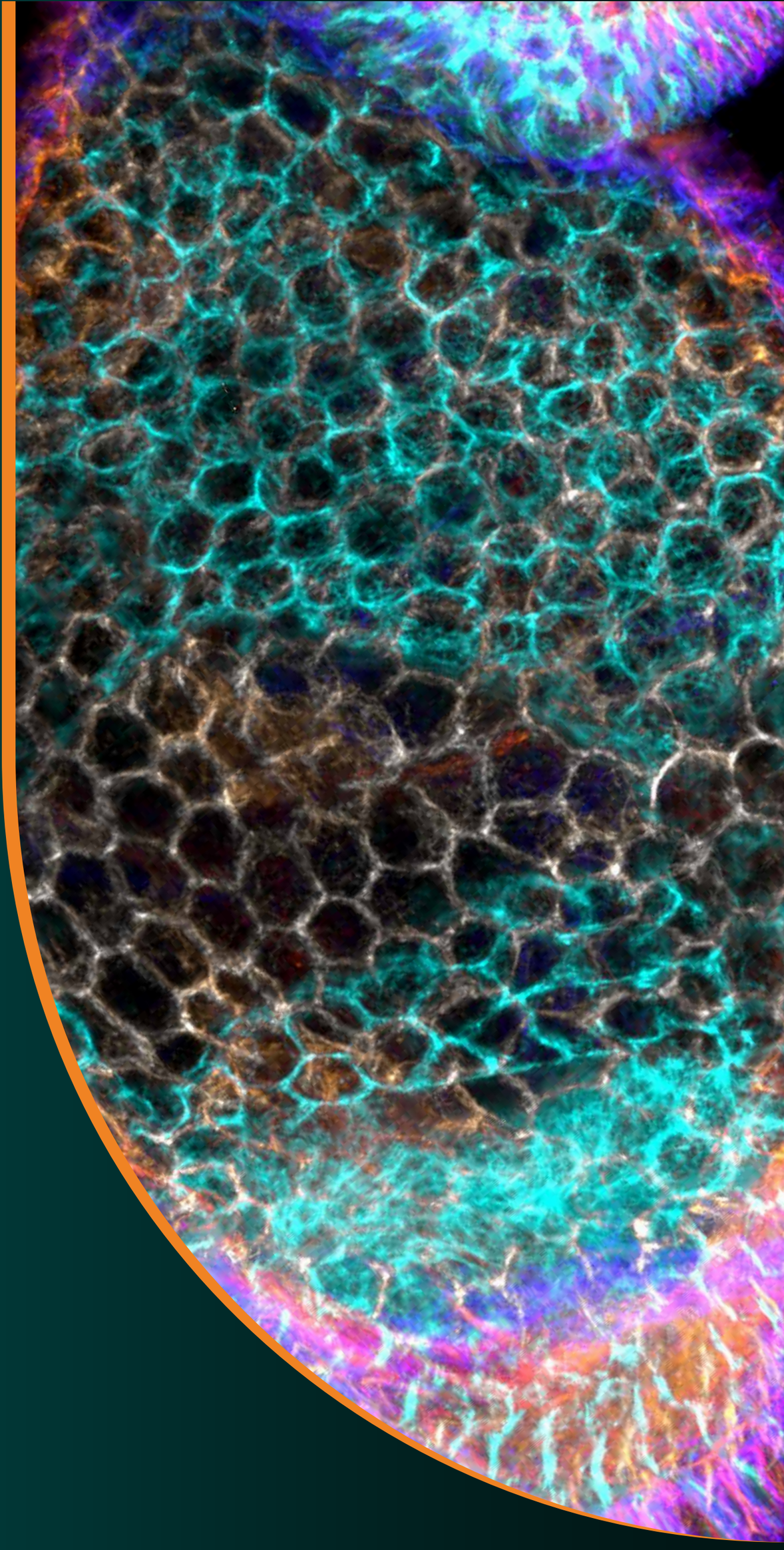
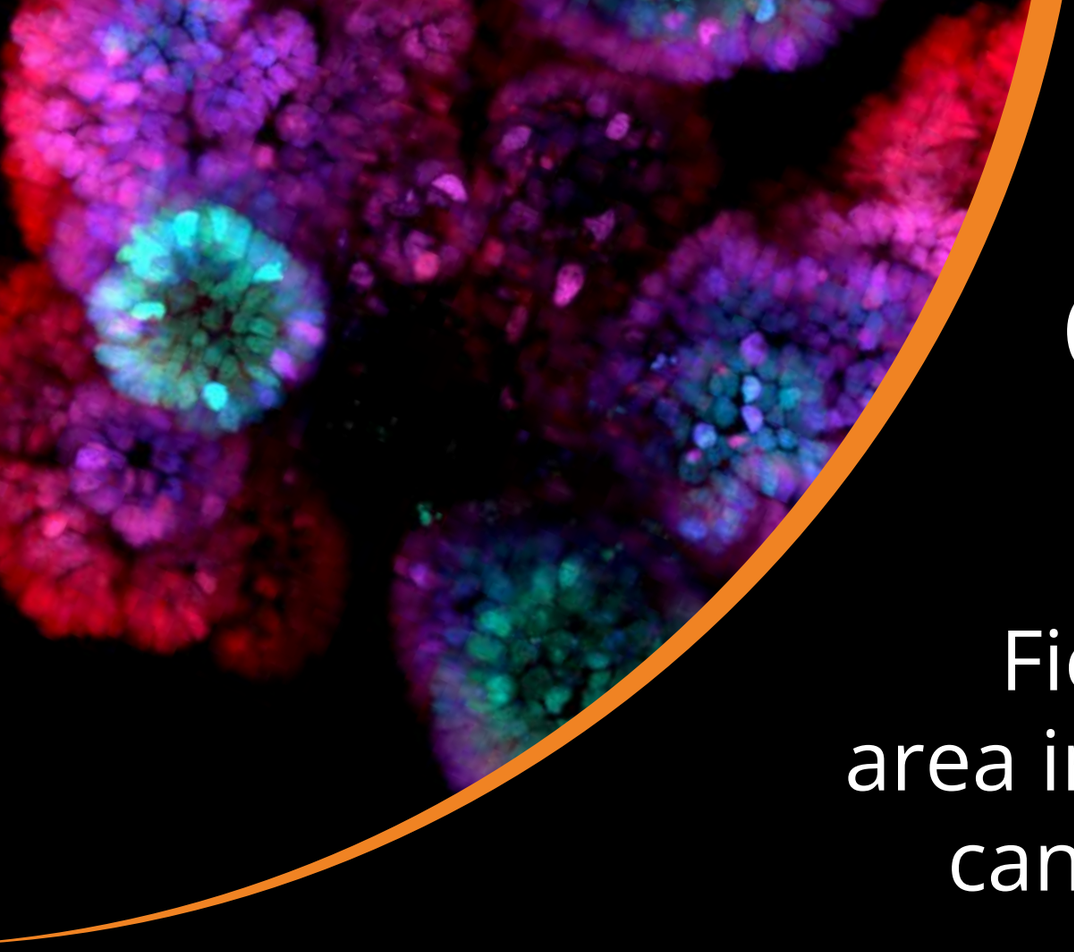
Confocal nl



