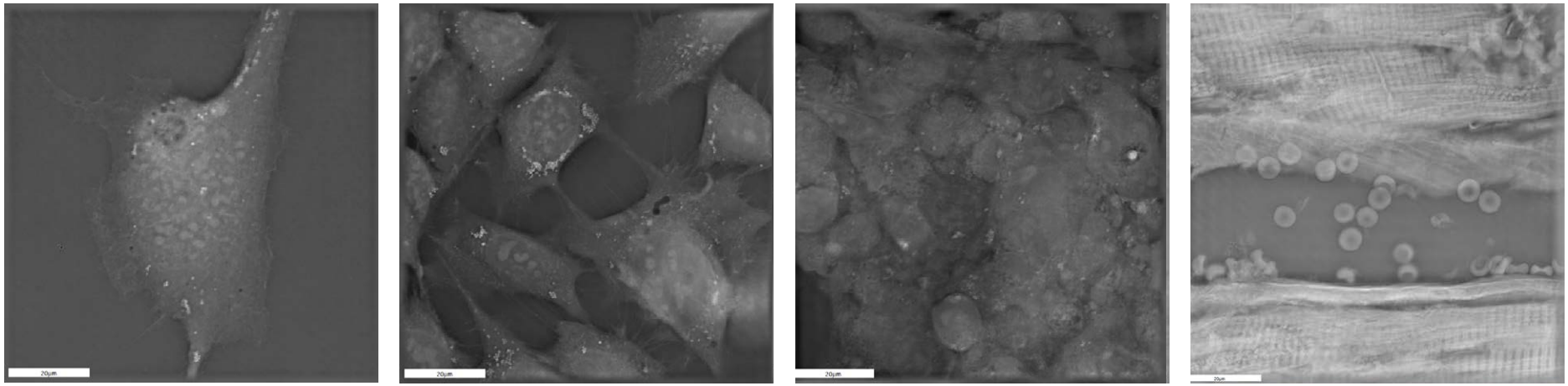
NANOLIVE
Looking inside life

Rules of Thumb

7 RULES OF THUMB FOR SAMPLE PREPARATION

1. We do cell imaging *in vitro*:
   single live & fixed cells, live & fixed cell cultures at low & high confluency (< 25 µm thick), tissue slices (~ 10 µm thick).



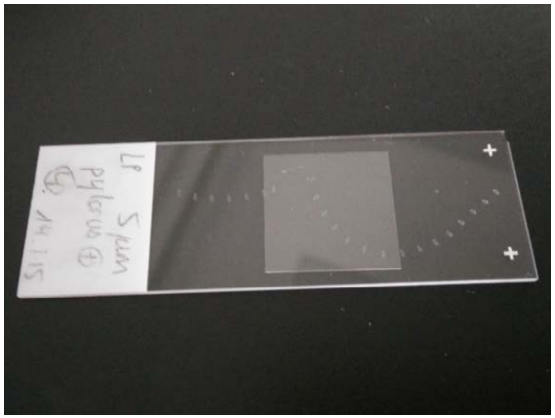
Cells 2D refractive index maps obtained with the 3D Cell Explorer. From left: single live cell, cell culture, high confluent cell culture, mouse miocardial tissue.

2. Image your cells on optically transparent dishes or coverslips (130-170 µm thick bottom).

Picture of typical culture dish

Schematic view of culture dish with critical bottom surface

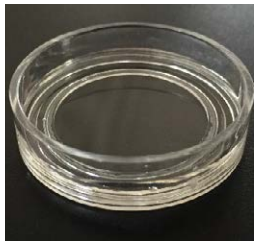
Thickness of the critical bottom surface: 130 - 170 µm



Picture of typical slide with coverslip

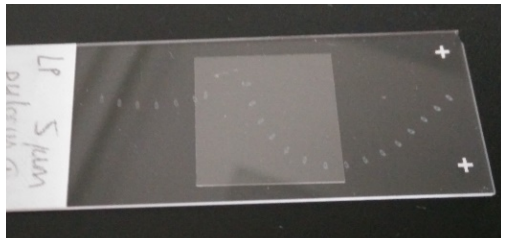
Schematic view of slide (top) and coverslip (bottom where cells are adhering) with critical bottom surface

