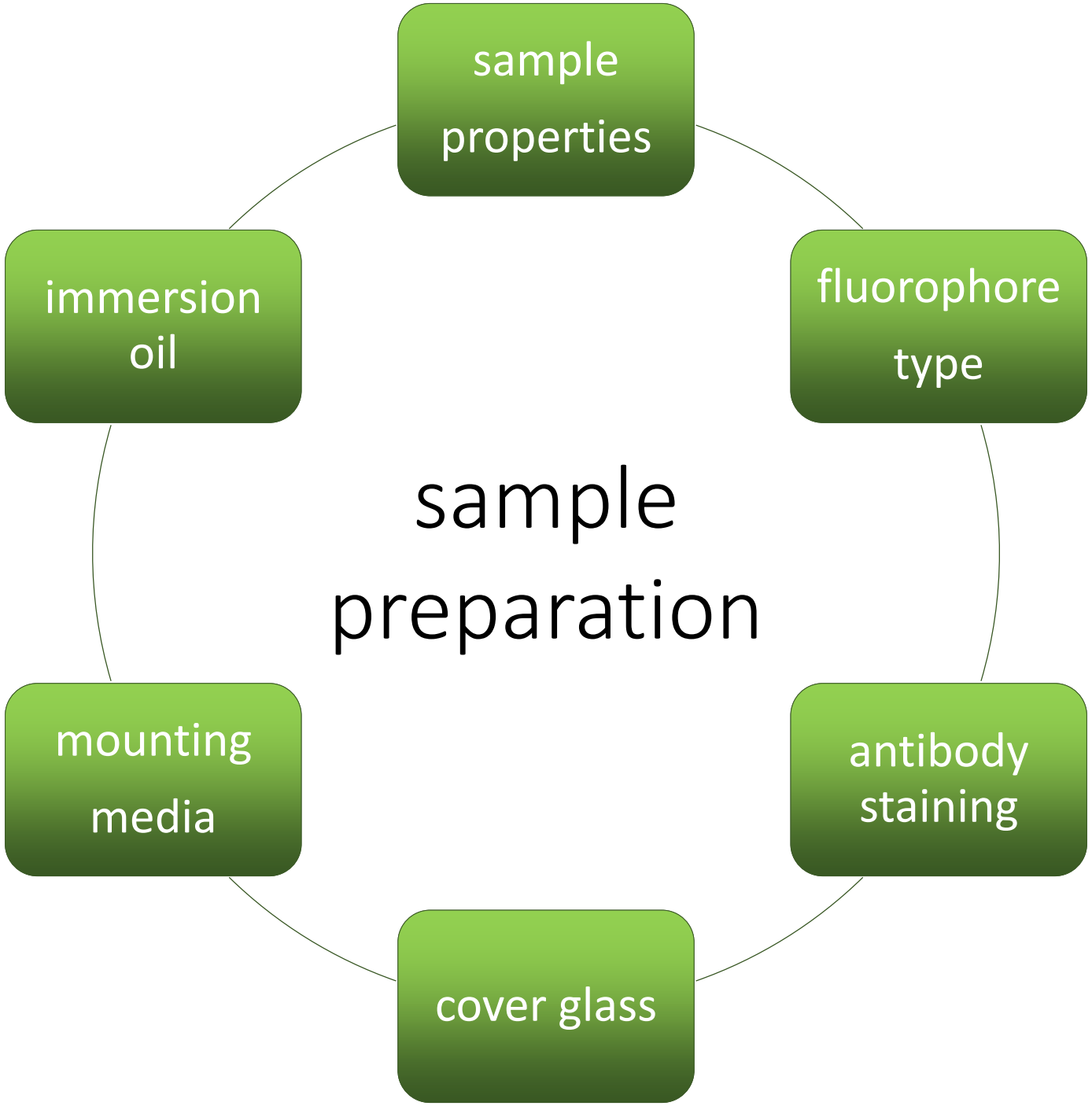
Sample preparation & limitations in SR

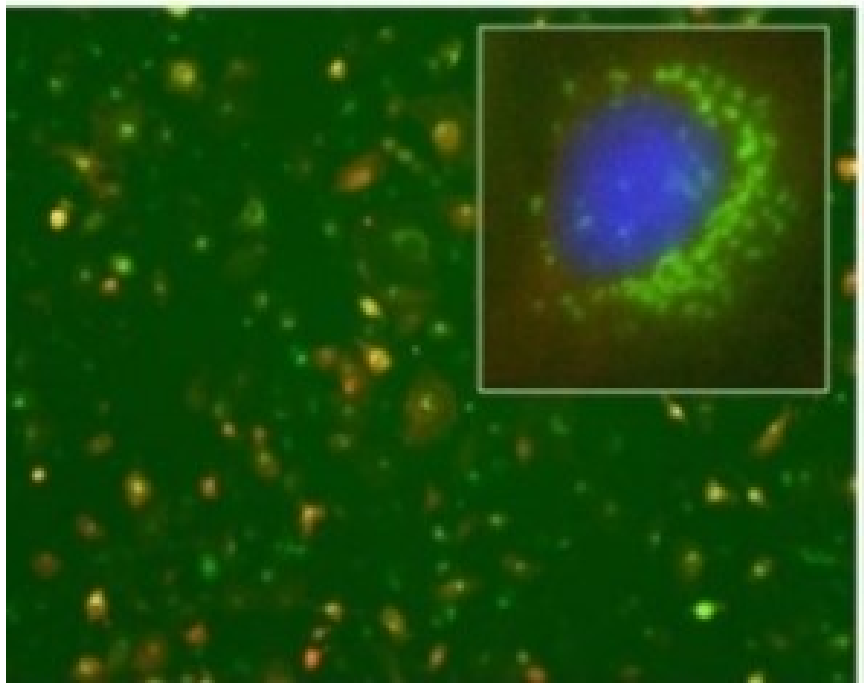
Anna Malinová

Prague, November 15, 2017

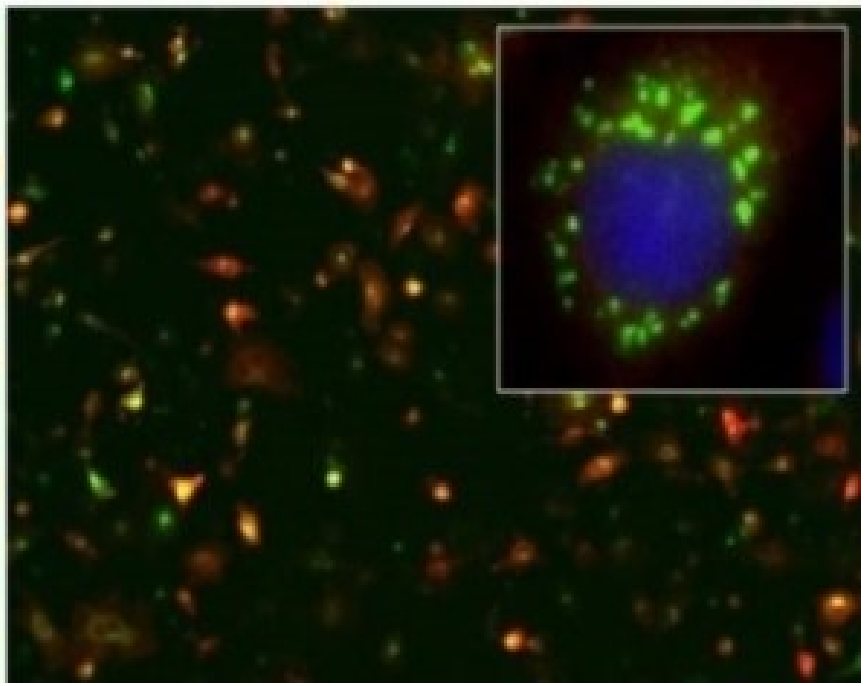

sample properties
fluorophore type
antibody staining
cover glass
mounting media
immersion oil
sample preparation