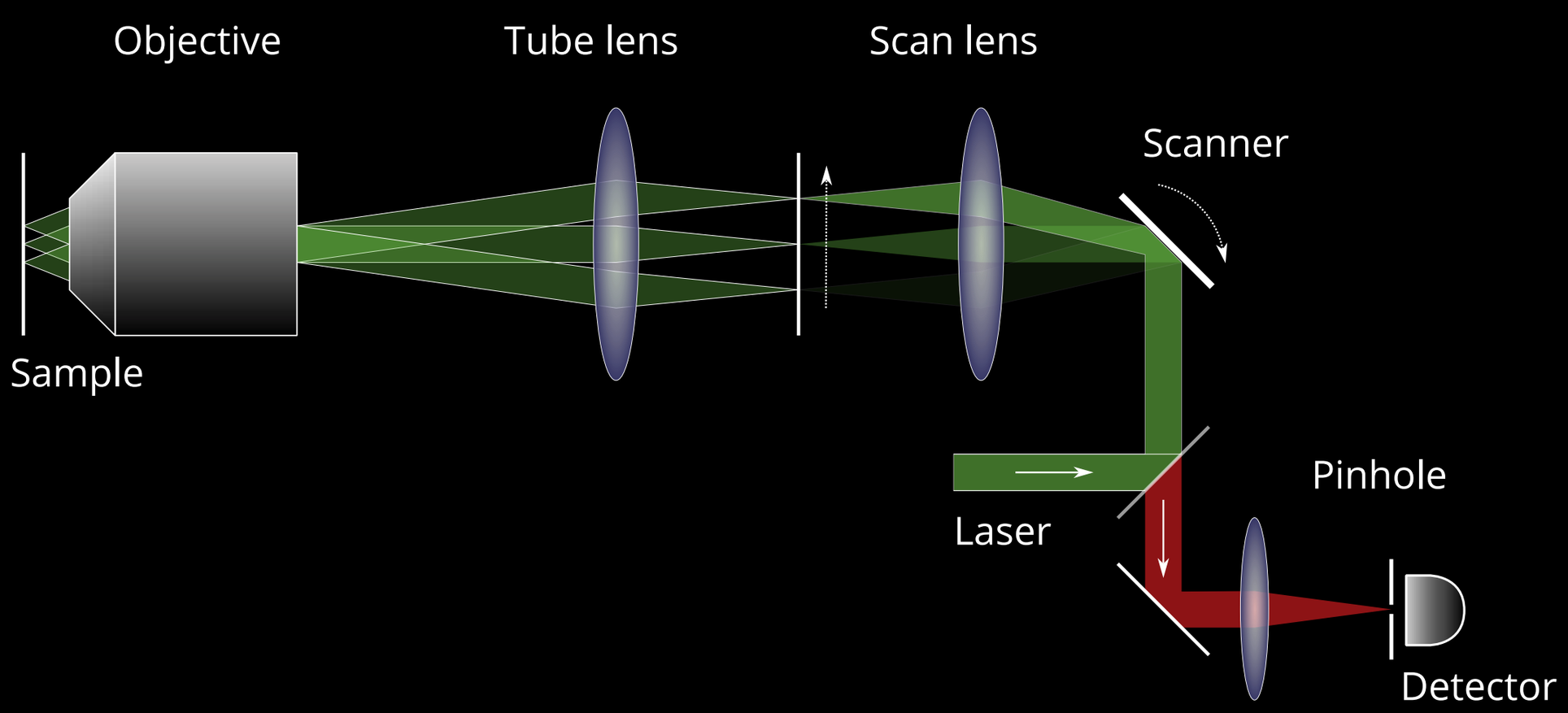
FIELD OF VIEW IN RESCAN CONFOCAL MICROSCOPY



Field Of View in Confocal Microscopy

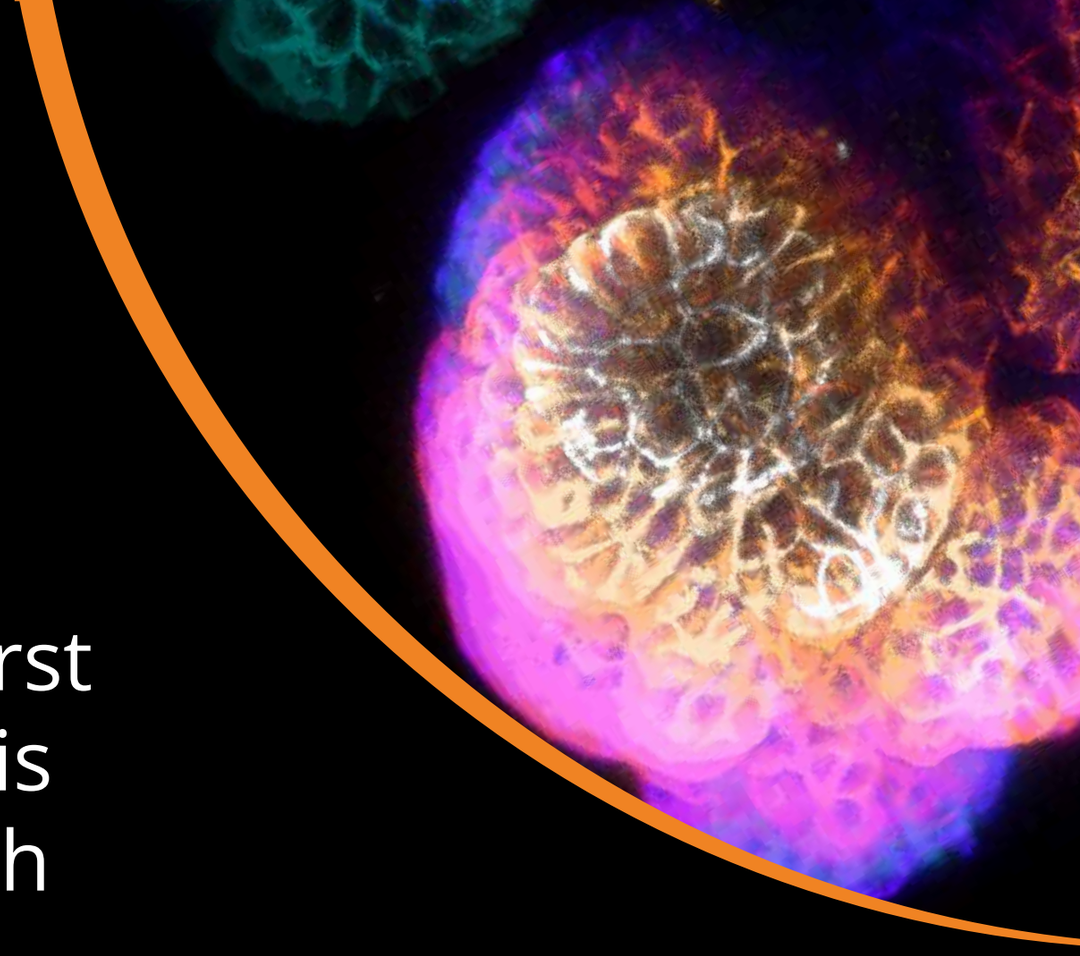
Field Of View (**FOV**) describes the area in the sample that a microscope can image. This area is also known as FOV_{sample}.

Additionally, FOV represents the portion of the object that fills the sensor of a detector ($FOV_{detector}$).

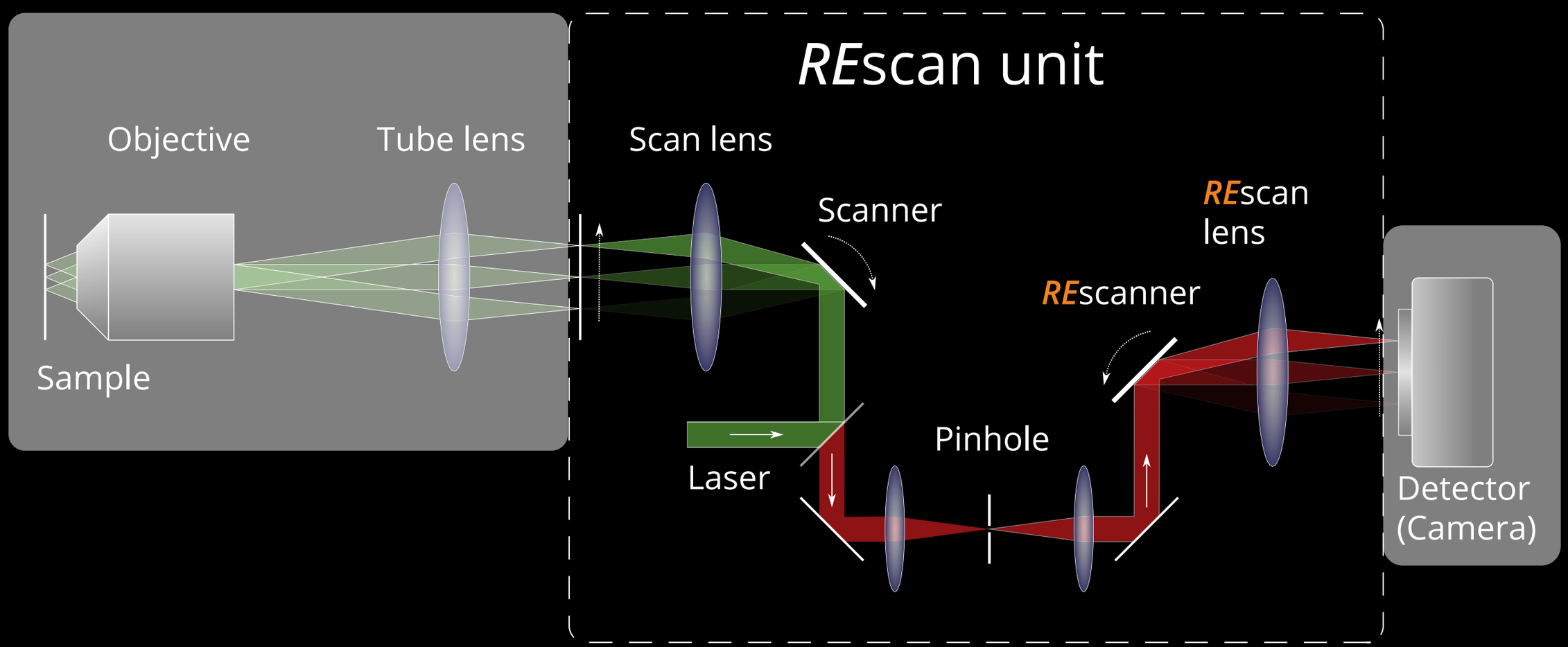


In standard confocal microscopy FOV_{sample} is usually equal to $FOV_{detector}$ and is often expressed in **pixels**.