3. Keep it clean & check visually that the surfaces are proper and transparent.



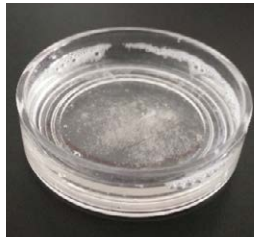
Picture of proper culture dish

White light view of proper sample

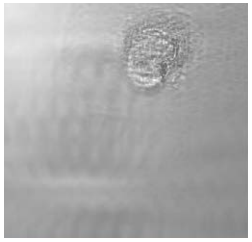


Picture of proper slide with coverslip

Schematic view of proper culture dish



Unobservable dirty culture dish



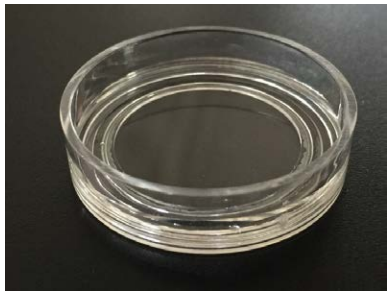
White light view of unobservable dirty sample

Unobservable dirty slide with coverslip

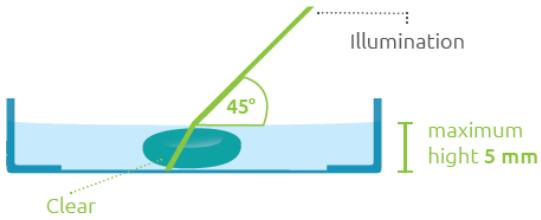
Schematic view of unobservable dirty culture dish with typical sources of dirt

4. Image your cells in aqueous media (dry samples can not be imaged)

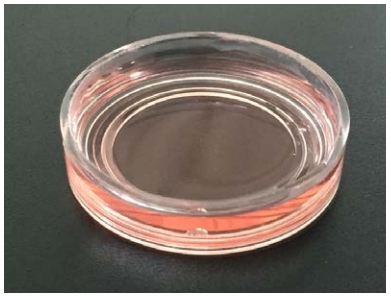
5. No scattering mounting medium is preferred. There are exceptions (e.g. phenol red media and agar).



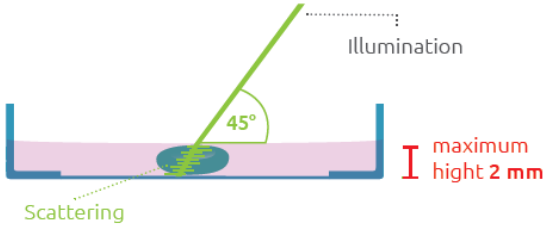
Picture of culture dish
with transparent medium



