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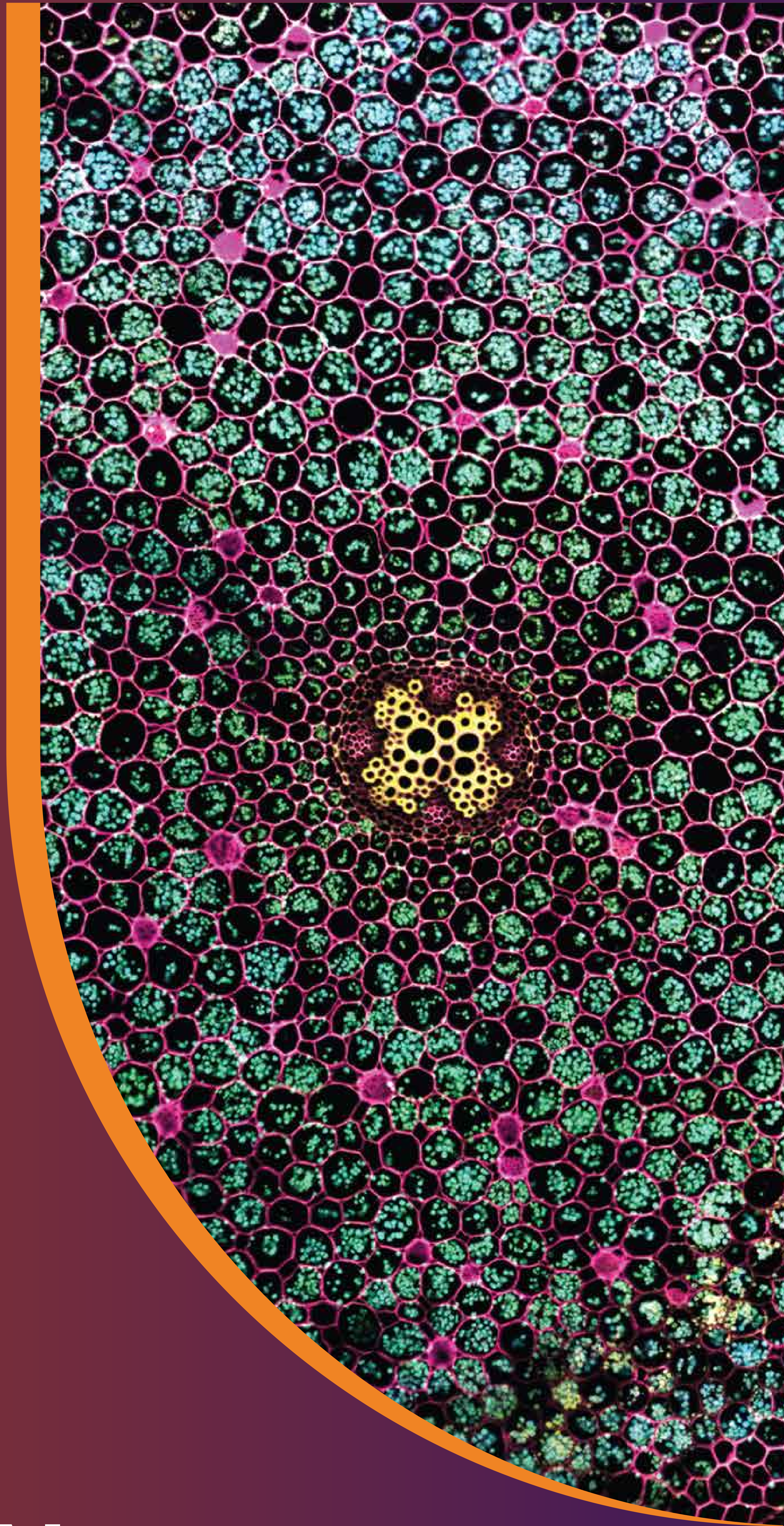
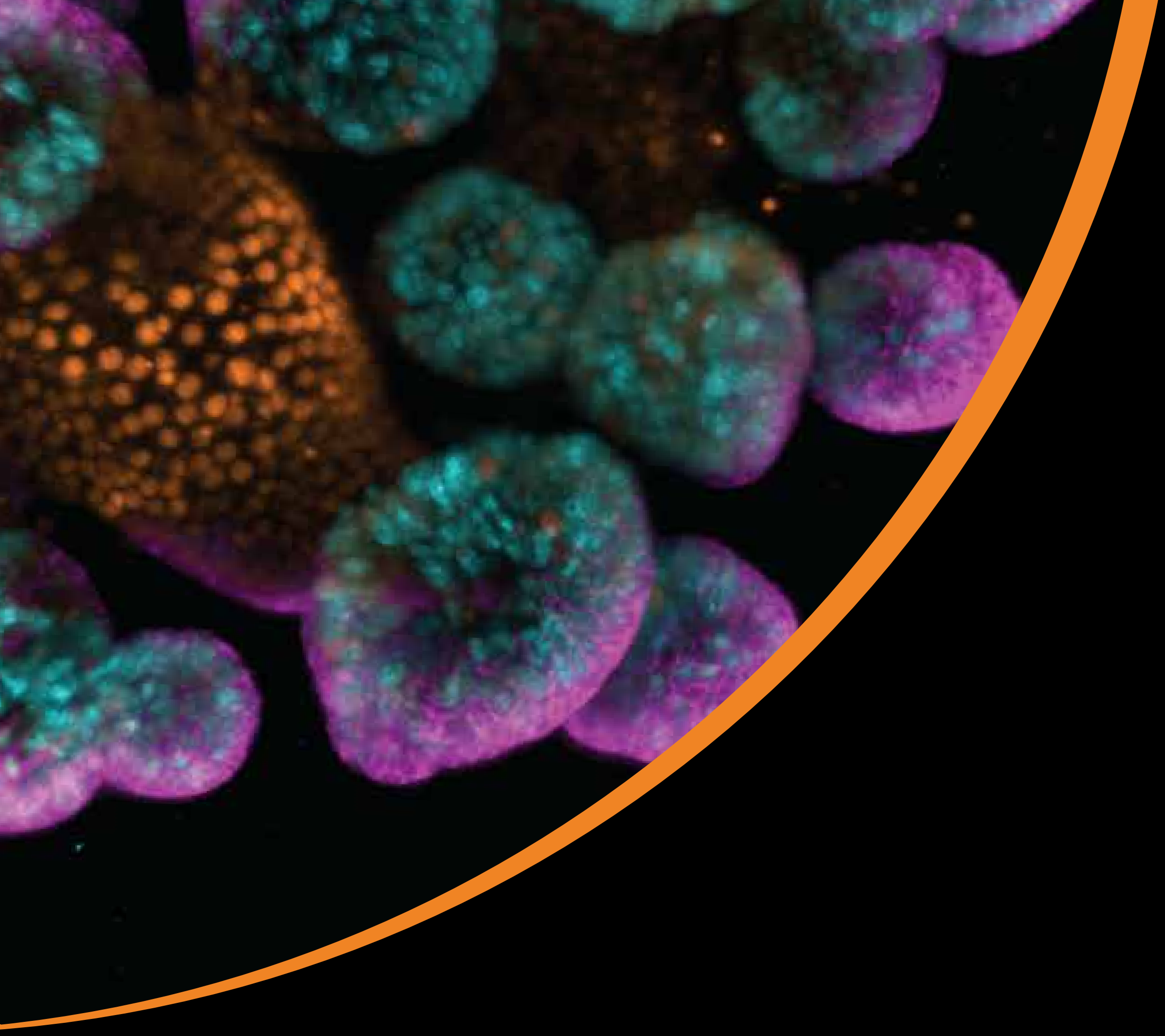