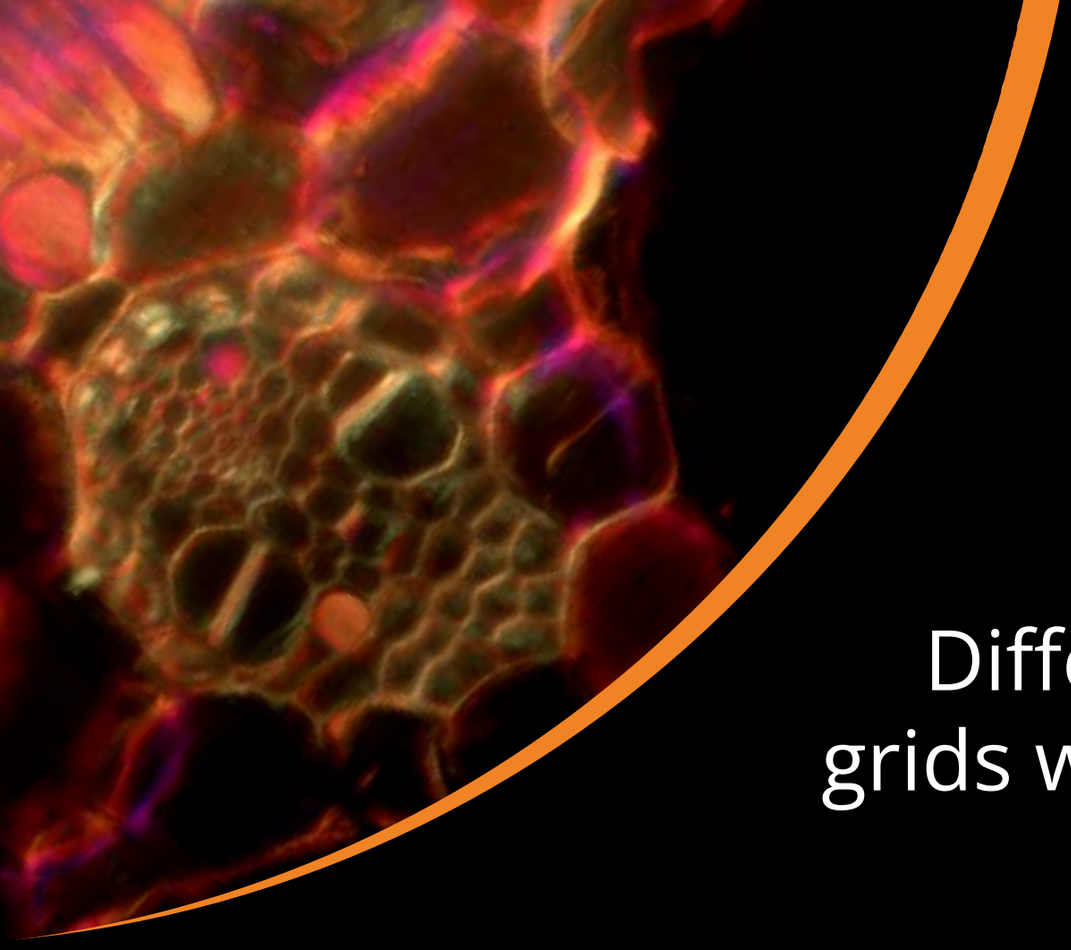
Field Of View in *RE*scan



In ***RE*scan** confocal, after the first scan of the sample, the image is rescanned onto a camera which works as a detector.



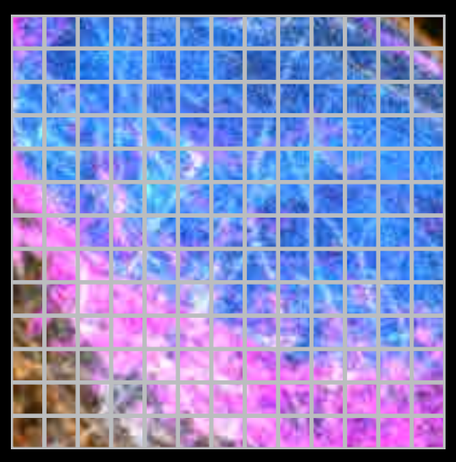
Here, two issues arise if the FOV is expressed in pixels.



Issue 1: Pixel size varies

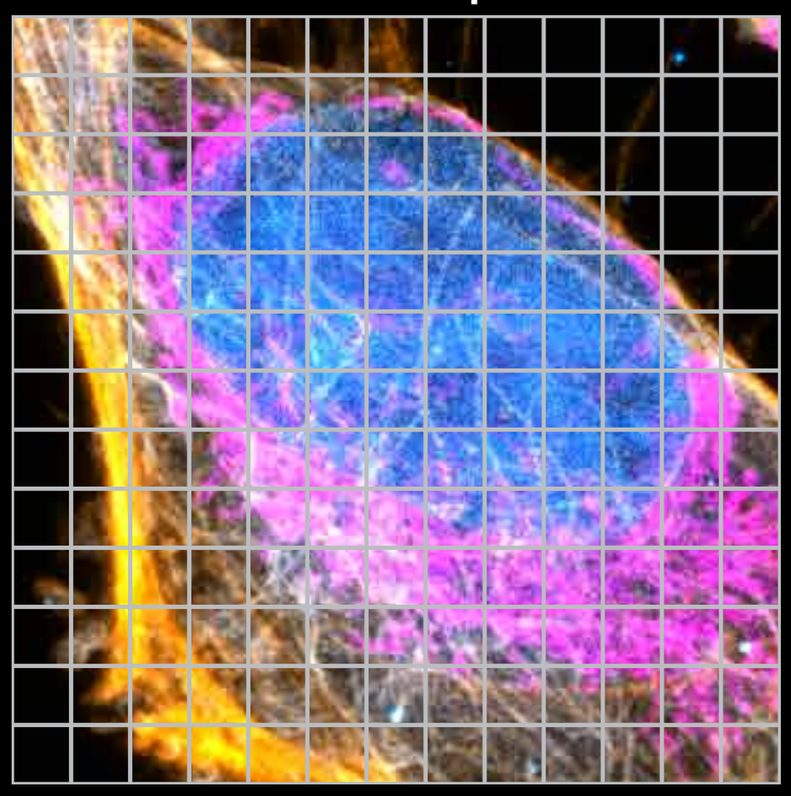
Different cameras might have pixel grids with the same number of pixels, but different pixel sizes.

