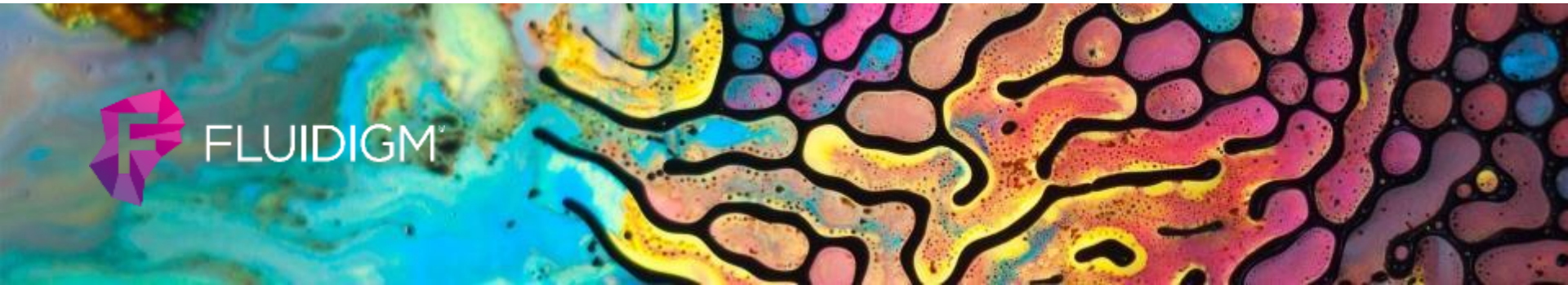


Hyperion Imaging System

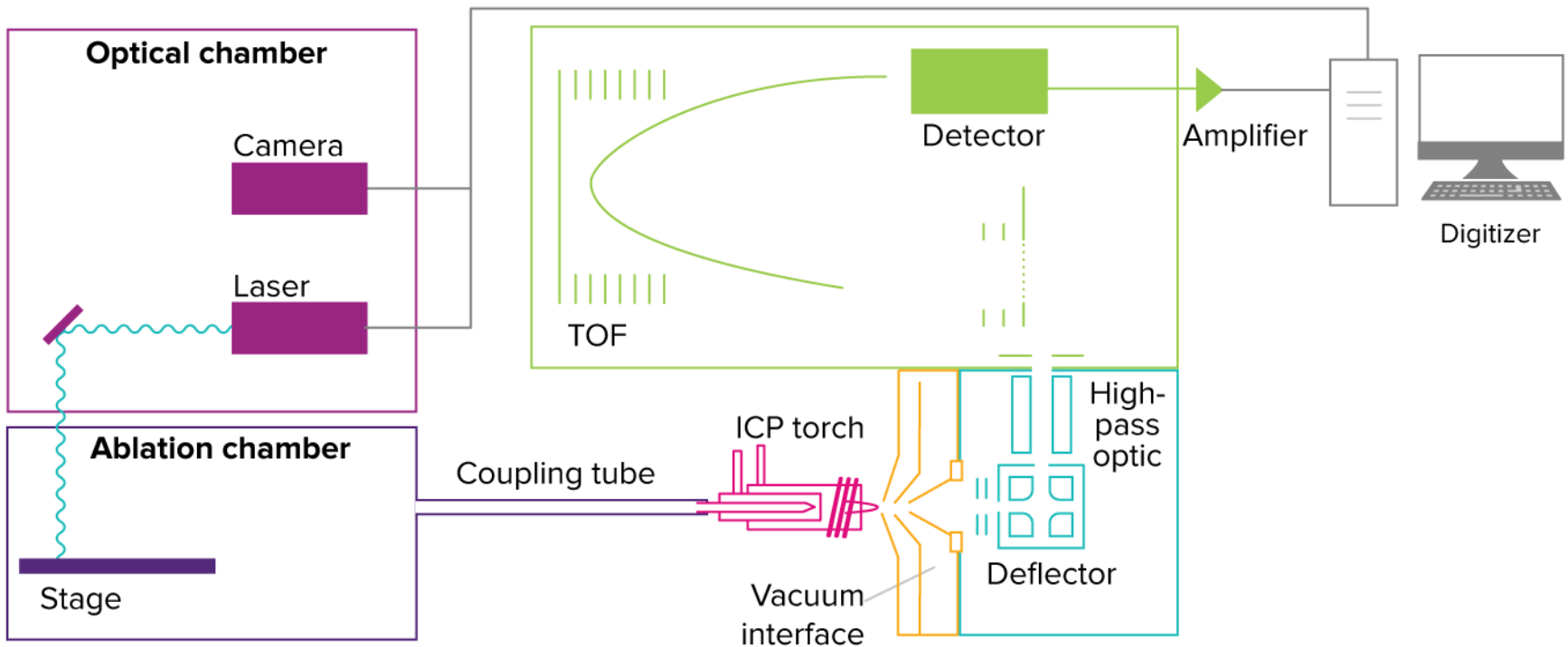
Introduction to Sample Acquisition



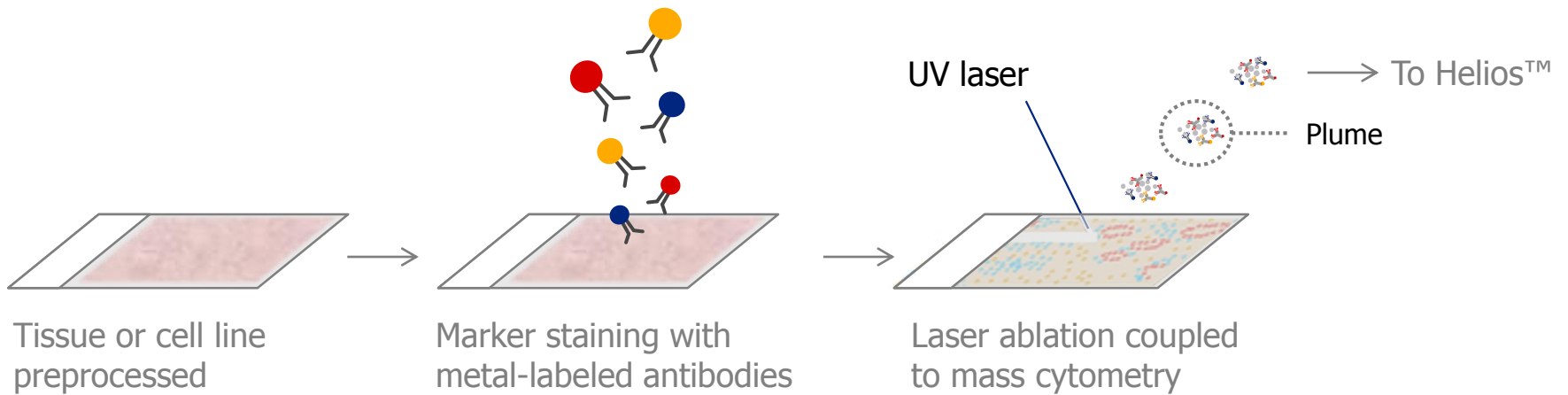
FLUIDIGM



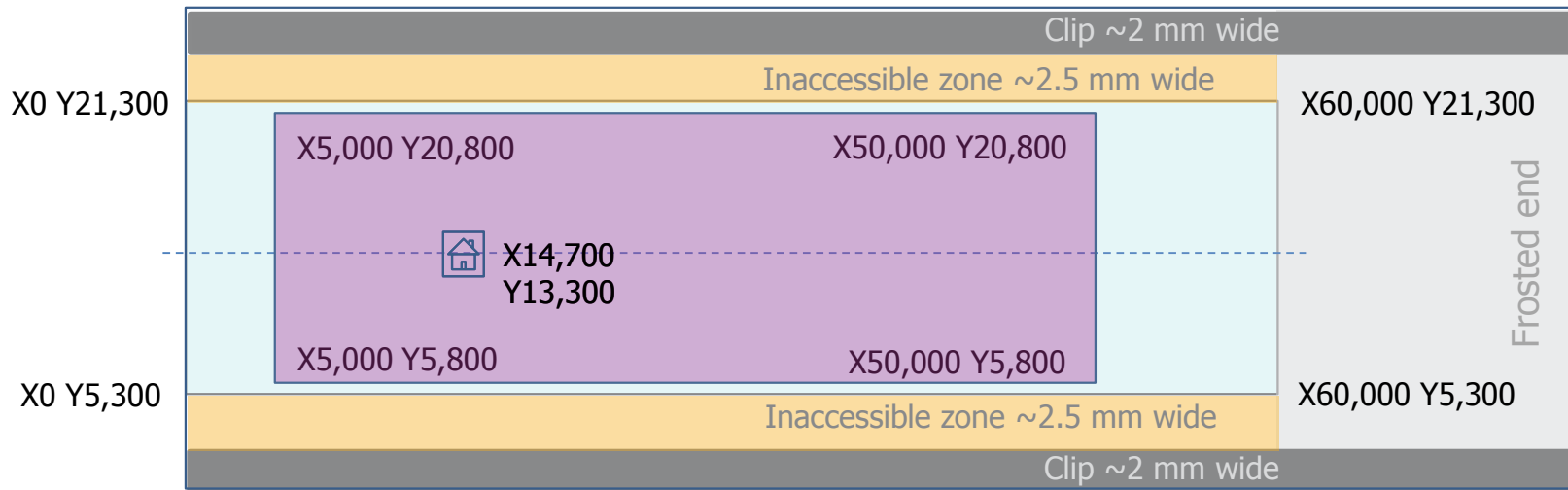