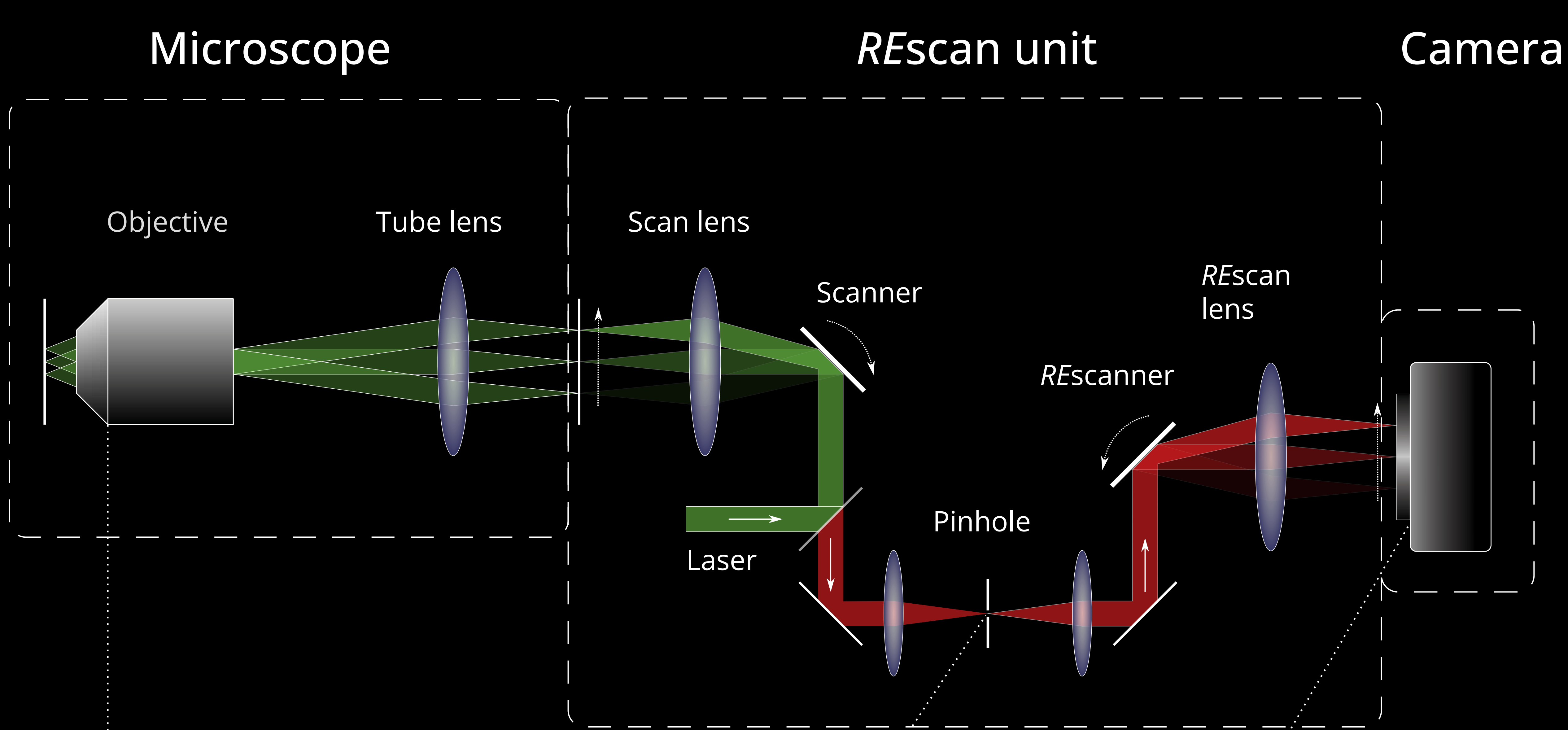
Quick Knowledge PART 4

RESCAN
NA
MAGNIFICATION
PINHOLE SIZE
PIXEL SIZE



*RE*scan confocal microscopy

The *RE*scan setup has three main parts



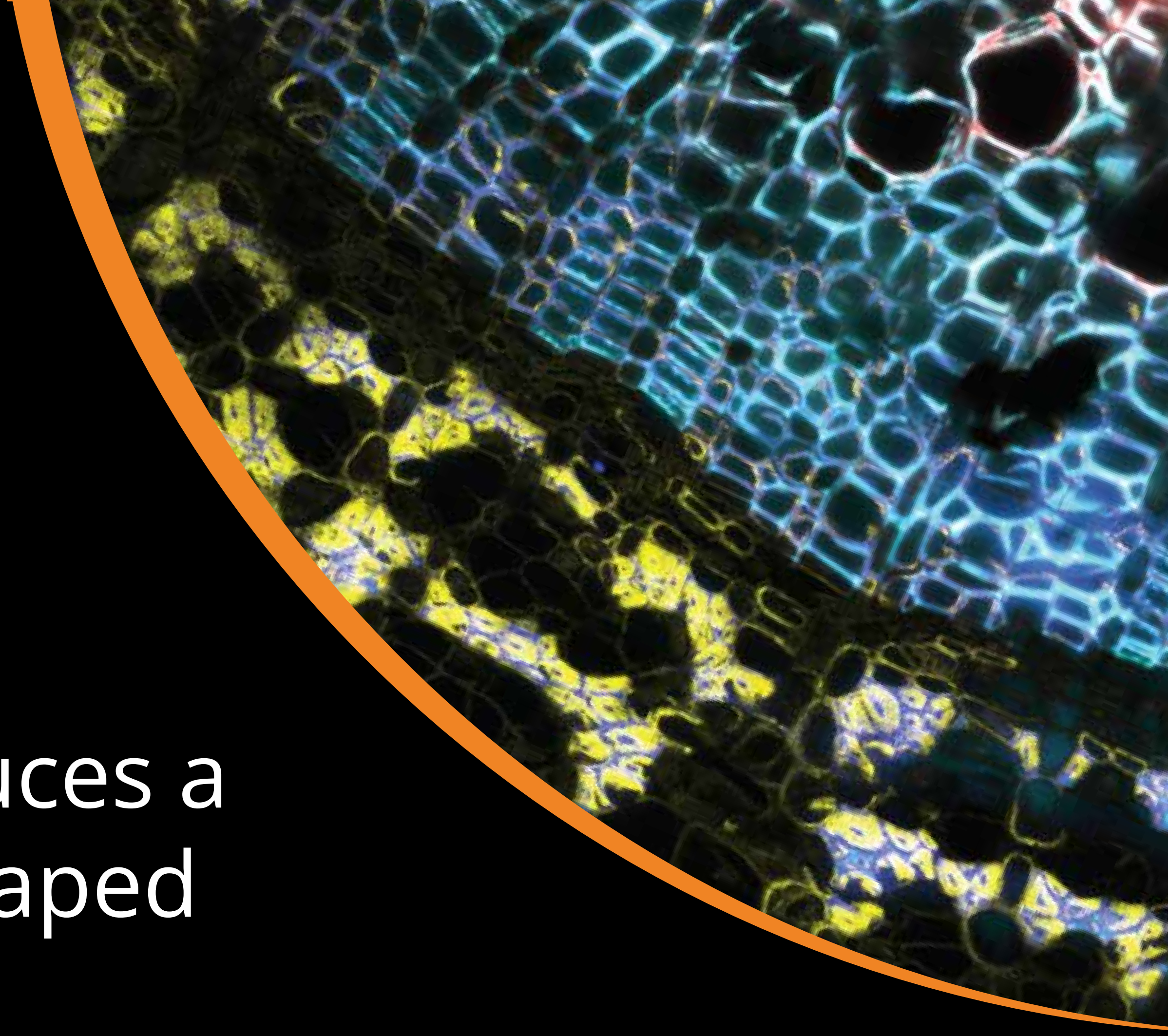
Magnification and **NA** of the objective

Optimum **size** of the **pinhole** (or slit)

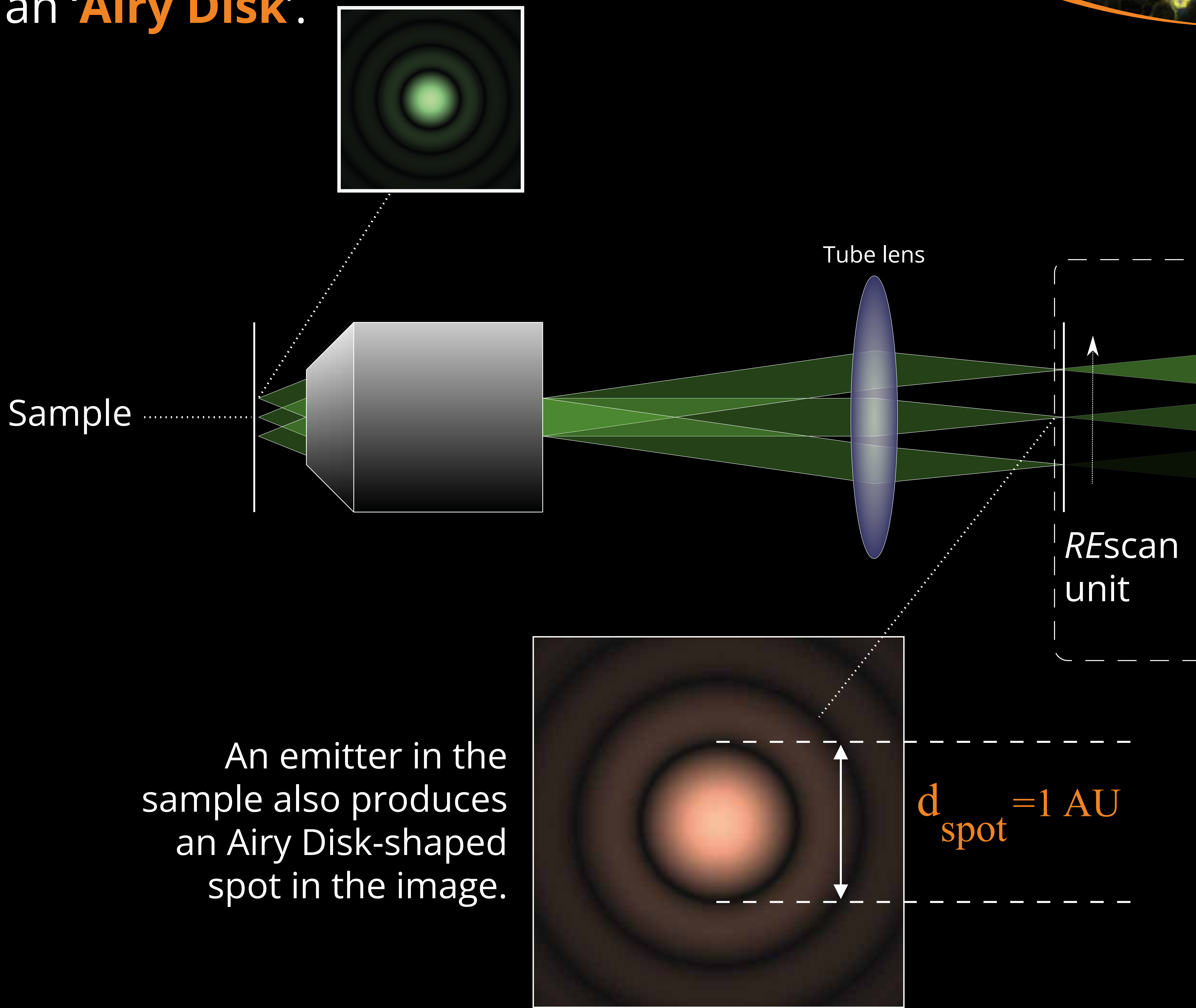
Size of the camera **pixels**

All influence each other.

