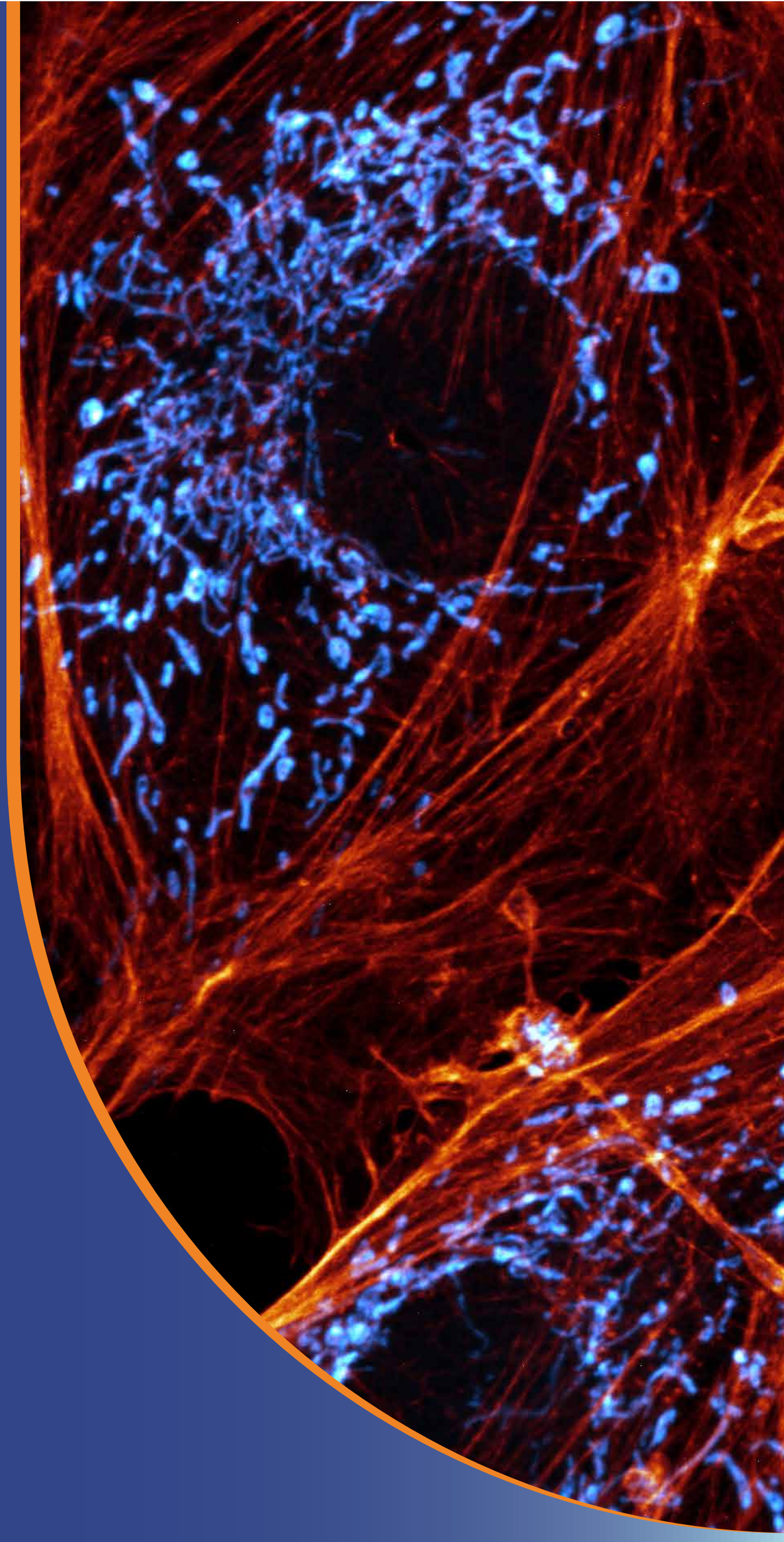
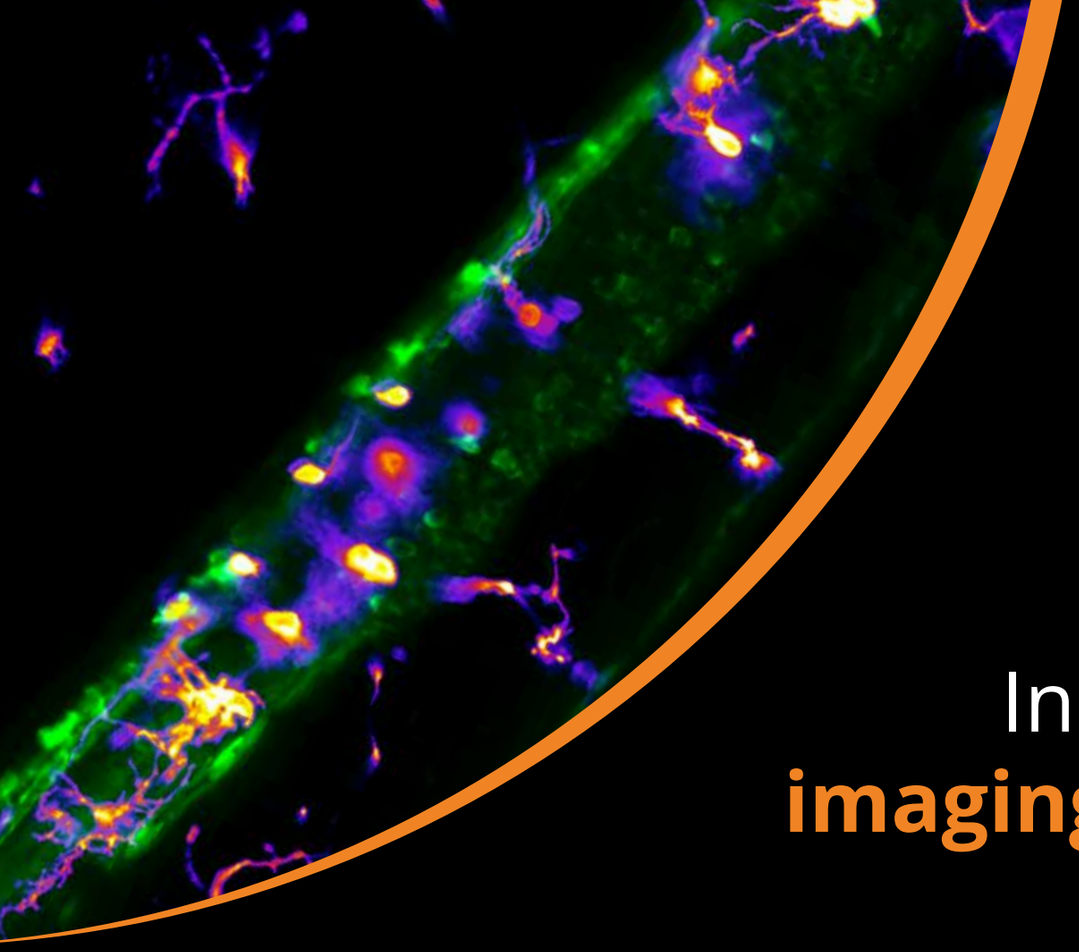
Confocal nl

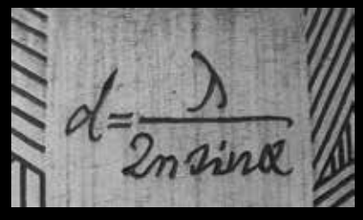


DEEP LIVE CELL IMAGING IN SUPER RESOLUTION



Super resolution

In theory, super resolution (**SR**) is **imaging beyond the diffraction limit** (d), defined by Abbe's law as:

