno-Fluorescence IF Staining and Imaging Workshop

Having challenges of IF staining?
Join our workshop for solutions! All Welcome!

Morning tea and lunch provided.
Contact Hong to register:
Email: hong.yu@sydney.edu.au;
Ph: 8627 3211

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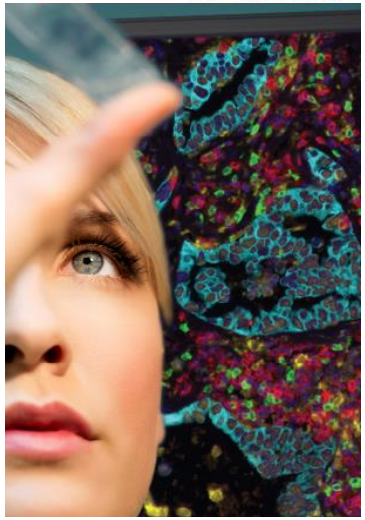

Topics

- Basics of fluorescence imaging & IF
- Signal enhancing and background reduction
- Stains for cell health and function
- Novel Opal technique- dramatically improves staining and allows multiple primary antibodies of the same species
- Making most of the exiting Nuance imaging system to get rid of your autofluorescence background
- Vendor training/demo of the Nuance

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Program (24 July 15)

10-10.30am	Getting great data from your fluorescence staining by Dr Jad El-Hoss, Thermo Fisher
10.30-11am	Real research stories: challenges of IF staining in liver and fungal studies by Dr Mahmoud Azar, STL WMI Dr Sophie Lev, CIDM WMI
11-11.30am	Morning tea, WMI Café area Sponsored by Thermo Fisher
11.30-12pm	Strategies for multiplexed IF staining: Opal kit and multispectral imaging Dr Justin Ross, PerkinElmer
12-1.00pm	Lunch WMI Café area Sponsored by Perkin Elmer
1.30-3.00pm	Demo and training on Nuance multispectral imaging system Room J2.08 Cell Imaging Facility
1.30-3.00pm	Product display and Q&A time Thermo Fisher Shared area Cell Imaging Facility

10am-3pm 24 July 2015, WMI L2 Conference Room C2.20, WRH Cell Imaging Facility

Program

10.00-10.20 Welcome. IF basics and some considerations for IF staining microscopy
Dr Hong Yu

10.20-10.45 Getting great data from your fluorescence staining
Dr Jad El-Hoss, Thermo Fisher

10.45-11.15 Real research stories: challenges of IF staining in liver and fungal studies
Dr Mahmoud Azar, STL WMI
Dr Sophie Lev, CIDM WMI

11.15-11.35 Morning Tea, WMI Café area
Sponsored by Thermo Fisher

11.35-12.00 Strategies for multiplexed IF staining: Opal kit and multispectral imaging
Dr Justin Ross, PerkinElmer

12.00-12.05 Closing remarks and expression of interest for future seminars
Dr Laurence Cantrill

12.05-13.00 Lunch WMI Café area
Sponsored by Perkin Elmer

13.30-16.30 Tours, Demo and training on Nuance/Mantra multispectral imaging system
Dr Justin Ross, Perkin Elmer; Room J2.08 Cell Imaging Facility
Product display and Q&A time
Dr Jad El-Hoss, Thermo Fisher
Shared area Cell Imaging Facility

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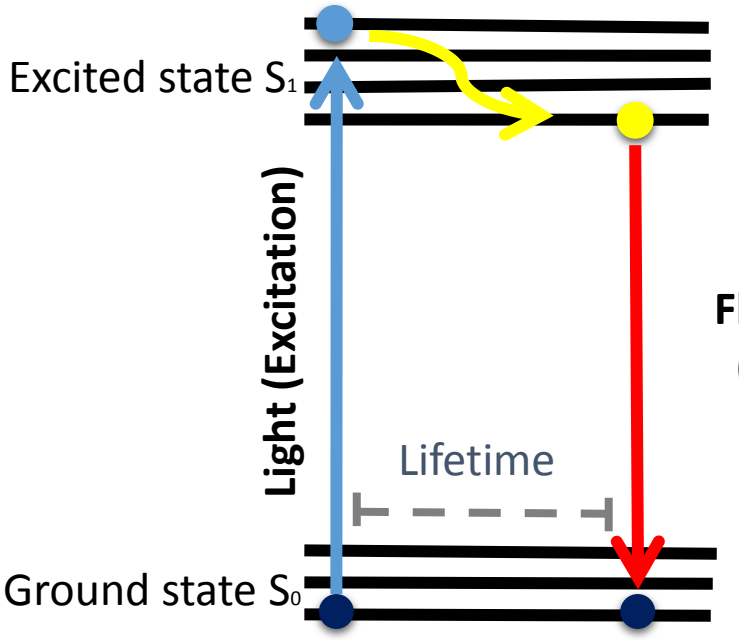
Theory of fluorescence



