# Spatial Transcriptomics Analysis of Lung Cancer Samples to Identify the Impact of Chronic Stress

Visium Pipeline Workflow & Key Findings
Ritupam Sarma
March 2025

# Introduction

BACKGROUND ON LUNG CANCER & STRESS RESPONSE

OBJECTIVE: COMPARE GENE EXPRESSION IN LUNG CANCER SAMPLES SUBJECTED TO DIFFERENT STRESS LEVELS.

METHODOLOGY: SPATIAL TRANSCRIPTOMICS USING 10X GENOMICS VISIUM & SEURAT PIPELINE

# Background

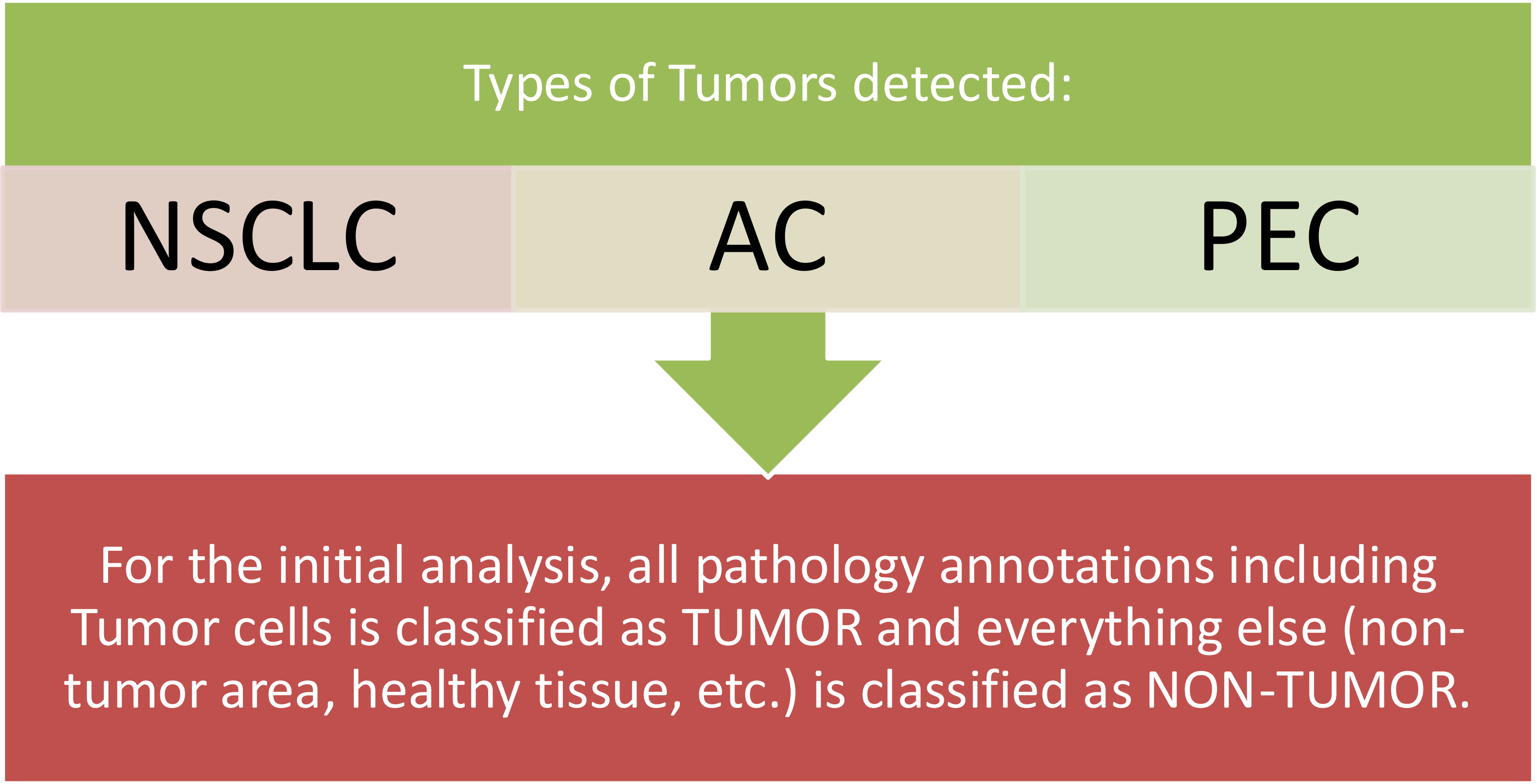
Stress in this project includes anything that has a chronic effect on the amygdala activity. It includes conditions like anxiety and depression.