12x12 px



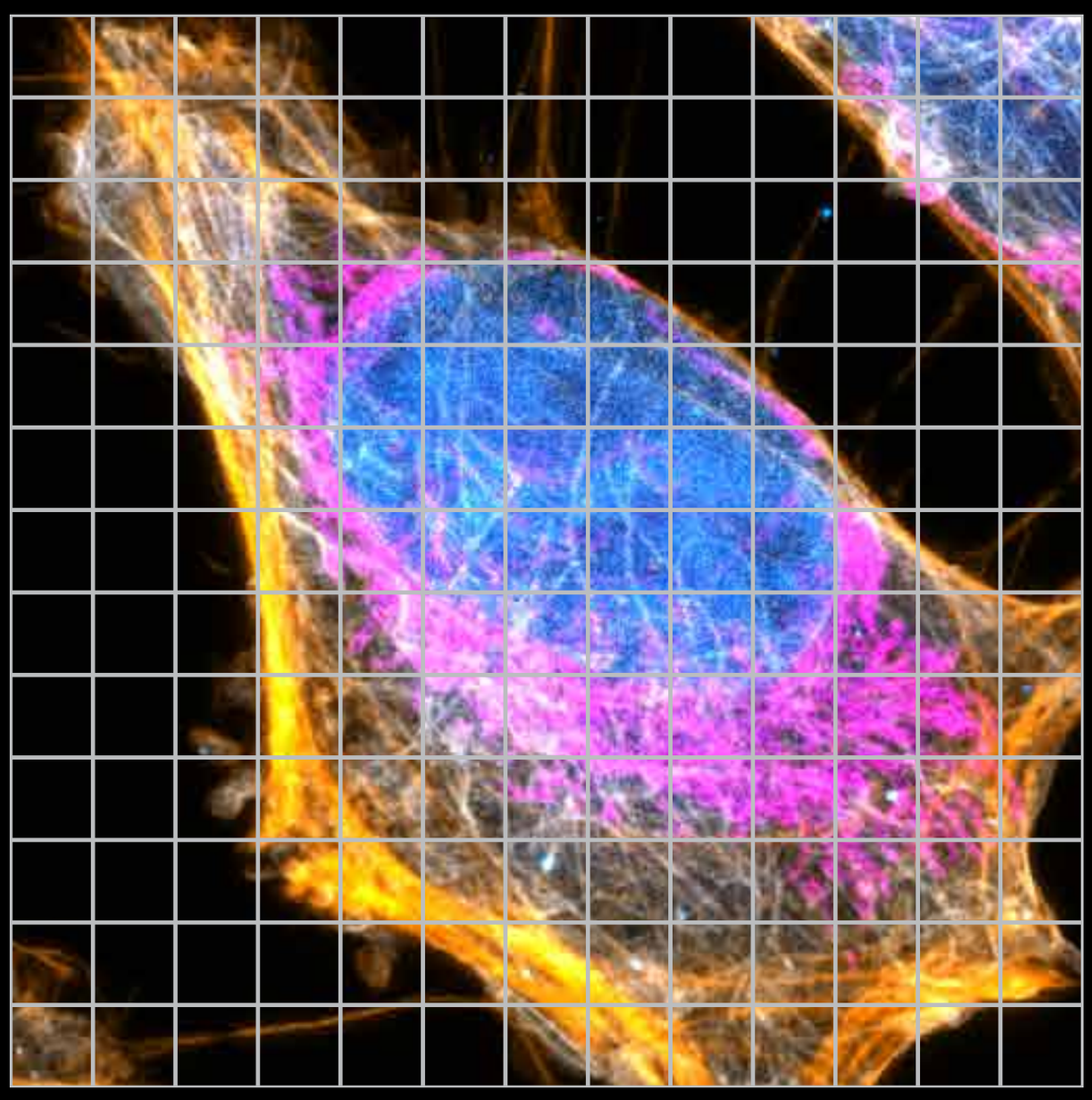
2.6 µm per px

12x12 px



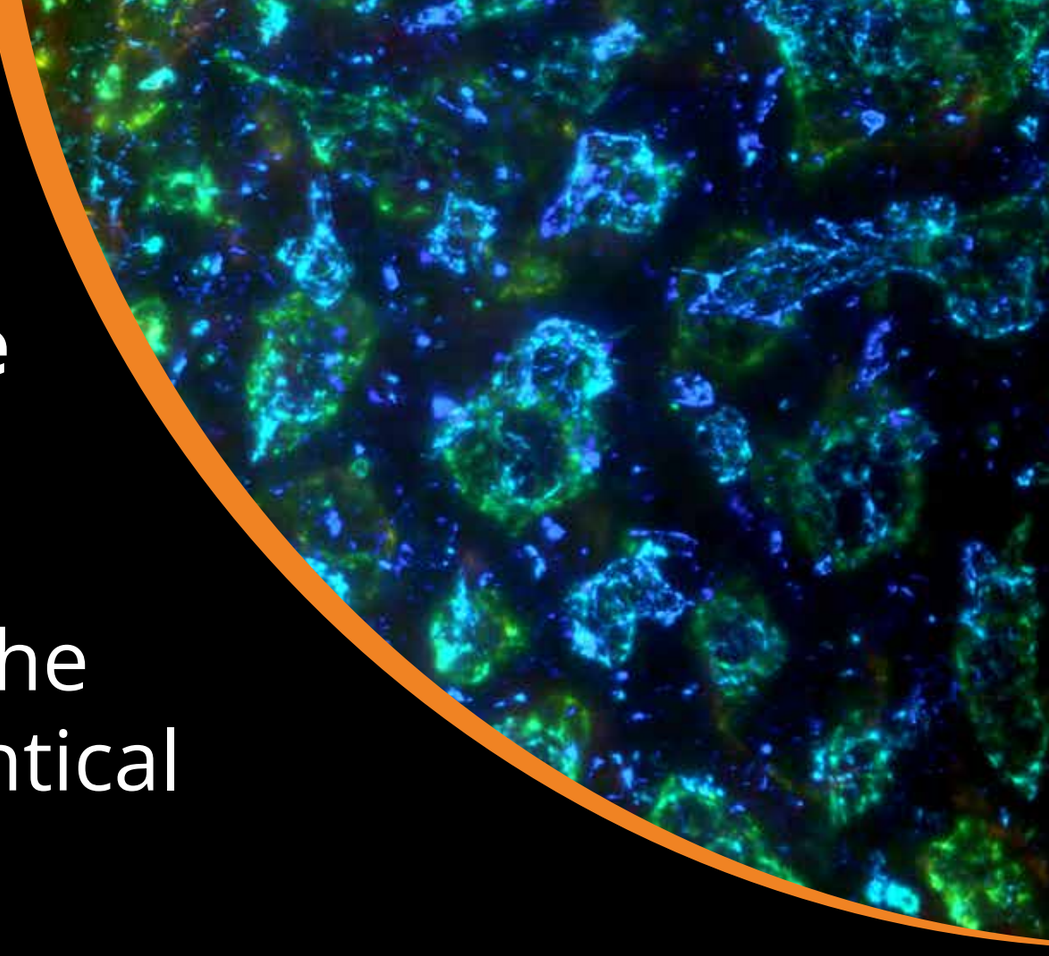
4.6 µm per px

12x12 px

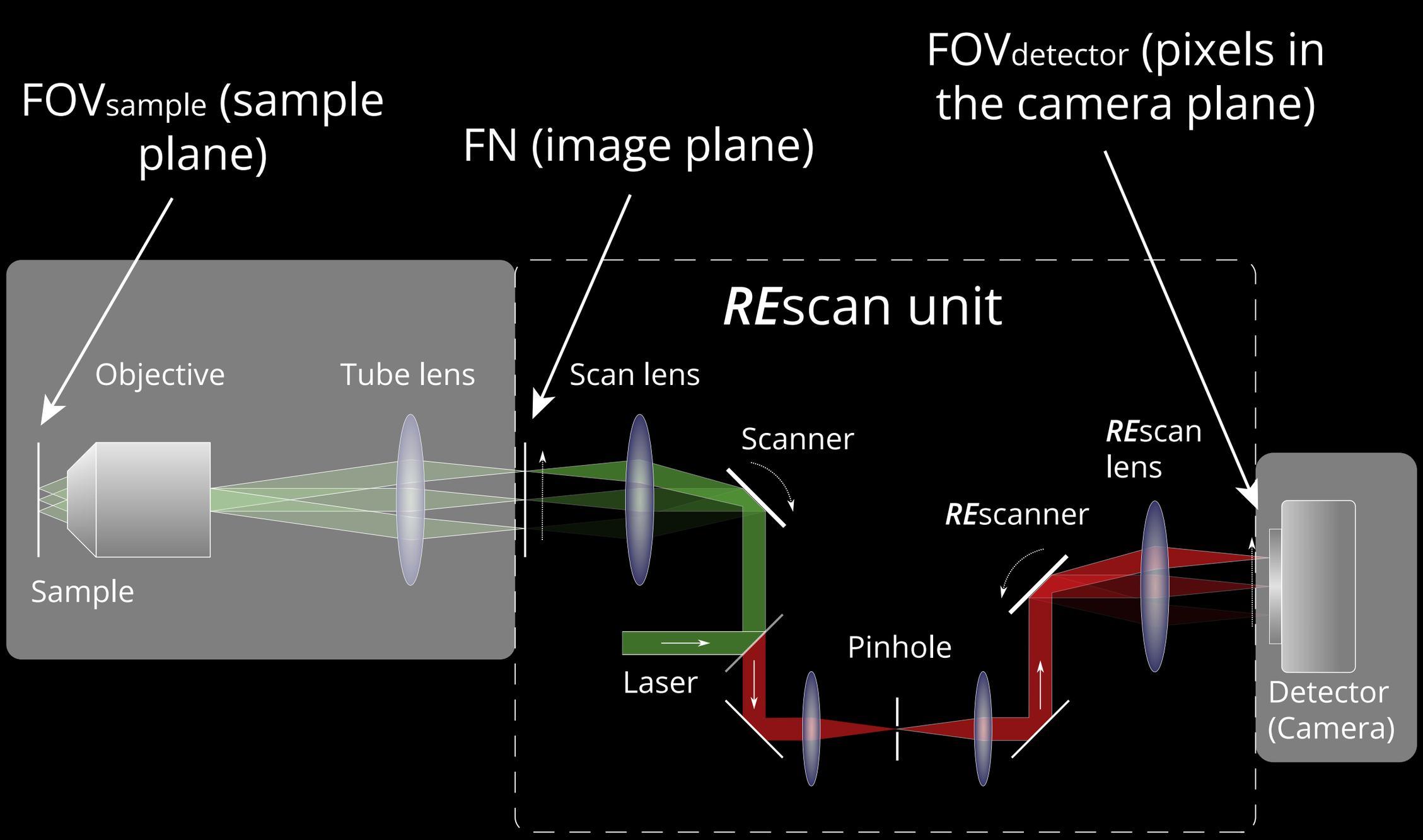


6.5 µm per px

Issue 2:
$FOV_{detector} \neq FOV_{sample}$



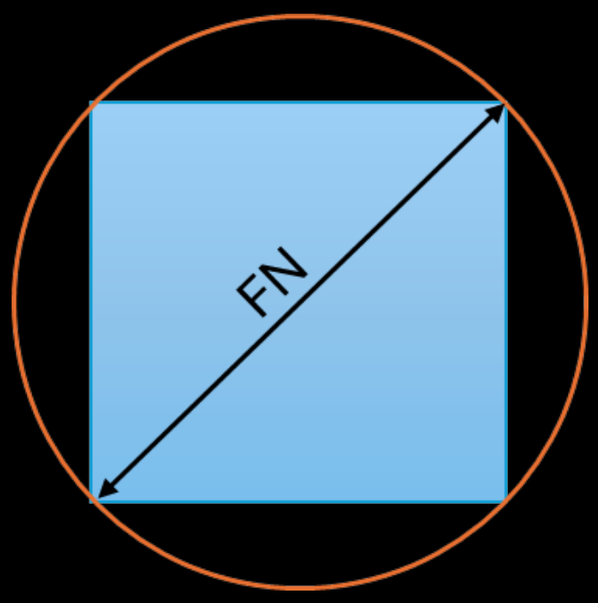
In ***RE***scan confocal, the size of the camera plane is not always identical to the FOV in the sample.