fixed sampl / live cell

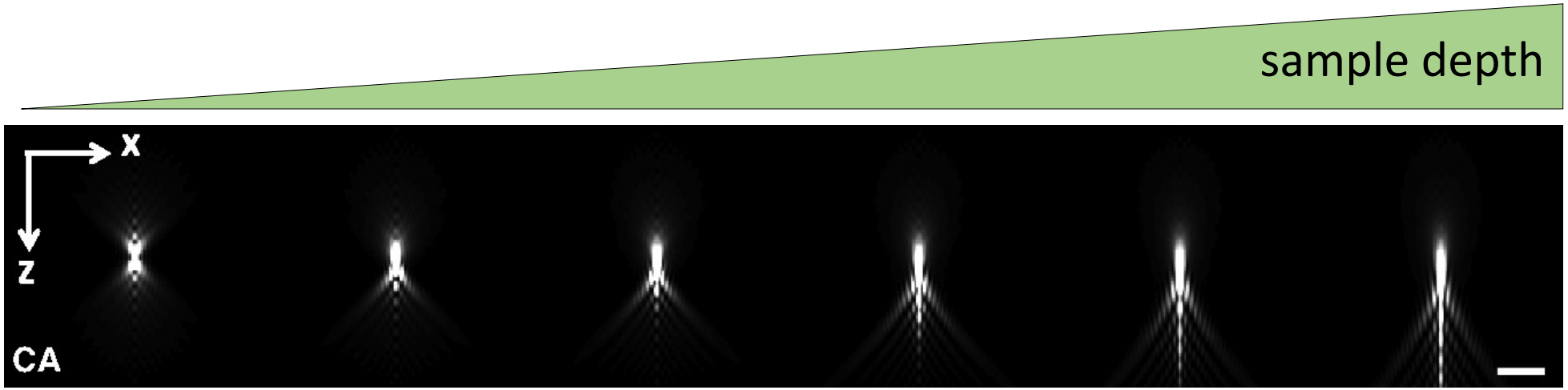
DMEM



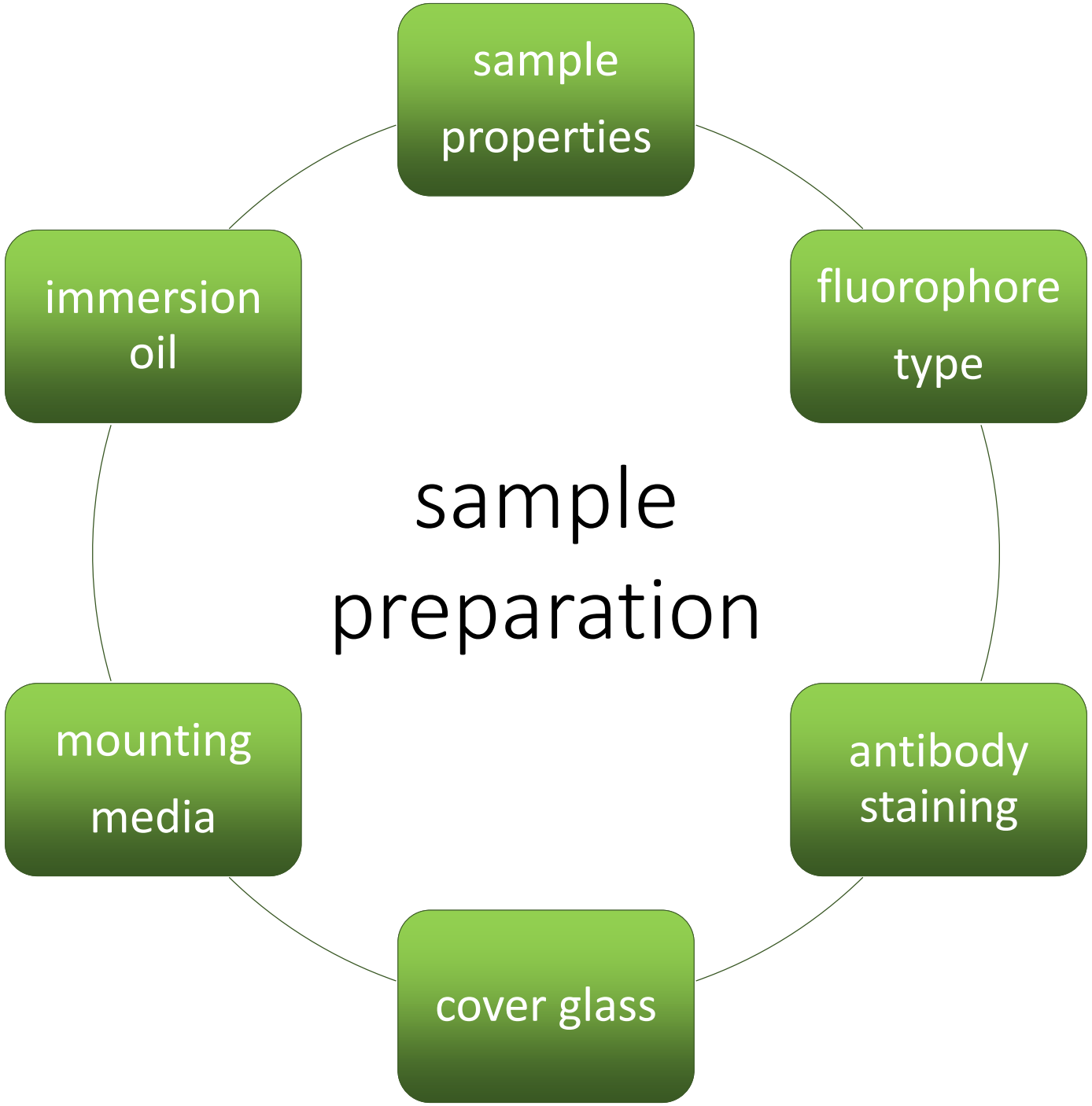
FluoroBrite™ DMEM



sample thickness



Ghosh *et al.,* 2015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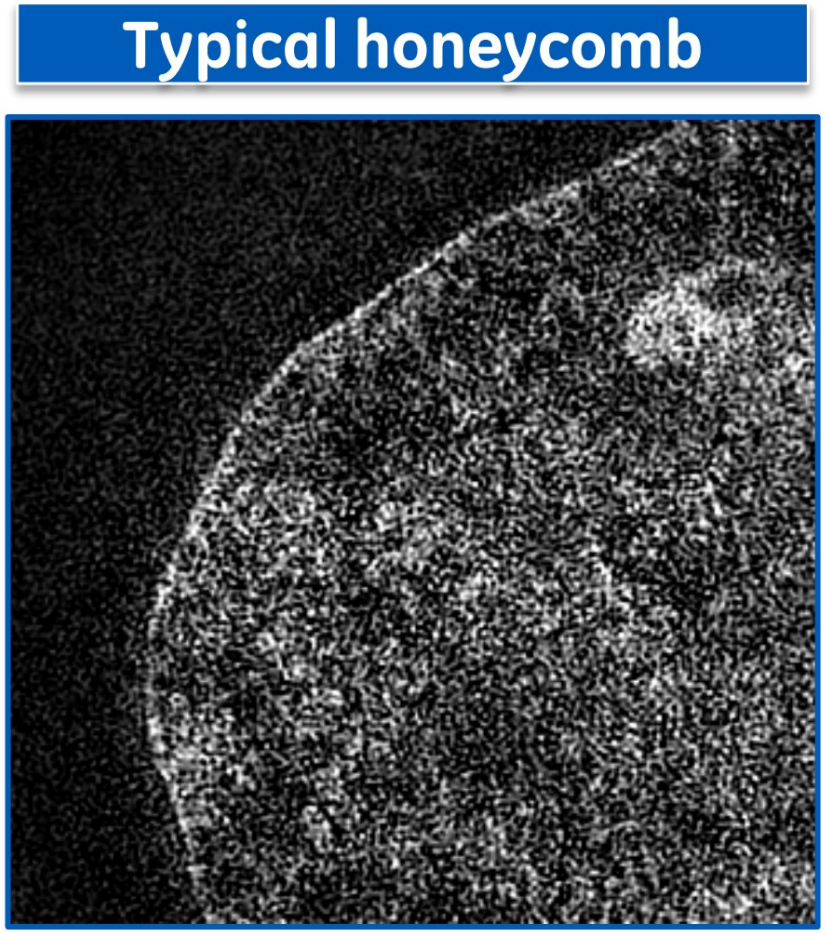
fluorophores