Hyperion™ Imaging System overview



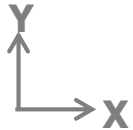
Laser ablation overview



Slide orientation



X0 Y0



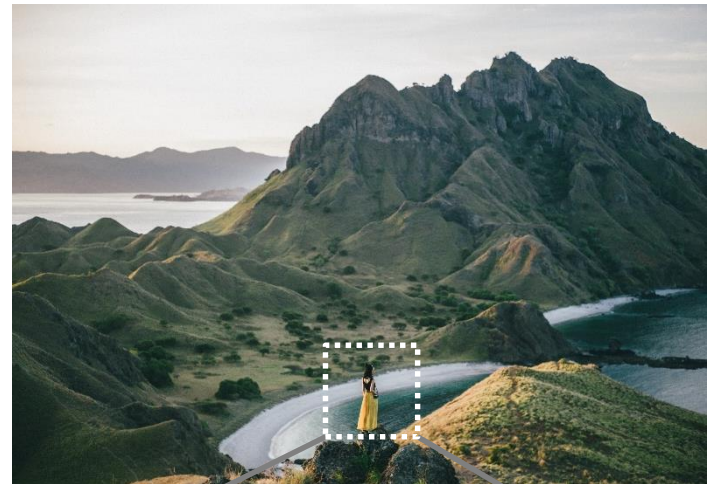
Dimensions of accessible area: 60,000 μm (X) by 16,000 μm (Y)

Dimensions of Fluidigm
validated addressable area: 45,000 μm (X) by 15,000 μm (Y)

Panorama and region of interest

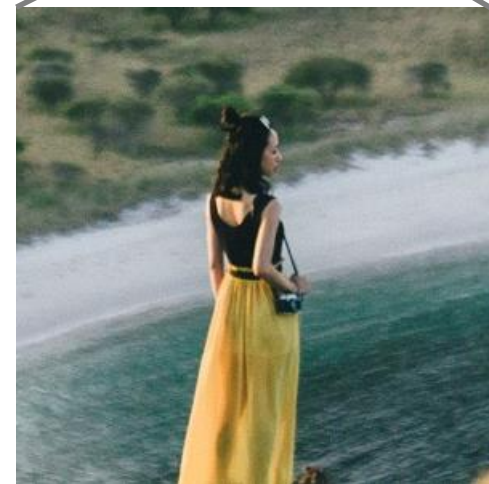
Panorama

- A high-level image of a section of your slide (or all of it)
- Panorama is just an image.
- Panorama does not get ablated.



