Institut de Recherche en Cancérologie
I R C Montpellier

HAU901I

Single-cell transcriptomics

Spatial transcriptomics & proteomics

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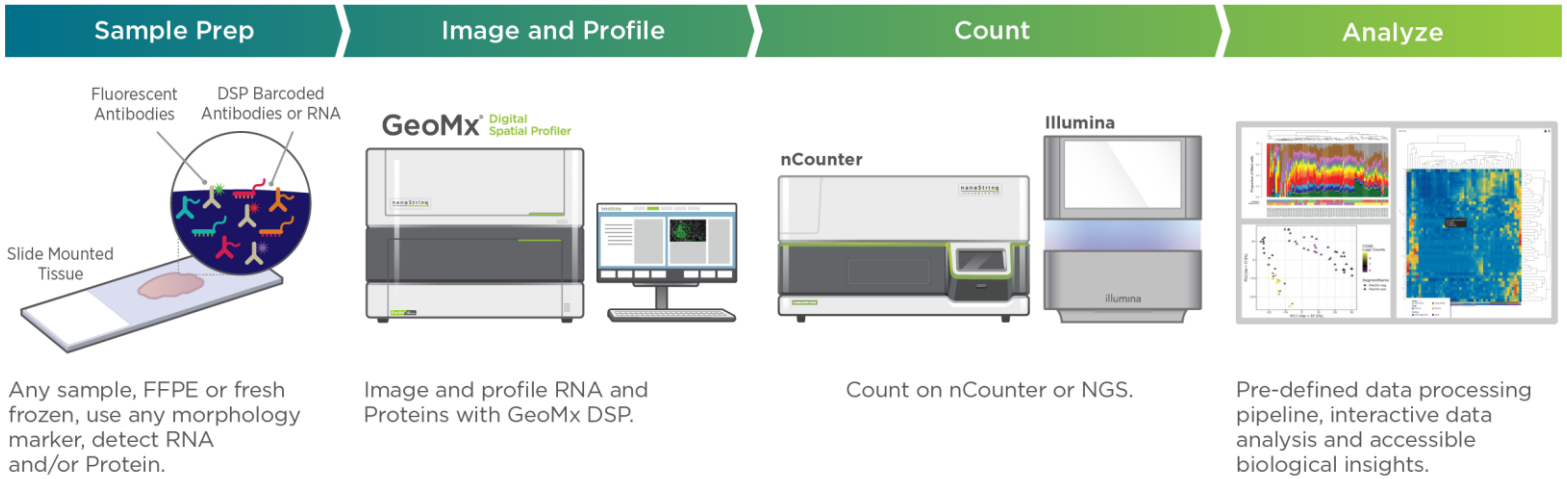
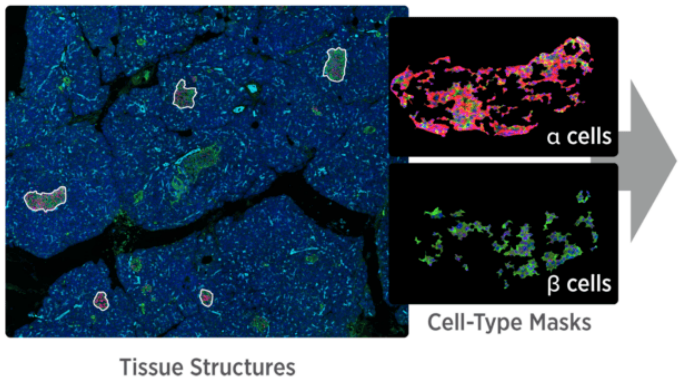
Overview