20 10x Visium samples.

Stress Levels determined by Questionnaires filled out by patients, and Blood glucocorticoid levels.

Loss of one high stress sample due to detachment.

# Tumor Classification



Types of Tumors detected:

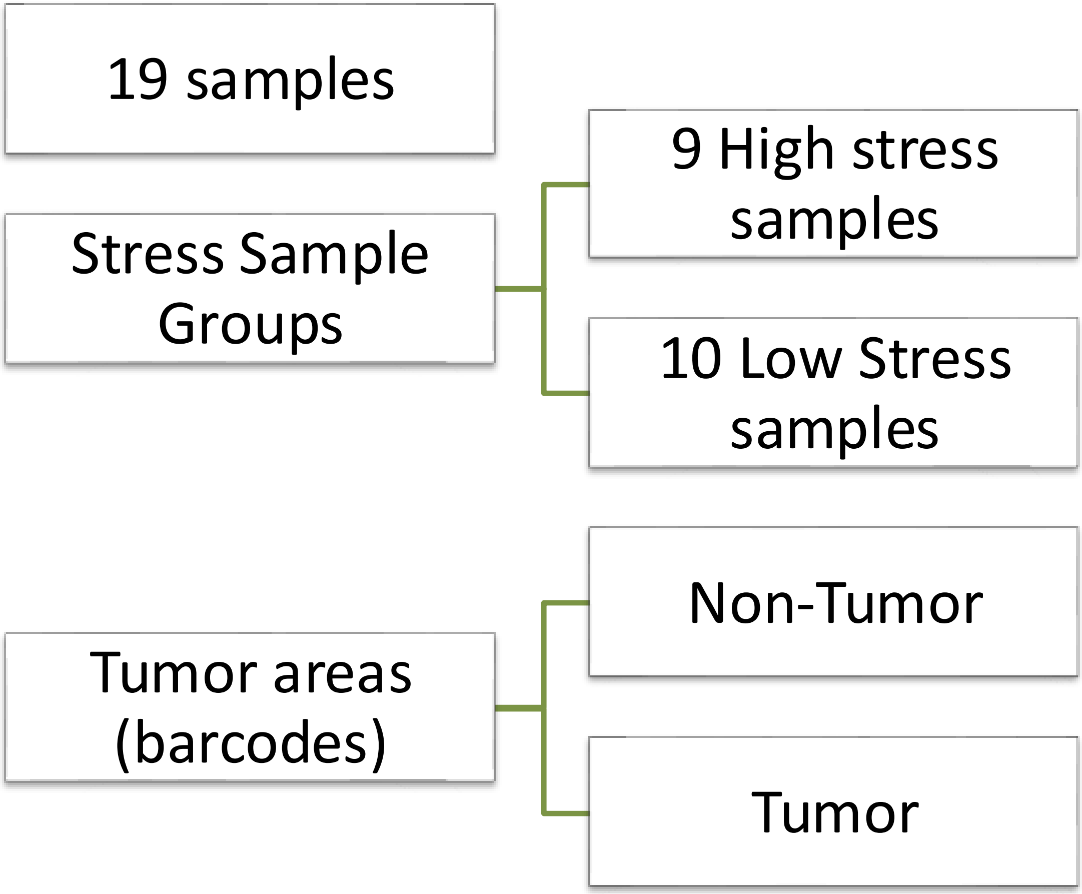
NSCLC

AC

PEC

For the initial analysis, all pathology annotations including Tumor cells is classified as TUMOR and everything else (non-tumor area, healthy tissue, etc.) is classified as NON-TUMOR.

# Stress and Tumor Groups



19 samples

Stress Sample Groups
- 9 High stress samples
- 10 Low Stress samples

Tumor areas (barcodes)
- Non-Tumor
- Tumor

# Key Data Stats

## Number of reads

- Range of reads per spot: between **19,144 (low PEC)** and **30,002 (high PEC).**
- Average: **25,581.**

## Number of genes detected

The median number of genes detected per spot varies widely (**426 to 5,650**).