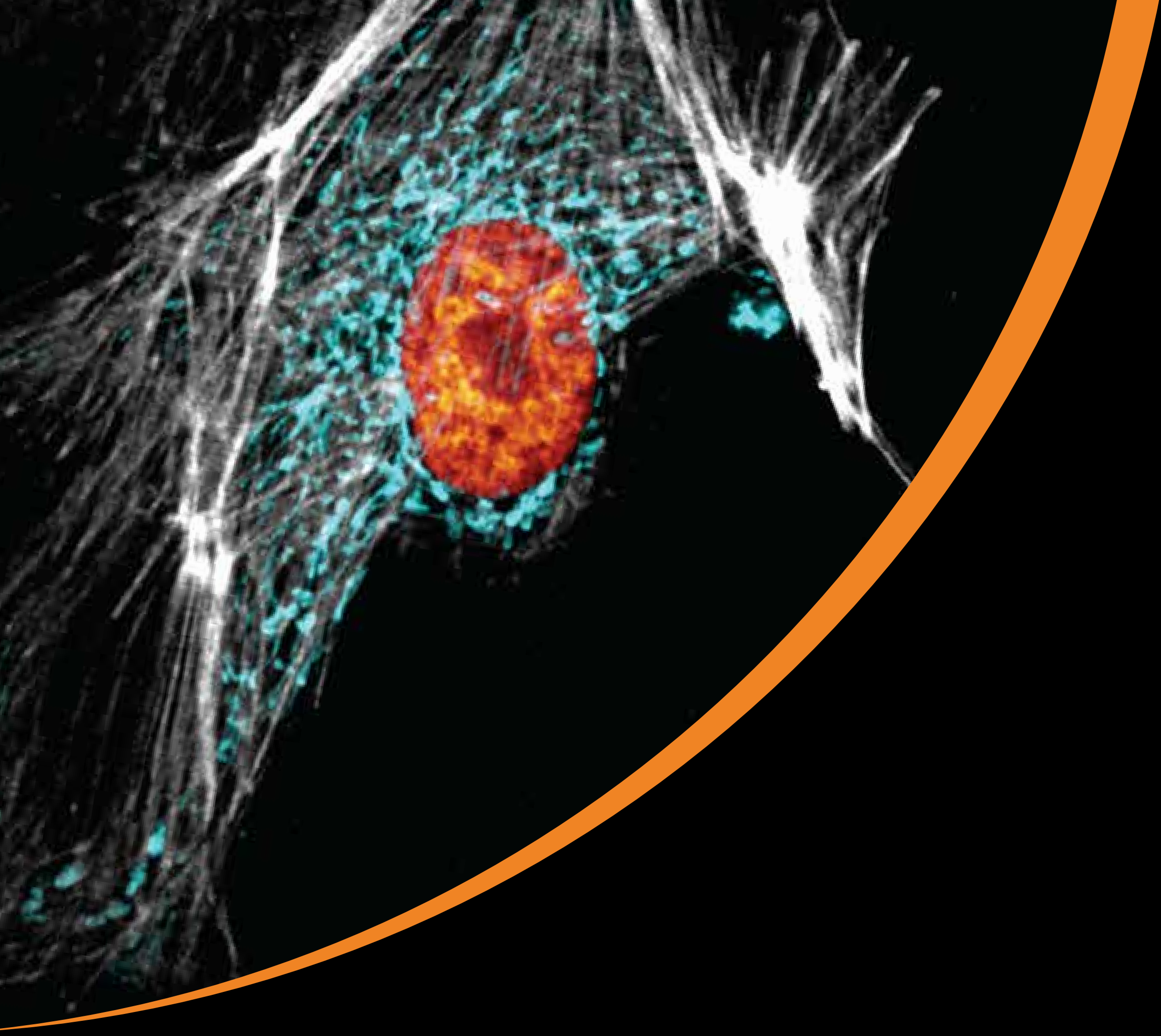
First of all: the Airy Disk



The scanning laser beam produces a **spot** in the sample, which is shaped like an ‘**Airy Disk**’.

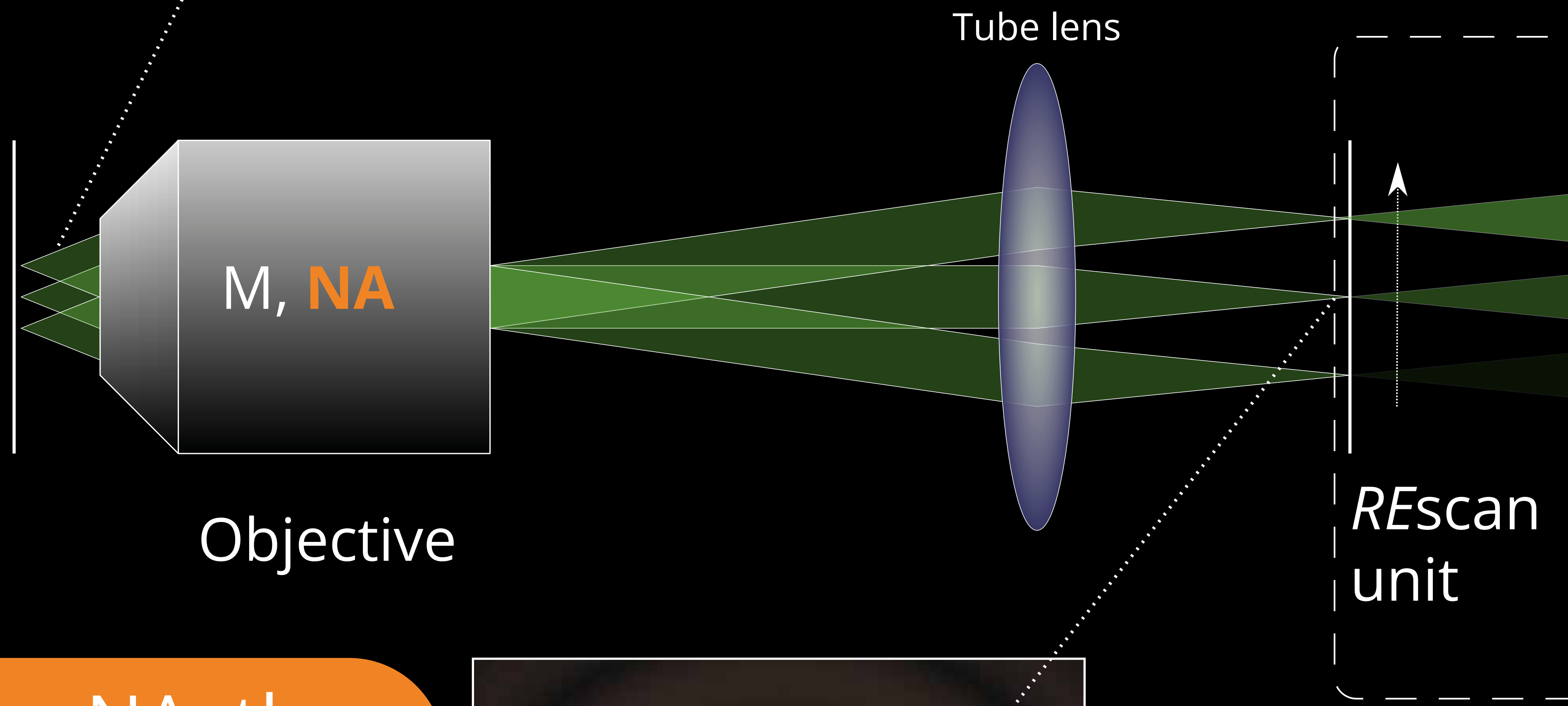


The diameter of the central peak of the spot is called one ‘**Airy Unit**’, or **1 AU**, and it depends on the objective NA.