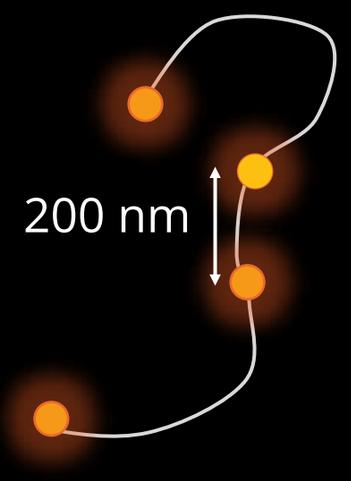
$$d = \frac{\lambda}{2NA}$$ or 

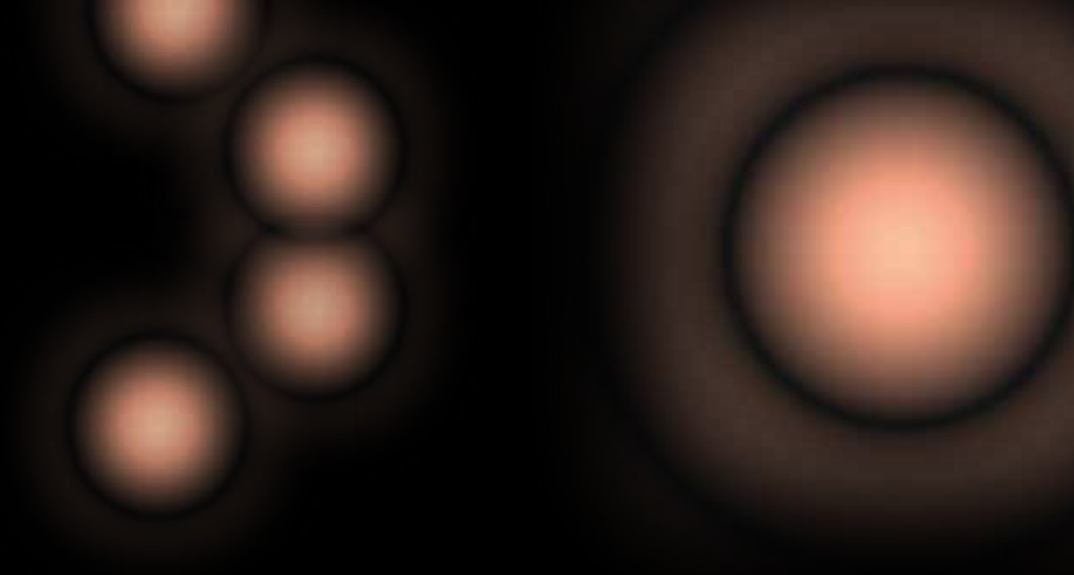
Ernst Abbe memorial Jena, Germany

Where d is the minimum resolvable distance, λ is the wavelength, NA is the numerical aperture, n is the refractive index and α is the maximum collection angle.

In practice, SR techniques are those that provide **resolution better than 200 nm...**

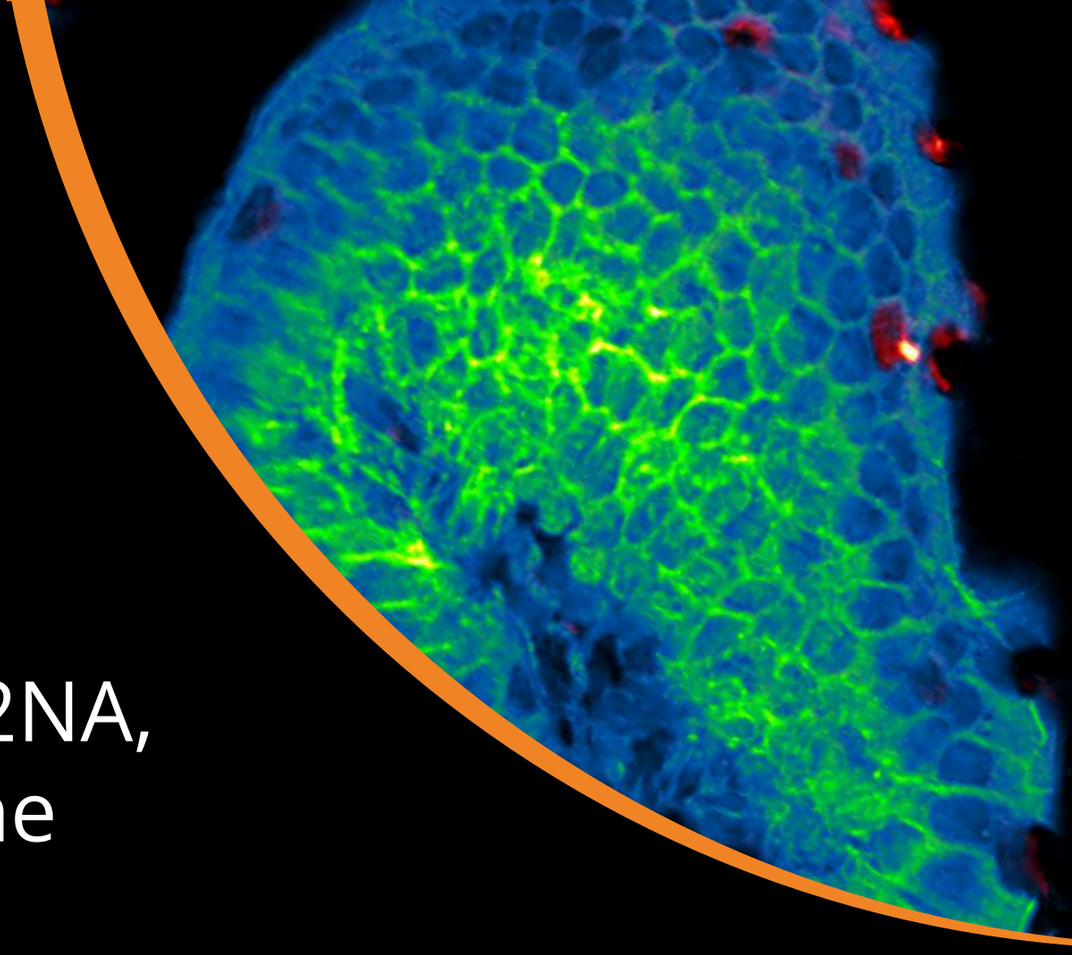
Sample | Super resolved image | Diffraction limited image