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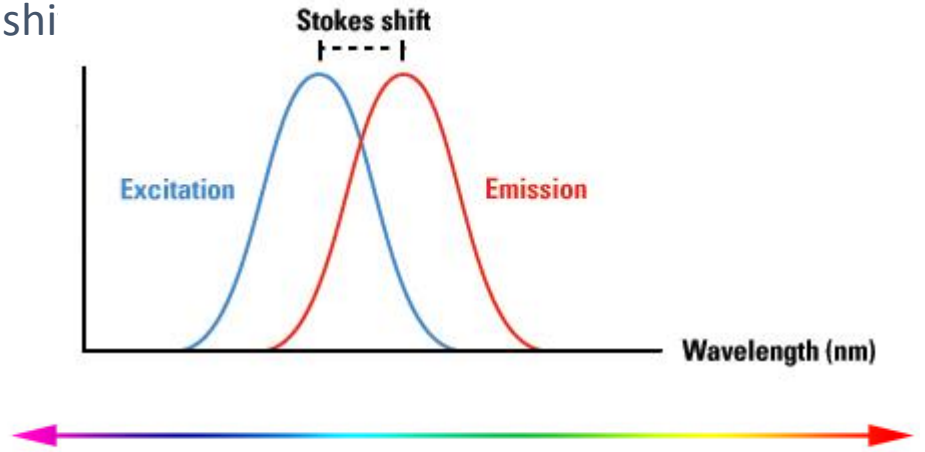
Fluorescence (Emission)



Shorter wavelength
Higher energy
Weaker penetration

Longer wavelength
Less energy
Better penetration

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Selection of fluorescent probes for IF staining

- Great Stokes shift
- Avoid crosstalk---SpectraViewer
- Thick/thin sample---
  longer/shorter wavelength
- Shorter wavelength better resolution
  Resolution = (0.61 x λ)/ **NA**