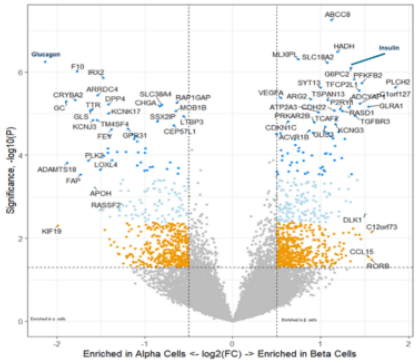
+ Spatial biology is attracting a lot of attention
+ It enables the mapping of tissue organization at the molecular level with an unprecedented precision
+ Main technologies are
  - Spatial transcriptomics, based on probes or sequencing
  - Spatial proteomics, based on specific antibodies and fluorescence or mass spectrometry
  - Spatial metabolomics, based on mass spectrometry imaging ; more marginal so far
+ Trend to develop devices acquiring RNA and protein data from the same sample
+ These technologies exist at different resolutions, from subcellular to medium-scale, multicellular
+ They can measure rather large pieces of tissues (1-2 cm^2) or small regions of interest (ROIs, 1-2 mm^2)

nanoString GeoMx

+ Define ROIs in FFPE or frozen tissue sections, close to micro-dissection
+ Very low resolution, but entire transcriptome (18k genes) and the possibility to also follow up to 100 proteins with Ab from the same ROIs
+ A few Abs (3-4) are used to define the ROIs

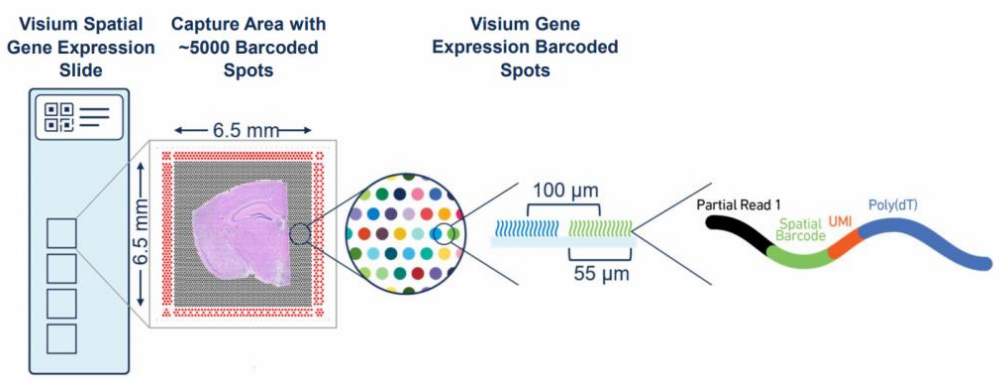


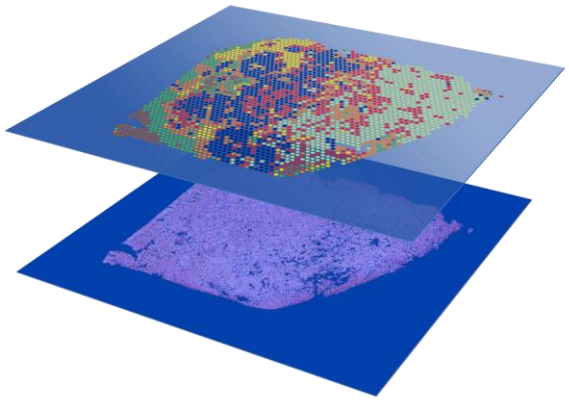




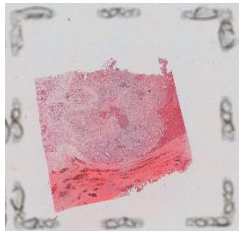
10x Genomics Visium

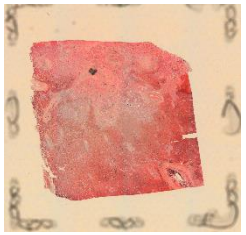
+ Application of a grid, each grid point is associated to a specific barcode, FFPE & frozen samples
+ Rely on scRNA-seq technology
+ ~1,000 genes *per* spot, unbiased