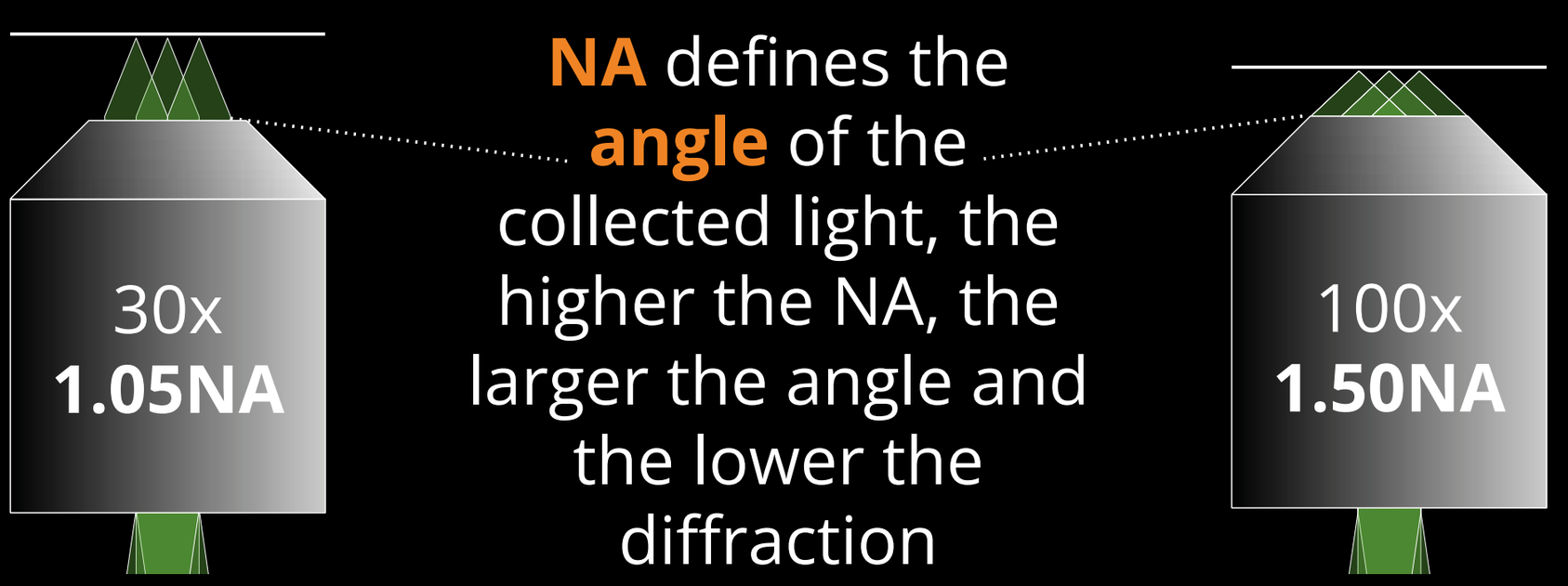


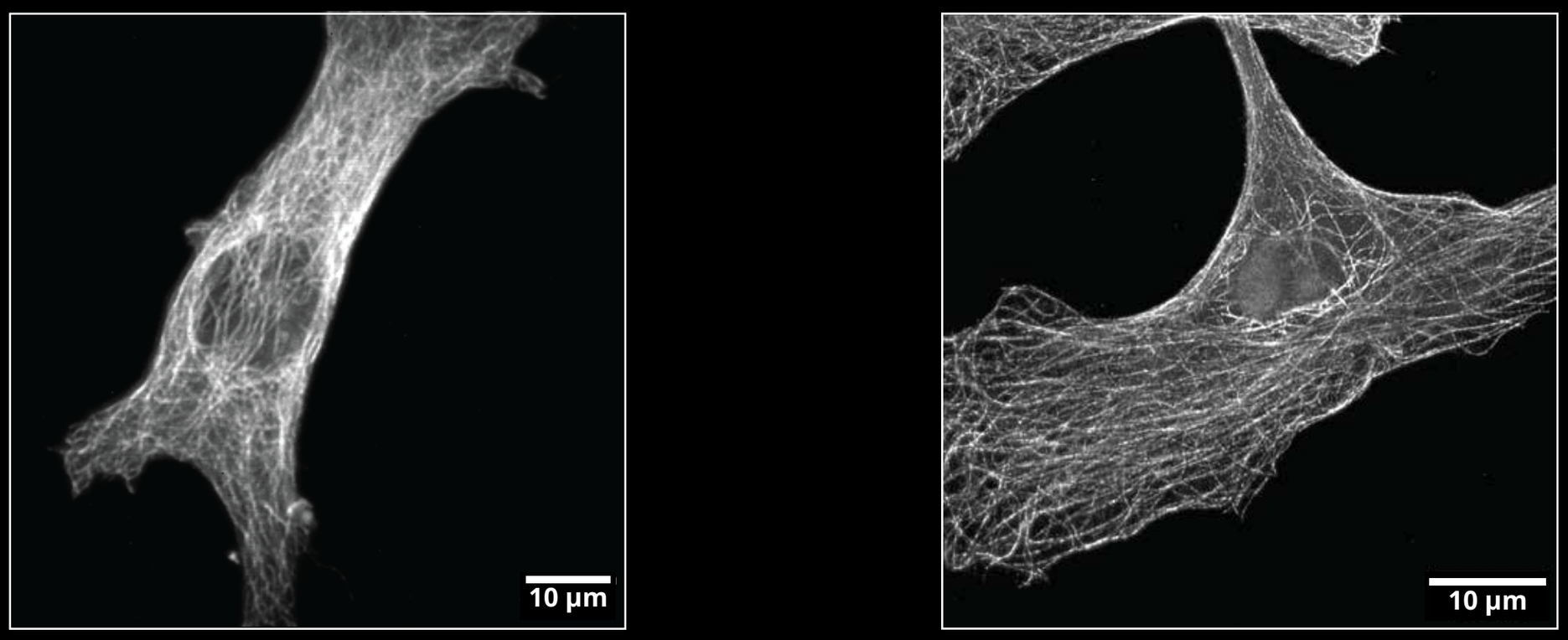
...when imaged with an objective with high NA and refractive index of oil. Usually, these objectives have high magnification and short working distance (WD).

SR dependent on NA

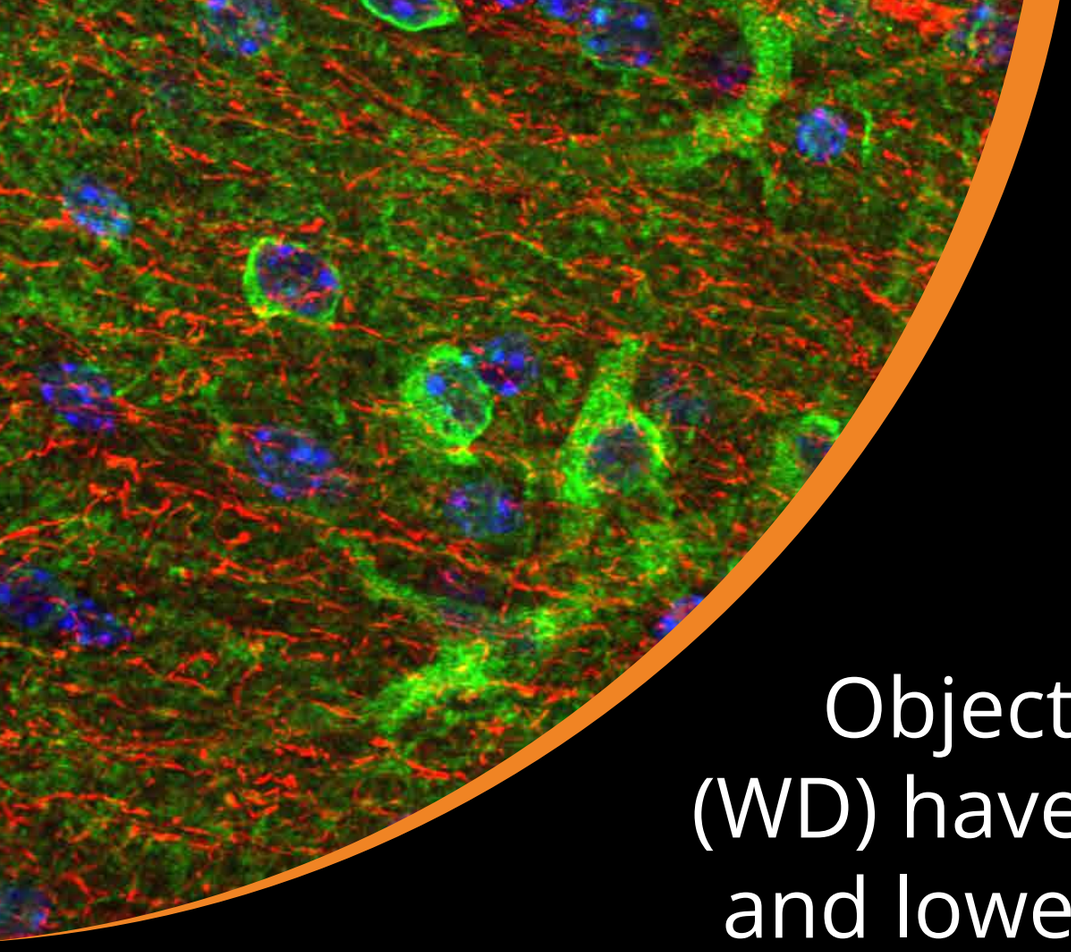


According to Abbe’s law, d = λ/2NA, the higher the NA, the better the resolution.



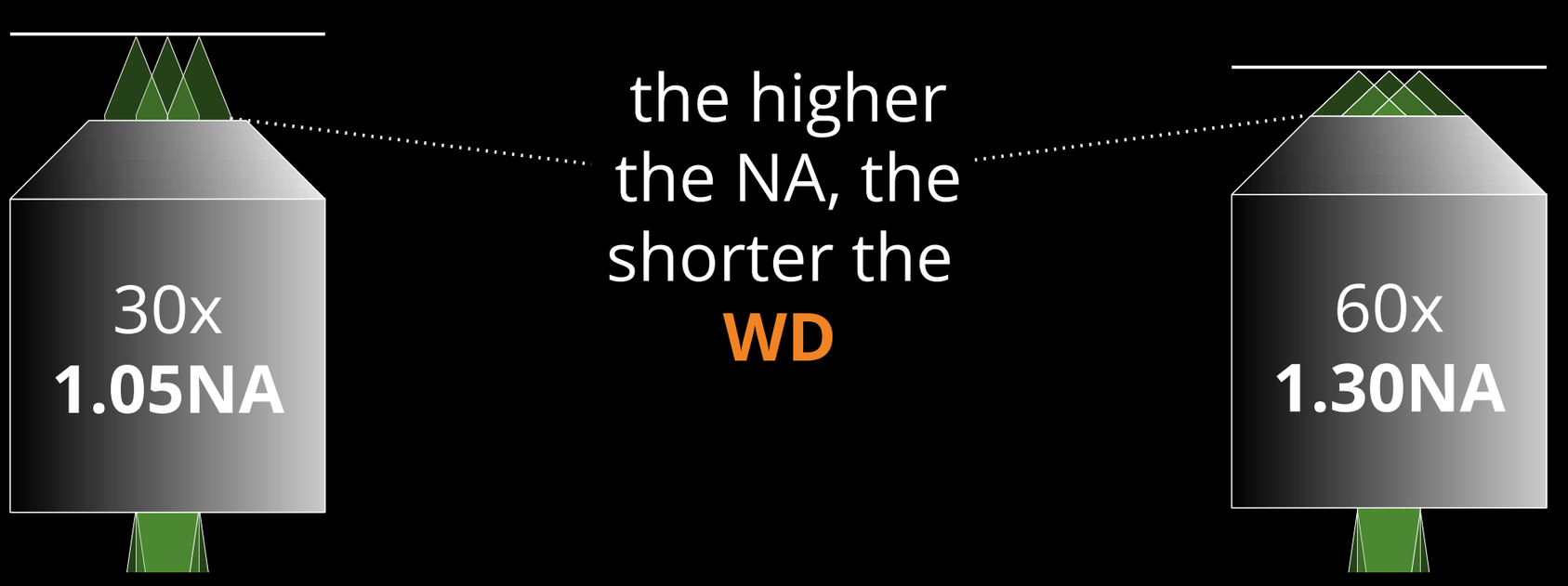


Fibroblasts imaged in SR using Point *RE*scan GAIA



Depth of SR dependent on WD

Objectives with long working distance (WD) have in general lower magnification and lower NA than those with short WD.