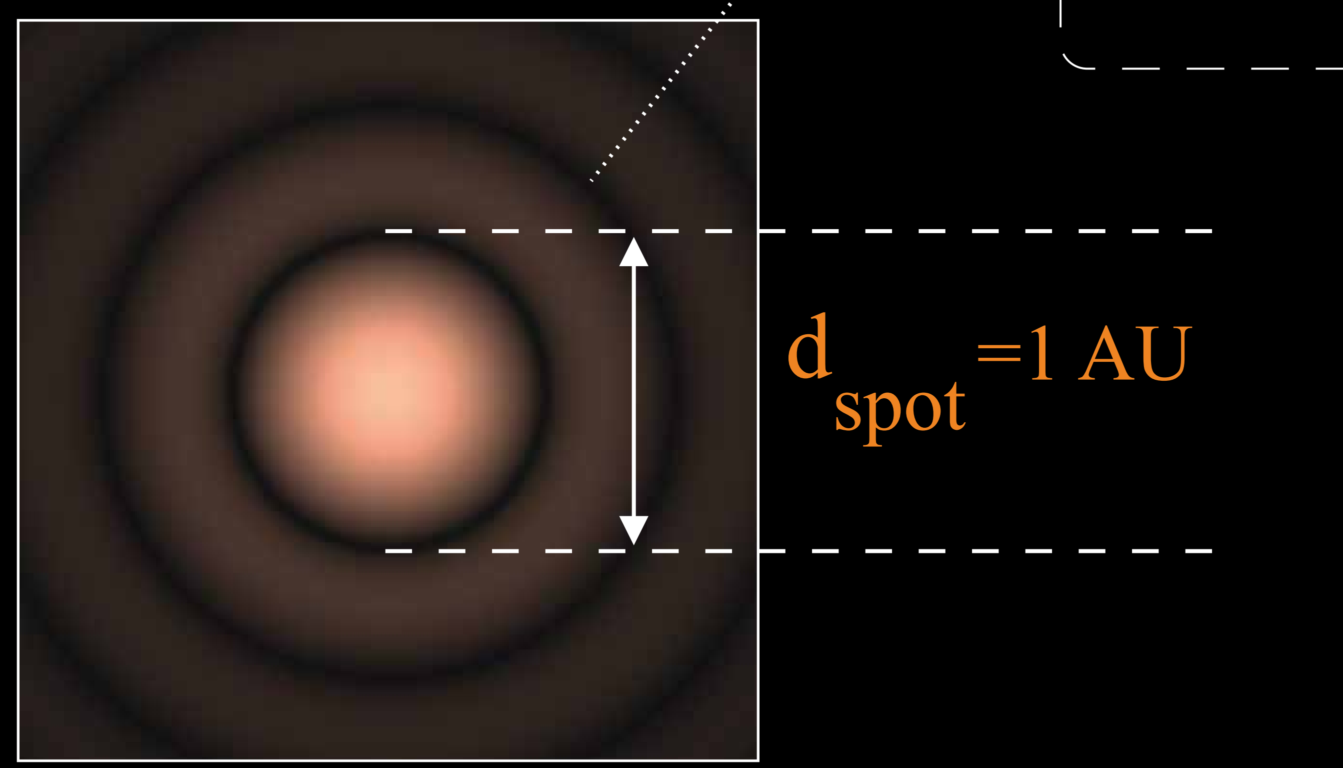
Objective: NA

The **NA** (numerical aperture) gives the largest **angle** in which the objective collects light.



The **higher** the NA, the **smaller** the spot.

(and the better the resolution)

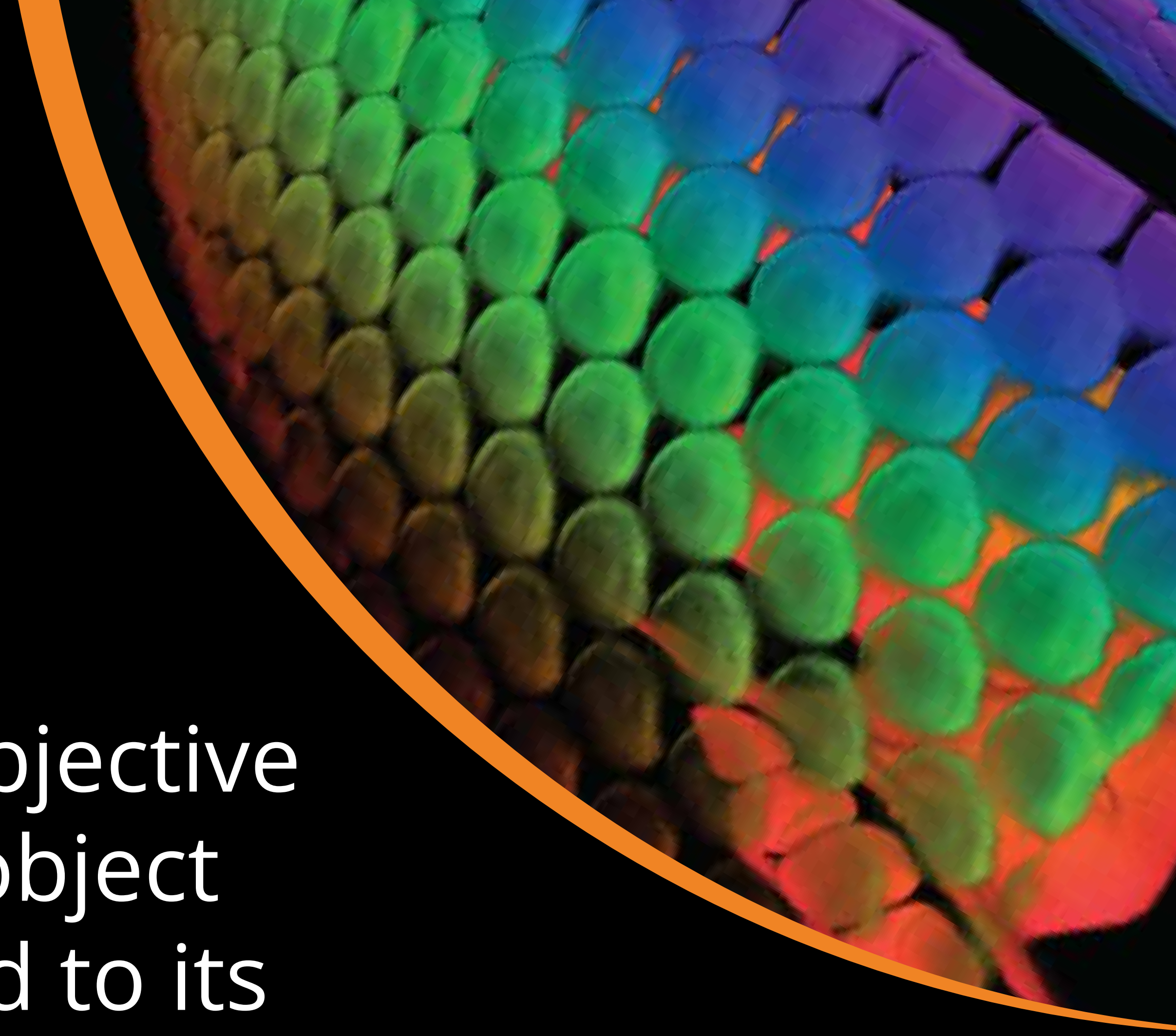


$$d_{spot} = 1.22 \cdot \frac{\lambda}{NA}$$

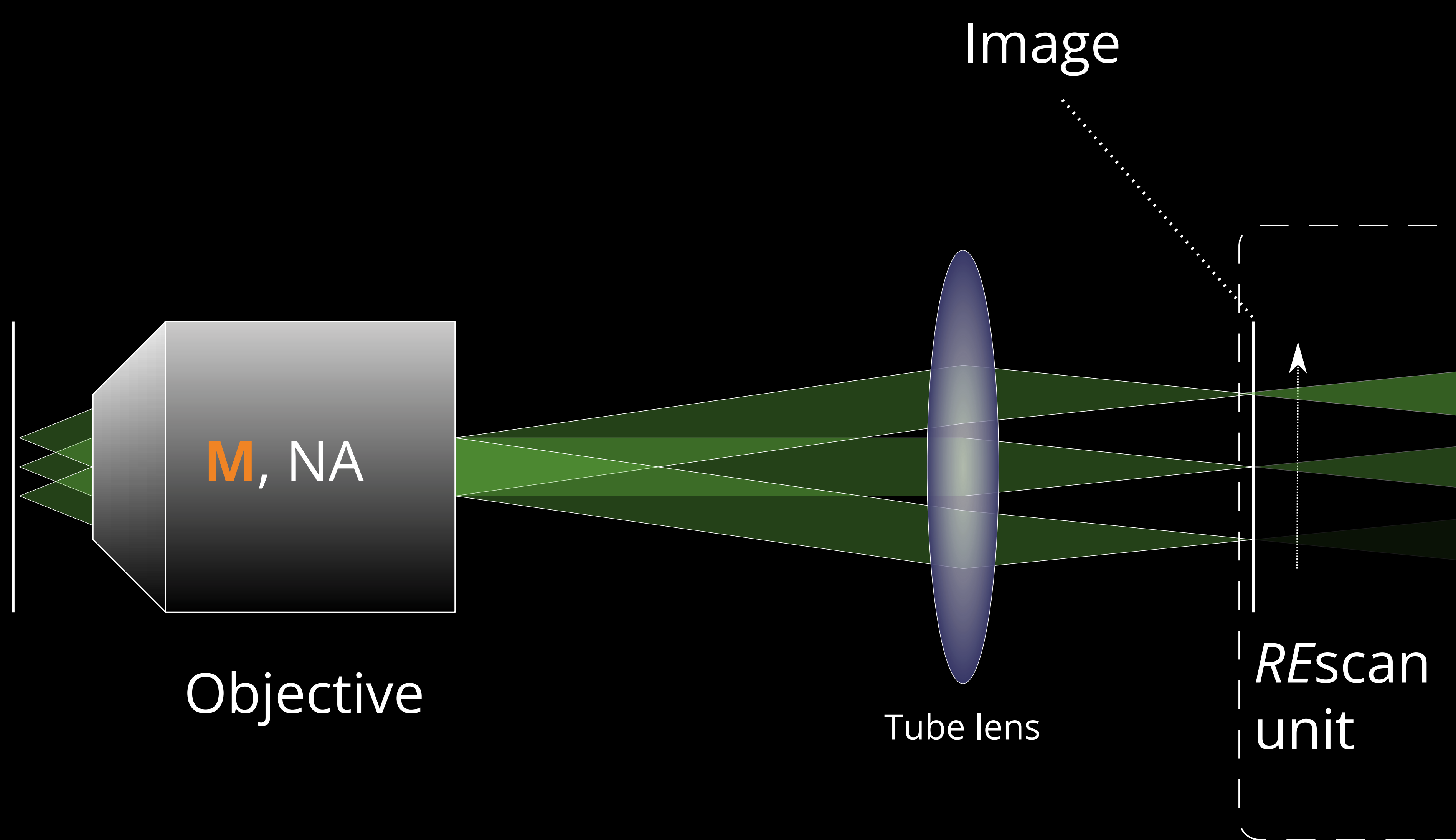
(Wavelength)