Tissue sections

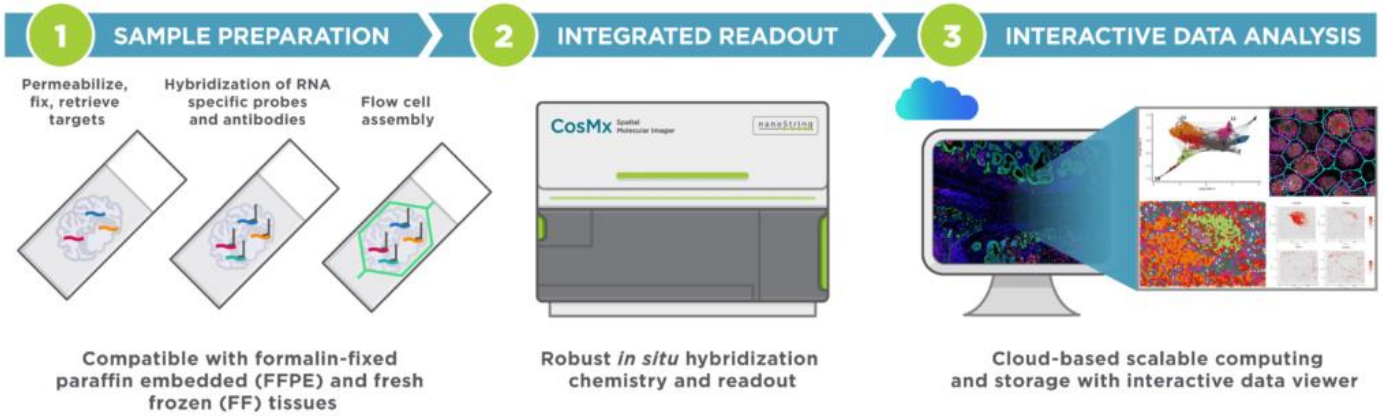


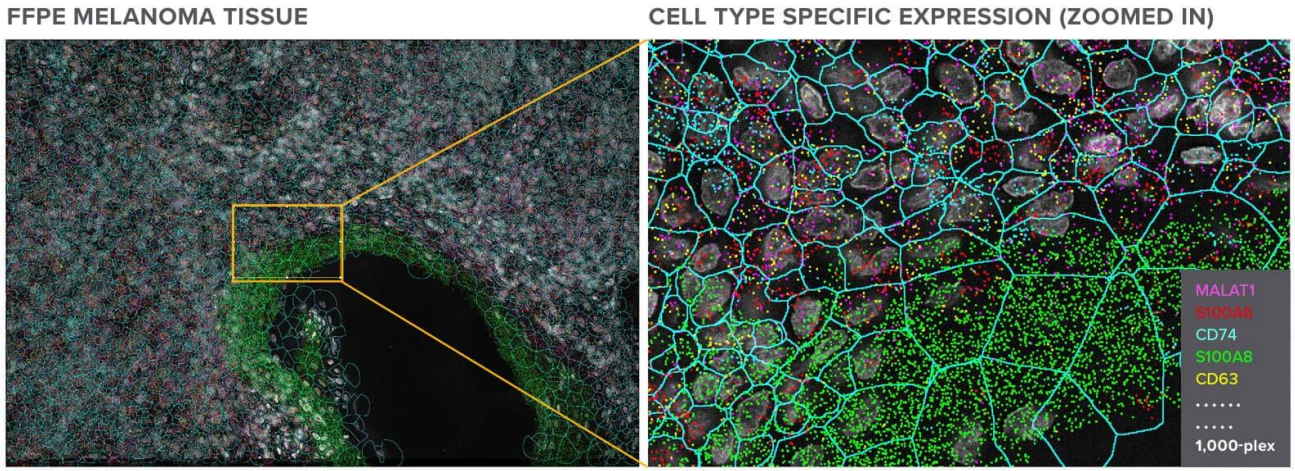


Colorectal liver metastases

nanoString CosMx SMI

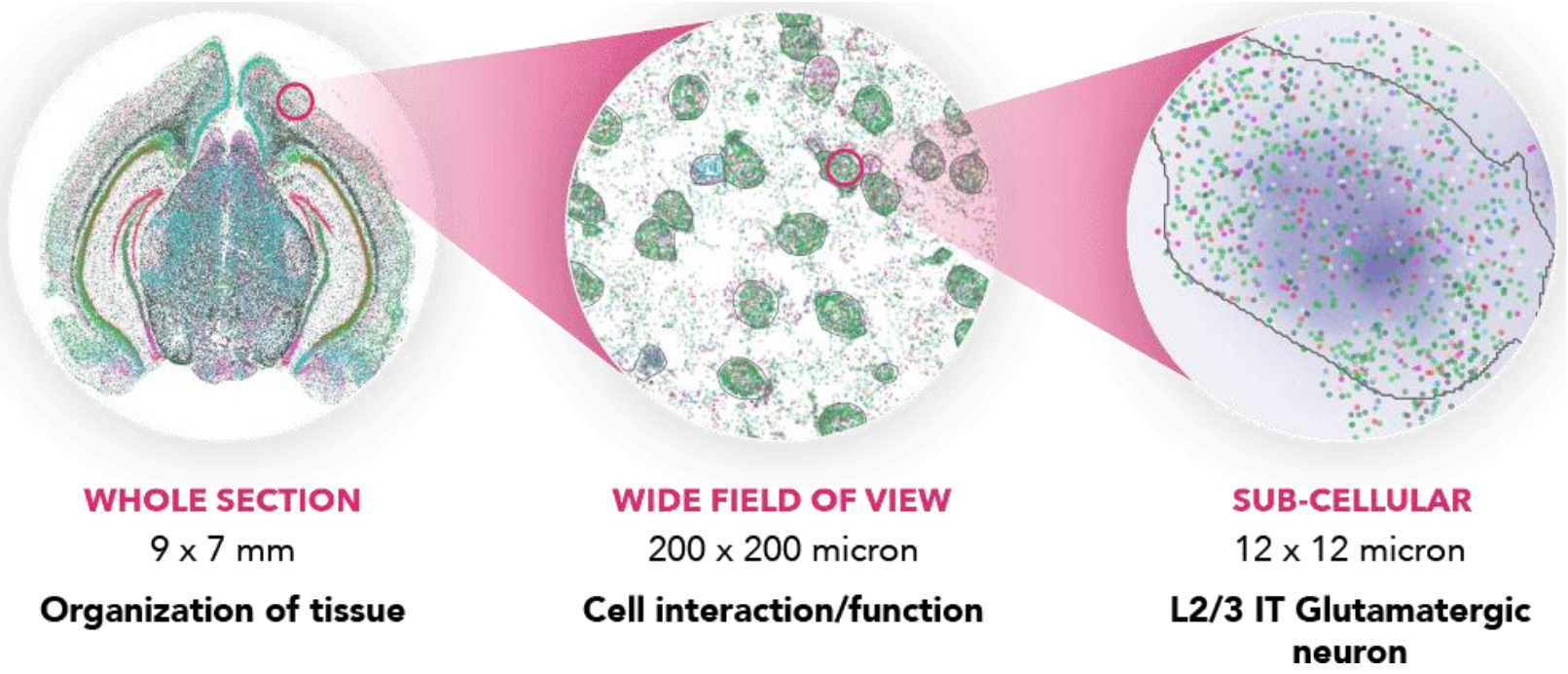
+ FFPE or frozen tissues, subcellular resolution
+ Up to 1,000 RNA and 64 proteins





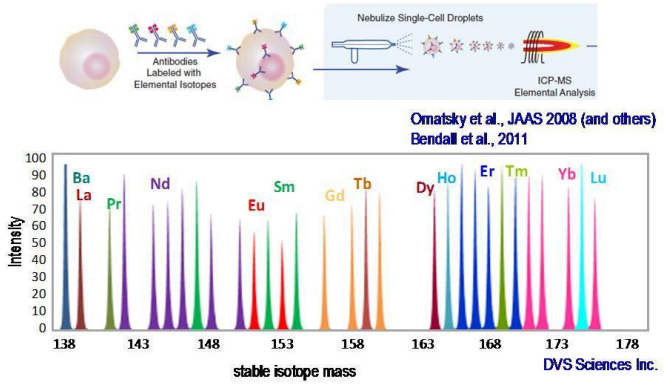
Other transcriptomic platforms with subcellular resolution

+ RNA-probe-based technologies
+ FFPE & frozen samples
+ 10x Genomics Xenium, up to 1,000 transcripts
+ vizgen MERSCOPE, up to 500 genes