# Workflow Overview

1. Data Pre-processing

2. Normalization & Feature Selection

3. PCA & Clustering

4. UMAP Visualization

5. Differential Gene Expression Analysis

6. Marker Gene Identification

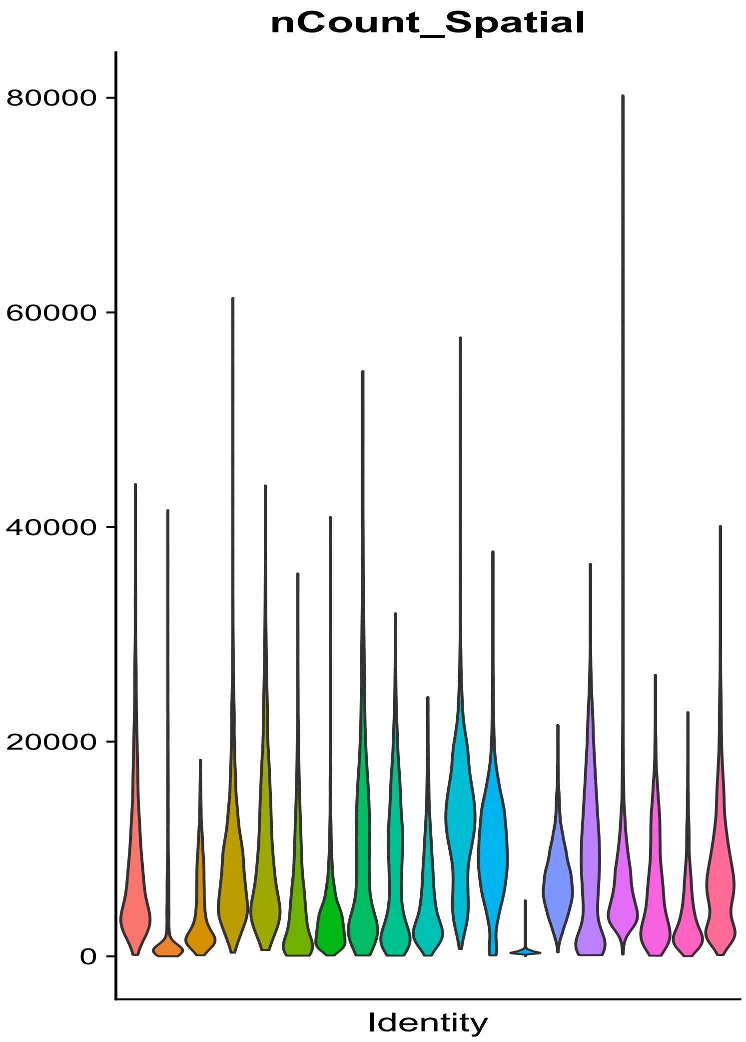
# Data Preprocessing

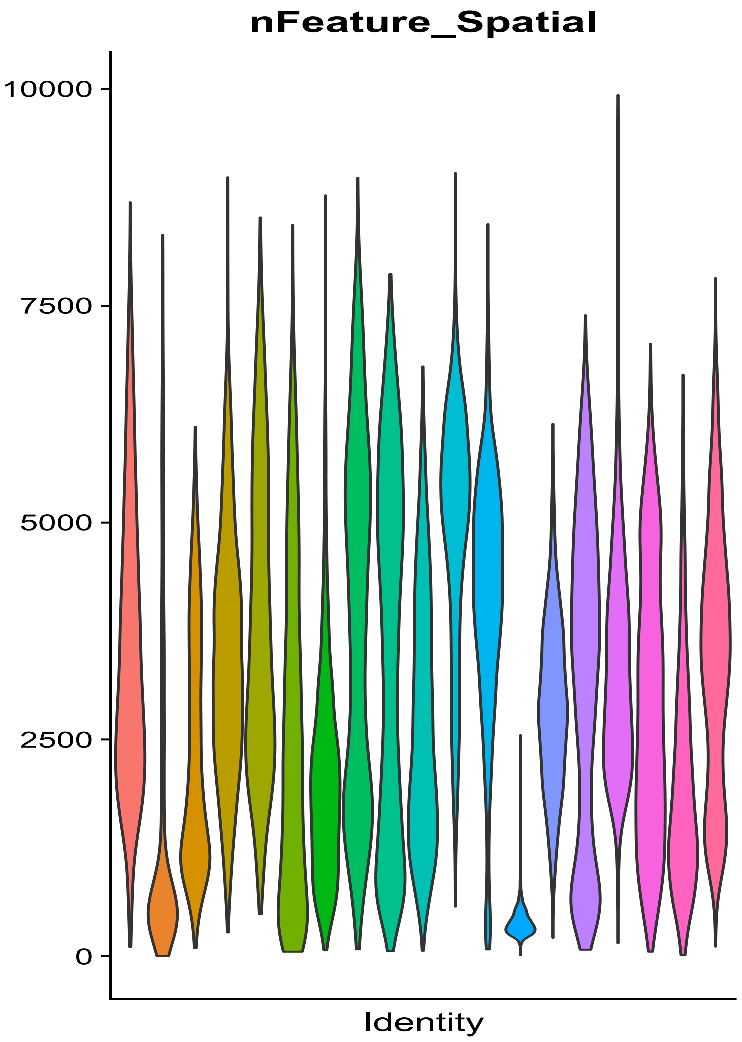
**Quality Control:** Removed spots with zero reads.