(NA of objective)

Objective: Magnification



The **magnification** (M) of an objective indicates how much larger an object appears in the image compared to its actual size.

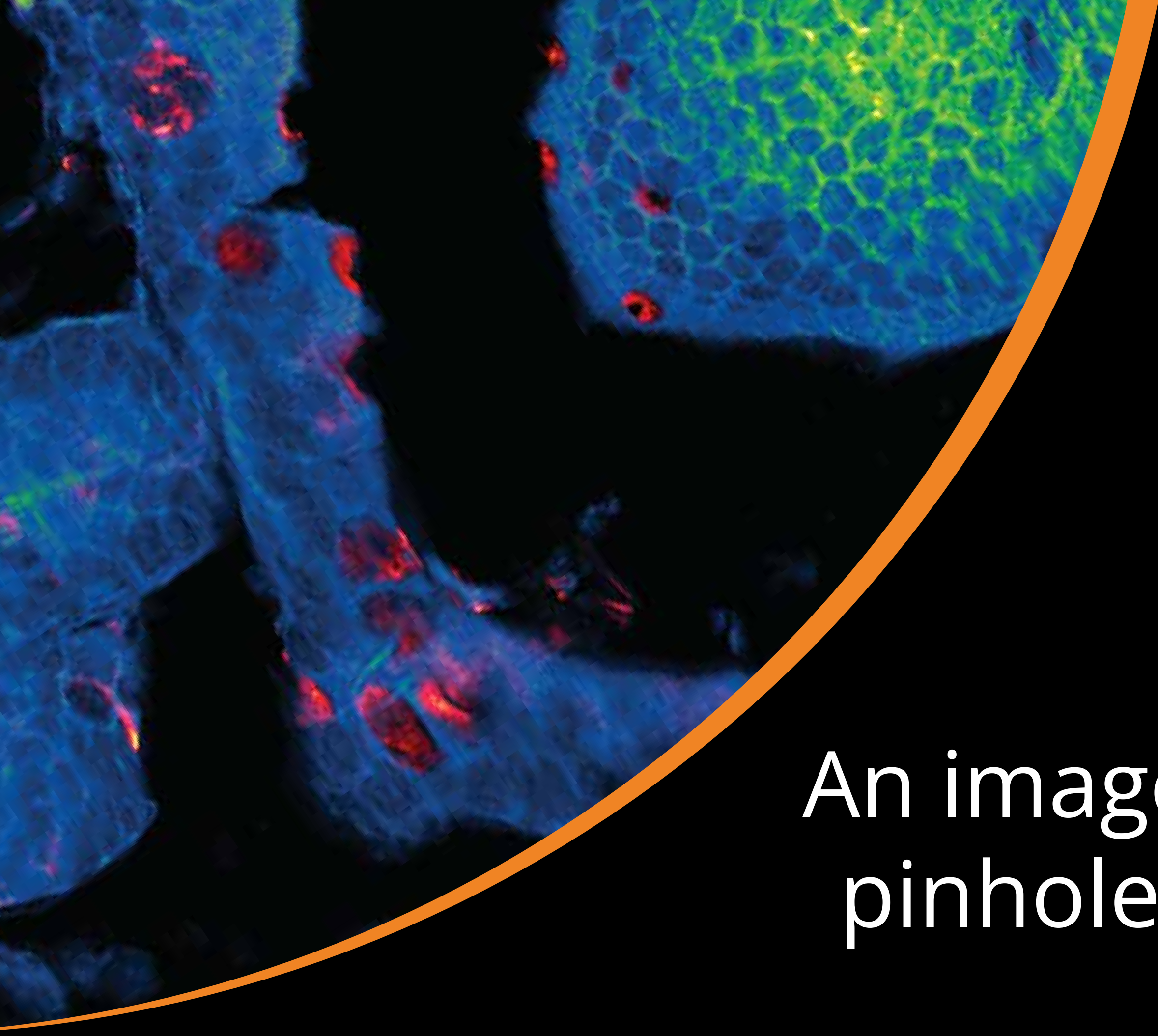


Magnification can be defined in relation to the field of view (FoV):

$$M = \frac{\text{FoV Image}}{\text{FoV Sample}}$$

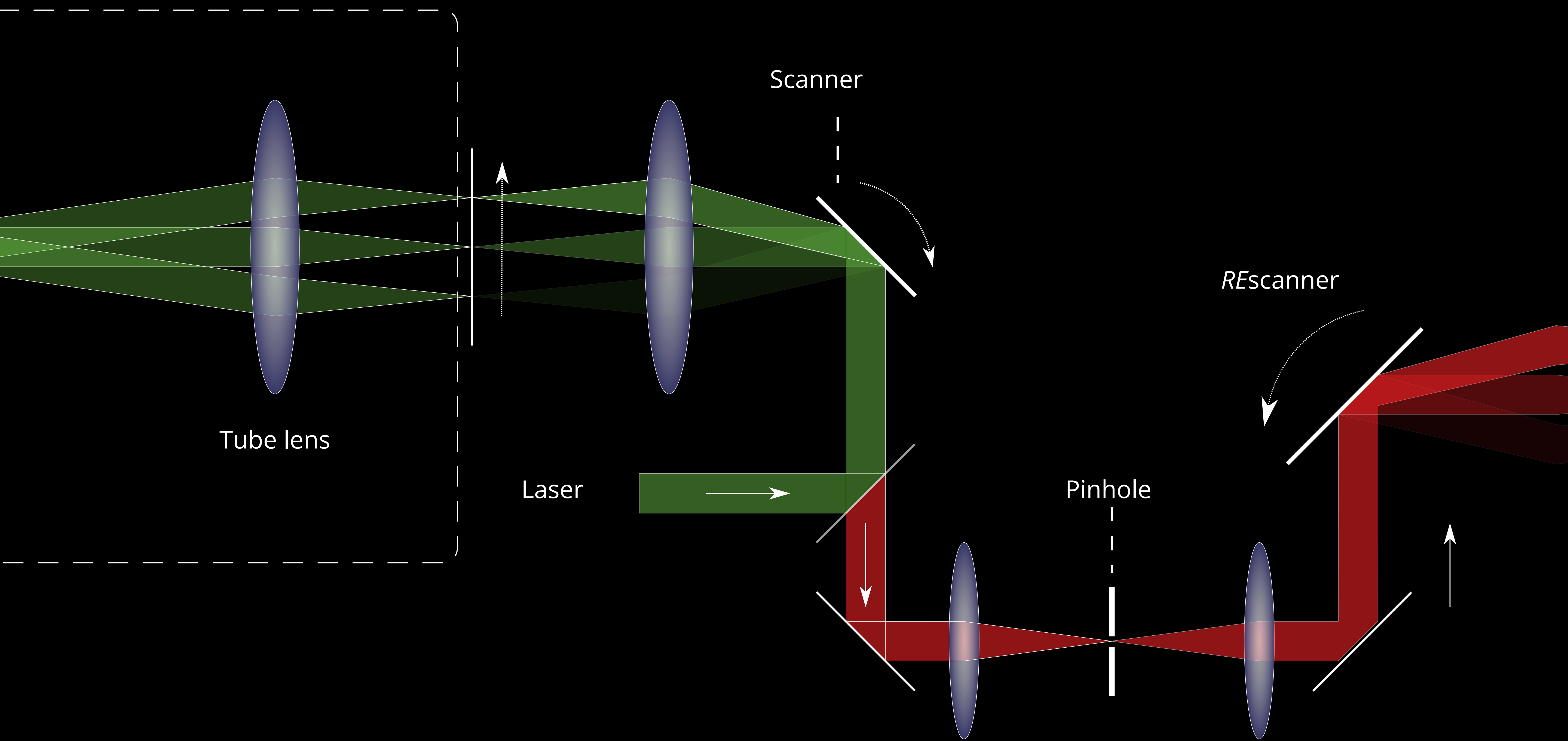
The spot size in the image (1 AU) is also magnified:

$$d_{spot} = 1.22 \cdot \frac{\lambda}{NA} \cdot M$$



Pinhole size

An image of the spot is projected on the pinhole, circular or slit, via the optics of the *RE*scan unit.