Region of interest (ROI)

- A part of your panorama that you are interested in ablating
- ROI gets ablated.



Defining a panorama

Slide 25000x75000, (11,313 , 6.660) μm

Add Panorama

X1 μm 28,188 Width μm 3,375
Y1 μm 16,160 Height μm 5,500

Add

Creating... Cancel

Description	Status	Sele
Pano 01	Done	<input type="checkbox"/>
Pano 02	Creating	<input type="checkbox"/>

Panorama

Pano 01, (30,254 , 15,432) μm , Zoom: 0.13X

1000 μm

Add ROI

X (μm) 29601
Y (μm) 15030
Width (μm) 1500
Height (μm) 2000

Add

ROI 02
x: 38800 y: 15960 μm
w: 1500 h: 1999 μm
Duration: 18.16 s

Acquisitions (1)

Region of interest

Slide 25000x75000, (11,313 , 6.660) μm

Add Panorama

X1 μm 28,188 Width μm 3,375
Y1 μm 16,160 Height μm 5,500

Add

Creating... Cancel

Description	Status	Sele
Pano 01	Done	<input checked="" type="checkbox"/>
Pano 02	Creating	<input type="checkbox"/>

Panorama

Pano 01, (30,254 , 15,432) μm , Zoom: 0.13X

1000 μm

Add ROI

X (μm) 29601
Y (μm) 15030
Width (μm) 1500
Height (μm) 2000

Add

ROI 02
X: 48800 Y: 15960 μm
W: 1500 H: 1999 μm
Duration: 18.16 s

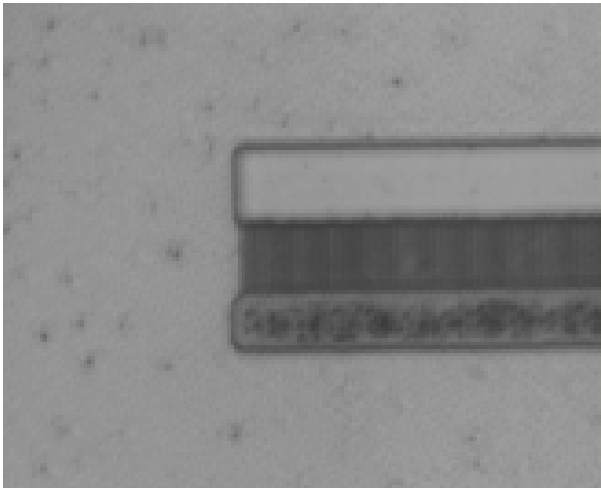
Acquisitions (1)

Ablation energy

Ablation energy: Energy of the laser beam delivered to the sample. It can be varied by the energy attenuator.

Laser is calibrated to ablate tuning film using the attenuator settings of 0 dB. This setting will have to be increased over time as the laser ages.

Ablation energy



Unablated region

Optimal: clear (bright) ablation region

Too high: cutting through glass

Too low: leaving ghost material behind

It is important to check ablation energy to ensure proper sample ablation.

- Use an area that is not needed for data collection.
- Ensure that the sample is ablated completely.
- Do not overablate samples and etch the glass slide.

Summary

Certain regions of a slide cannot be ablated due to restrictions governing the movement of the stage.

Select a region of your sample to generate a panorama.

Within the panorama, select an ROI to ablate.

Ensure that the proper ablation energy is used.

**Simplify the complex
quest to understand and
apply biology.**



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