0.8mmWD

0.3mmWD

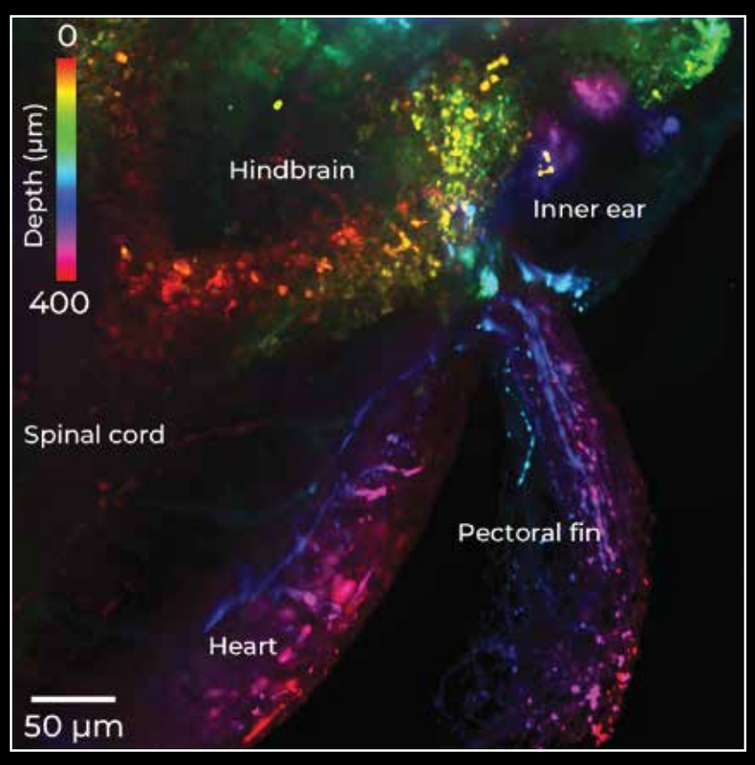


Image depth 400 µm

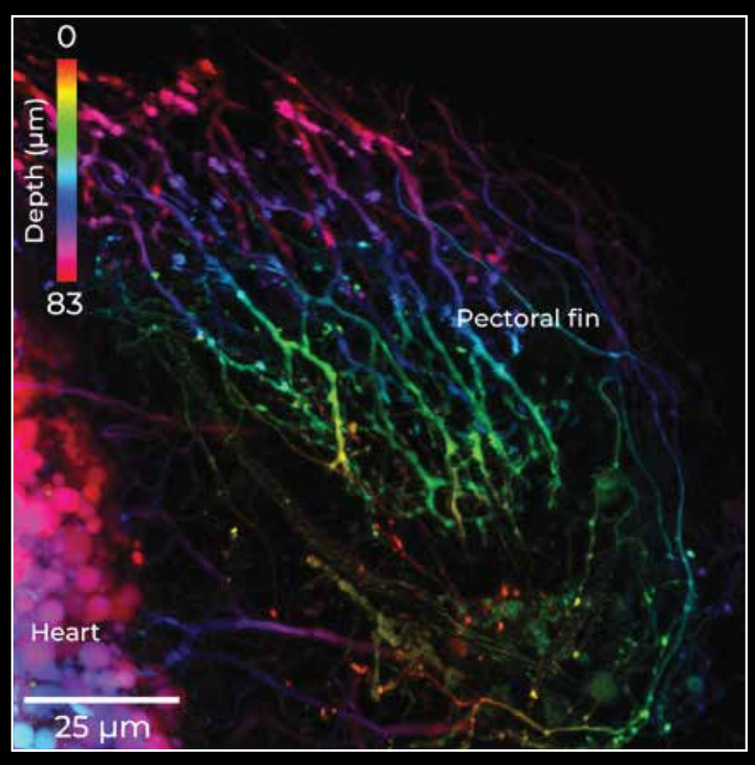
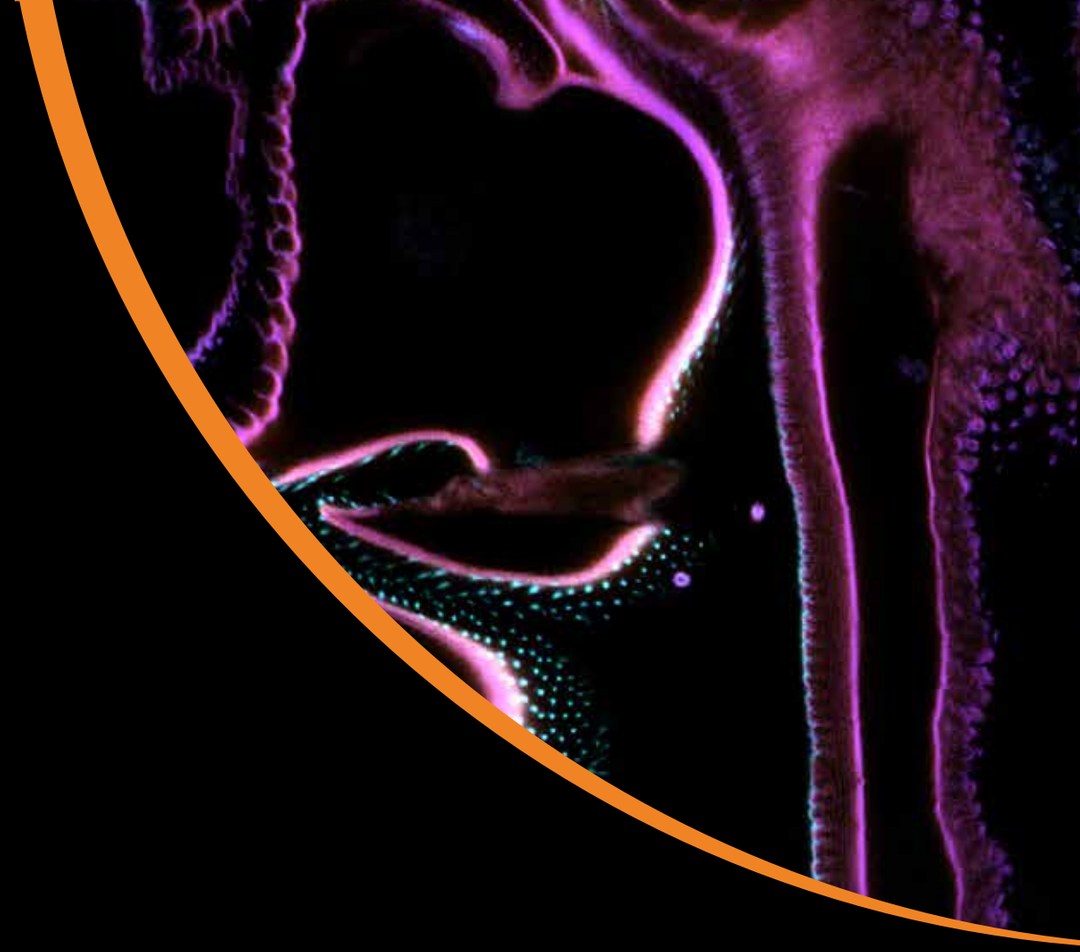


Image depth 83 µm

Living zebrafish imaged in SR using Point *RE*scan GAIA

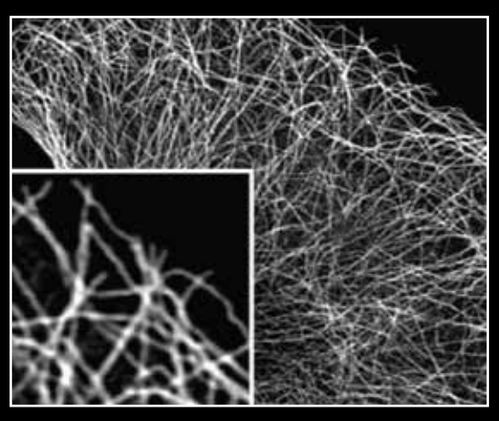
Types of SR techniques



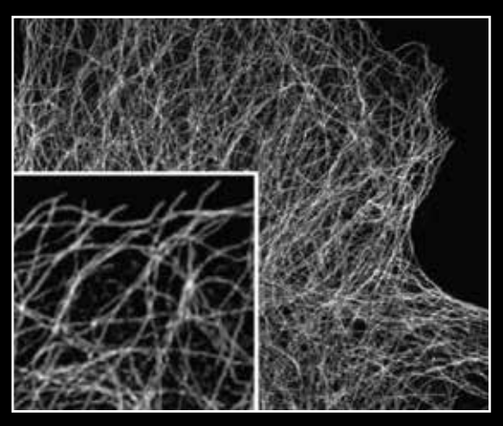
Based on resolution, there are two groups of SR techniques:

1. Providing resolution 100-200 nm:

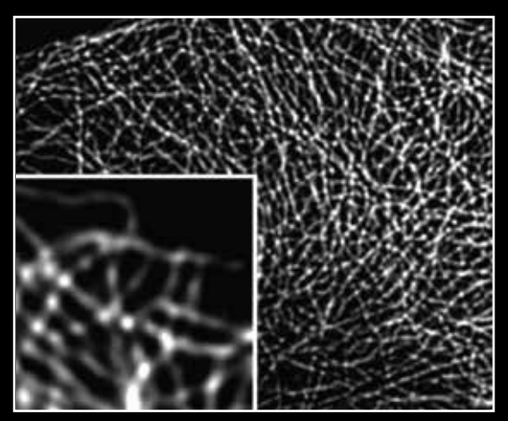
PR (Pixel reassignment)
d = 120-150 nm



SIM
d = 100-130 nm

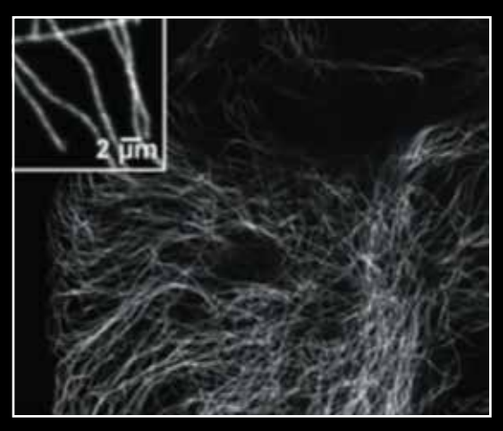


FB (Fluctuation based)
d = ca. 150 nm

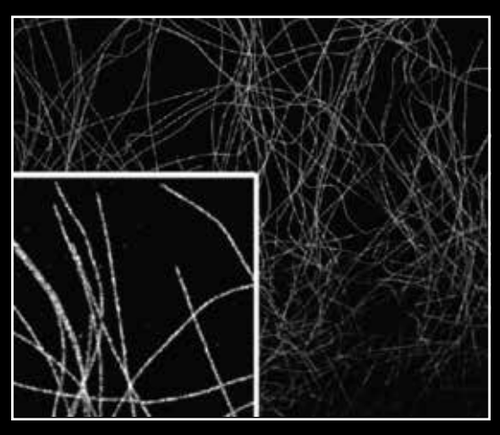


2. Providing resolution beyond 100 nm:

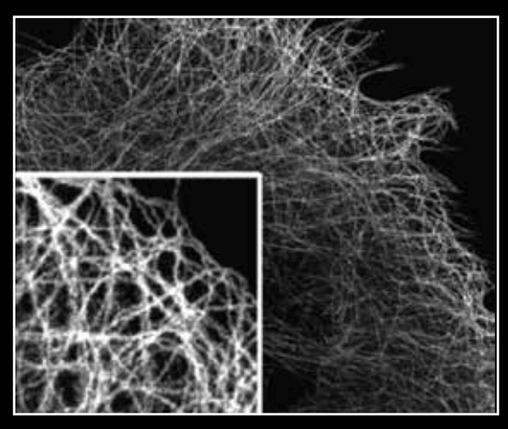
ExM (Expansion microscopy)
d = 50 nm



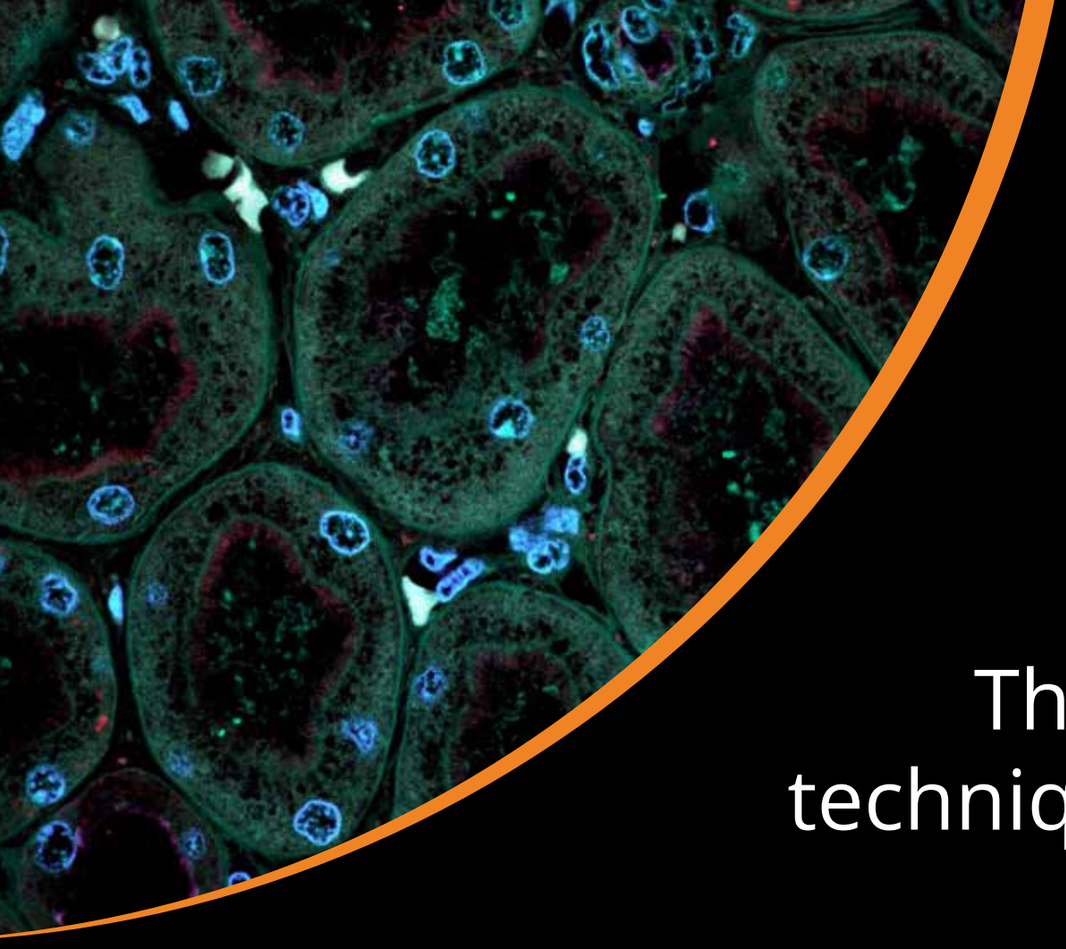
SMLM
d = 10-20 nm



STED
d = 50-100 nm



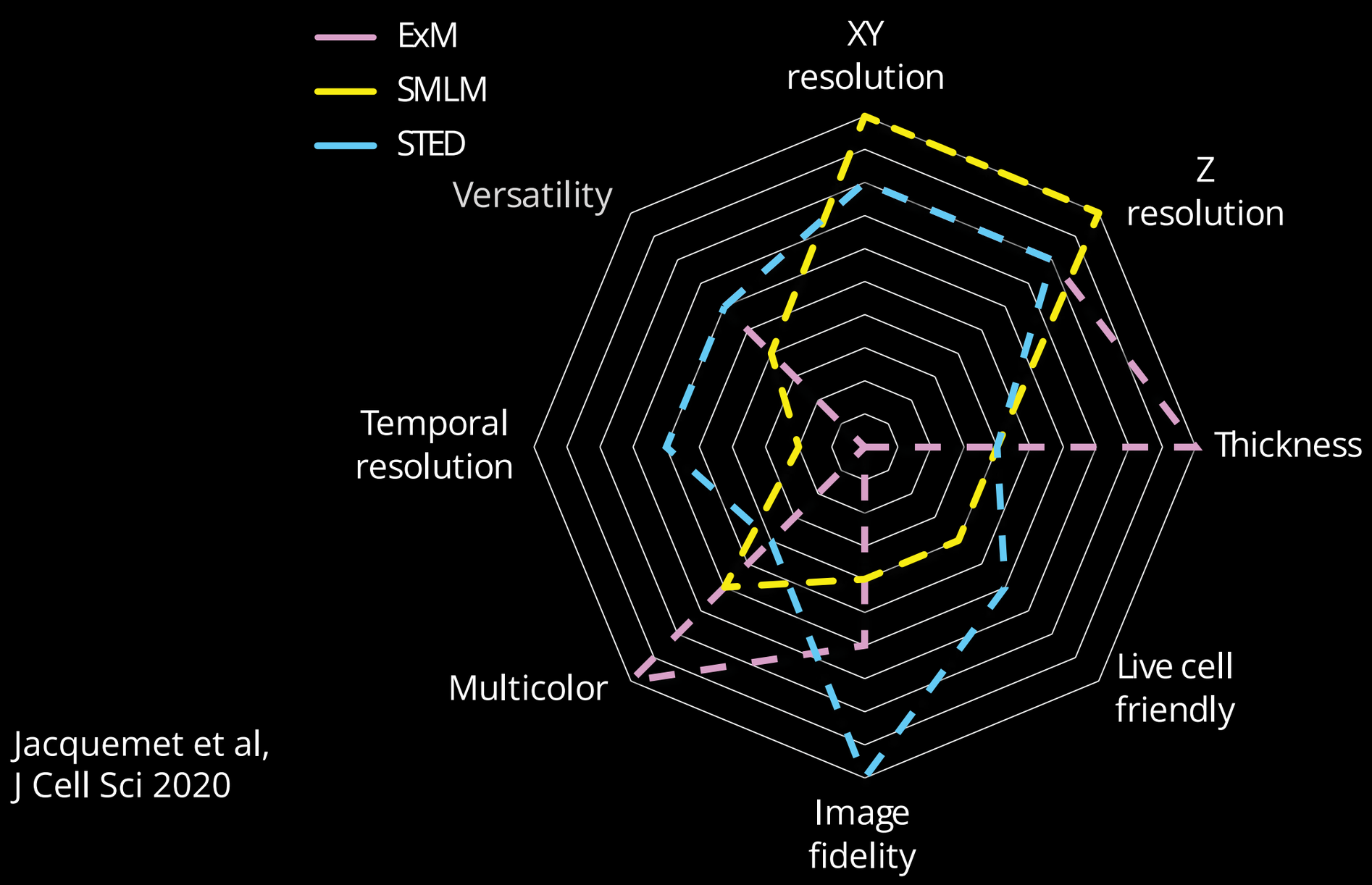
Jacquemet et al, J Cell Sci 2020
Zhang et al, Curr Protoc Neurosci 2020