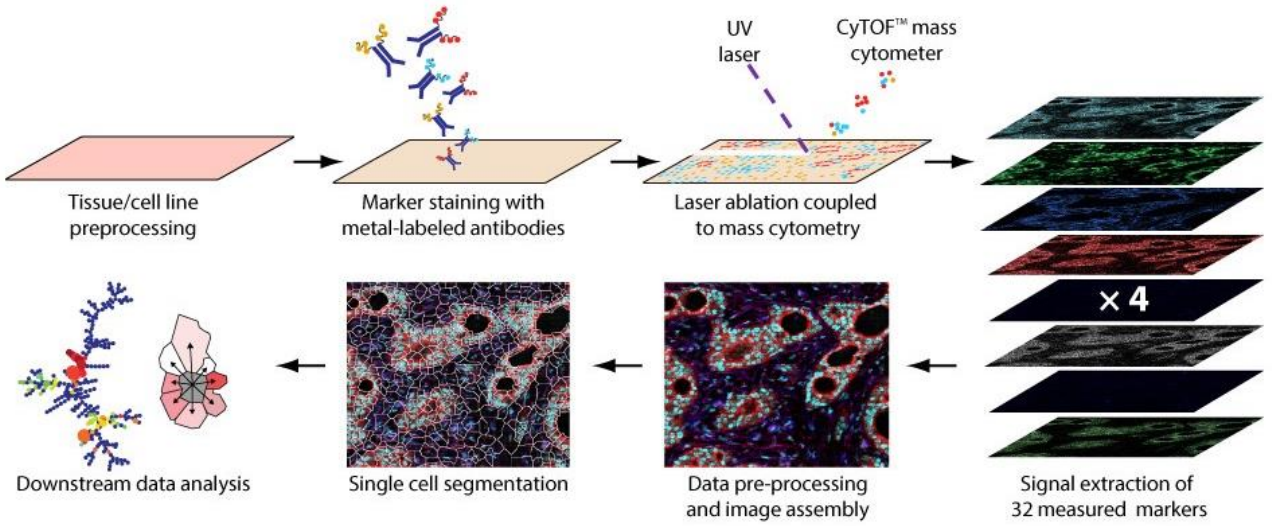


Spatial proteomics by mass spectrometry: CyTOF

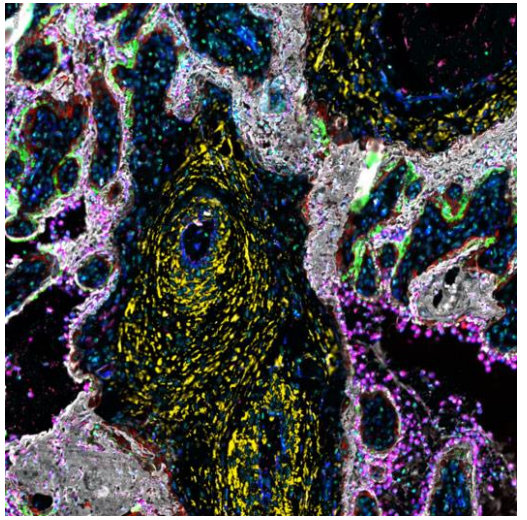
+ Principle: up to 40 Abs are linked to rare metals with distinct masses
+ Signals at corresponding masses inform on the cell surface markers
+ Can be used as a highly multiplexed flow-cytometry device