**Normalization:** Log-normalization and scaling using Seurat

**Feature Selection:** Top 2000 variable features using VST

# Violin Plots per sample





# Dimensionality Reduction

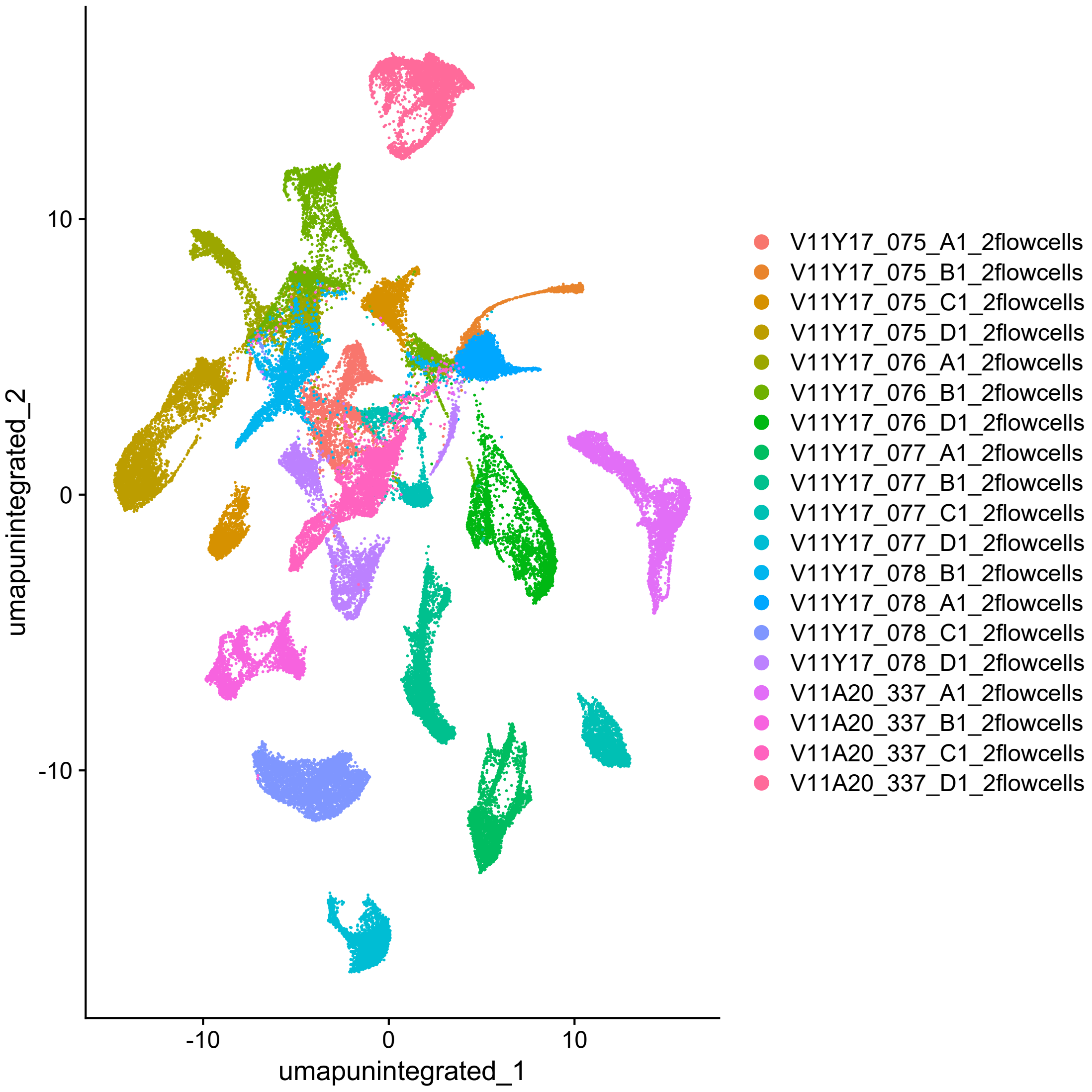
Principal Component Analysis

Elbow Plot used to determine the number of PCs