SIM

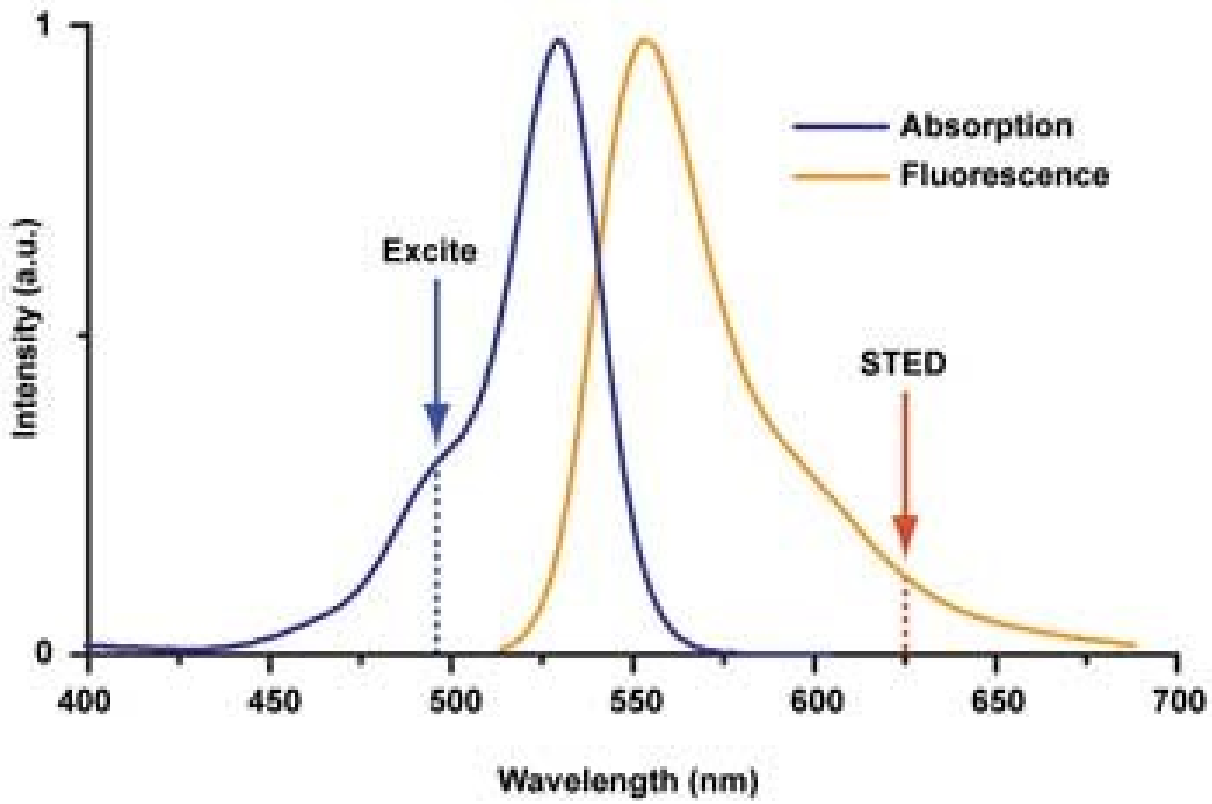
- traditional fluorophores
- high brightness, low background
- low photobleaching
- up to 4 colors



Diffuse labeling and high S/N will give artifacts!

fluorophores STED

- emission at STED λ
- not absorption at STED λ
- multicolour labeling



fluorophores STED

- emission at STED λ
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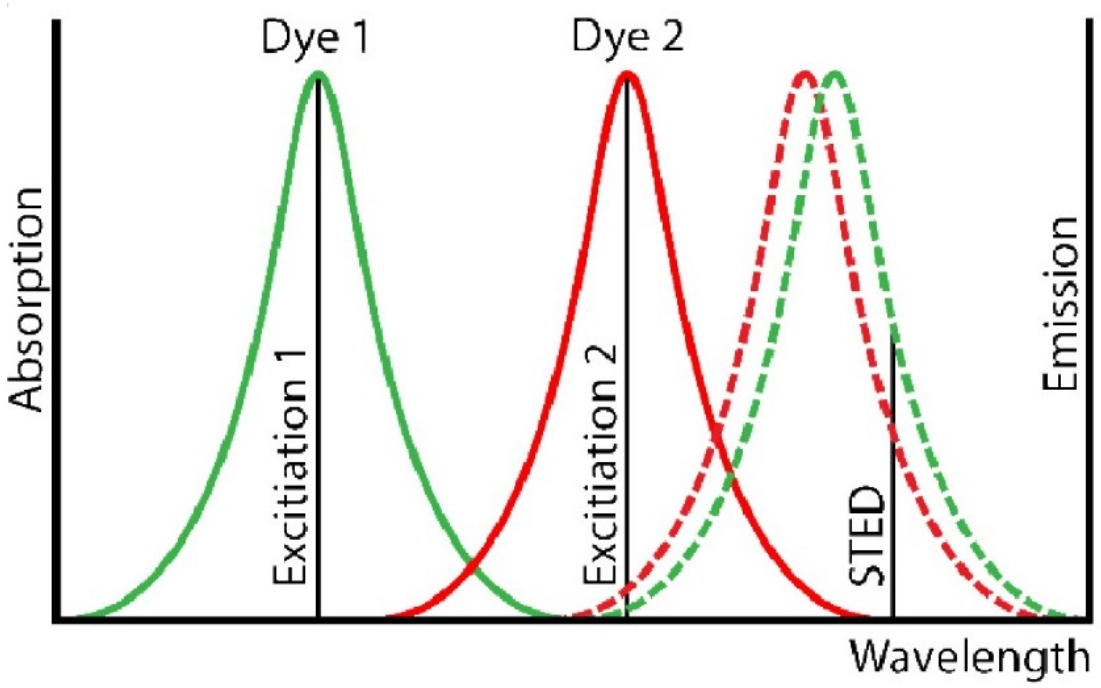
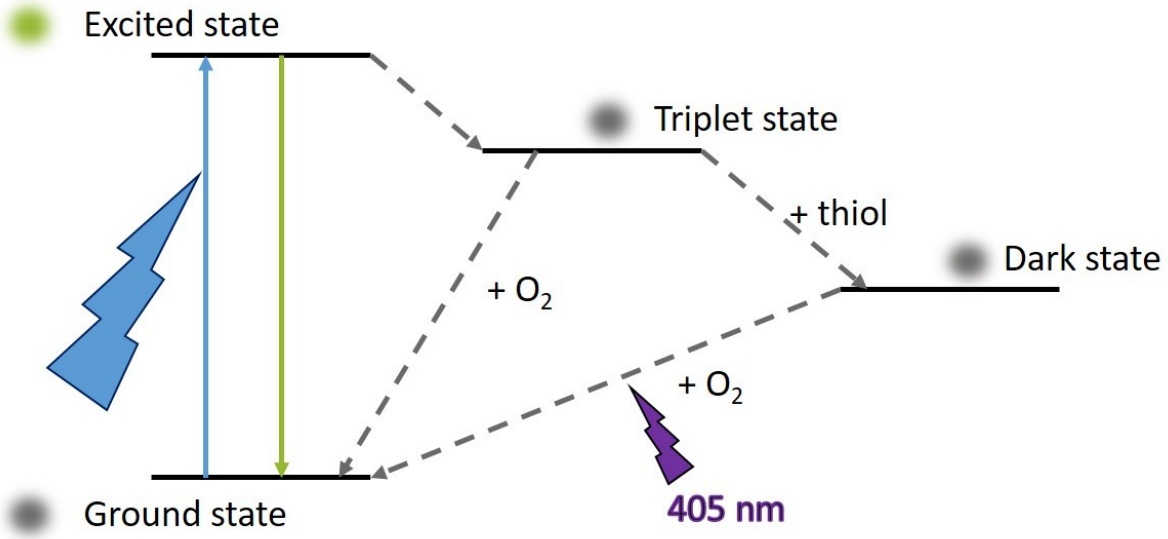


