# Pyxo

Stellaromics

Discover the only technology that creates fully annotated, single-cell maps of 3D gene expression in thick tissue sections up to 100µm. Built on STARmap and RIBOmap technologies.





Stellaromics' Pyxa platform delivers true 3D spatial multi-omics in thick tissue sections. Stellaromics offers a streamlined end-to-end workflow from kit-based sample preparation to automated fluidics, confocal volumetric imaging and integrated GPU-enabled signal processing on Pyxa to intuitive 3D visualization and analysis with PyxaStudio™ software.

Stellaromics.com



# Pyxa is Spatial, Cubed

# **Convoluted Shapes**

2D Slide Viewer

**3D Reality** 

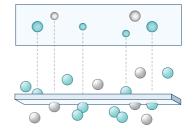
**Features** 

**Applications** 

Vasculature, neurons & fibrosis

NASH, other fibrosis, neuroscience, ophthalmology, organoids

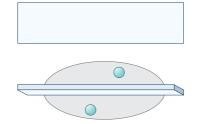
#### **Complex Distributions**



Spatial relationships between cell populations

Immuno-oncology, immunology, metabolic disease

## **Sparse Objects**



Rare cell populations in large volumes of tissue

Gene therapy, CRISPR, CAR-T

Pyxa from Stellaromics is the first platform to deliver true 3D analysis of thick tissue slices. Based on Stellaromics' patented STARmap technology, the Pyxa system measures RNA and RNA translation within intact tissue at the subcellular level.

Biological reality is governed by 3D cellular organization and interactions within the tissue microenvironment. Therefore, to truly comprehend these processes and develop effective interventions, a 3D perspective is required. This represents a move from observing isolated cells and thin 2D tissue sections to understanding the systemic architecture of disease, profoundly changing the types of questions researchers can ask and answer.

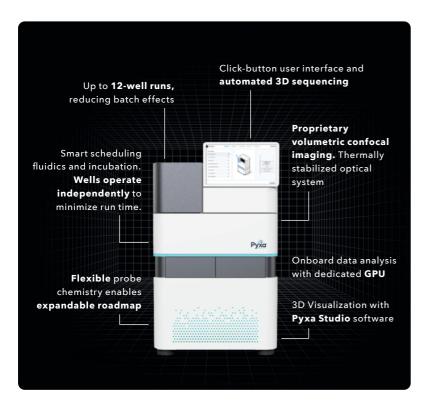
3D spatial transcriptomics allows scientists to survey a larger volume of cells within the same spatial area, increasing the odds of capturing a rare event such as gene edits, formation of neural connections, cellular differentiation, or immune cell activation. This technology has the power to rapidly advance numerous fields of research including neuroscience, oncology, developmental biology, and therapeutic development and drug delivery.

# **3D Spatial Transcriptomics**

- Enhanced Insights: 3D spatial data with Pyxa provides a deeper understanding of cell-cell interactions and rare events missed by 2D assays.
- Precision and Accuracy: Whole cell analysis
  ensures precise localization of gene expression,
  critical for detailed molecular studies. Unlock new
  data modalities such as cell morphology through
  the analysis of intact cells.
- Rare Event Detection: Increased tissue volume imaged improves capture of rare cell populations and rare perturbations.
- Versatility: Curated and customizable gene panels for multiple assays make Pyxa<sup>™</sup> adaptable to a wide range of research applications.
- Ease of Use: Onboard analysis and automated workflows simplify operations, making advanced spatial transcriptomics accessible to more researchers.



# **Analyze Tissue with Unprecedented Depth**



- Tissue Thickness: up to 100μm
- Resolution: Subcellular transcript localization at minimum resolution of 350nm lateral and 1µm axial
- Gene Panels: Hundreds of validated genes, with customization options available
- Sample Processing: 12-well plate format
- Workflow: Automated sample imaging and on-instrument data analysis
- Data analysis: PyxaStudio and compatibility with open-source tools for quantitative analysis

# **Key Features of Pyxa**

- 3D Spatial Transcriptomics: Detailed visualization and analysis of gene expression within thick tissue sections, providing insights into cellular interactions and tissue architecture.
- STARmap Assay: Providing comprehensive spatial transcriptomics, along with nuclear detection.
- State of the Art: Custom optics and proprietary multi-channel confocal imaging system uniquely enable high plex, thick tissue analysis.
- Subcellular Resolution: Transcript localization at the subcellular level, allowing for precise identification and localization of gene expression patterns.

- 12-Well Plate Format: Efficient sample processing using a 12-well plate, compatible with high-throughput workflows.
- Curated and Custom Kits: Flexible panel selection for a variety of species and tissues.
- Automated 3D Imaging: Fully automated and integrated fluidics. Includes walkaway sample loading and processing.
- Click-button data analysis: Primary analysis performed on-instrument.
- Visualization Software: Custom-built 3D visualization software, PyxaStudio, included at no additional charge.