Dimensionality Reduction and clustering

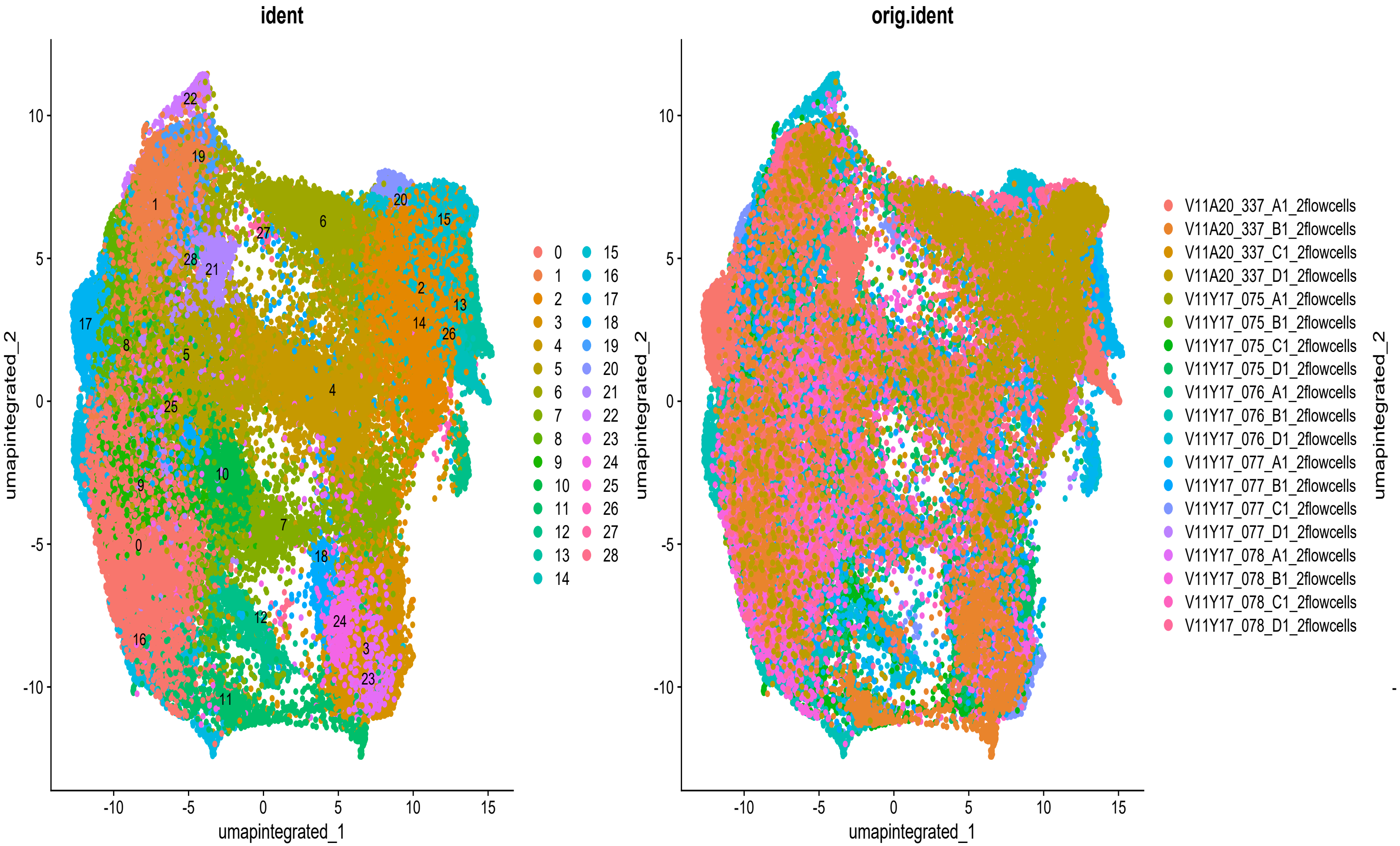
# Unintegrated UMAP

Clustering without batch correction



# Integrated UMAP

Used **Harmony** for batch correction:
reduced batch effects, and
Allowed biologically meaningful clustering.