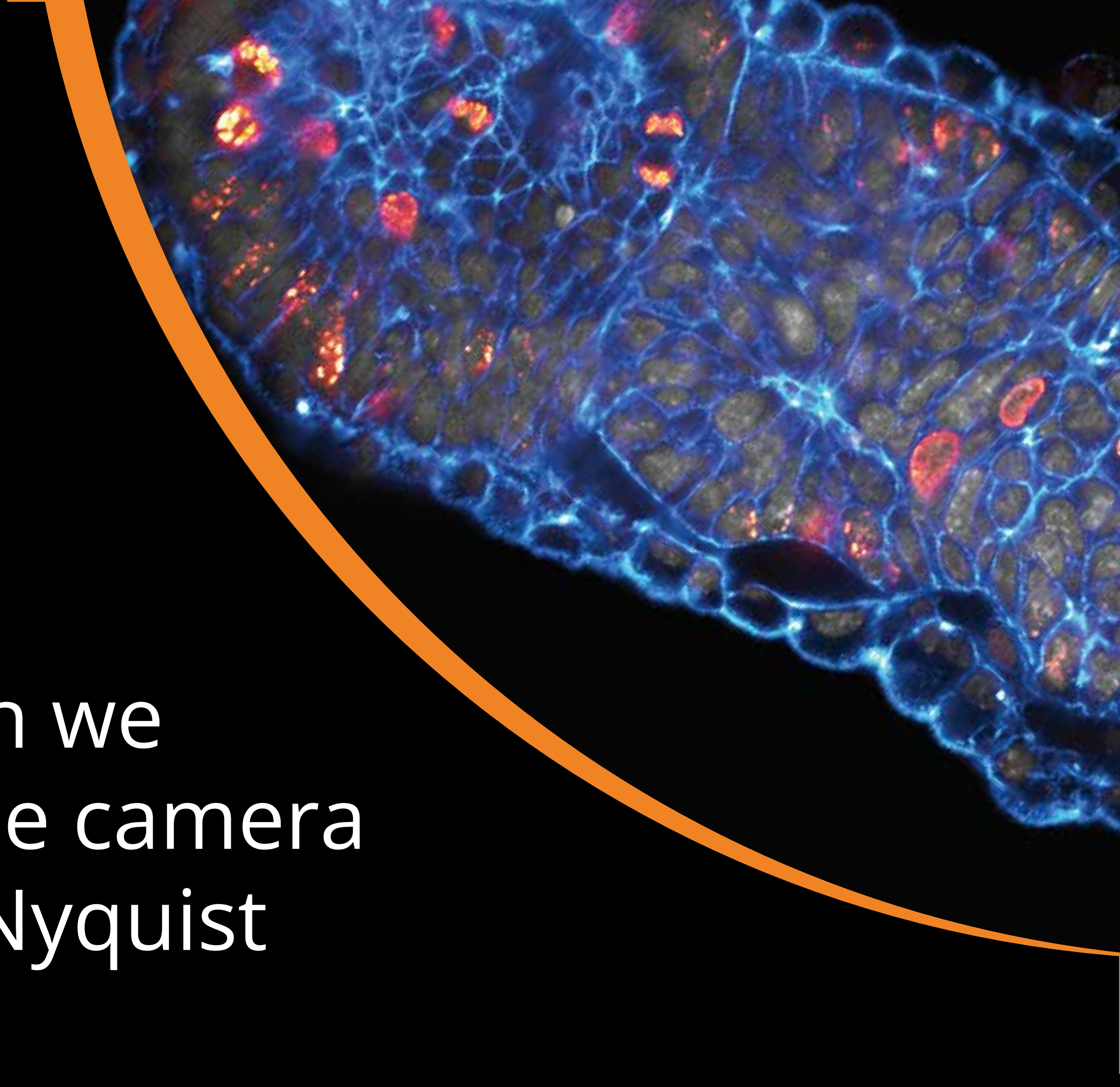


The optimal trade-off between confocality and signal intensity without losing resolution in *RE*scan is achieved when the **pinhole size** is **1.5** AU.

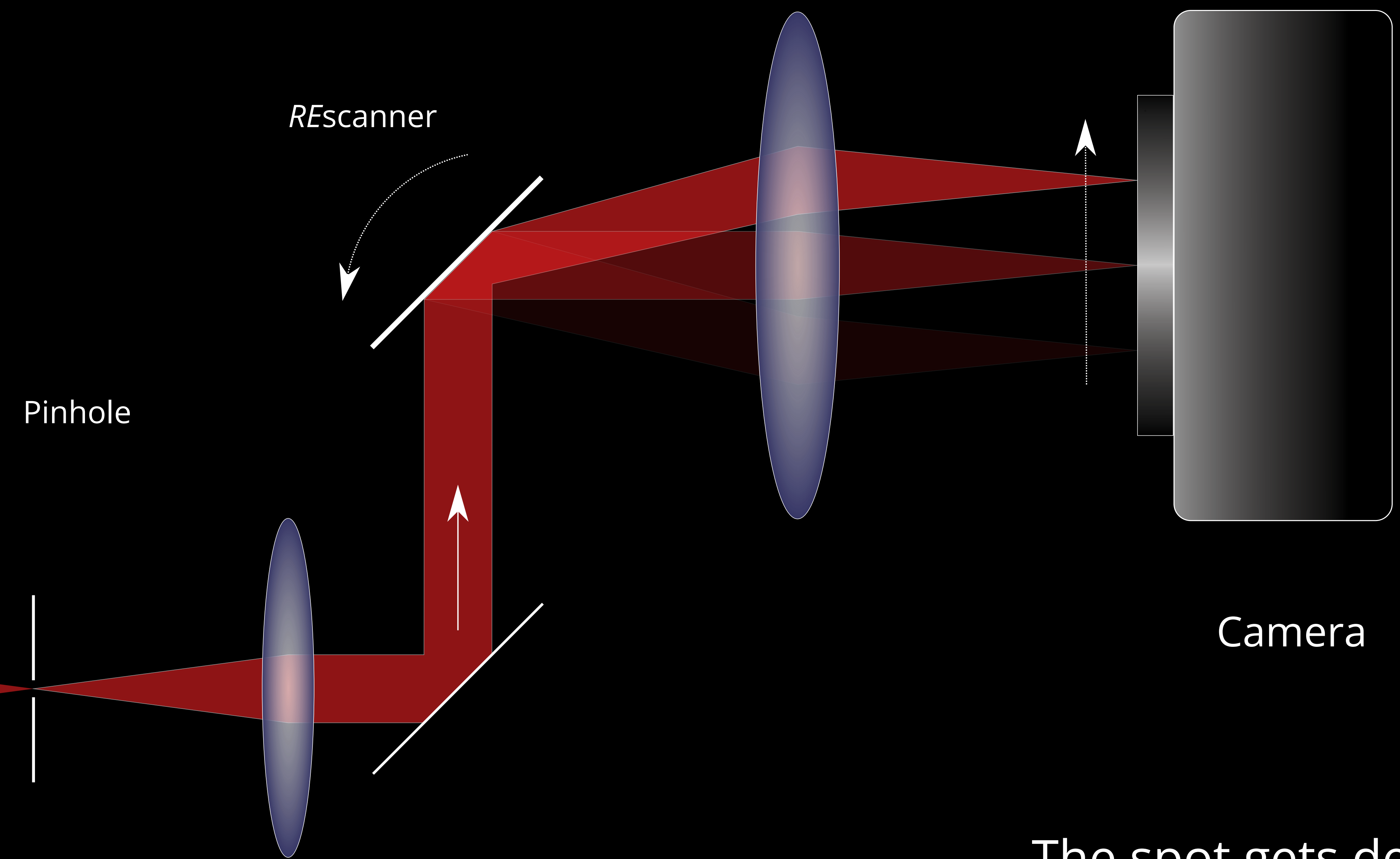
$$d_{pinhole} \approx 1.5 \cdot 1.22 \cdot \frac{\lambda}{NA} \cdot M \cdot M_{internal}$$

This formula applies for both circular and slit pinholes in **Point *RE*scan** and **Line *RE*scan**, respectively.