FOV can also be expressed as the Field Number (**FN**) in the image plane in mm.

The solution: FOV in units of length

The FOV_{sample} is the Field Number divided by the objective magnification (**M**).

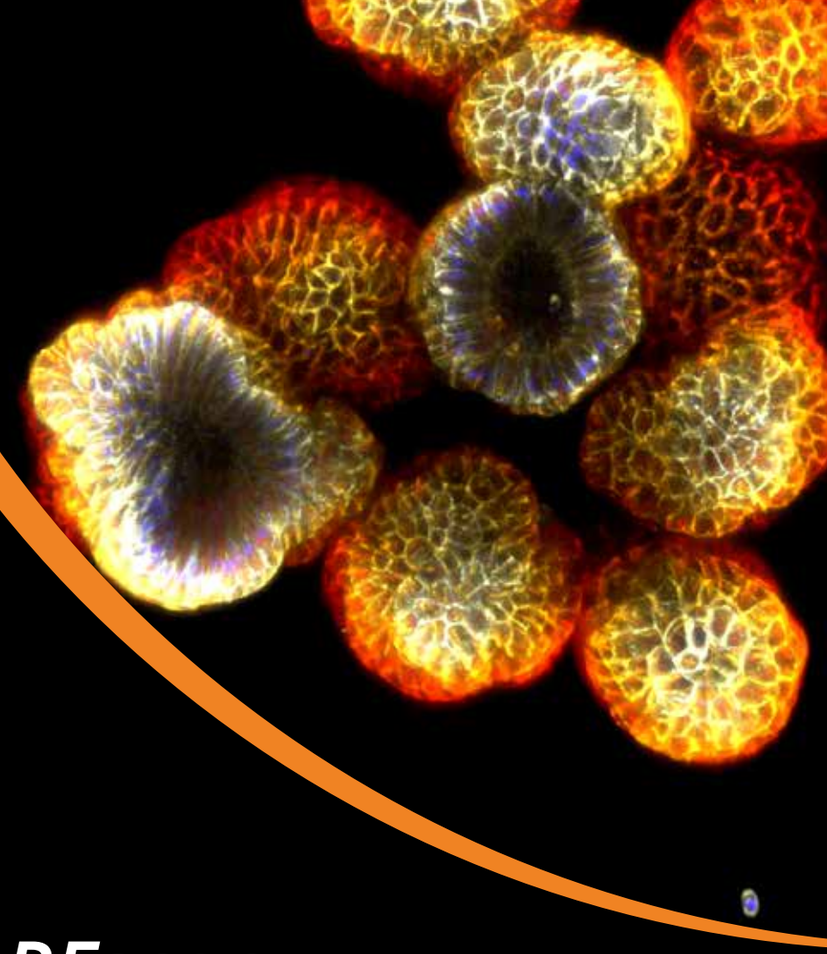


$$FOV_{sample} = \frac{FN}{M}$$

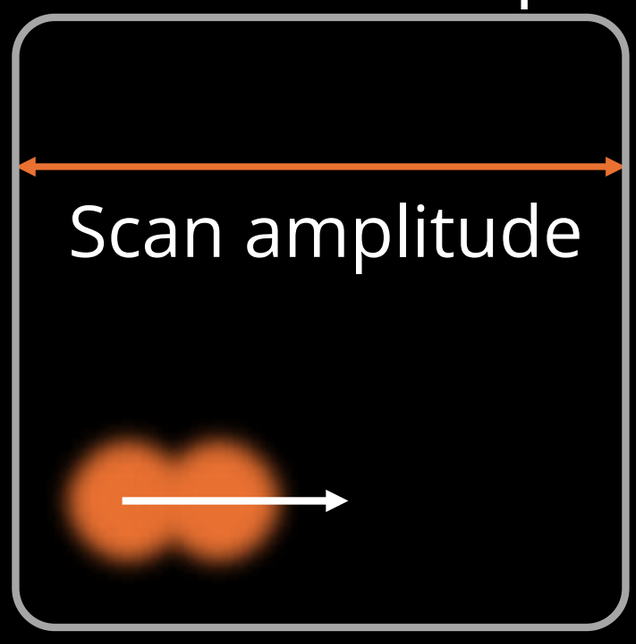
FN	FOV in mm²	Number of 6.5 µm pixels	Number of 4.6 µm pixels
12.5	**8.9 x 8.9**	1369 x 1369	1935 x 1935
18.8	**13.3 x 13.3**	**2048 x 2048**	2898 x 2898
22	15.6 x 15.6	2400 x 2400	3392 x 3392
25	17.7 x 17.7	2728 x 2728	3855 x 3855

Different ***RE***scan systems handle FOV_{sample} in distinct ways. For example, this table applies to GAIA but not to RCM2.