SR techniques not suitable for living cells

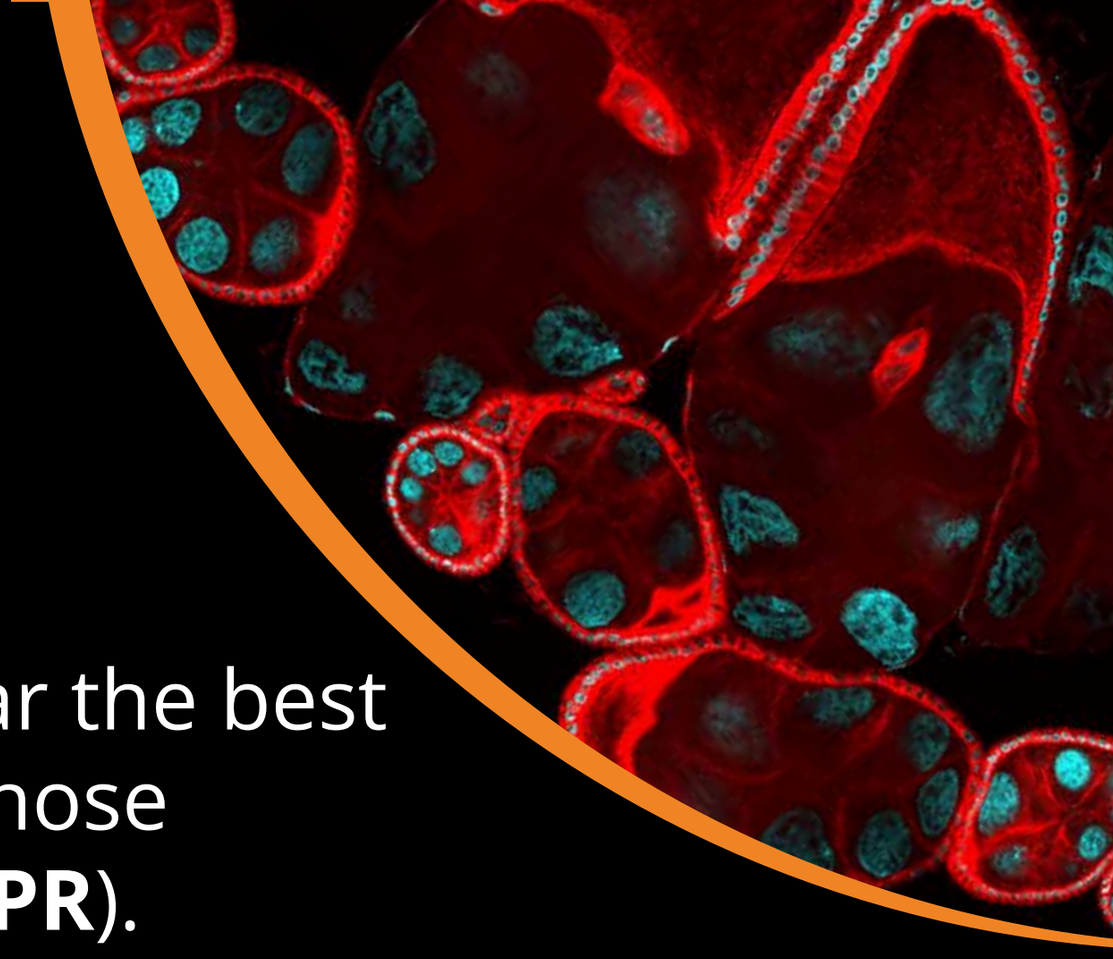
The better the resolution of an SR technique, the less suitable for live cell imaging that technique is.

	Live cell friendly
ExM	0/5, impossible due to the sample prep
SMLM	2/5, almost impossible due to the sample prep, long acquisition and high laser powers
STED	3/5, possible only over very short periods due to high laser powers