Camera pixels

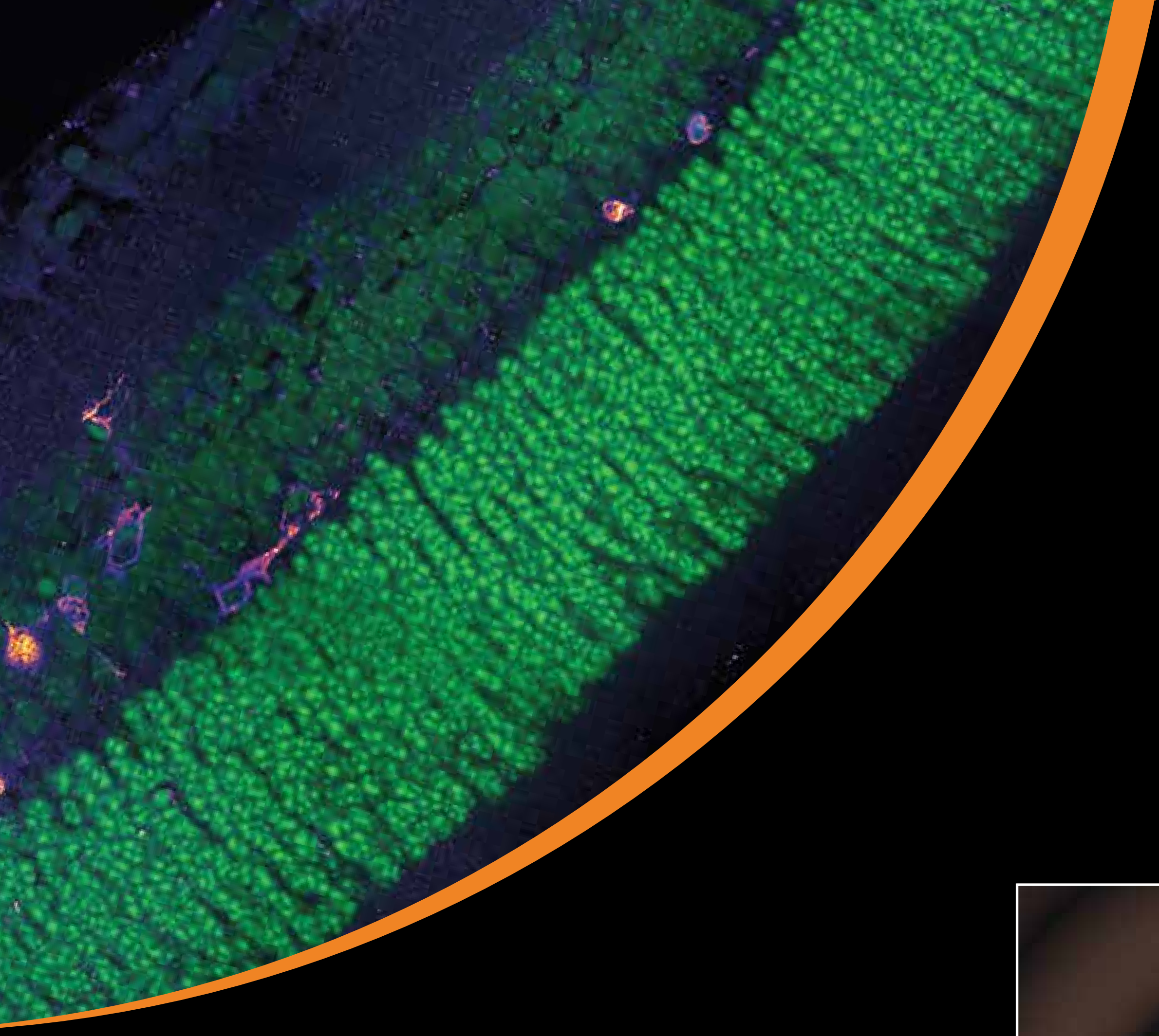


To obtain maximum resolution we need at least four **pixels** on the camera within 1 AU, according to the Nyquist theorem.



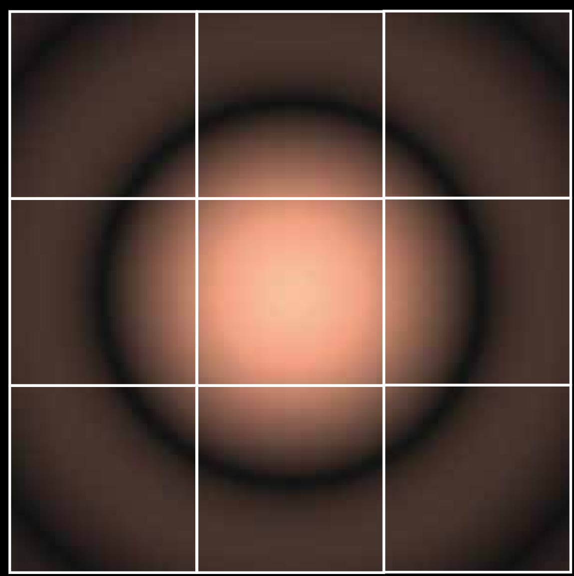
The spot gets demagnified for super resolution imaging.

$$d_{pixel} \leq \frac{1}{4} \cdot 1.22 \cdot \frac{\lambda}{NA} \cdot M \cdot M_{spot}$$