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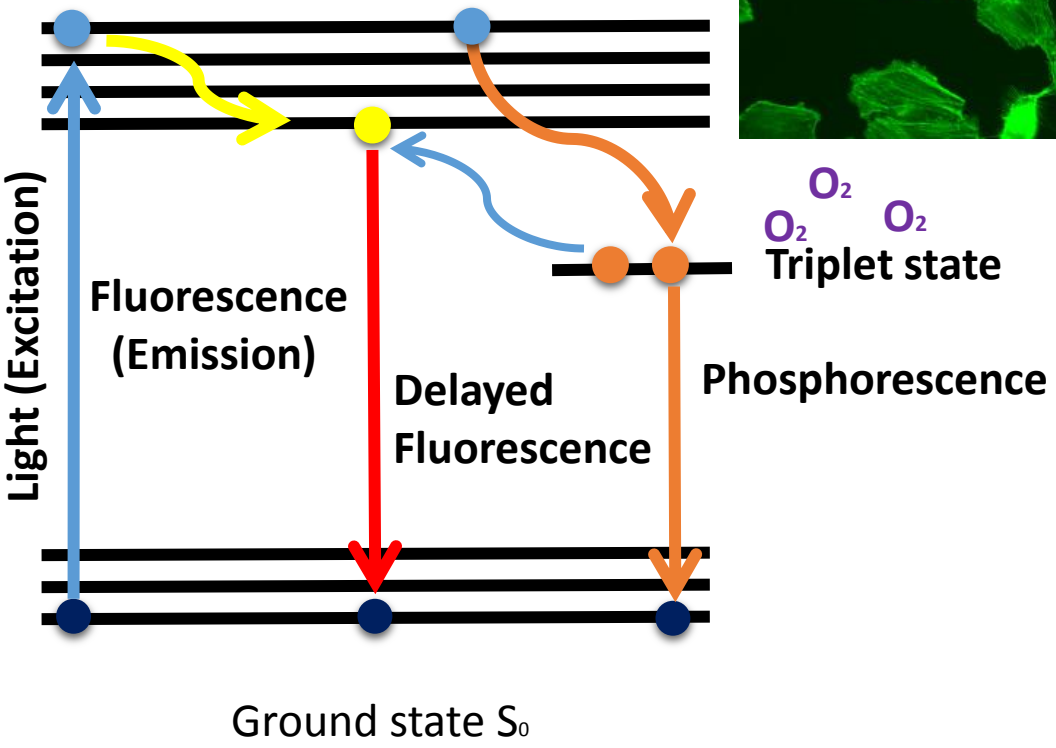
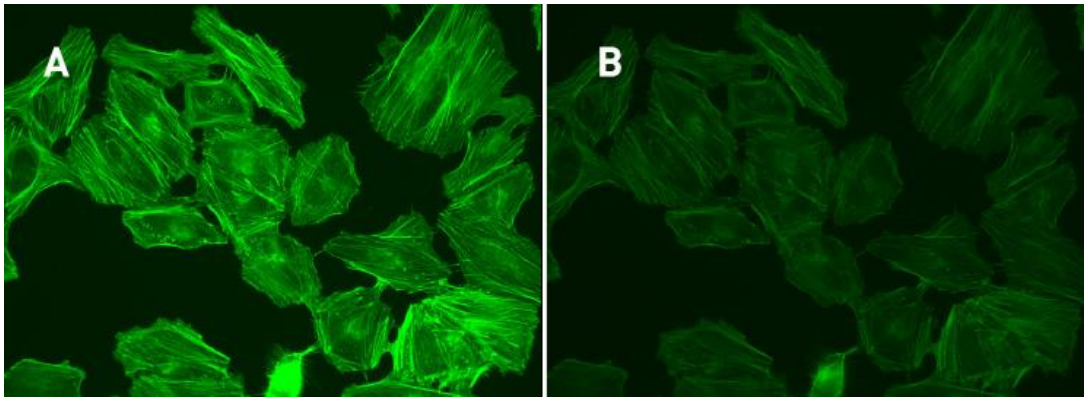
Photobleaching and solutions





Protecting against photobleaching

- Use anti-fade agents to de-oxygenate your sample
- Reduce excitation intensity or exposure time
- Store your slides at low temperature

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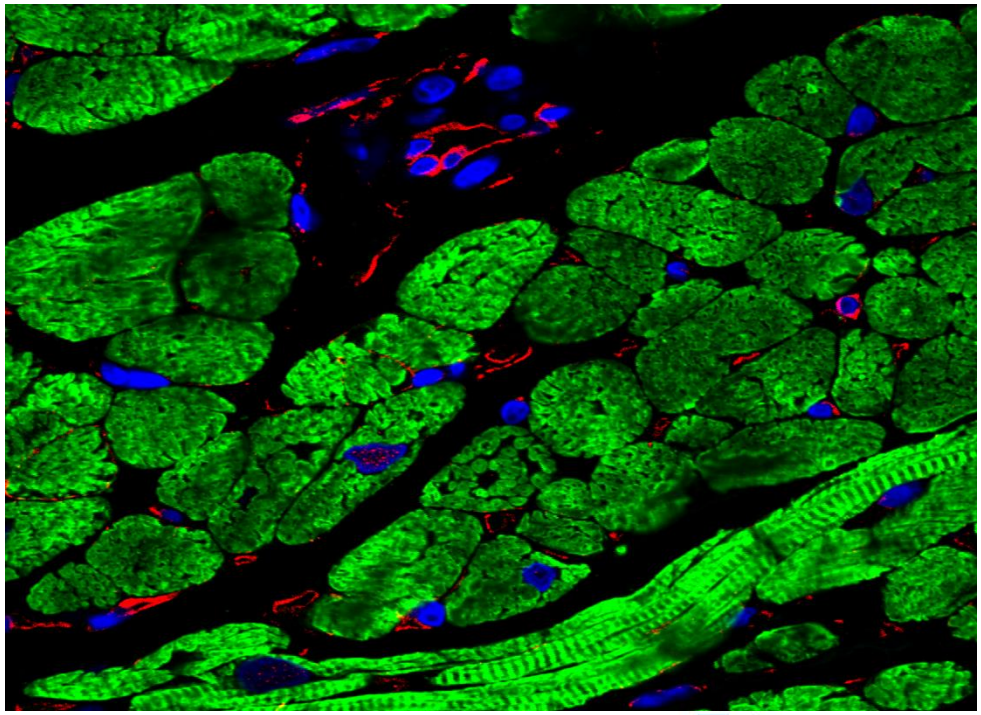
Autofluorescence (AF)