*RE*scan in RCM2

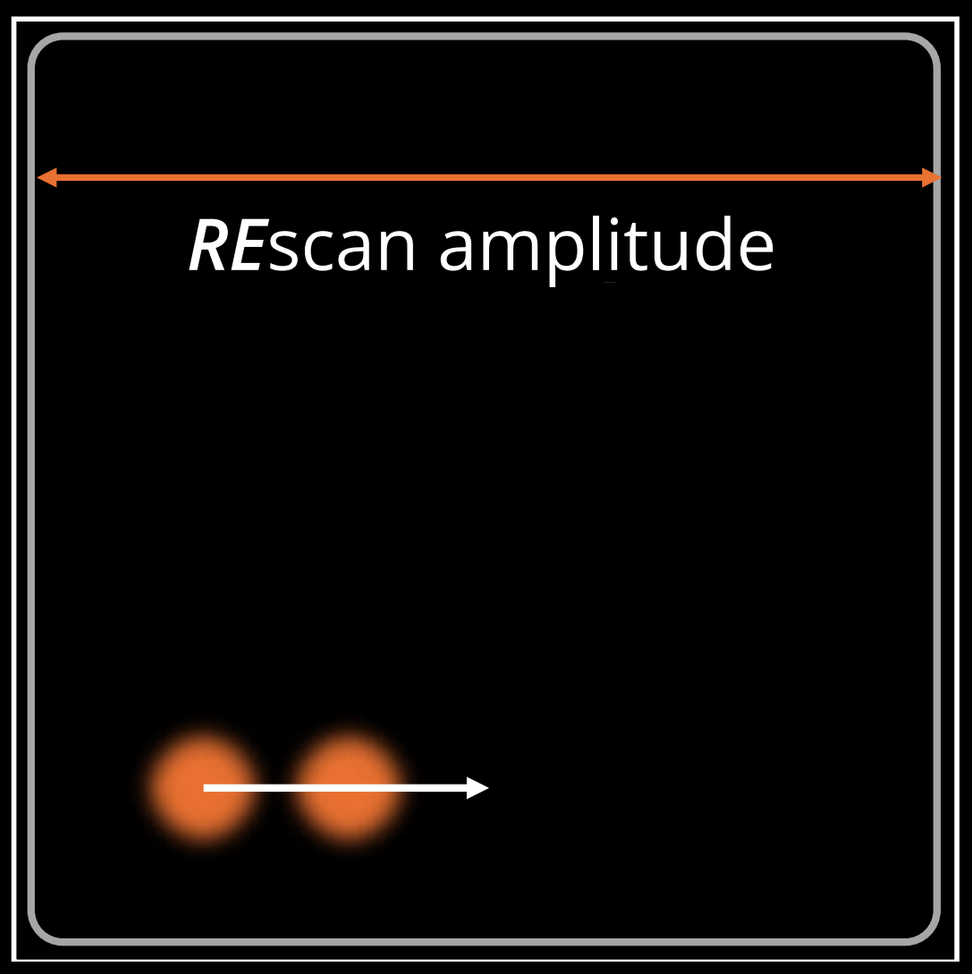


Scan on sample

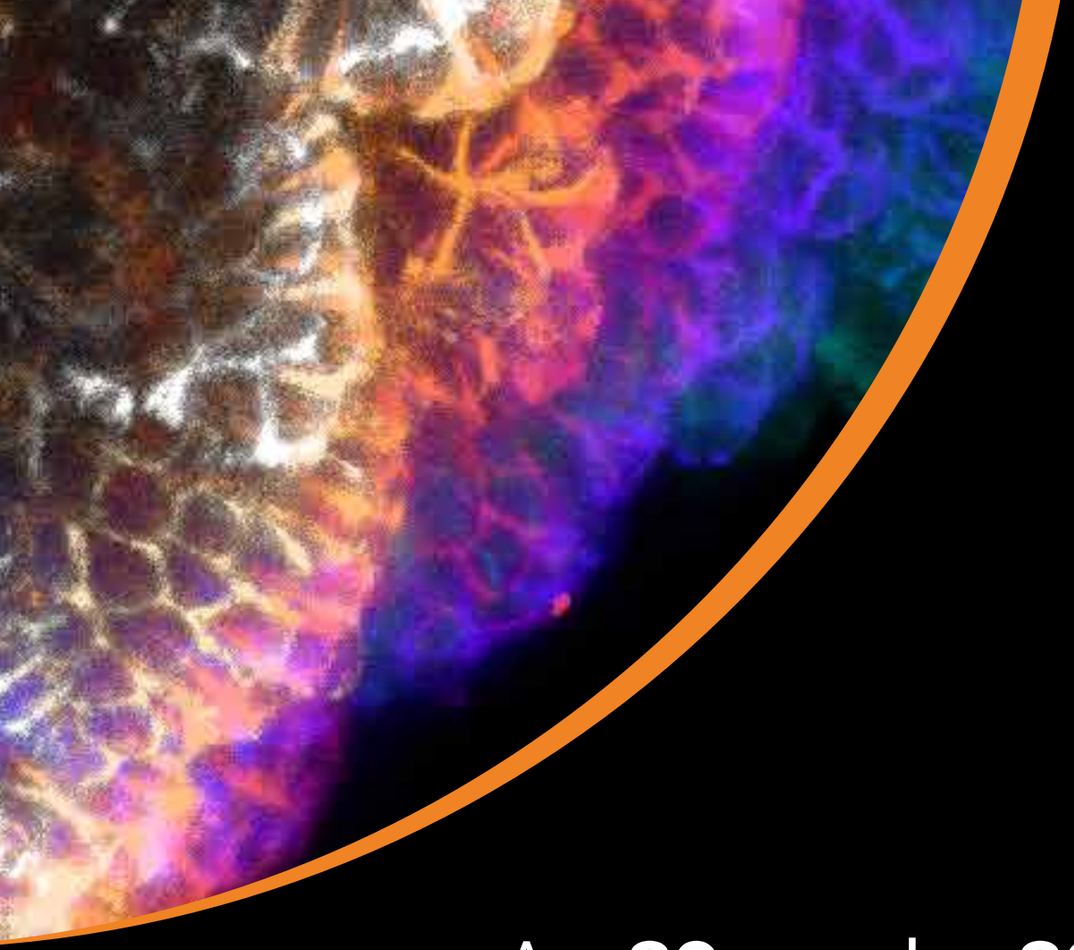


In RCM2, the ***RE***scan amplitude is larger than the scan amplitude, creating a super resolution image, yet limiting FOV_{sample}.

REscan on camera



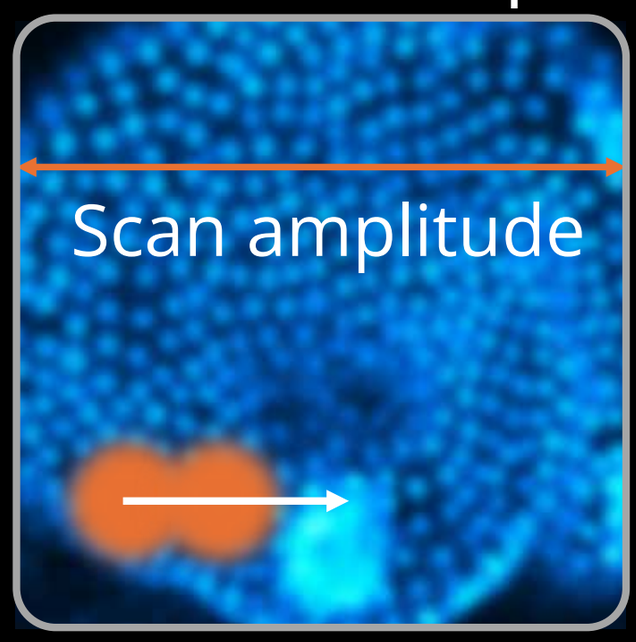
Here, the image plane is limited to **FN12.5** even though the camera plane is **FN18.8**.



FOV in RCM2

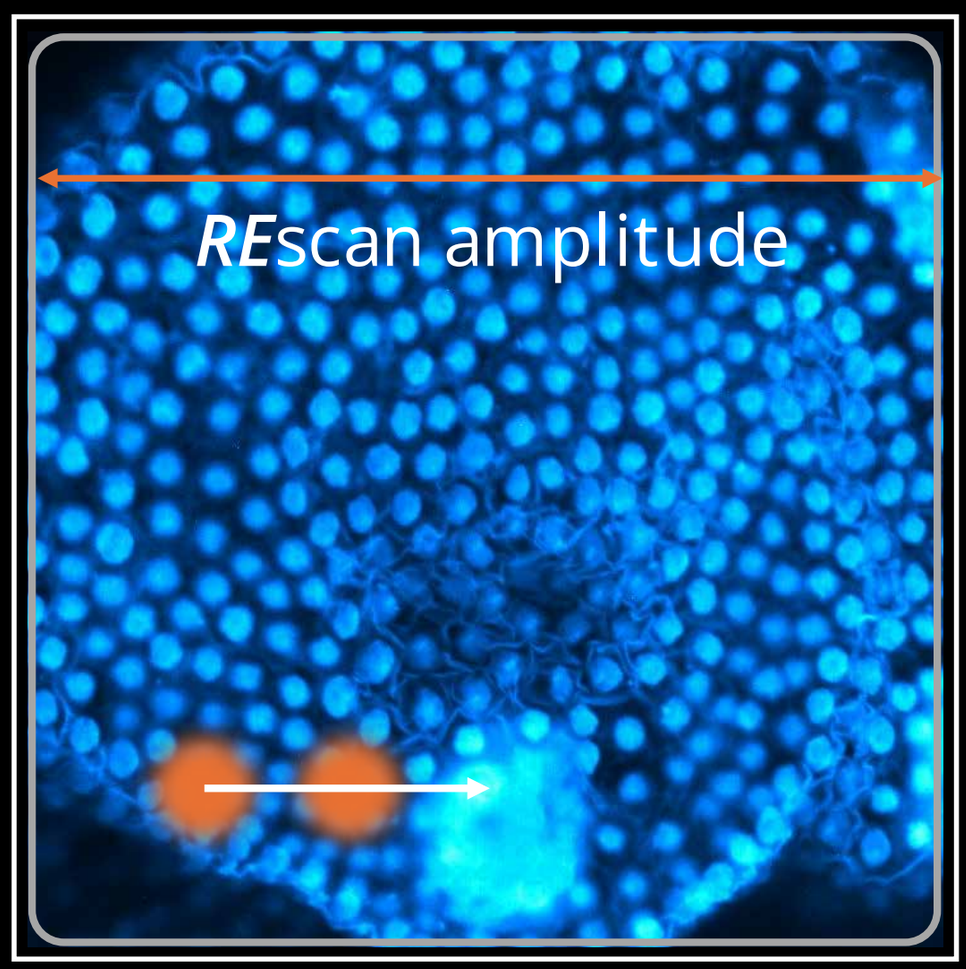
An **89 µm** by **89 µm** surface on the sample plane is scanned, when a 100x objective is used.

Scan on sample



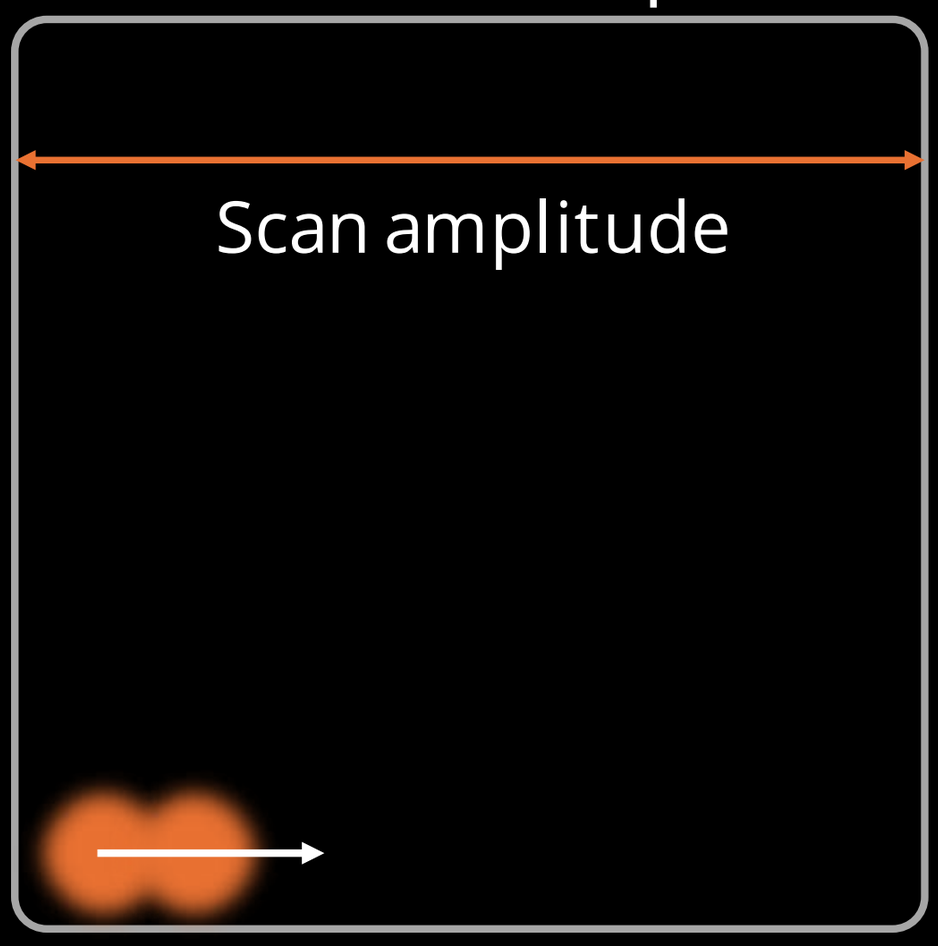
This is rescanned onto **2048** by **2048** pixels on the camera with a pixel size of 6.5 µm.