large Stokes shift dye (green)
small Stokes shift dye (red)
1 STED laser

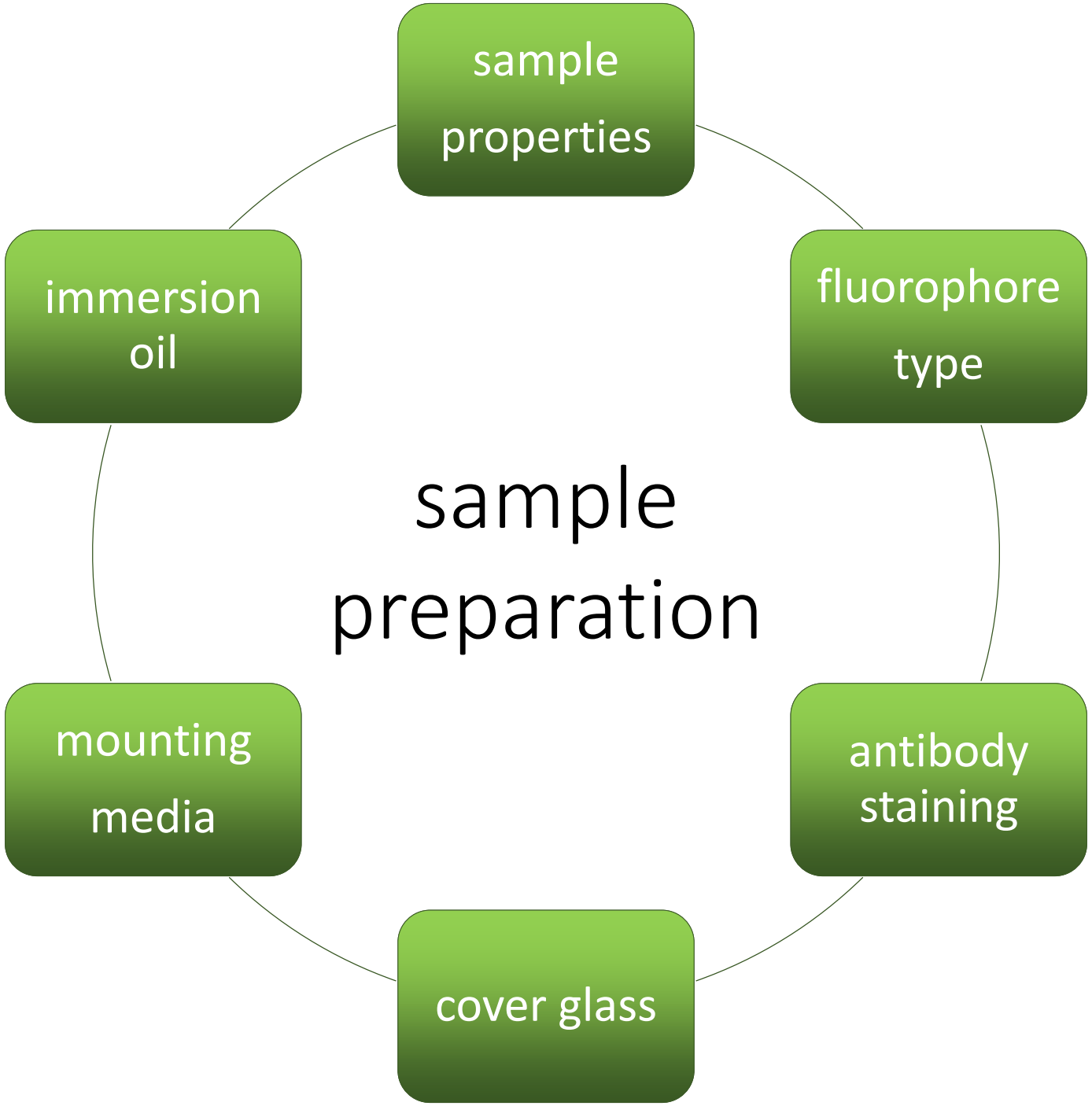
fluorophores
SMLM

Alexa Fluor 647

- blinking fluorophores (photoactivable/switchable or standard dyes)
- low bleaching
- high ability to go to dark state
- high number of switching cycles
- blinking buffer (for standard dyes)
  - thiols (MEA, cysteamin)
  - glucose oxidase



adopted from M. Olšinová presentation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cover glass
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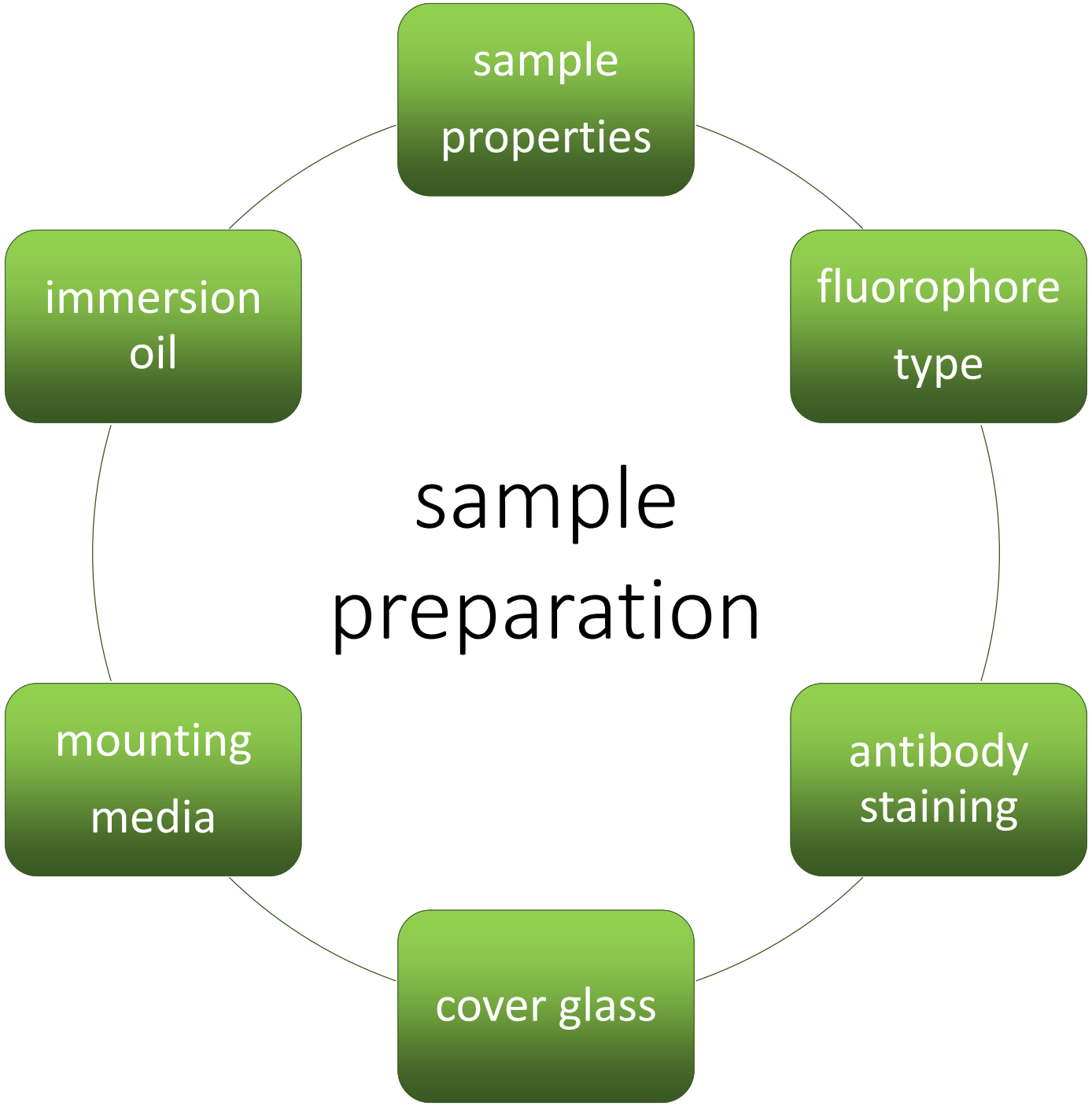
immunofluorescence labeling

- **fixation** (perservation of cellular structures)
  - 2% PFA (cross-linking), methanol...
- **permeabilization** (allow antibodies to penetrate cells)
  - higher concentration/longer incubation → more accesible epitope/destroys structures
- **blocking** (binding of unspecific epitopes)
- **1° & 2° antibody staining**
  - higher concentrations/longer incubation for SR
  - but carefully with increased background