- AF Fixatives—wash with 0.1% borohydride in PBS for 30 min
- AF--- worst @ 488nm; use other channels i.e. blue, red, or far red
- AF quenchers: Sudan Black, Trypan Blue, Trueblack™ (Biotium)
- Nuance multispectral imaging system



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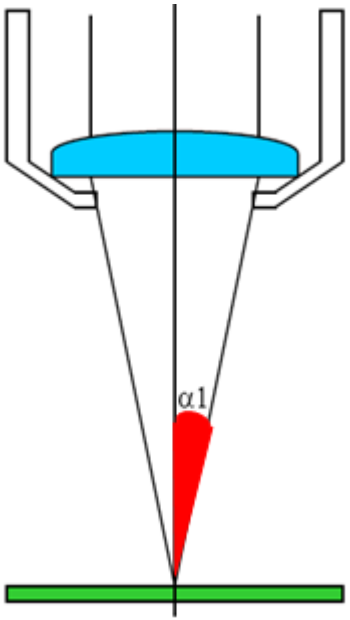
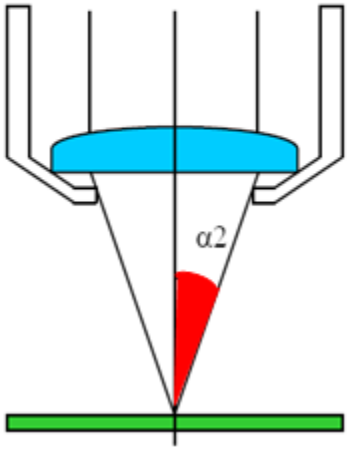
Numerical Aperture (NA) of a lens

$$NA = n \sin \alpha$$

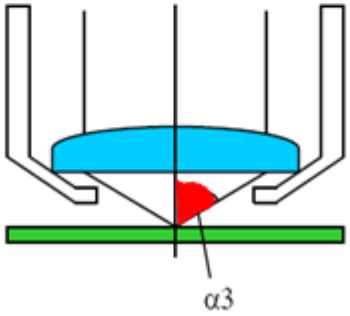
α: one-half angular aperture
n: refractive index