Nyquist sampling

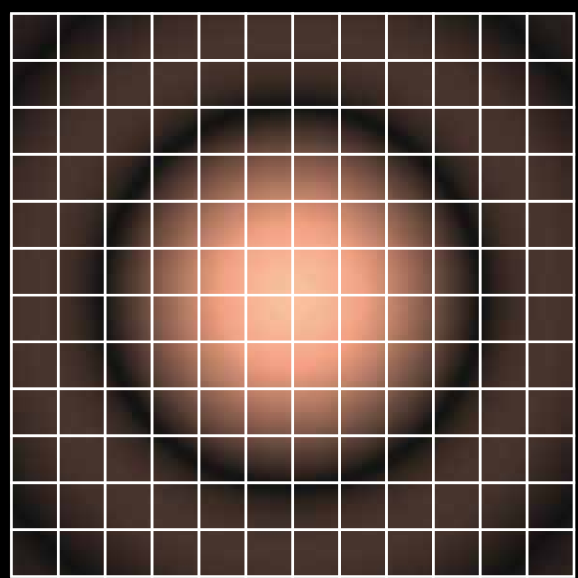
Undersampling



Lost resolution

Watching a 4k movie on a Gameboy

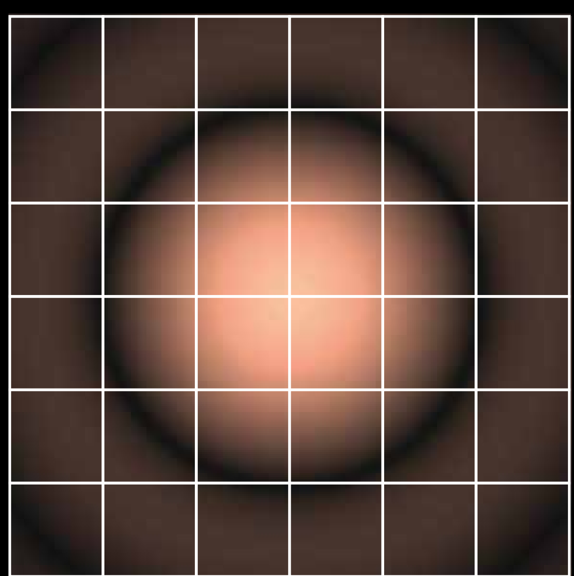
Oversampling



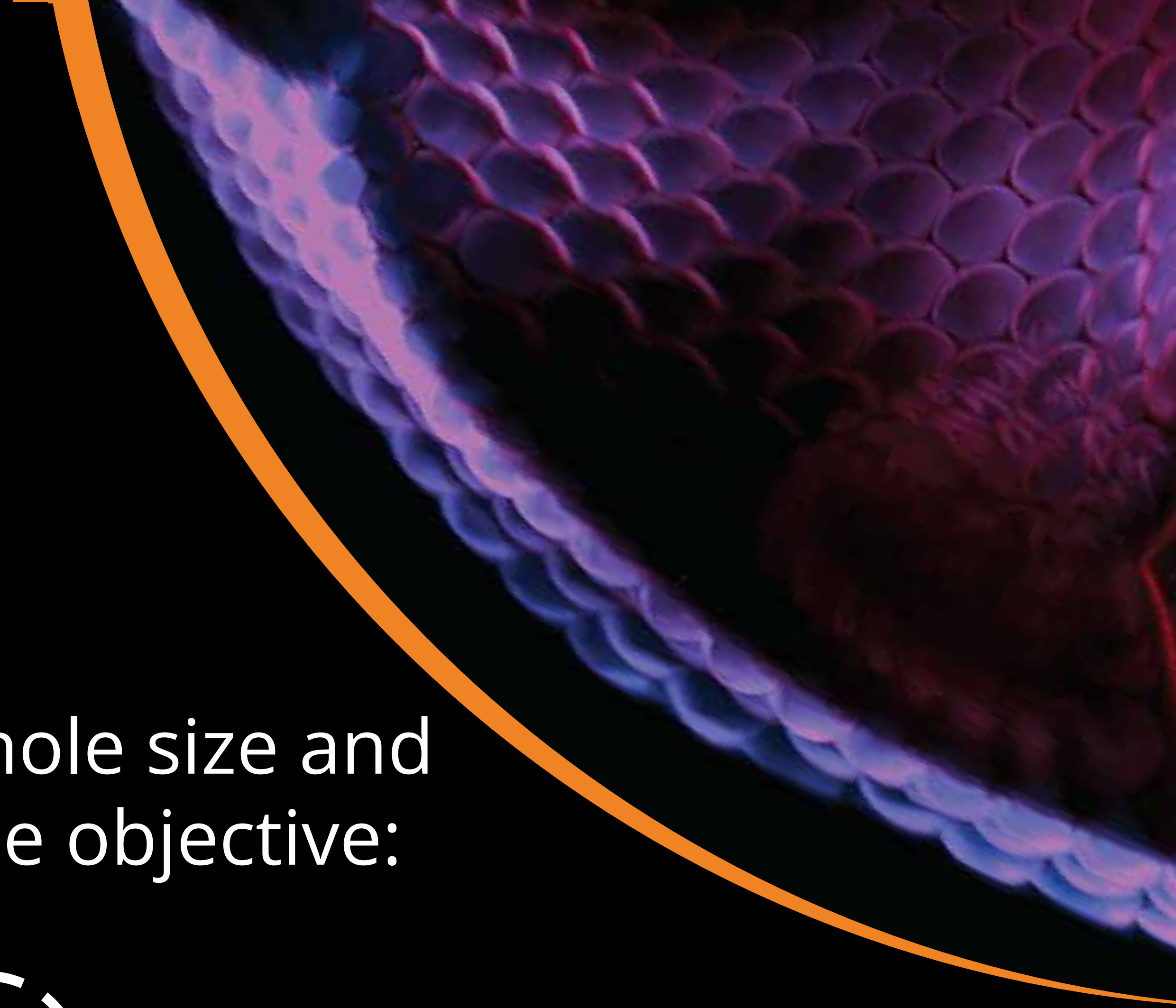
A waste of pixels

Watching a VHS tape on a 4k monitor

Perfect Nyquist sampling



4 pixels within 1 AU

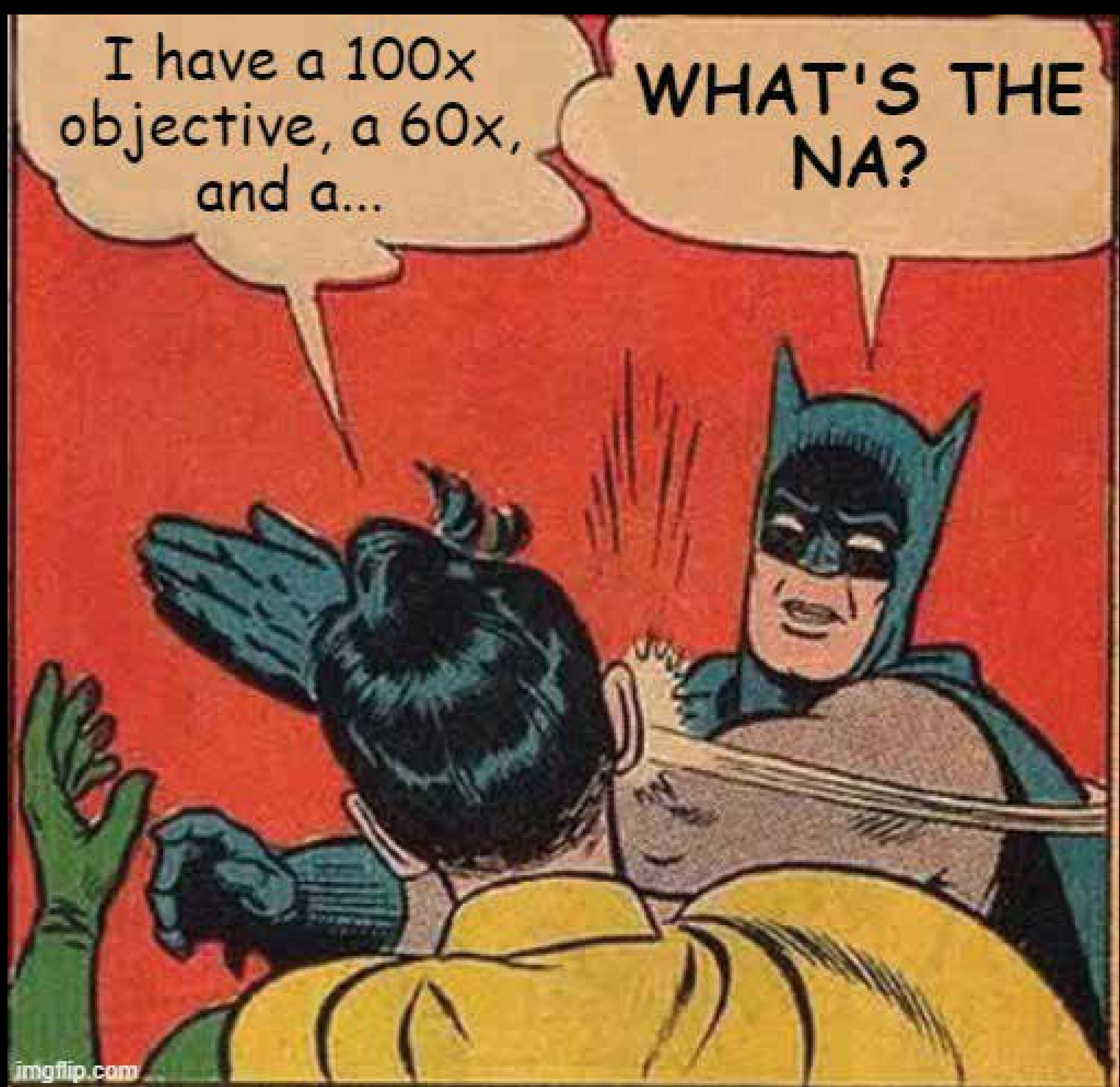


Optimising your system

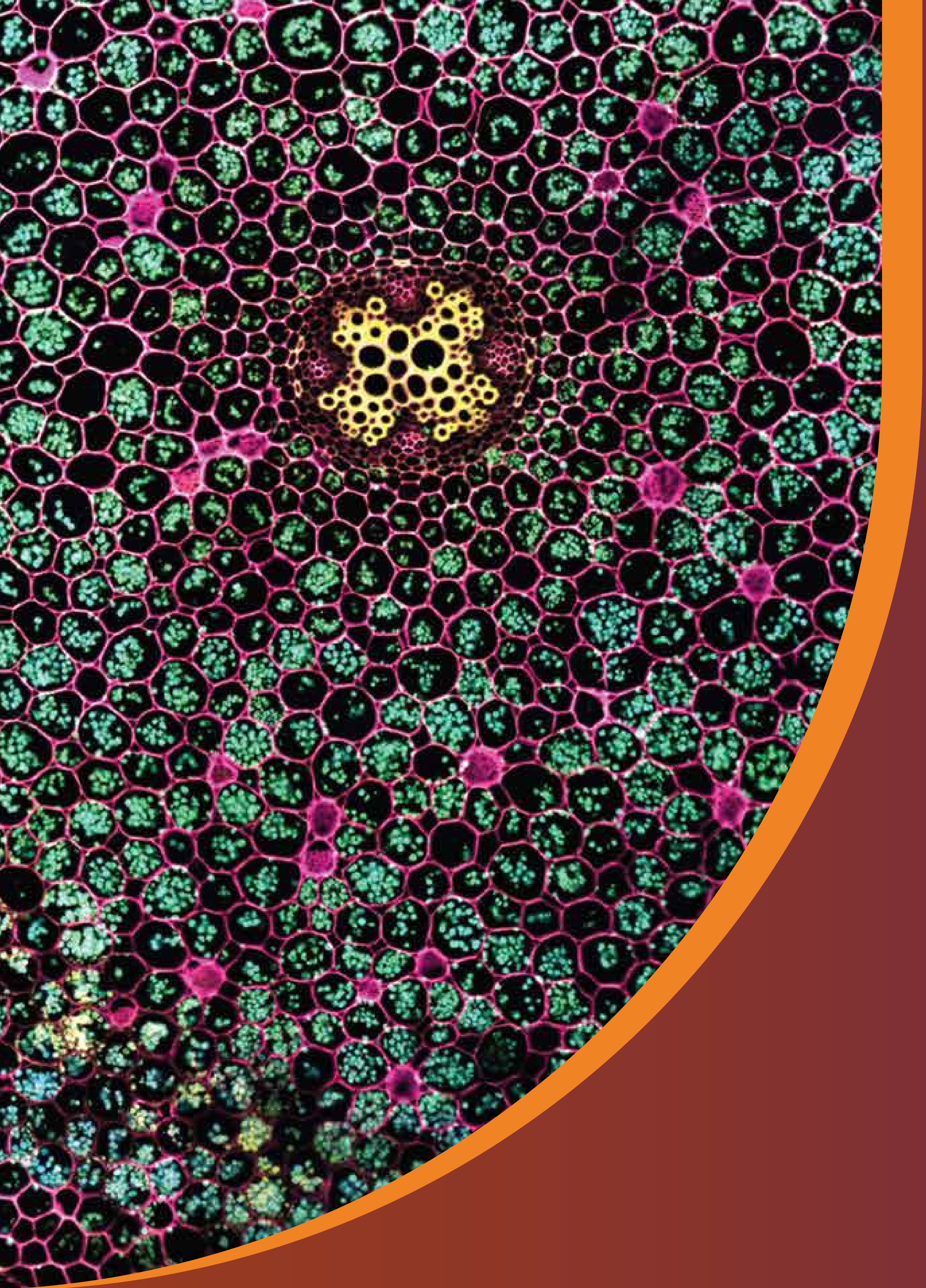
The choice of both the optimal pinhole size and the optimal pixel size depend on the objective:

$$d_{pinhole} \approx 1.5 \cdot 1.22 \cdot \frac{\lambda}{NA} \cdot M \cdot M_{internal}$$

$$d_{pixel} \leq \frac{1}{4} \cdot 1.22 \cdot \frac{\lambda}{NA} \cdot M \cdot M_{spot}$$



Make sure to take into account both M and NA for the correct choice!



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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).