→

- **washing with PBS**
  - decreasing background
- final **washing with water**
  - salt removing
- **drying**
- **mounting**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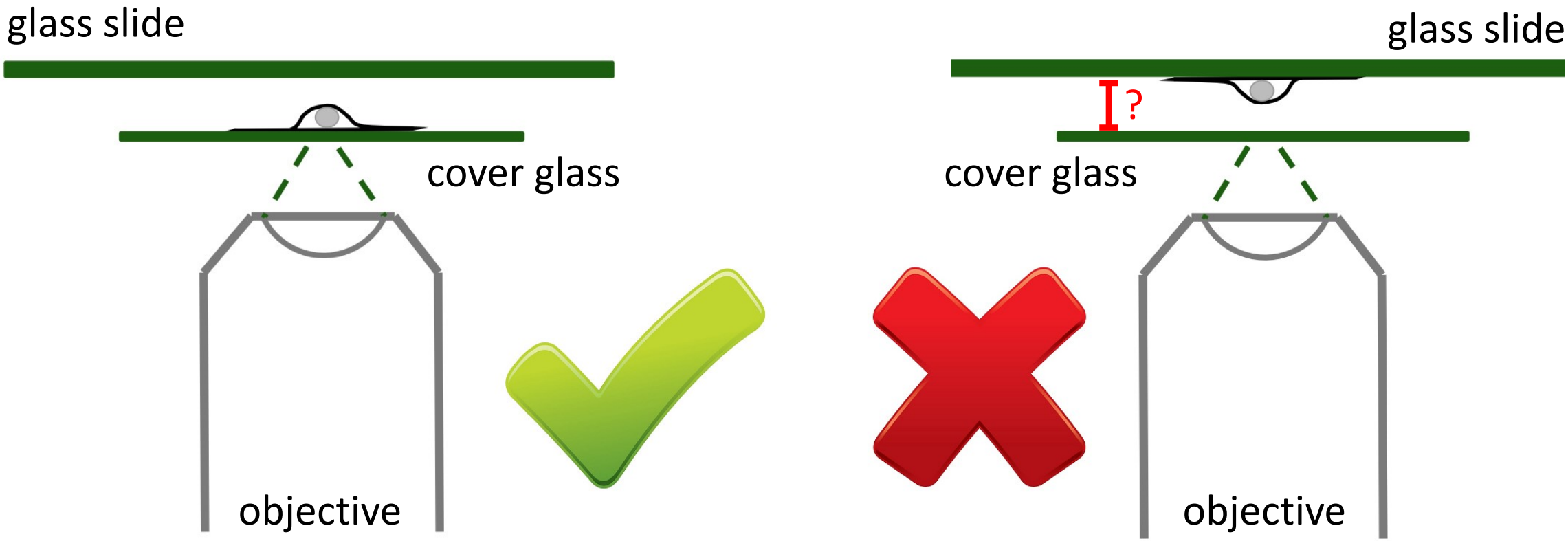
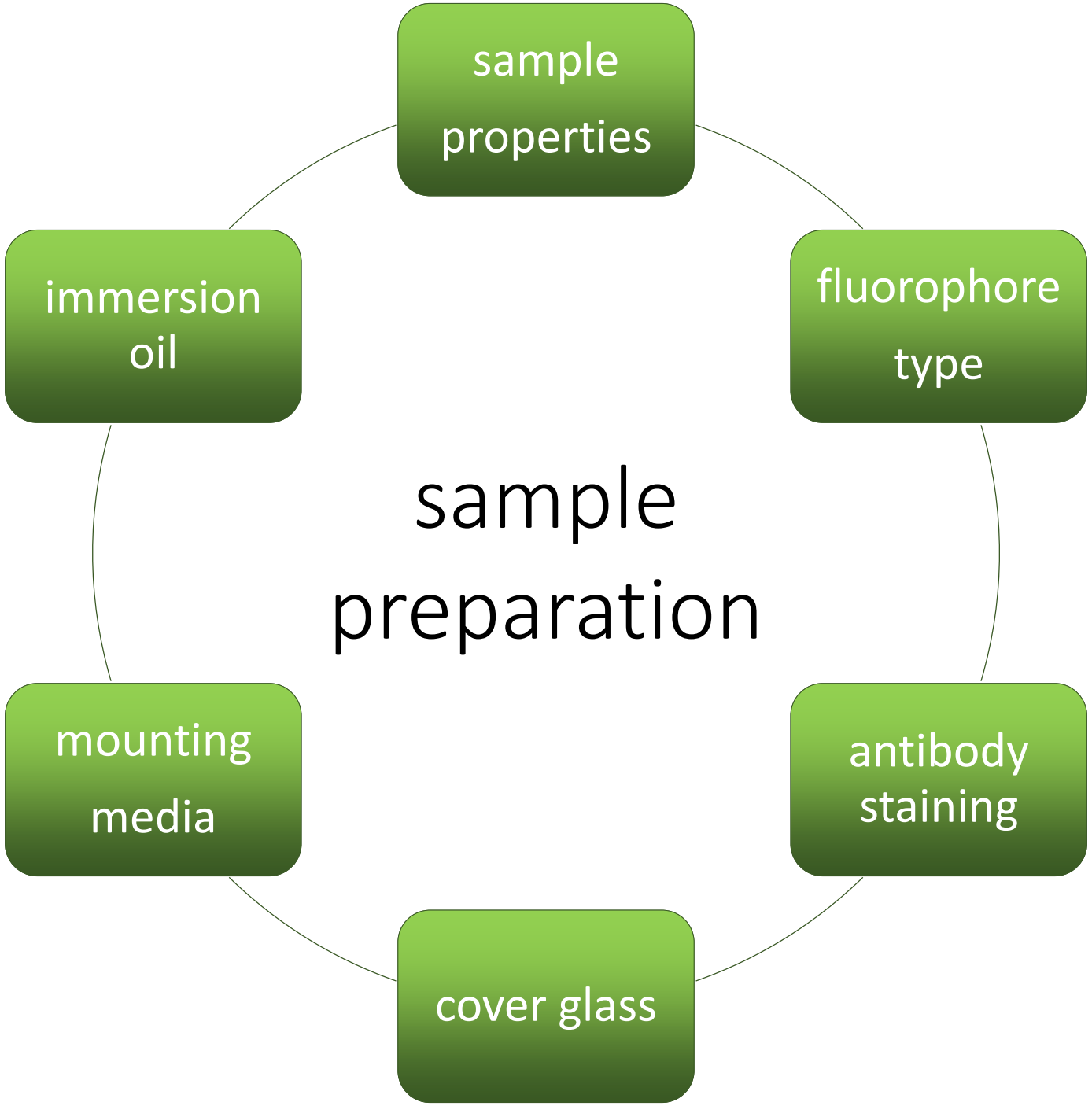
cover glass


glass slide
cover glass
objective
?
glass slide
cover glass
objective

cover glass - thickness

Precision cover glasses thickness No. 1.5H (170 µm ± 5 µm) for high performance microscopes

Microscopes are optimized for #1.5 cover glass!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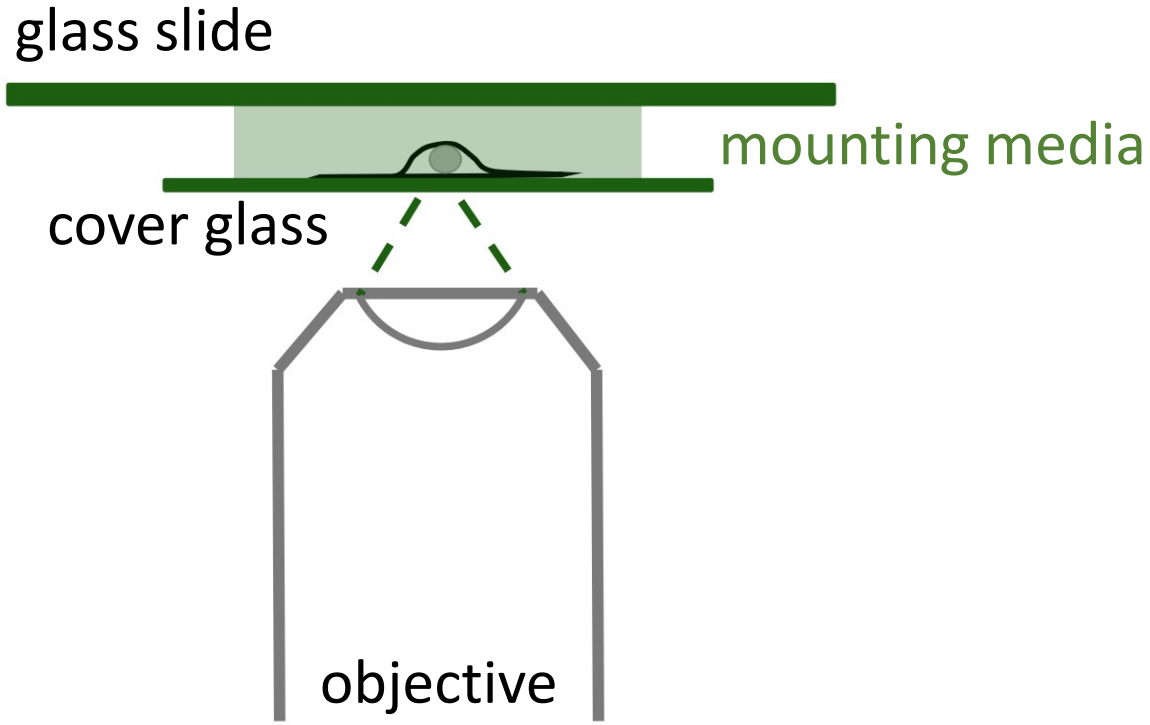
mounting media