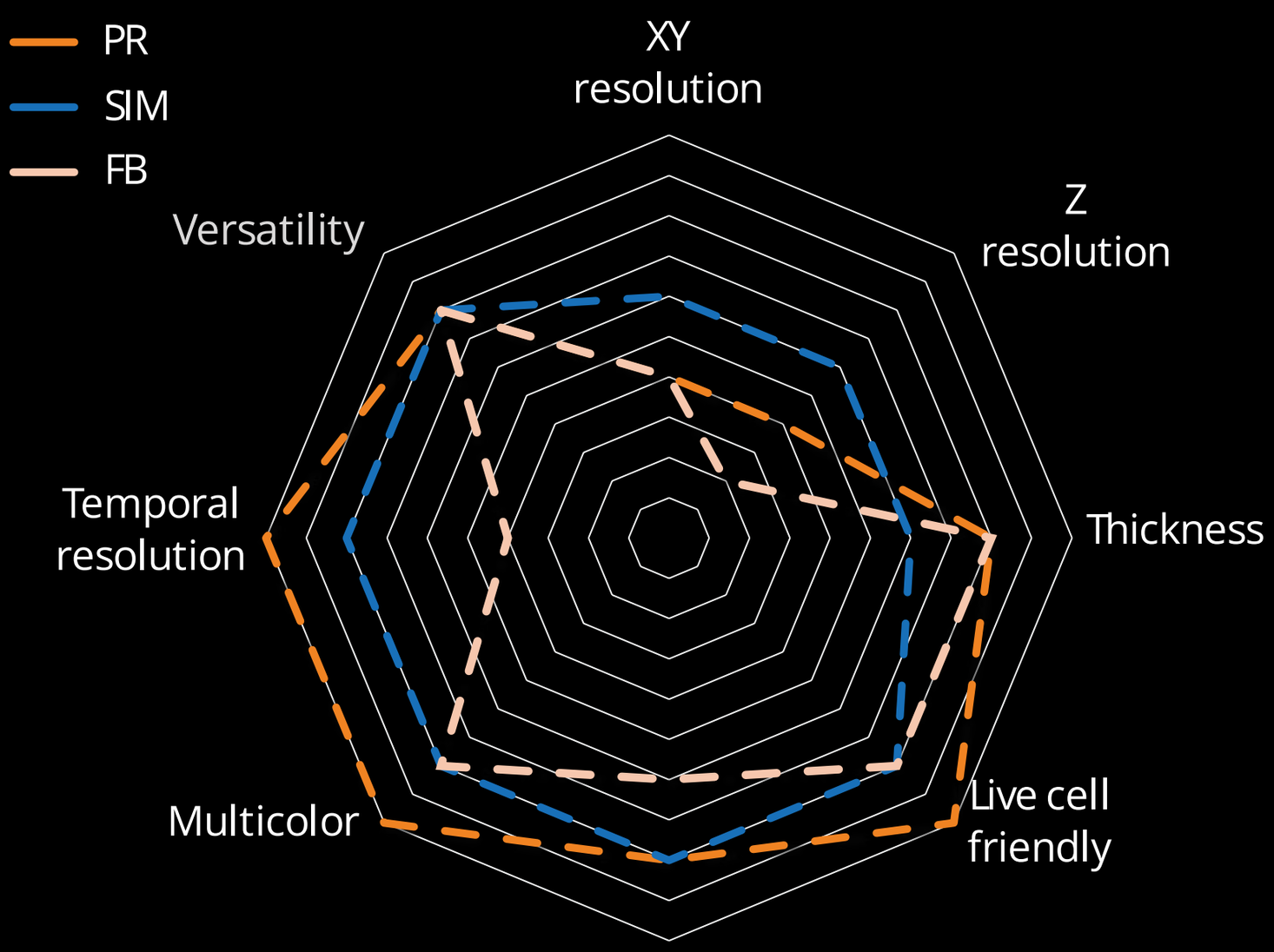
Jacquemet et al,
J Cell Sci 2020

SR techniques for live cell imaging

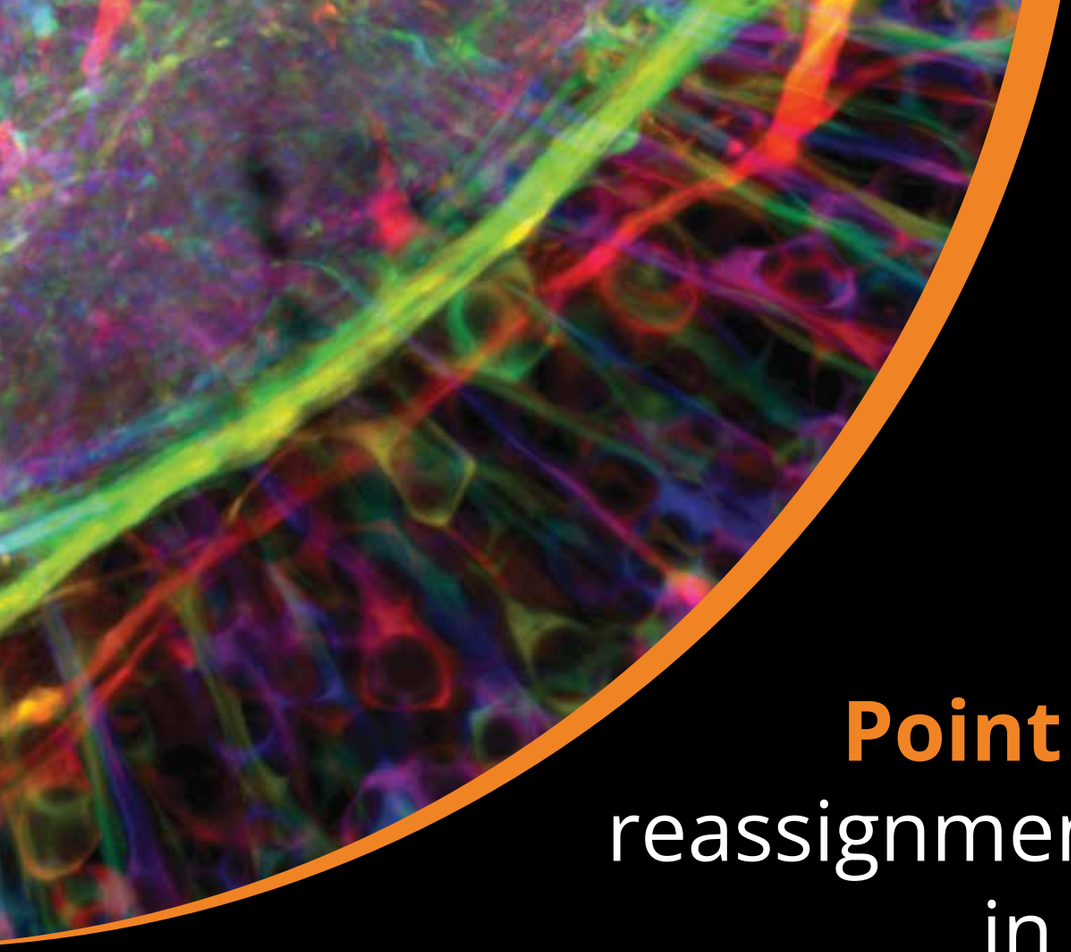


Among all SR techniques, by far the best ones for live cell imaging are those based on pixel reassignment (**PR**).

	Live cell friendly
PR	**5/5, best suitable for live cell imaging**
SIM	4/5, most common 3D SIM is more phototoxic than TIRF SIM that is incapable for deep imaging
FB	4/5, phototoxicity varies depending on the method (SOFI, SRRF)



Jacquemet et al,
J Cell Sci 2020

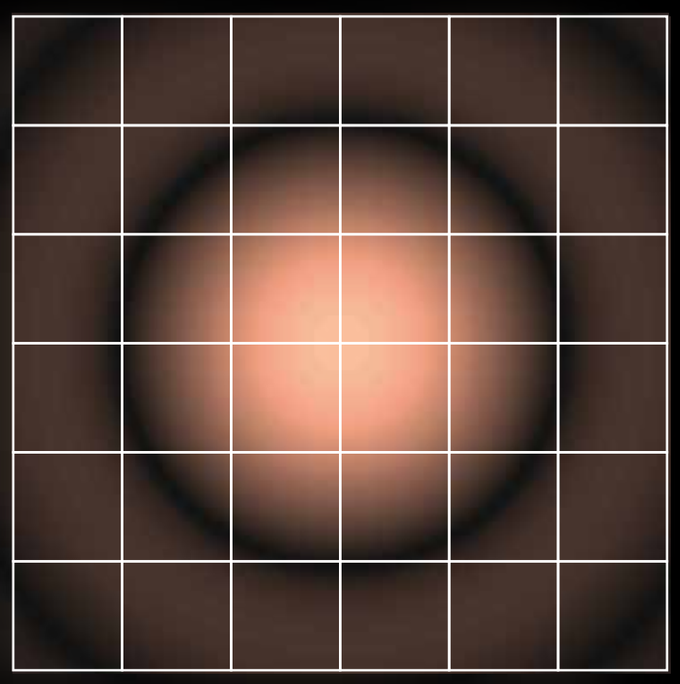


Deep live cell imaging using PR techniques

Point *RE*scan stands out among pixel reassignment techniques for its advantages in both deep and live cell imaging.

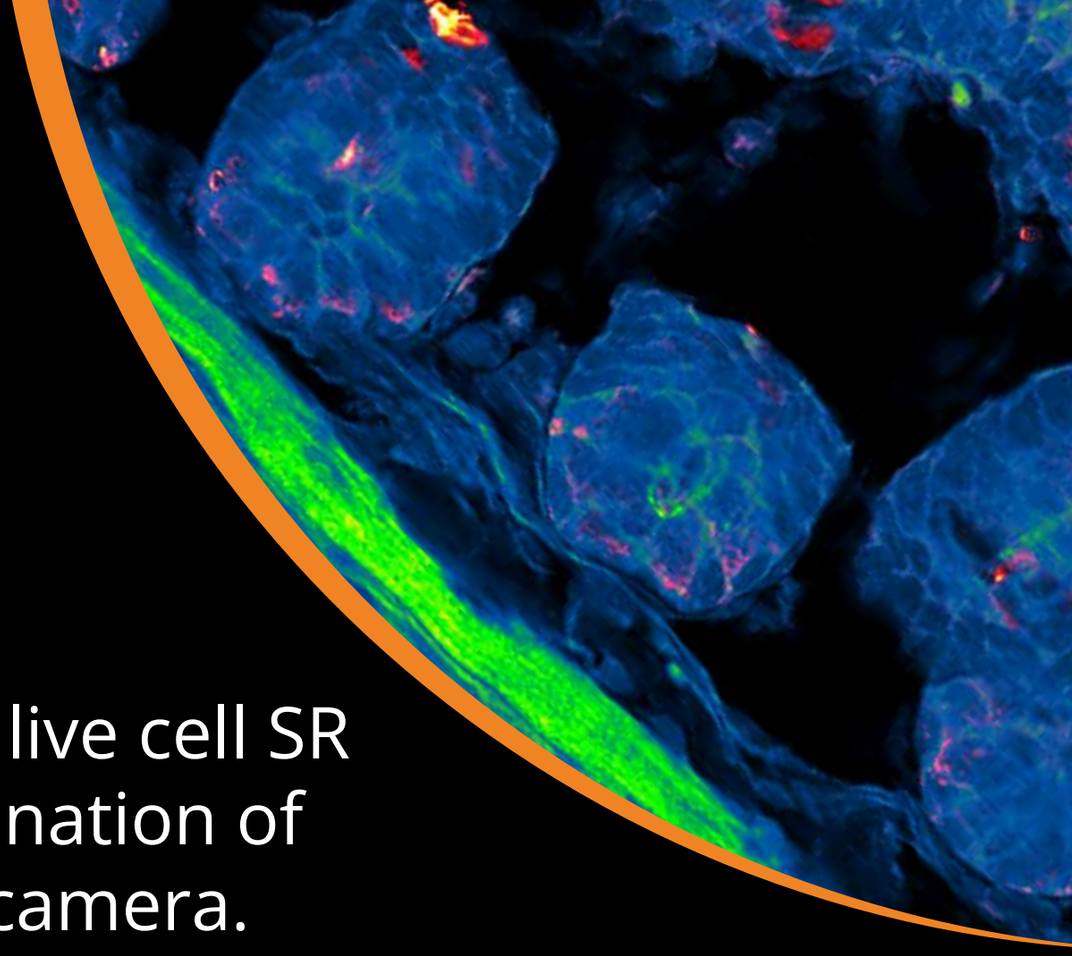
1. Being optimized for the widest range of objectives gives ***RE*scan advantage in deep imaging** as it can use objectives with longer WD.

