



# OpenNano

Laura Beltrame, Niamh Callinan Keenan,  
Charalampos Anagnostakis, Cristiano Cordì  
Supervisor: Robert Forsyth

KU LEUVEN

OPENNANO

Faculty of Bioscience Engineering  
Master of Bioinformatics

## Introduction

**NanoString's GeoMx Digital Spatial Profiler (DSP)** technology enables tissue imaging and multiplexed RNA or protein profiling within defined Regions Of Interest (ROIs). [1]

The existing data analysis tools, the GeoMxTools R package and the NanoString DSP Analysis suite, face limitations in flexibility, parameter customization, and reliance on proprietary standards.

To address these constraints, **OpenNano** was developed as the first open-source Python package for GeoMx DSP, offering enhanced flexibility, usability, and scalability.

Validation was performed using the dataset from Kremsky et al.'s study on spaceflight-induced gene expression in mouse brains.[2]



## Technical Workflow

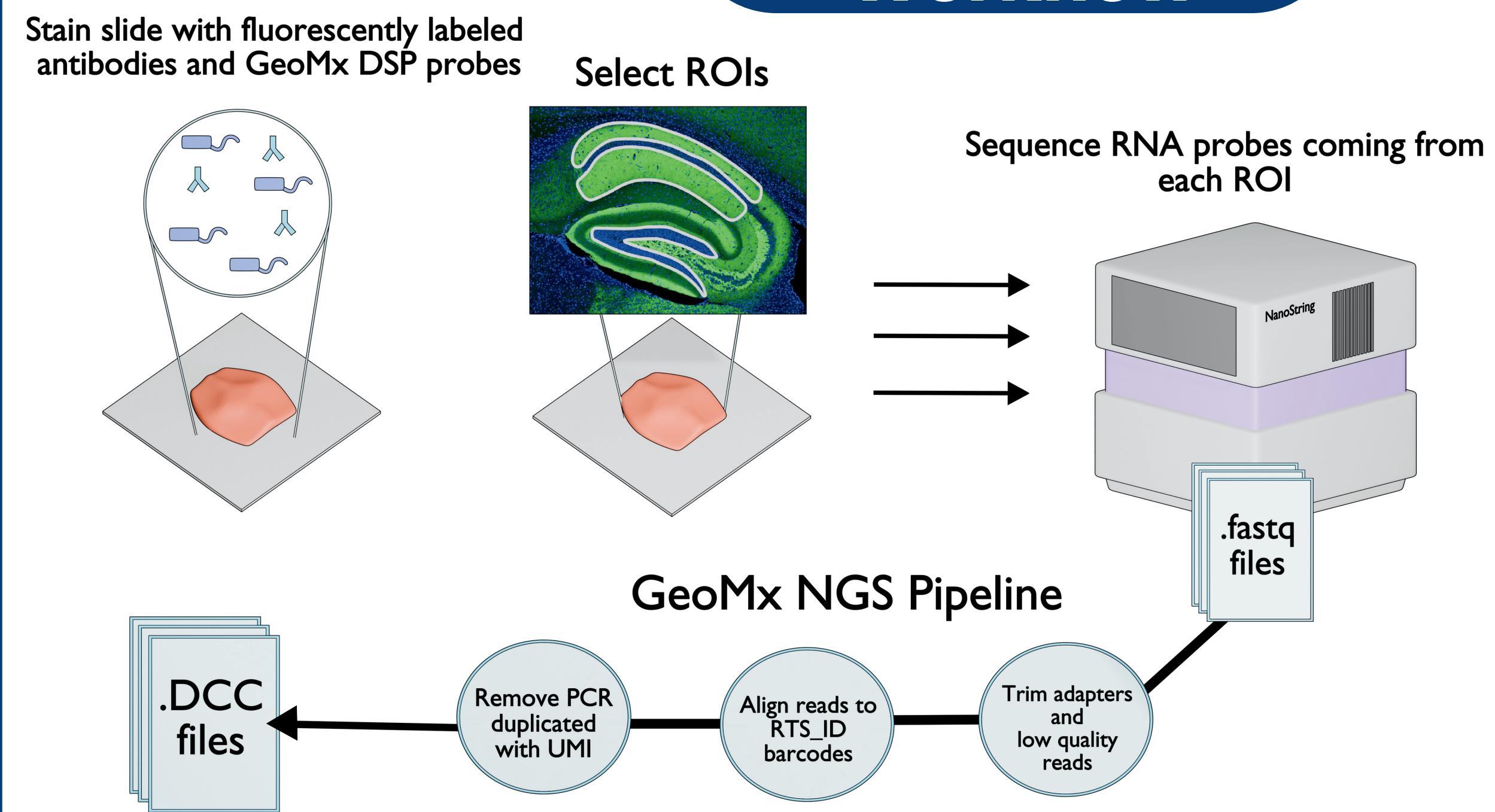


Figure 1. GeoMx DSP NGS pipeline

## Methods

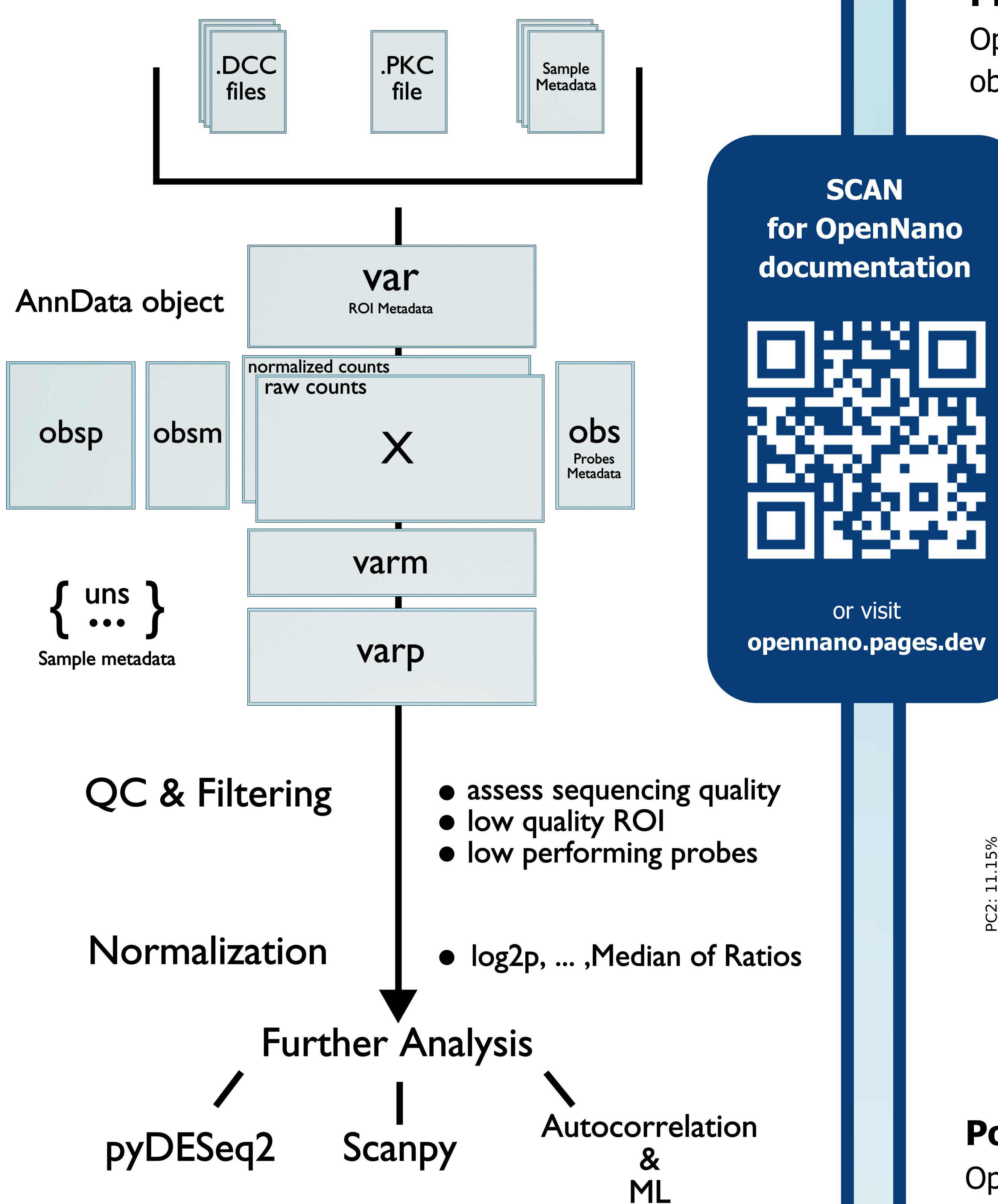


Figure 2. OpenNano pipeline

## Pre-Processing

OpenNano aggregates data from the dcc, pkc, and metadata files into an AnnData object that can be used for further analysis or is compatible with other packages.