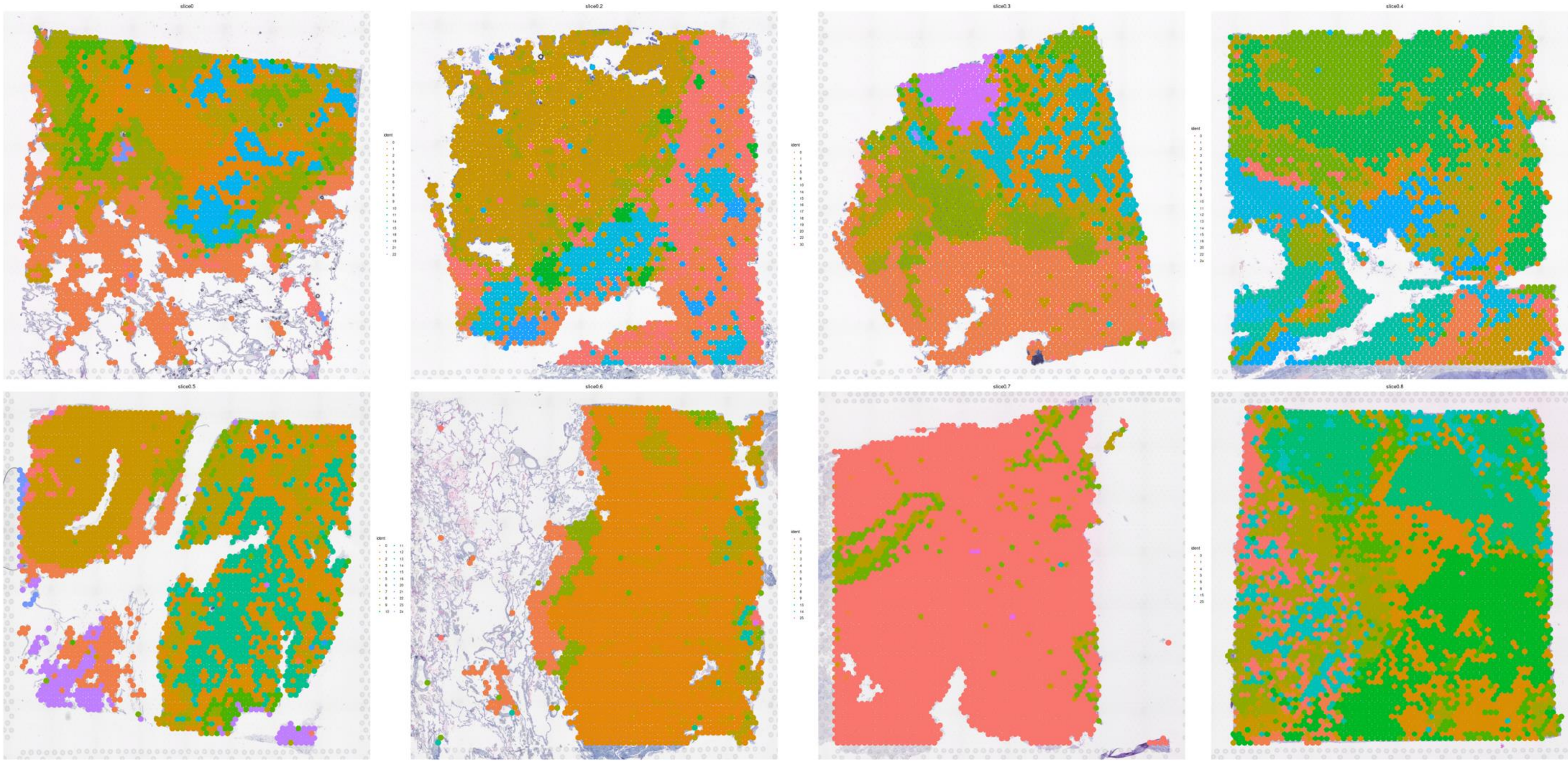


# Spatial Visualization

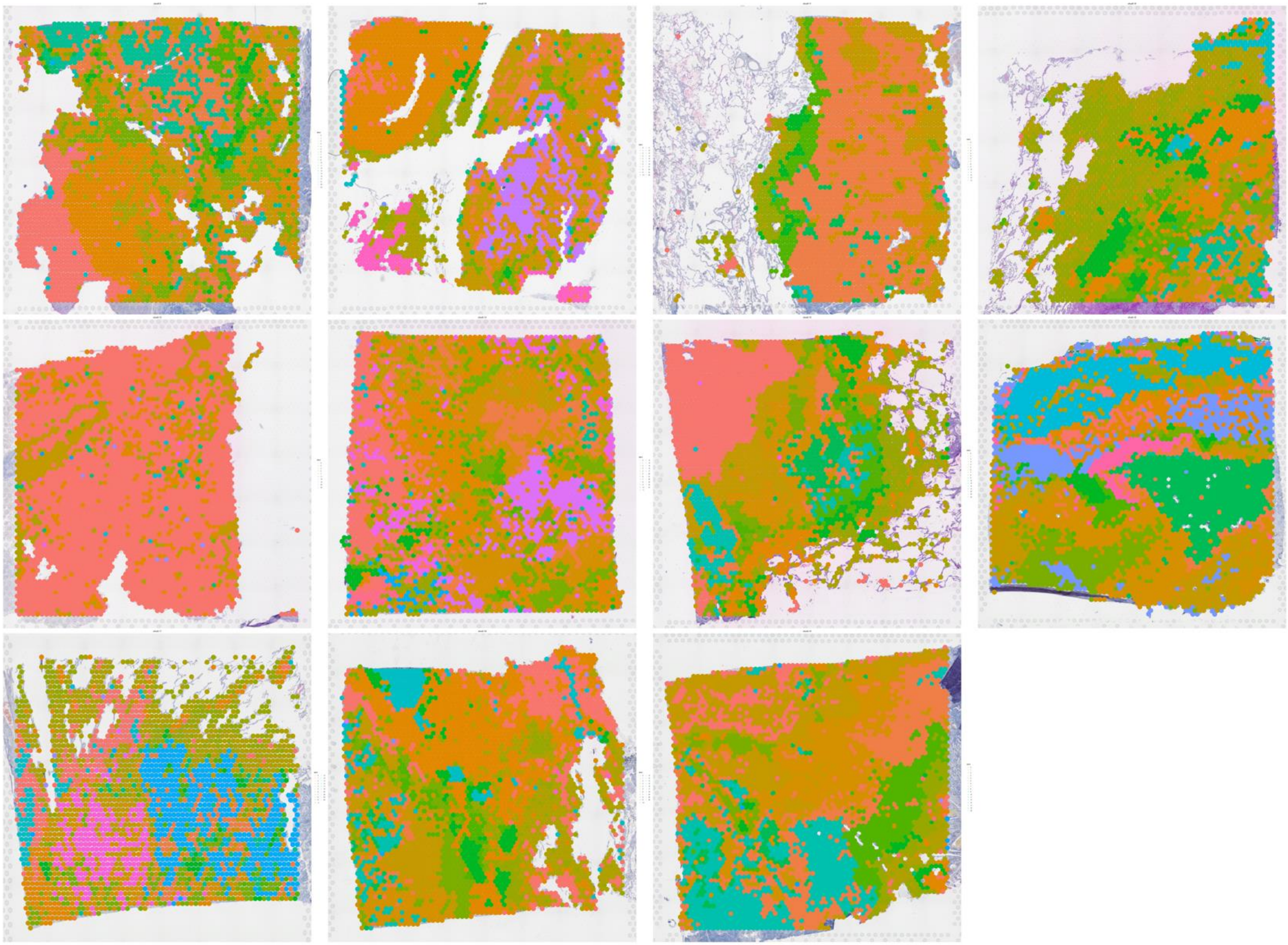
SpatialDimPlot of clusters on Visium slides

Overlay expression for spatial viewing.