- preservation of the sample
- base + antifade reagents
  - NPG, DABCO, PPD...
- don´t use MM with DAPI
- affect fluorophore properties
- hardening/non-hardening
- minimize RI mismatch



http://www.gelifesciences.com

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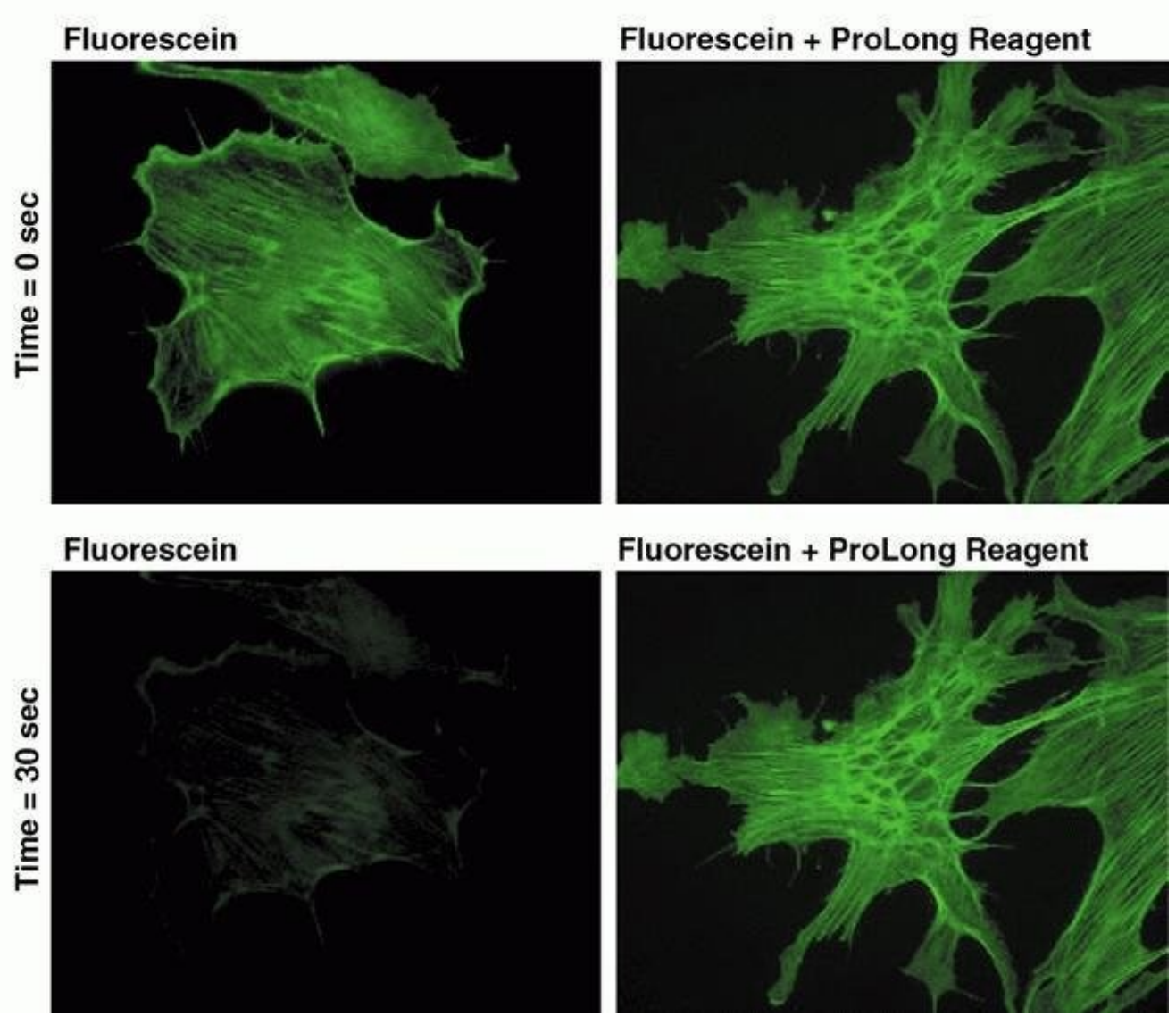

Ono *et al*., 2001

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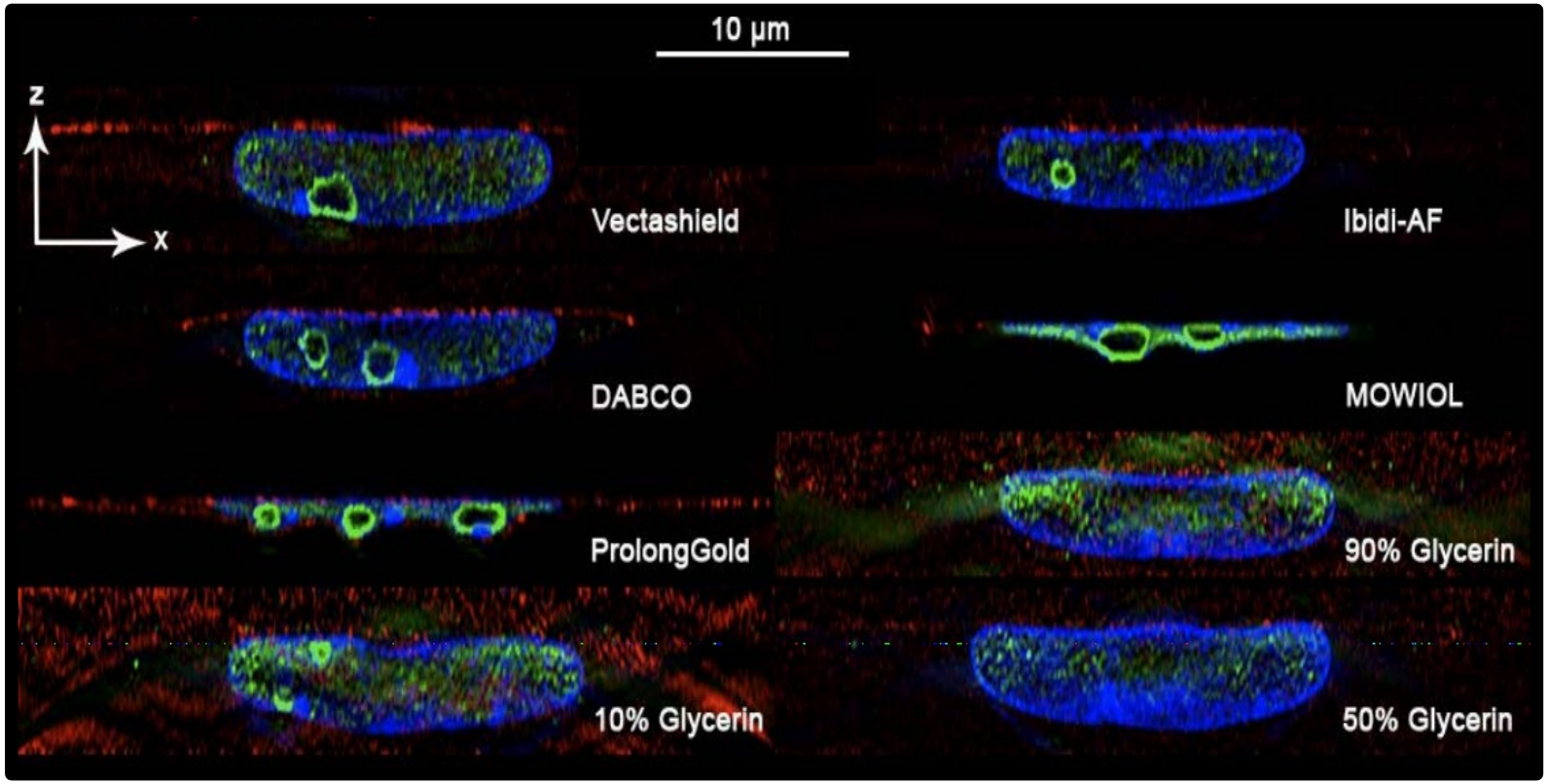
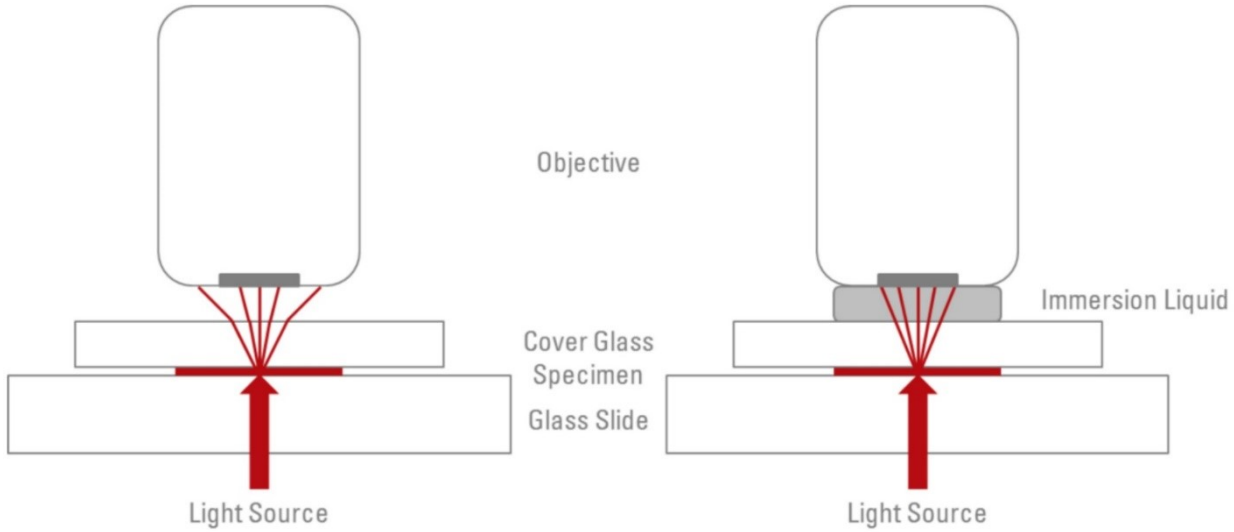


http://www.gelifesciences.com

mounting media
refractive index mismatch

By matching the RI of the mounting medium to that of the immersion liquid and cover glass, optical aberrations and scattering are minimized.