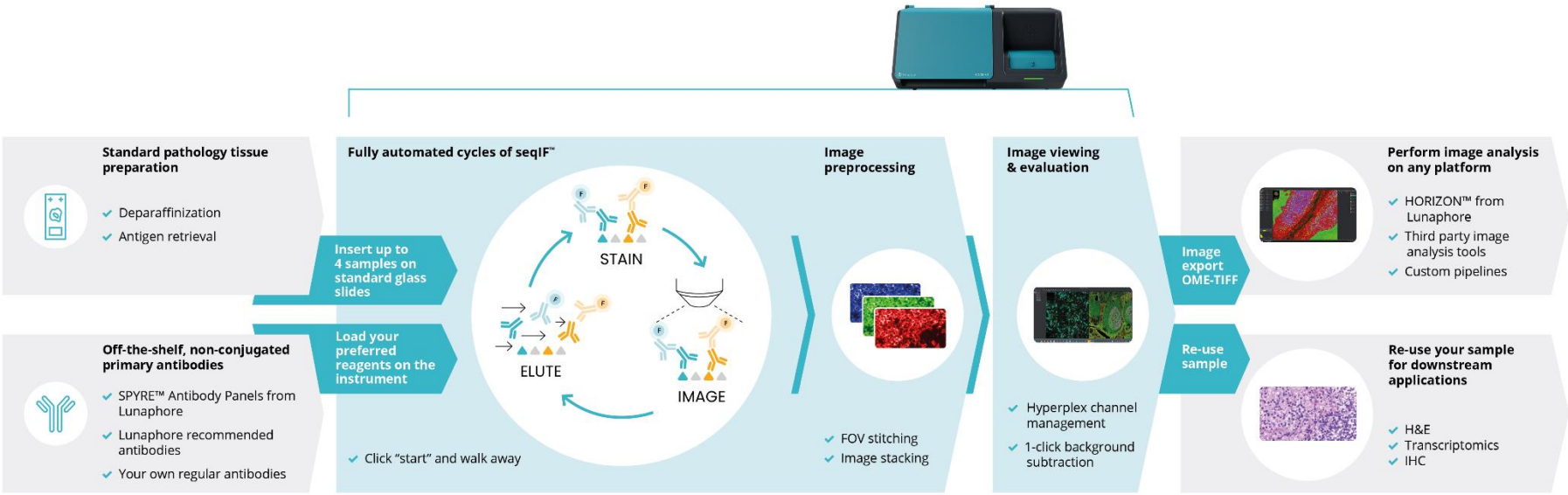
+ With the hyperion modeule, CyTOF can also be applied to frozen or FFPE tissue sections analyzing small ROIs on a grid with subcellular resolution



Multiplexed antibodies

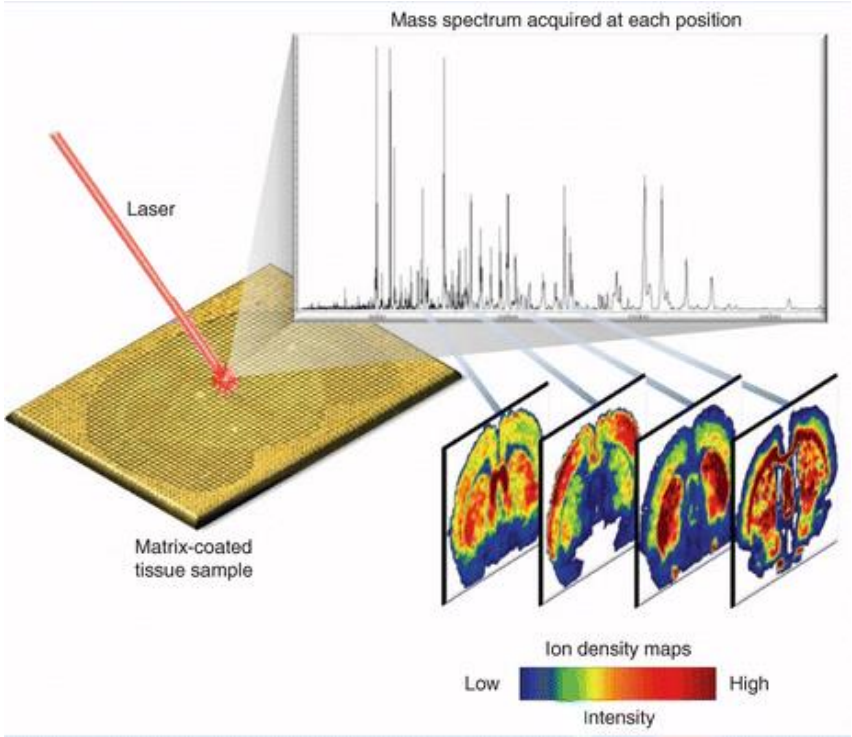


+ A number of companies have developed devices to perform multiplexed Ab fluorescence-based imaging
+ Usually, different cycles of staining with 40-50 Abs simultaneously
+ Washing or alternative methods are applied to release the Abs between cycles
+ Most technologies propose up to 200-300 Abs in multiple cycles of staining/washing, subcellular resolution on rather large sections (1-2 cm^2)