# Offering a Streamlined End-to-end Workflow

Step 1

#### **Tissue Sectioning**

Tissue sectioning

Placement into 12 well plate

Tissue is fixed and attached to each well

Sample preparation in 12-well microplate format increases throughput (each well area is nearly equivalent to a standard 2D slide). Samples can be stored and shipped after preparation.

### **STARmap Sample Prep**

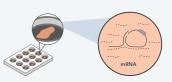
Step 2

Thick tissue SNAIL probe hybridization and ligation

DNA amplicon generation

Tissue is embedded into a 3D hydrogel

Tissue is cleared for sequencing





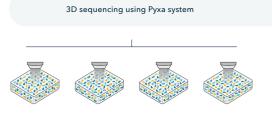




Pyxa chemistry leverages specific amplification of nucleic acids via intramolecular ligation via paired primer and padlock probes (SNAIL probes) to convert target RNA molecules into DNA amplicons with gene-unique codes. This enables highly multiplexed RNA detection in tissue hydrogels by multiple rounds of sequencing by ligation.

## **Automated Imaging and Processing**

Step 3



Primary image processing on-instrument



Processed data for analysis

	Cell 1	Cell 2	 Cell X
Gene A	3	0	11
Gene B	0	5	3
Gene X	1	7	0
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SEDAL sequencing uses reading probes to decode bases, and fluorescence.

# Visualization and Data Analysis

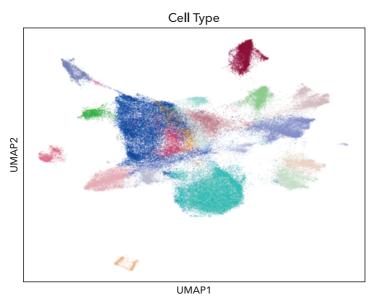
Step 4

Thick tissue SNAIL probe hybridization and ligation DNA amplicon generation Tissue is embedded into a 3D hydrogel for sequencing

Decoding probes transiently bind to the target DNA and ligate to form a stable product for imaging only when a perfect match occurs, which eliminates error accumulation.



# Immediate Access to 3D Insights



- Astrocyte
- Choroid plexus
- E\_Baiap3\_Tmem163
- E Cck Tcf4
- E\_Homer2\_Pou3f1
- E Pou3f1 Cck
- E\_Rora\_Zic1
- E\_Tbr1\_ld2
- E\_Tshz2\_Lamp5
- E\_Zic1\_Lef1
- E\_Zic1\_Syt9

- Endothelia
- Ependymal
- Hybrid\_El\_neurons
- I\_Chrm2\_Pvalb
- I\_Npy\_Lhx6
- I\_Ppp1r1b\_Gpr88
- Microglia
- Oligodendrocyte
- Oligodendrocyte progenitor cell
- Other

When you purchase a Pyxa or partner with us for a services project, your data can be seamlessly explored in PyxaStudio, our dedicated visualization tool for navigating 3D gene expression maps, annotating spatial patterns, and quantifying transcript localization in volumetric context. Key features of PyxaStudio include:

- Visualize the spatial distribution of cell types across the tissue
- Explore multiple cell layers in 3D
- Explore cell-cell interactions through cell/ transcript proximity in the 3D space

- Investigate subcellular transcript localization
- Filter by specific cell types or genes of interest
- Select individual cells or subclusters via the UMAP view
- Bookmark cells of interest
- Export a subset of the dataset for downstream analysis
- Share views directly with collaborators
- Share views directly with collaborators

#### Data files are compatible with numerous open-source tools for quantitative analysis

- Exploratory analysis of cellular spatial organization: Monkeybread (python), squidpy (python), Voyager (R and python), Giotto (R), StLearn (python)
- Exploratory analysis of single-cell gene expression: Seurat (R), scanpy (python)
- Analysis of spatially variable genes: Maxspin (python)

# **Technology Access Services**

Be the first to explore spatial biology's next dimension.

## Standard Service Offering – Mouse Neuroscience

- 250 gene mouse neural cell typing panel analysis of 20µm-100µm thick, fresh frozen mouse brain tissue
- Tissue sectioning, wetlab workflow, instrument processing, and primary data analysis performed by Stellaromics
- Minimum 4 samples processed;
   5-8mm² total area analyzed per sample
- Custom gene panel design available and custom scan configurations available by request

Working with a different tissue type? Talk to us!

#### **Data Deliverables**

- Comprehensive data report and technical presentation with Stellaromics Computational Biology team
- Cell by gene matrix including XYZ locations and subcellular localization.
- 3D cell mosaic and cell segmentation TIFF images
- Customized data and QC reports
- Guidance on open-source visualization and analysis tools
- Access to Stellaromics 3D visualization software, PyxaStudio<sup>™</sup>
- 8 week sample-to-answer turnaround time for standard projects