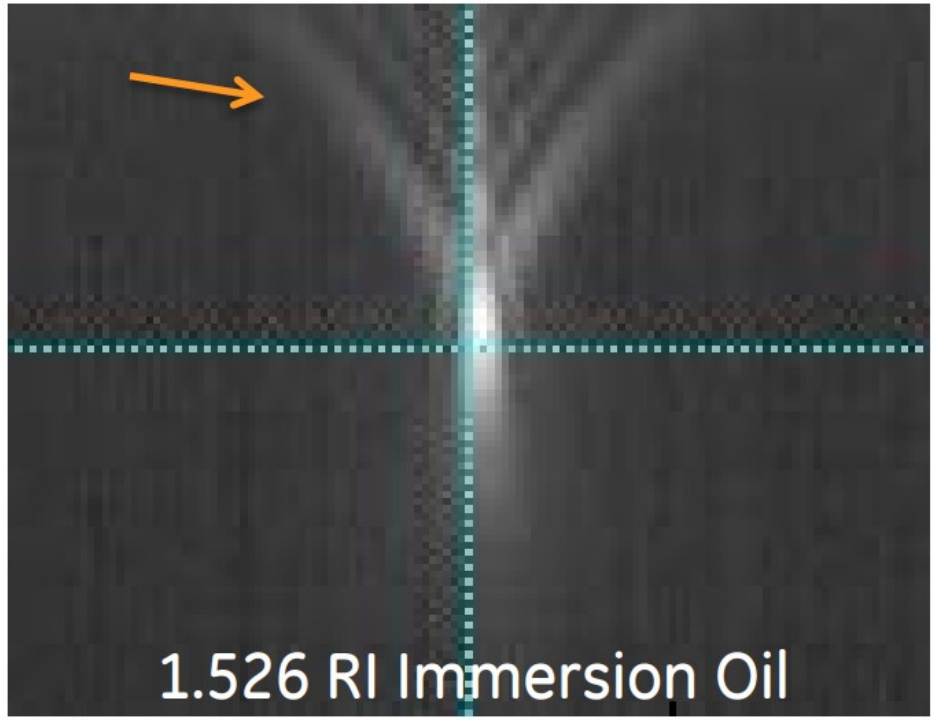
Air	1
Water	1.33
Glass	1.52
Oil	1.52
Fluoromount	1.39
Vectashield	1.44
Prolong	1.46
90% Glycerol	1.46
MOWIOL	1.49
Thiodiethanol (TDE)	1.52



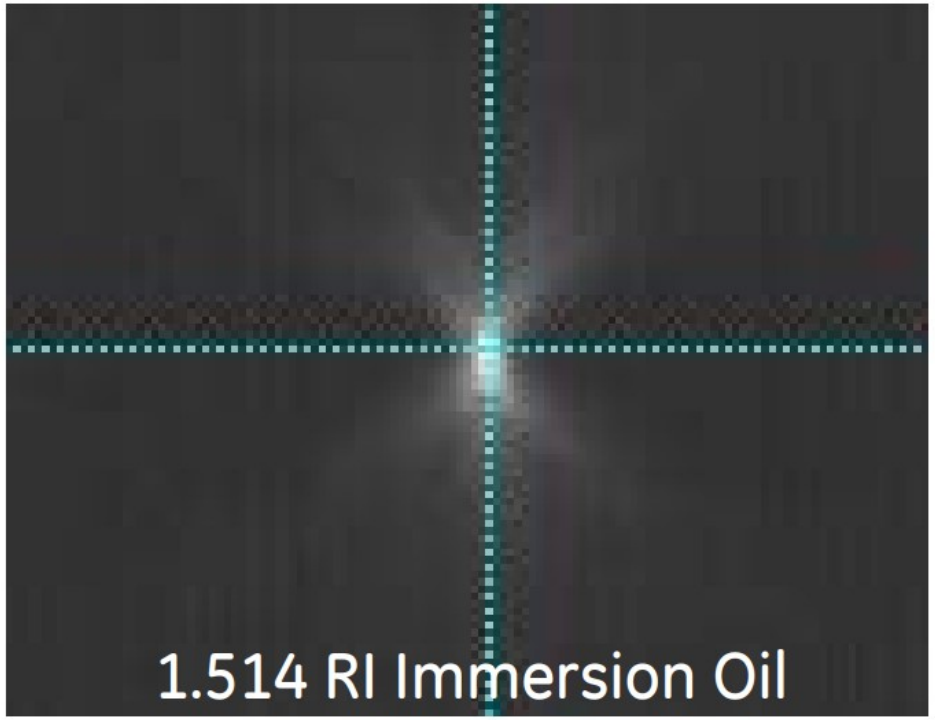
We recomand:
90% glycerol + NPG for antibodies staining
90% glycerol + DABCO for protein tags

immersion oil
refractive index mismatch

PSF with Mismatched oil



PSF with Matched oil



immersion oil

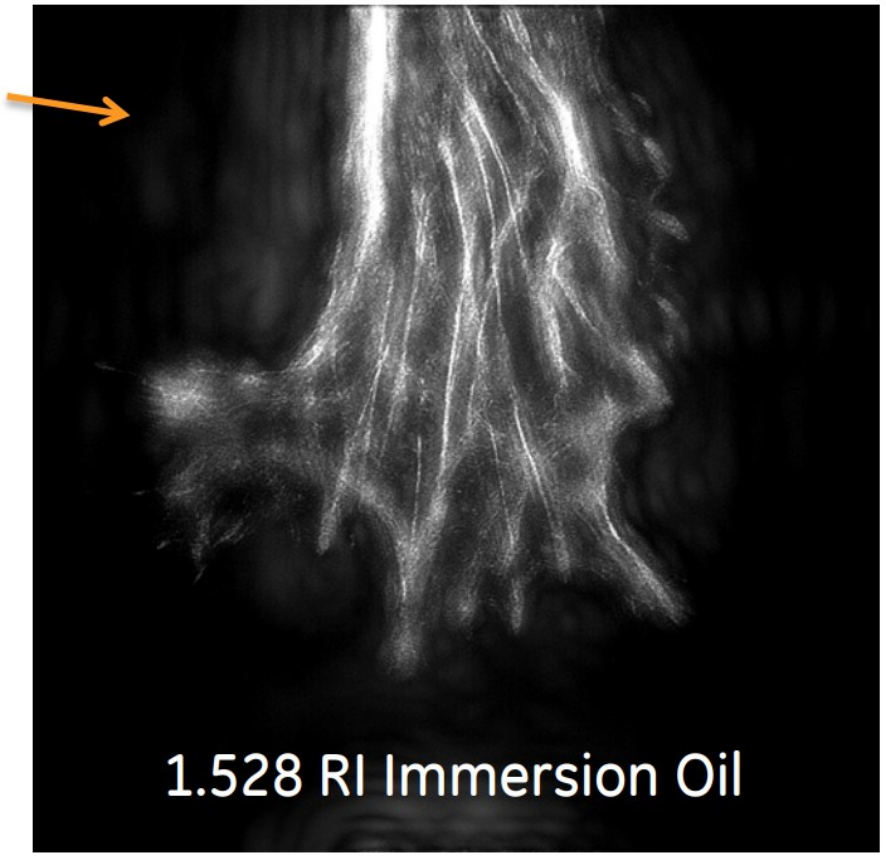
refractive index mismatch

SIM algorithm assumes a perfectly matched PSF!