10x Dry
NA 0.42

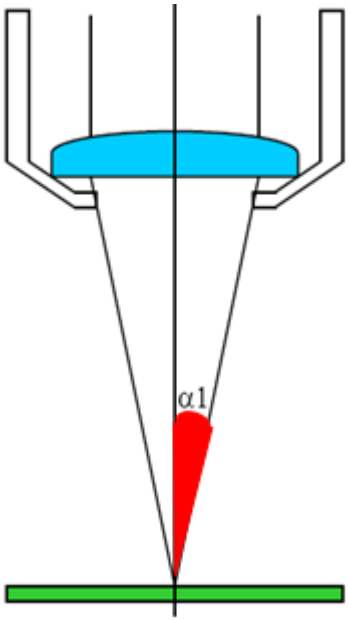


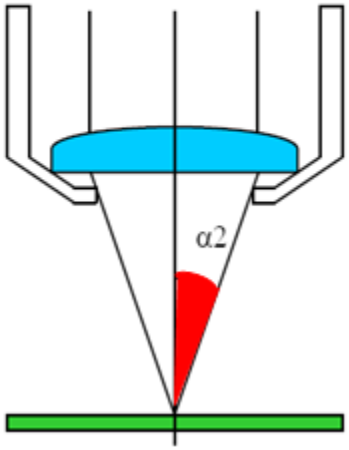
40x Water
NA 0.65

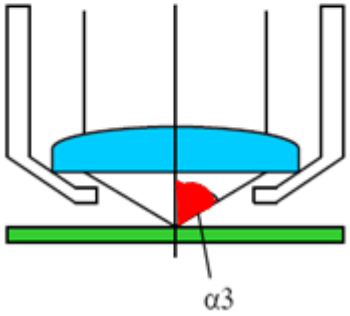


60x Oil
NA 1.42

Choosing the right objective







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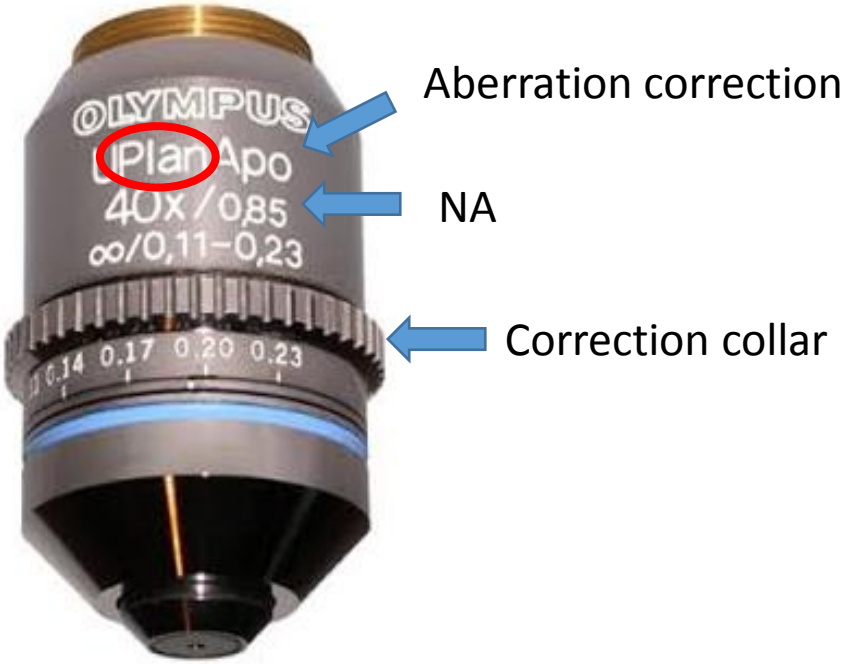
Choosing the right objective

- high NA lens
- Apochromatic lens
- Correction collar



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Interpreting IF results--controls

- **Negative controls—preclude false positive**
  - no staining at all—AF background
  - 2nd Ab only—non-specific staining
  - isotype control (monoclonal 1st Ab)
  - Absorption control—specific staining
  - knockout
- **Positive controls—verify protocol and 1st Ab**
  - known sample
  - immunoblots
  - Single staining
- **Include controls in scientific papers**

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Interpreting IF results—staining pattern

Location!

Staining pattern!