Schematic view of beam illumination in
transparent medium

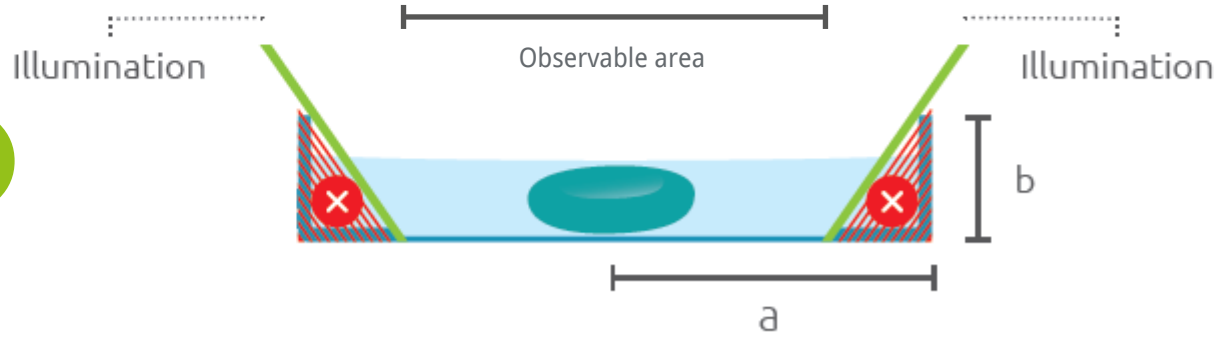


Picture of culture dish
with scattering medium



Schematic view of beam illumination in
scattering medium

6. Be aware of the critical well /dish aspect ratio.

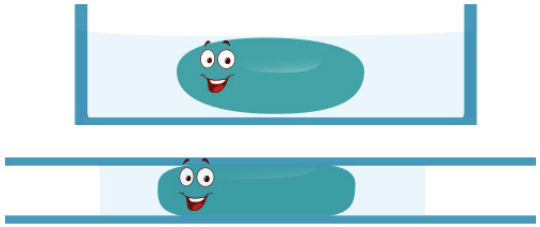


$$\frac{a}{b} > 1$$

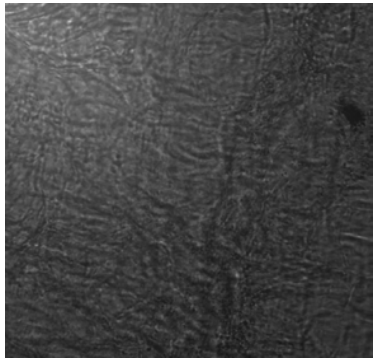
Critical aspect ratio for observable area