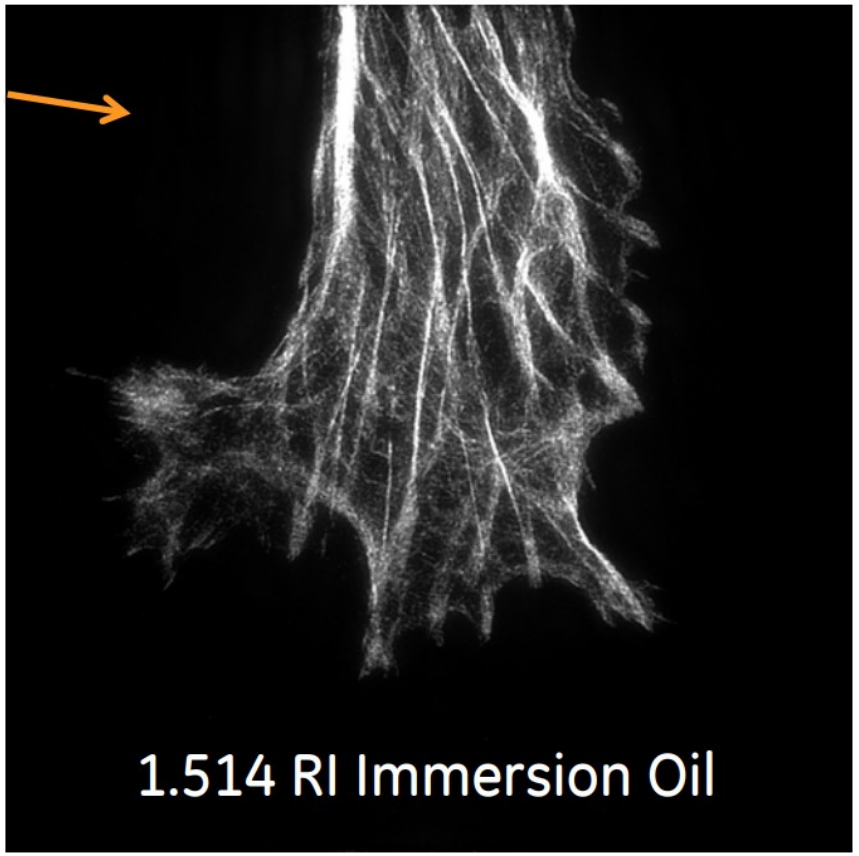
Out of focus light from mismatched PSF is considered a real signal and reconstructed.

immersion oil
refractive index mismatch

Mismatched oil



Matched oil

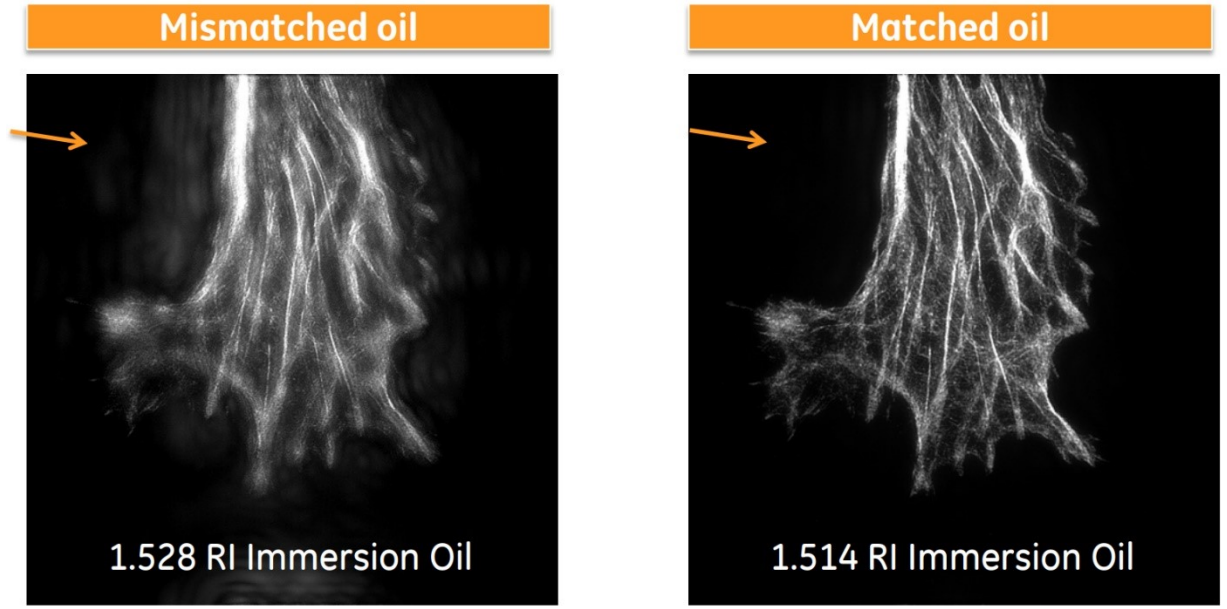


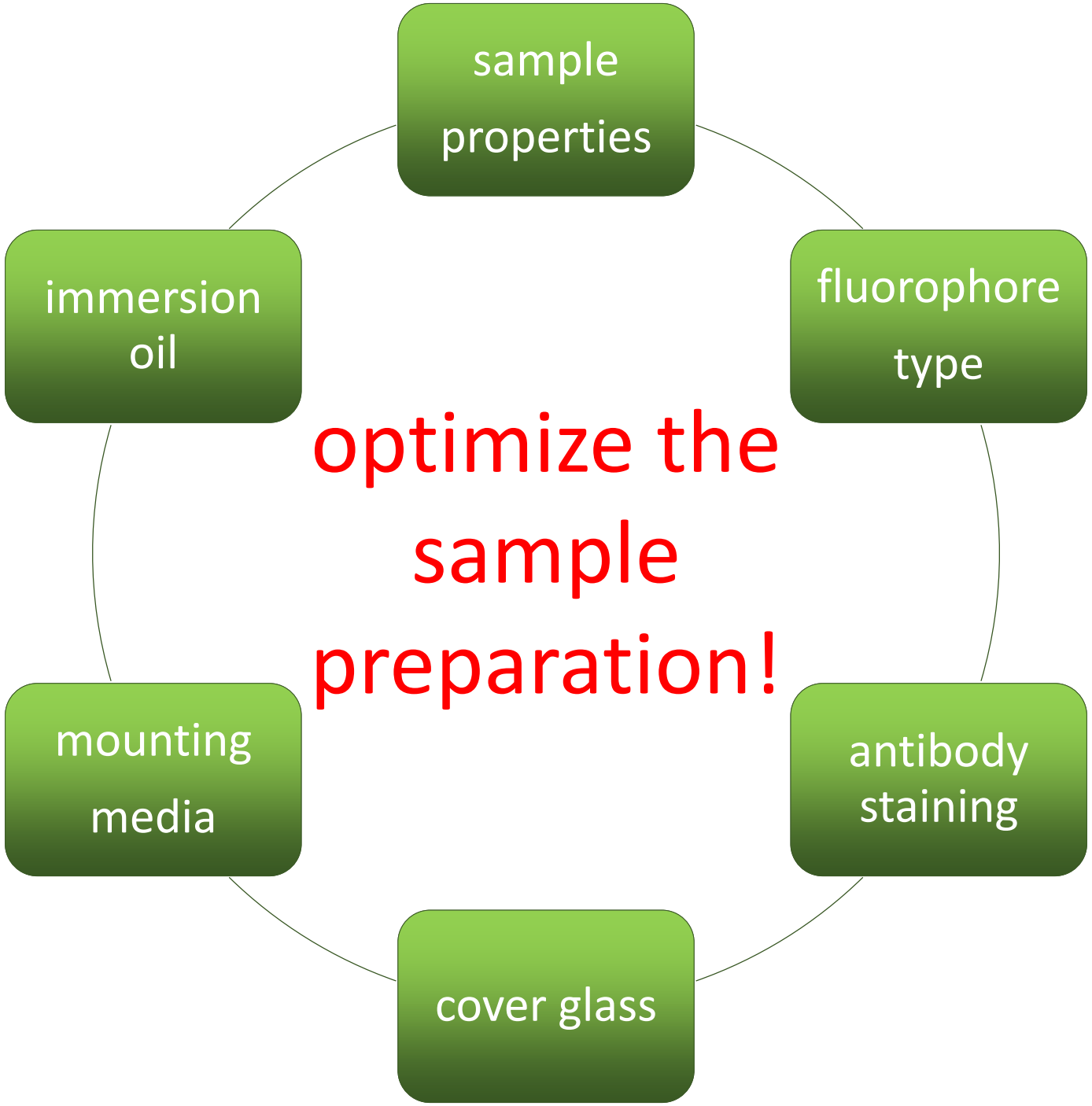
immersion oil
refractive index mismatch

multi-color samples
- the oil matched only for 1 λ
- suboptimal oil matching will create artifacts

⬇

compromize or choose one λ

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optimize the sample preparation!

praqolab

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Makers of the Huygens Software

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