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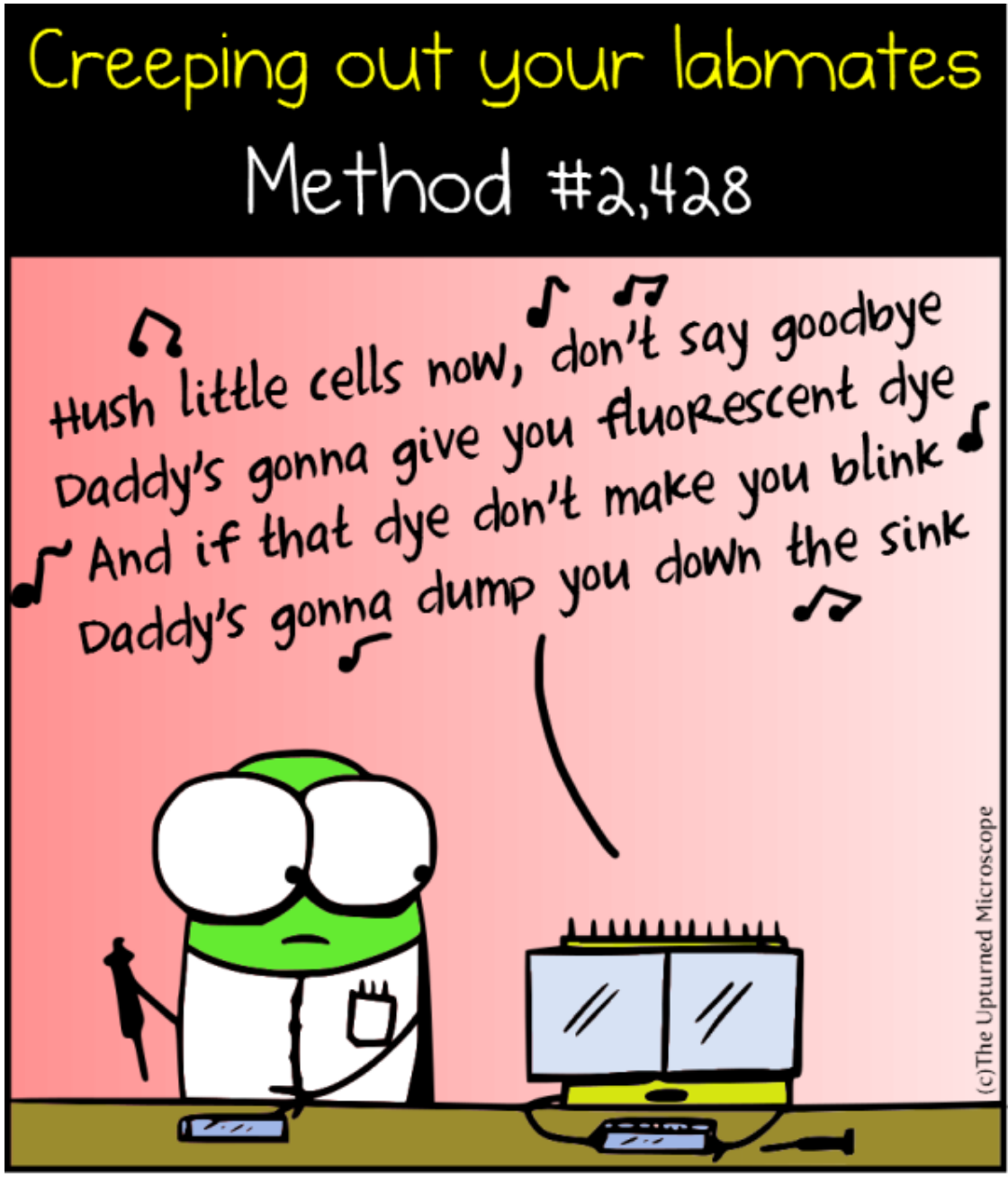
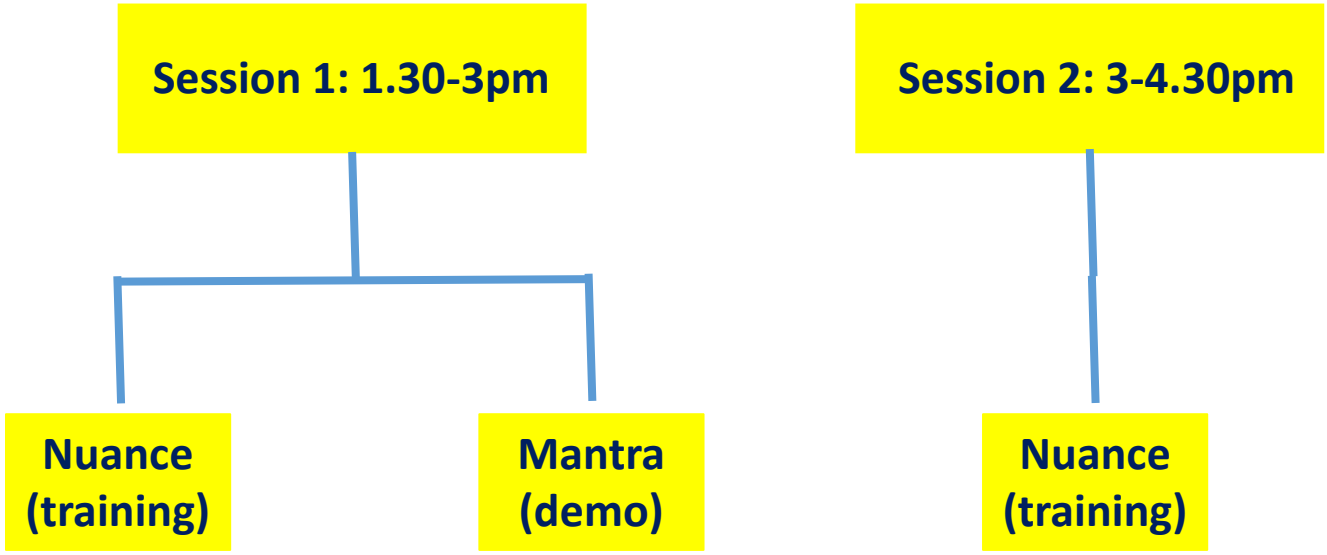
Thank you and Enjoy more talks!



Afternoon training and workshop



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What topics would you like for next seminar/workshop?

1. Foundational light microscopy
2. Confocal microscopy
3. LCM workshop
4. Live cell Imaging
5. Super-res microcopy---

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