REscan on camera

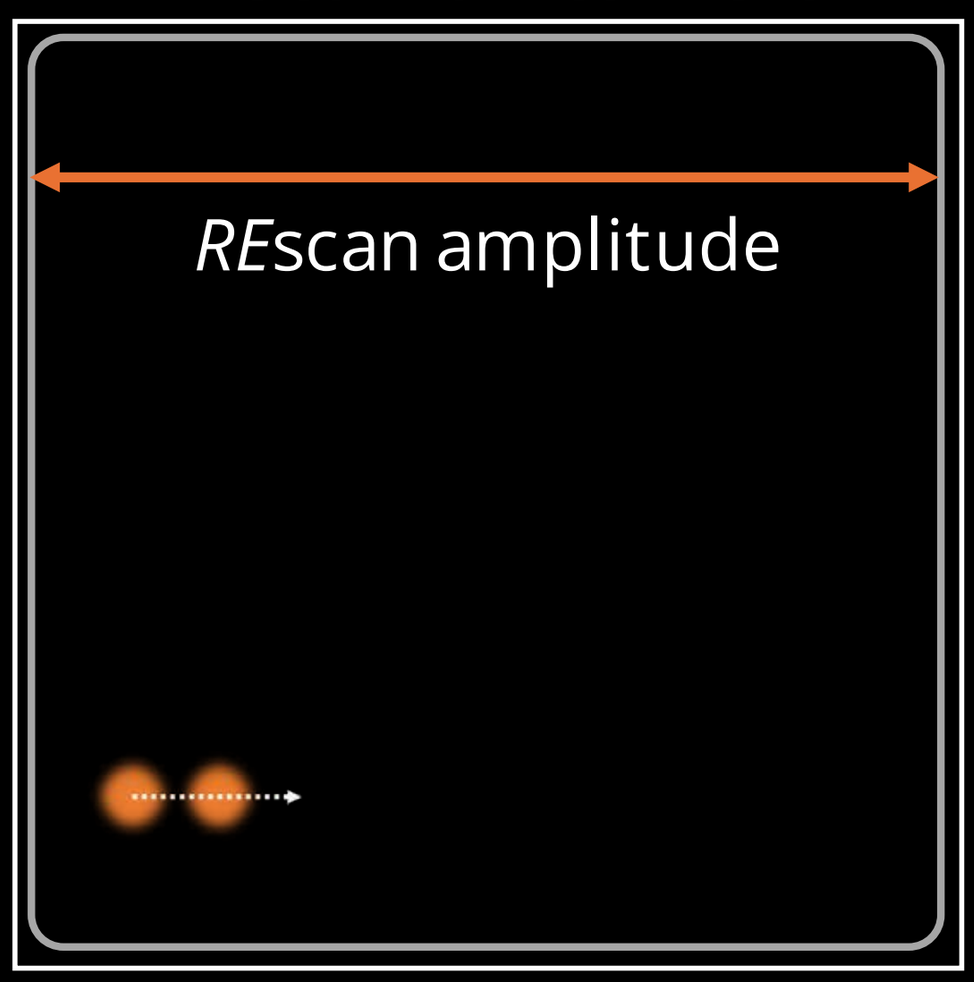


*RE*scan in GAIA

Scan on sample

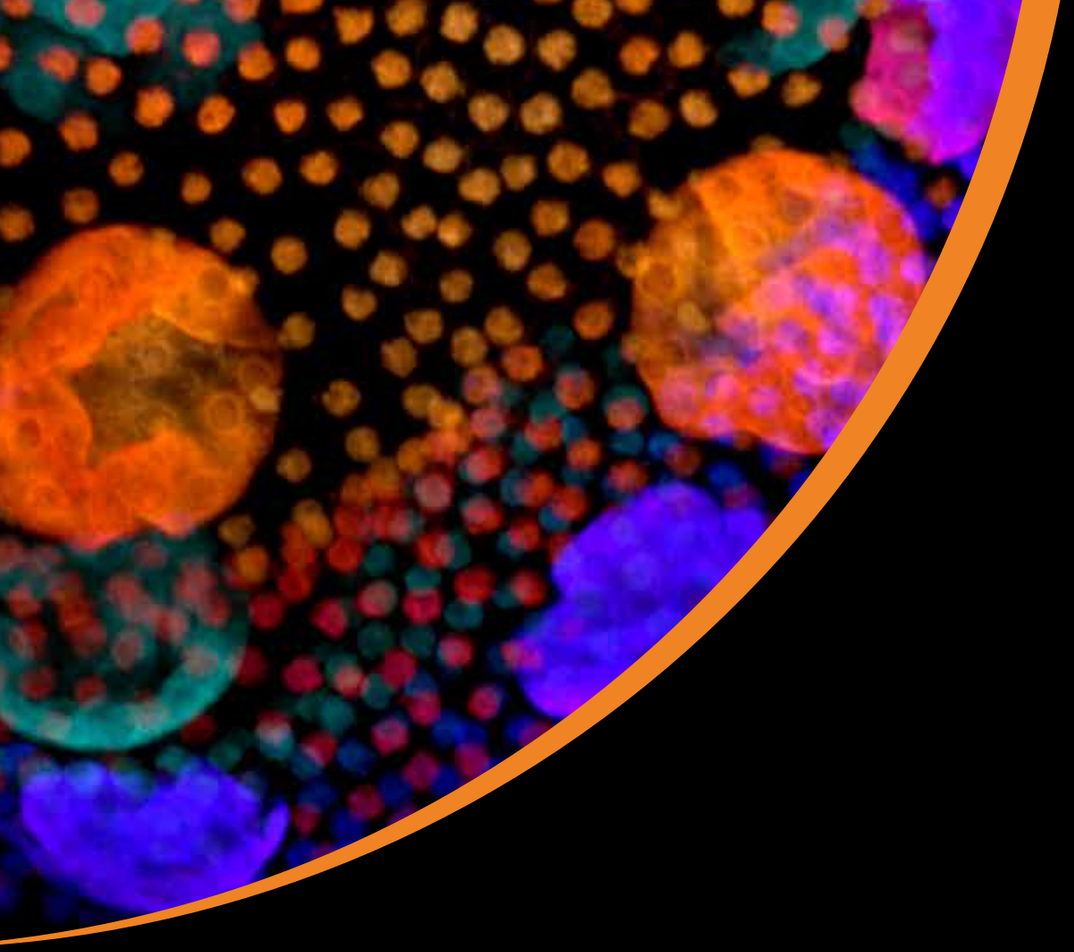


*RE*scan on camera



In GAIA, scan and ***RE***scan amplitude are always identical, creating super resolution images by using the complete **FN 18.8** of the microscope.