7. Avoid too thick (>25um) or too scattering/absorbing samples

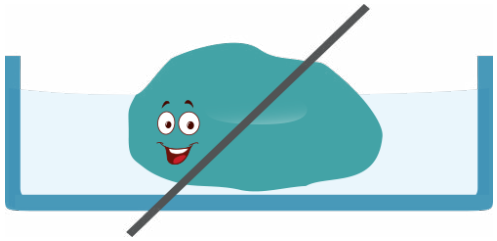
White light view of transparent cell



Schematic view of transparent cell



White light view of unobservable absorbing sample

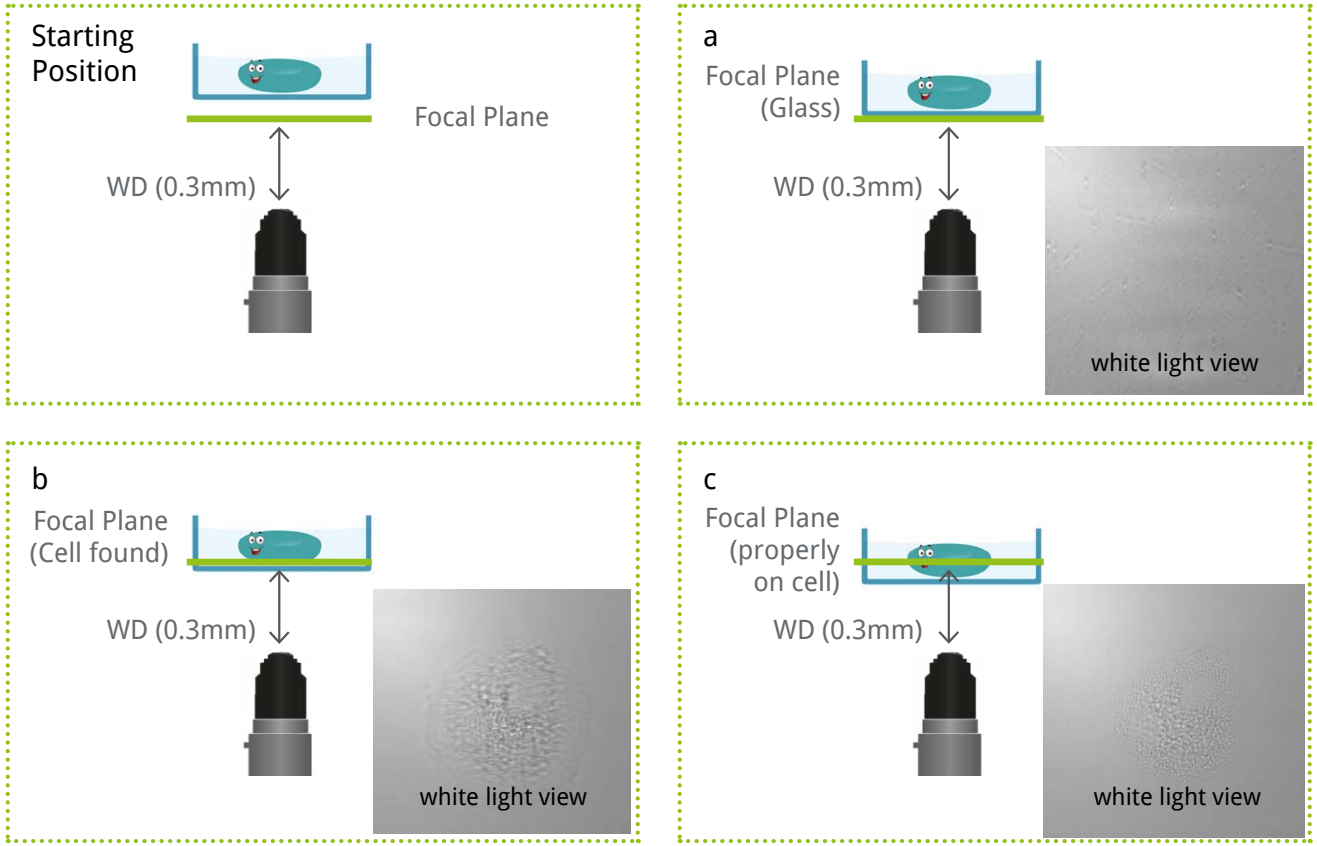


Schematic view of unobservable thick (>25um) cell

3 RULES OF THUMB FOR LIVE CELL IMAGING

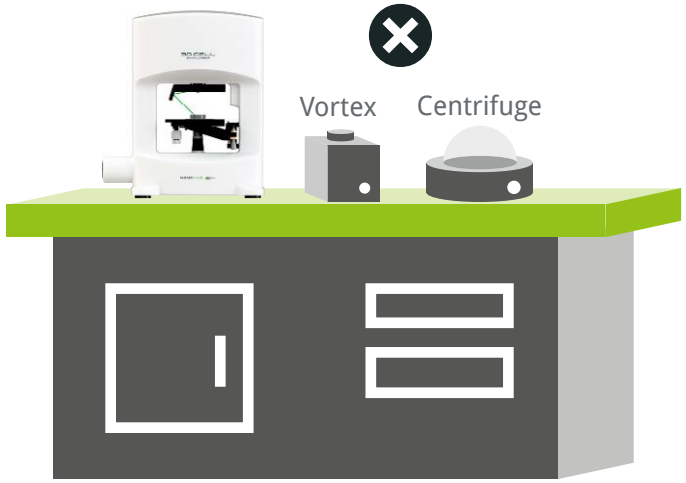
1. Focus on your cells (the Working Distance (WD) of the microscope objective is 0.3 mm)
   a. Find the bottom surface of your dish from the starting position (>0.5mm): use the z-knob to move the stage downwards until you find the first glass surface (Figure a)
   b. Keep moving down for 130 - 170 µm until you find your cells (Figure b)
   c. Put them in focus (tip: the less you see your cell the closer you are to the focus) (Figure c)