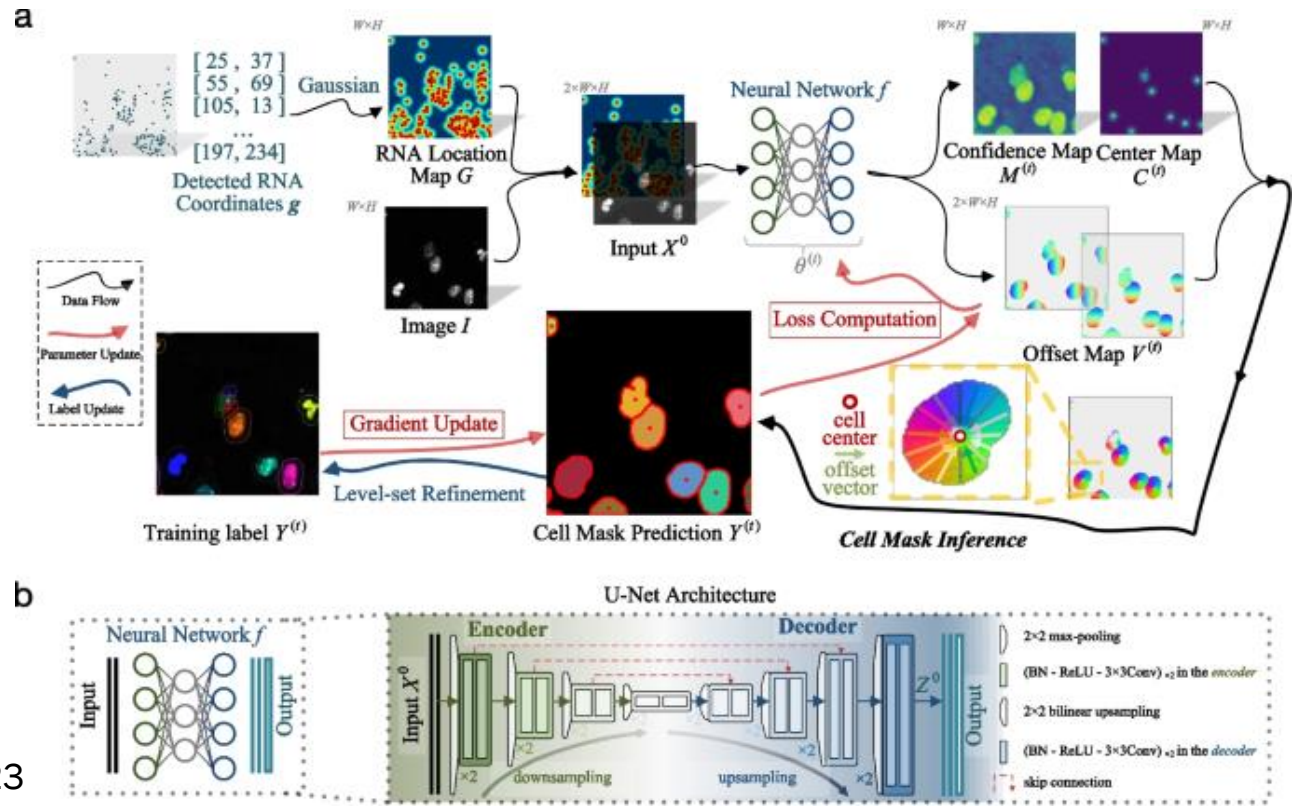
Mass spectrometry imaging (MSI)

+ Molecules must be ionized to be analyzed by mass spectrometry
+ An old technique calles matrix-assisted laser desorption ionization (MALDI) uses a laser to shoot and ionize material deposited on a metallic plate
+ This can be turned into an imaging device by having a tissue section on the metalic plate and shooting the laser on a grid
+ Ionized molecules are difficult to identify, they can be proteins or metabolites
+ Usually, they are lipids in majority
+ Hence, spatial metabolomics
+ Each mass is a molecule and peak intensities correlate with abundance
+ MSI can thus be regarded as multiplexed imaging



Cell segmentation software for subcellular resolution

+ A large number of sw packages are available to detect the individual cells from an image, and to assign an average expression to each transcript or protein
+ QuPath, Cellpose, GeneSegNet, CellSeg, StartDist, CellProfiler, etc.
+ Most tools rely on CNN
+ Output is typically a large table with cell coordinates and expression values for each quantified molecule



GeneSegNet
Wang, Genome Biol, 2023