2. More light through more open pinhole (≥1.5 AU) and higher quantum efficiency of a detector (96% QE) give ***RE*scan advantage in live cell imaging.**

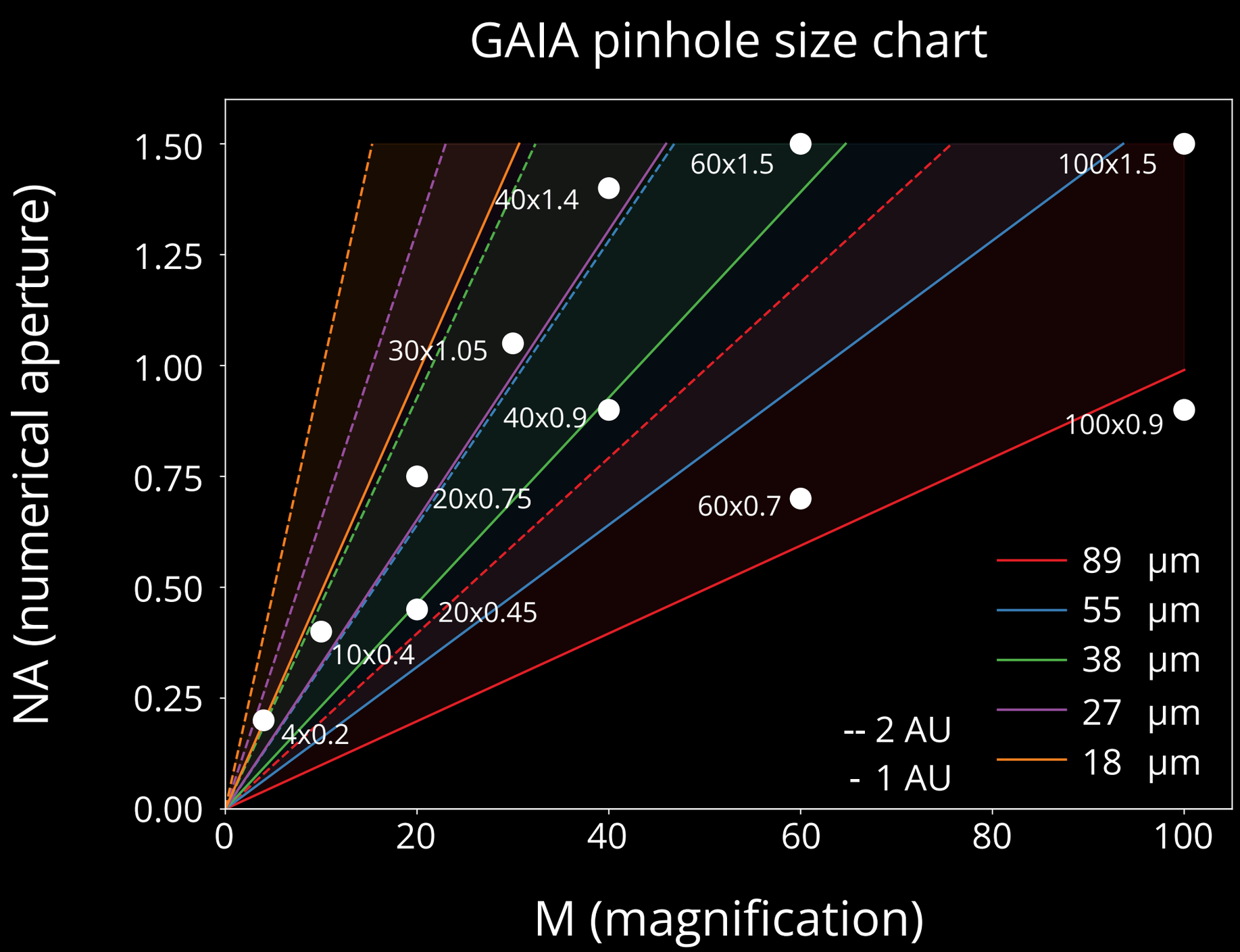


***RE*scan** collects more light without losing resolution.

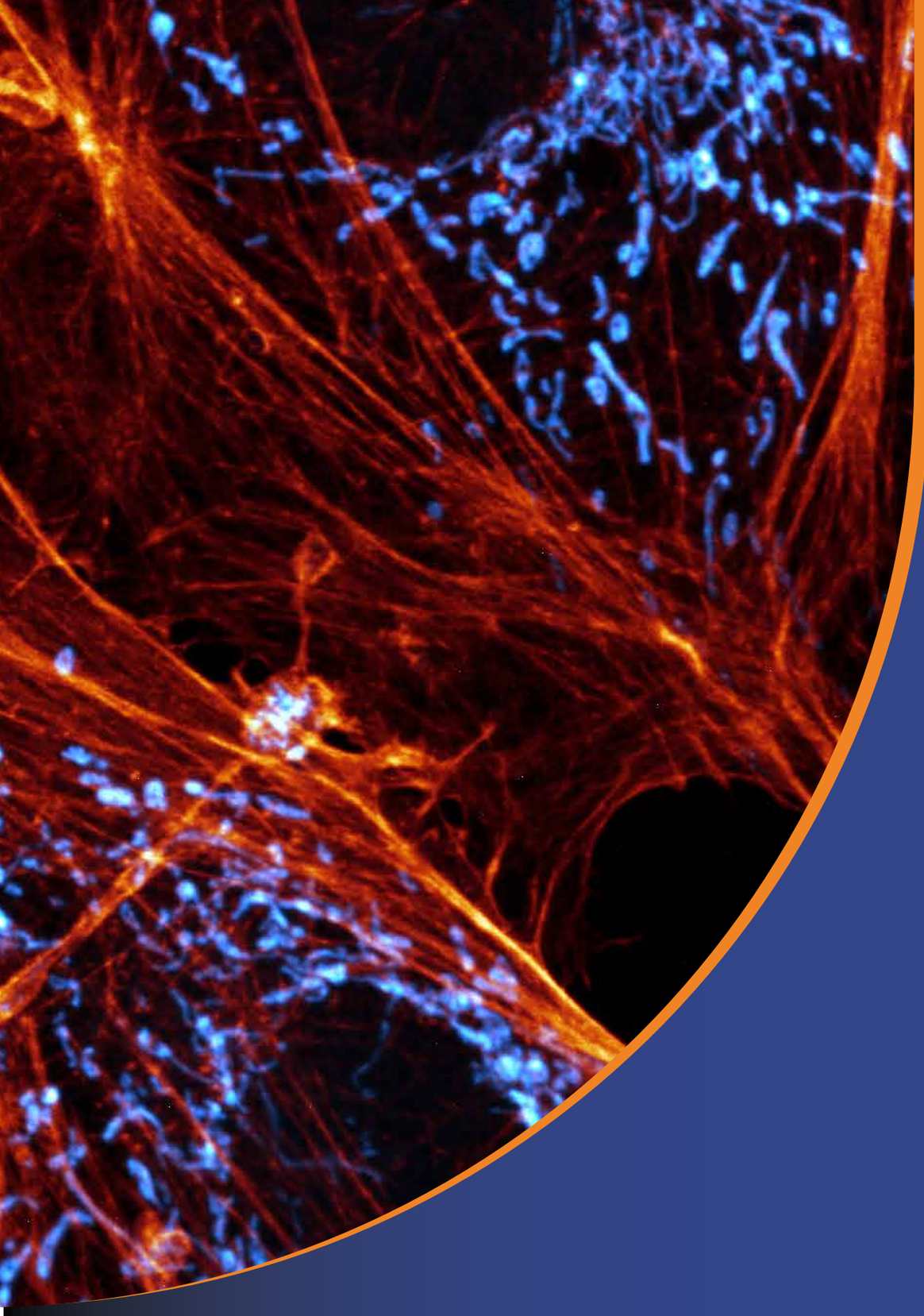
Deep live cell SR imaging



Solution to NA/WD problem in deep live cell SR imaging is finding the optimal combination of magnification, NA, pinhole size and camera.



GAIA is the solution that can provide live cell imaging in SR deeper than 500 µm in sample.



Confocal nl

Find out more at

www.confocal.nl

Achieve the ideal ratio of objective NA & magnification, and camera pixel size with our Line and Point *RE*scan systems. You can select the optimal pinhole size and image with a wide range of objectives (4x-100x).