Pictures of XYZ stage on 3D Cell Explorer

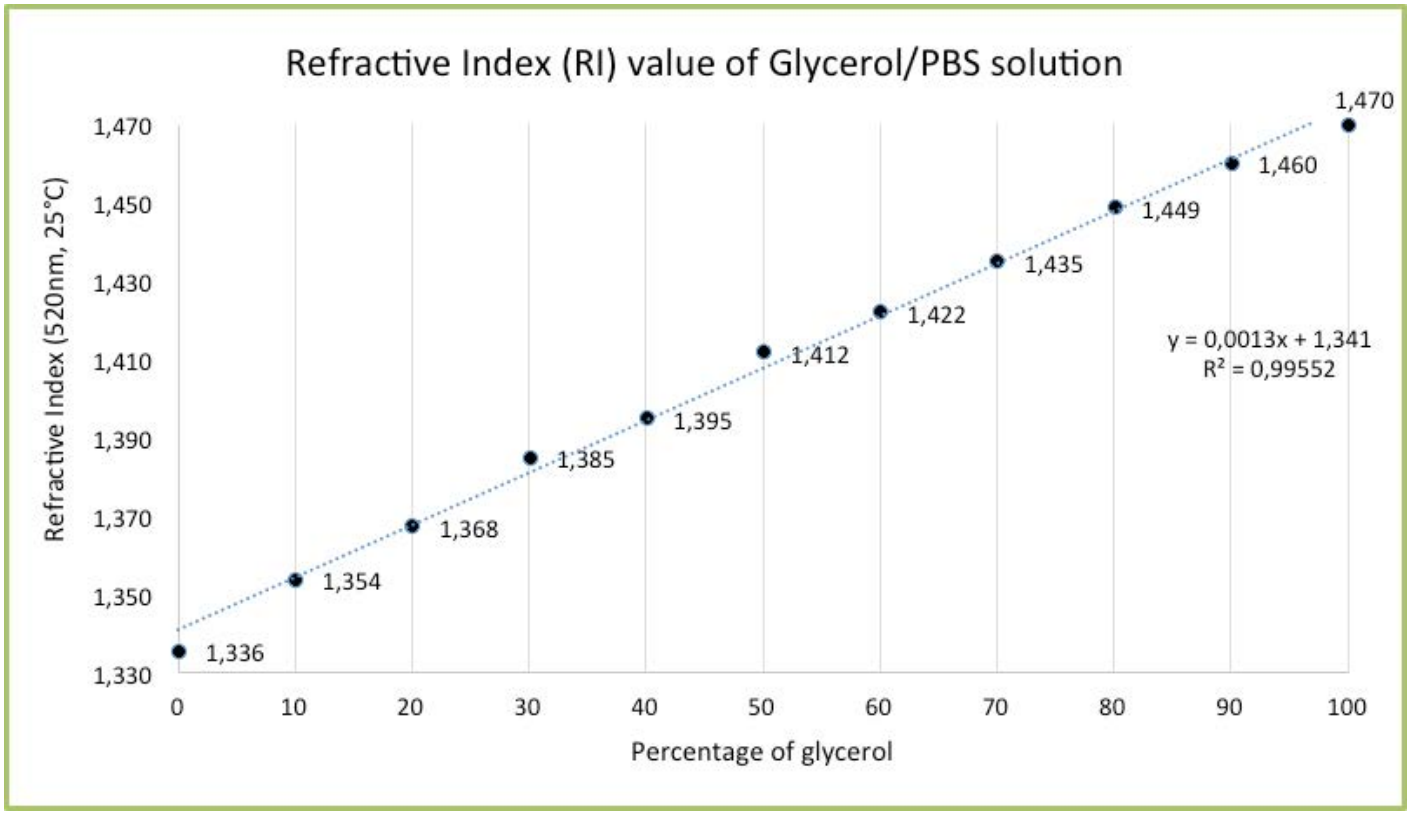


2. Place on a flat and solid surface.

3. Avoid vibration sources on the same working surface.



Appendix



Graph 1. Refractive Index (RI) value of Glycerol/PBS solution

Glycerol reference: G5516 – Sigma Aldrich
Instrument: AR4 Series Abbe Refractometer (KRUSS OPTRONIC)