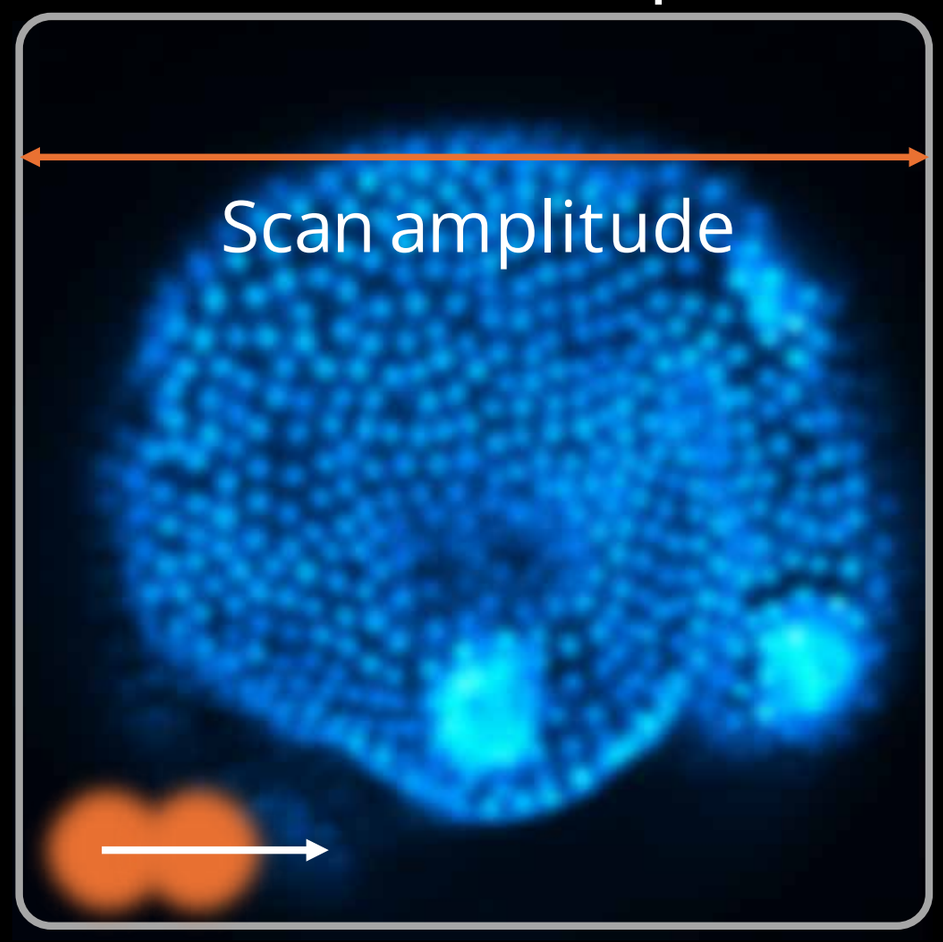
The rescanned spot on the camera is smaller than the scanned spot on the sample.



FOV in GAIA

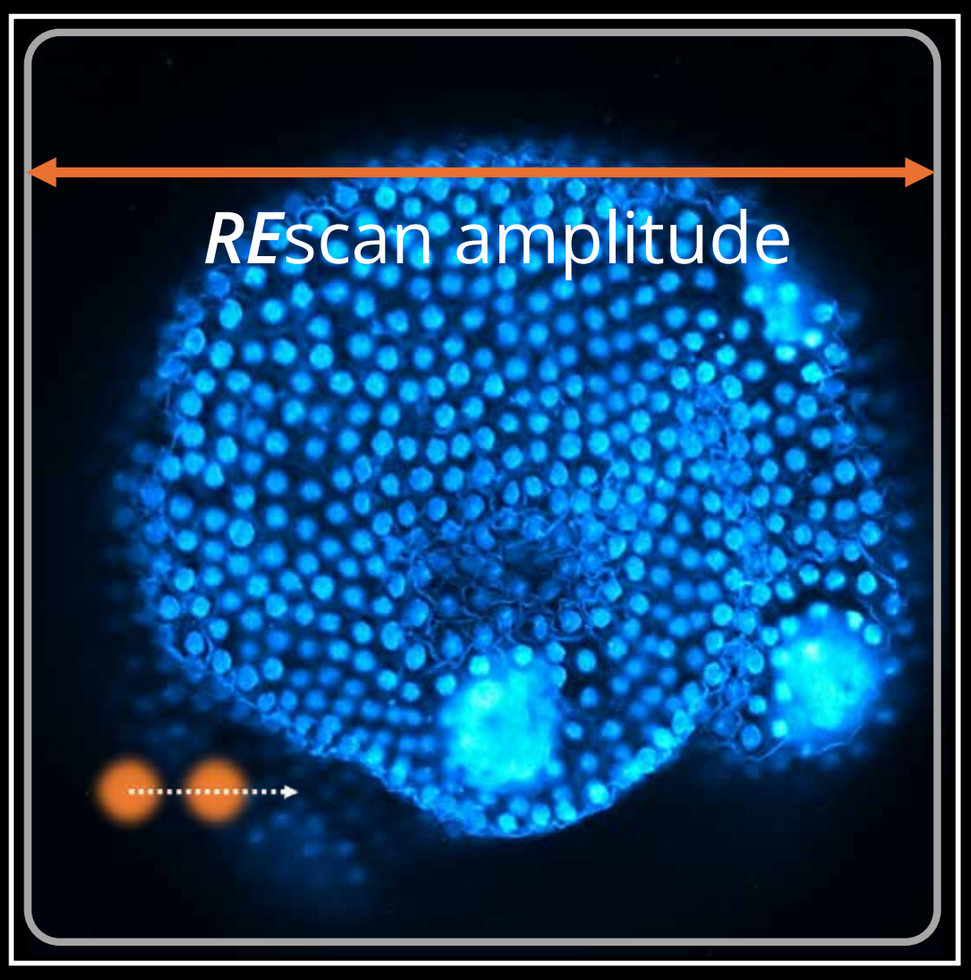
An **133 µm** by **133 µm** surface on the sample plane is scanned, when a 100x objective is used.

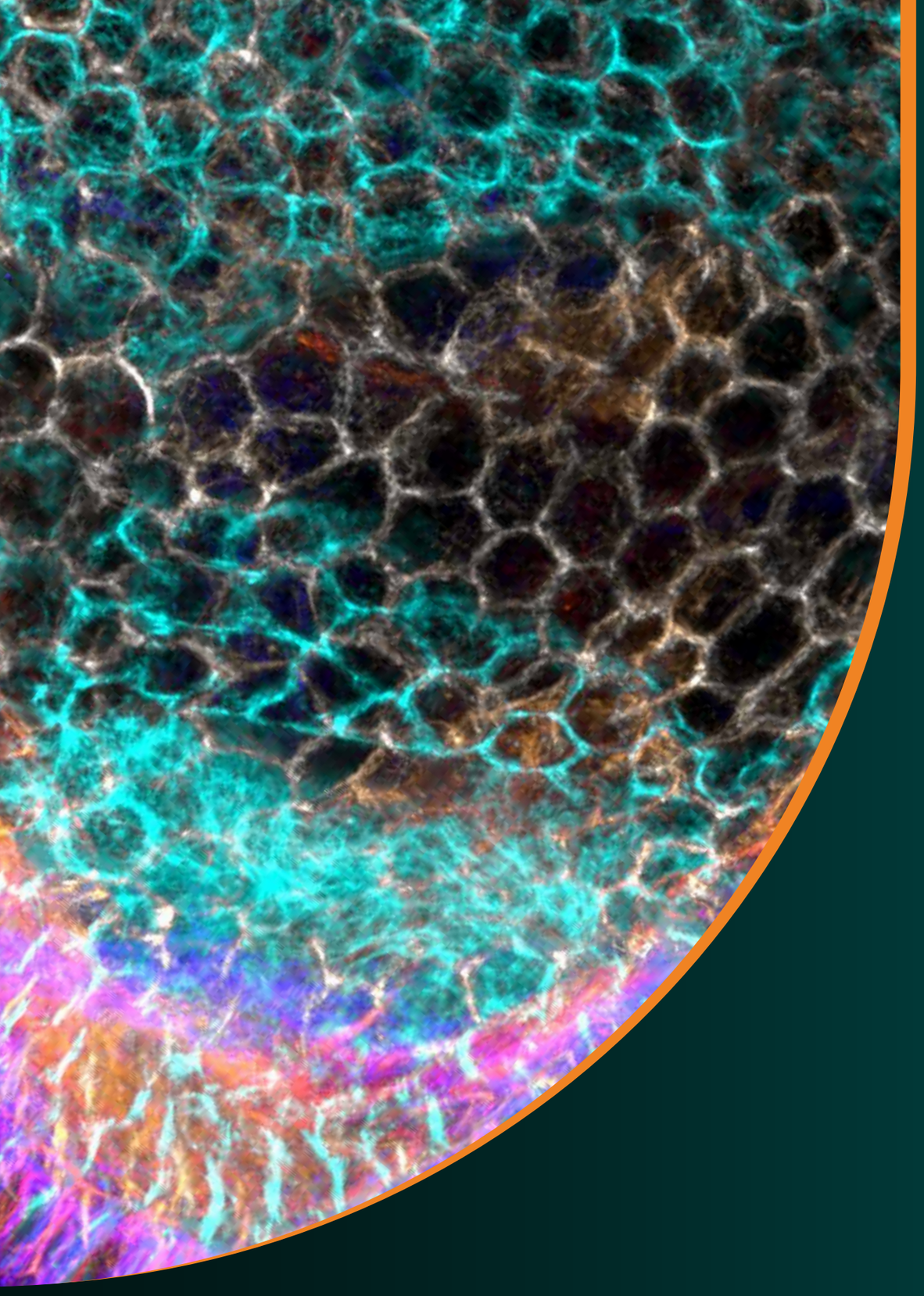
Scan on sample



This is rescanned onto **2048** by **2048** pixels on the camera, the **same FOV$_{detector}$** yet **larger FOV$_{sample}$** when compared to RCM2.

REscan on camera





Confocal nl

Find out more at

www.confocal.nl

Point *RE*scan

Line *RE*scan