## Quality Control

OpenNano provides a detailed report on the number of failed quality control tests as well as their corresponding segments, based on several parameters for the reads or segments. It is also possible to filter the AnnData object based on the expression levels of the negative probes. The results that we obtained are concordant with GeoMxTools' outputs, as both approaches followed a similar methodology.

## Normalization

The normalization step has five distinct methods for processing, ensuring the usability for downstream analyses. These methods include for instance: Median of Ratios and Variance Stabilizing Transformation (VST). With these two methods, we compared PCA results from OpenNano and GeoMxTools.

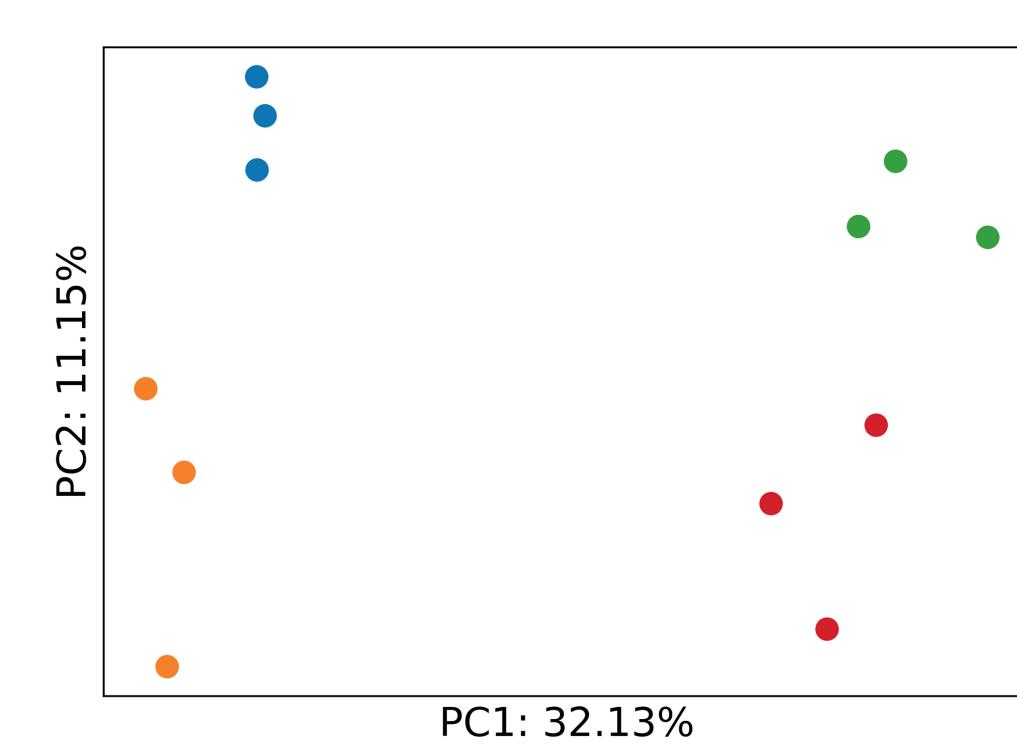


Figure 3. OpenNano PCA

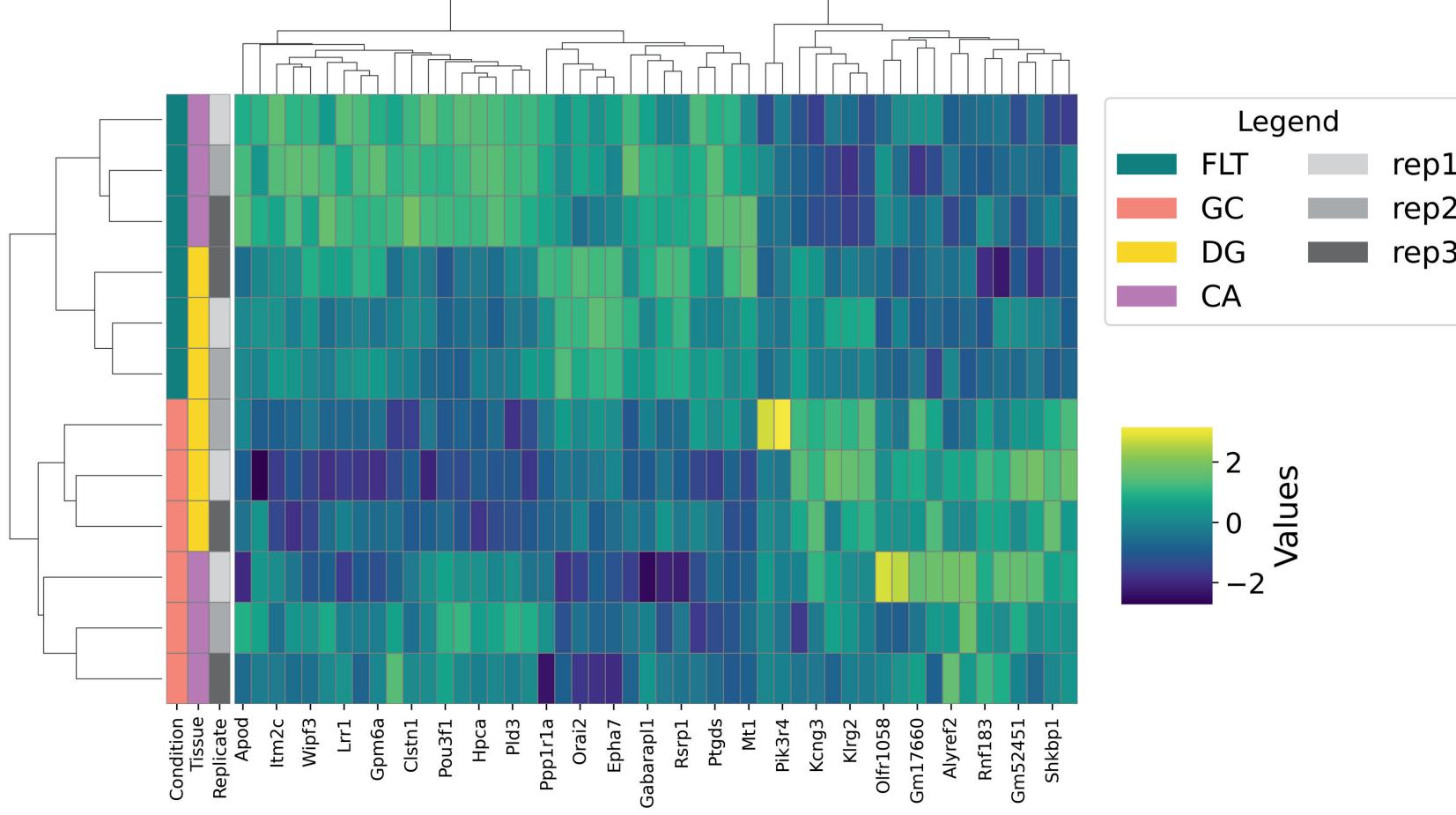


Figure 4. OpenNano Heatmap

## Post-Analysis

OpenNano provides the user with an AnnData object which can be used with compatible packages, like Scanpy or pyDESeq2.

## Conclusion

- Accessible analysis:** OpenNano automates GeoMx DSP data preprocessing and provides Python library compatibility.
- Key features:** Enhances reproducibility, scalability, and accessibility through modular design and bioinformatics integration.
- Future Directions:** Focus on visualization tools and scalability for large datasets.

**OpenNano** is a major step forward in making spatial transcriptomics accessible and continuous development and collaboration will ensure the fulfillment of the demands of the research community.

## References

1. GeoMx® DSP NGS Readout User Manual. (2022). Available at: <https://nanostring.com/wp-content/uploads/2022/06/MAN-10153-01-GeoMx-DSP-NGS-Readout-User-Manual.pdf>.
2. Kremsky, Isaac, et al. "Spaceflight-Induced Gene Expression Profiles in the Mouse Brain Are Attenuated by Treatment with the Antioxidant BuOE." International Journal of Molecular Sciences 24.17 (2023): 13569.