# Spatial Plot (8/19)



# Spatial Plot (11/19)



# Differential Expression Analysis

**Identified differentially expressed genes (DEGs)** between high and low stress groups, and between tumor and non-tumor barcodes.

**Findings are grouped into four categories:**

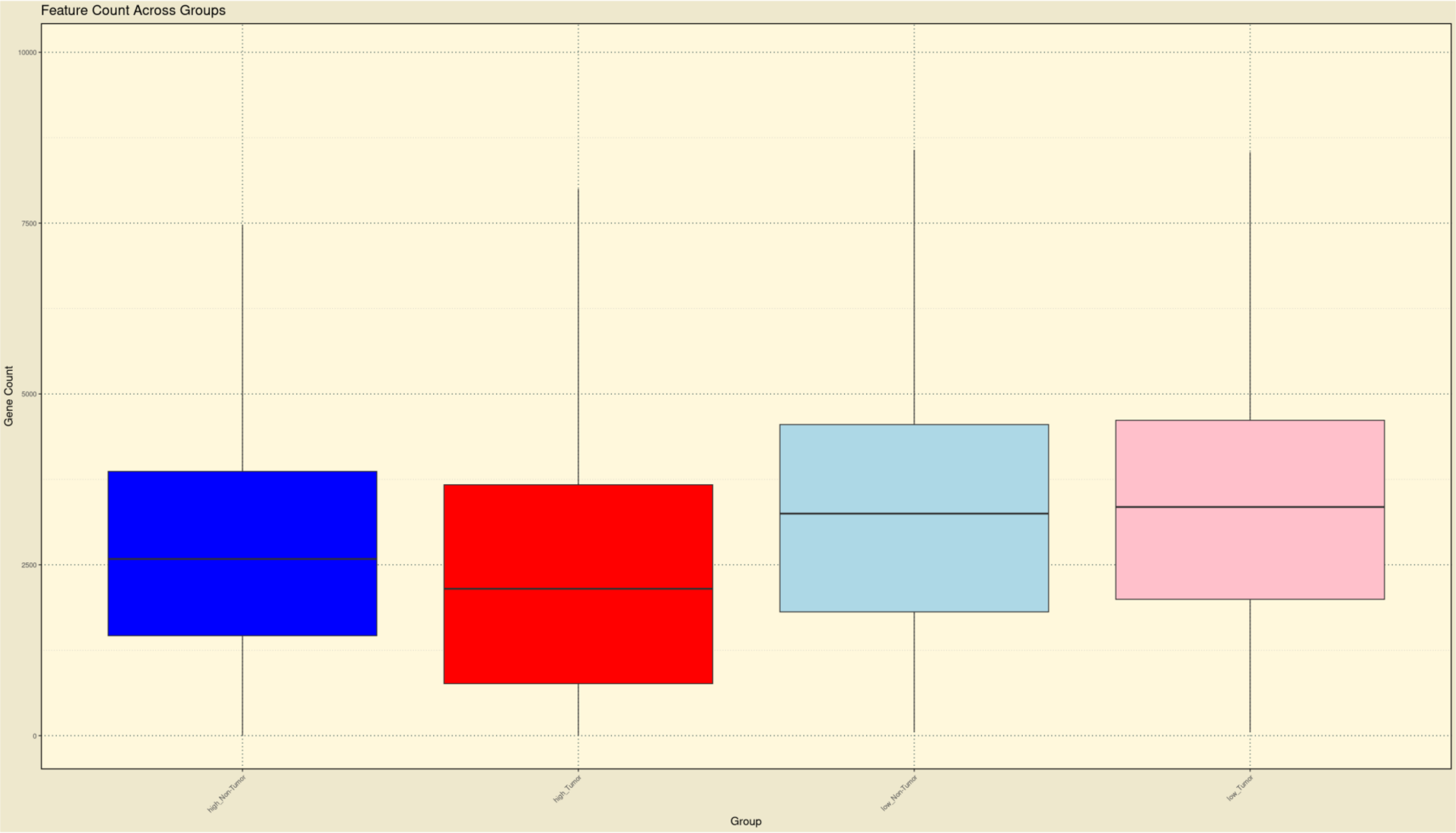
High stress, non-tumor

Low stress, non-tumor

High stress, tumor

Low stress, tumor

# Differential Gene Expression across the 4 Groups

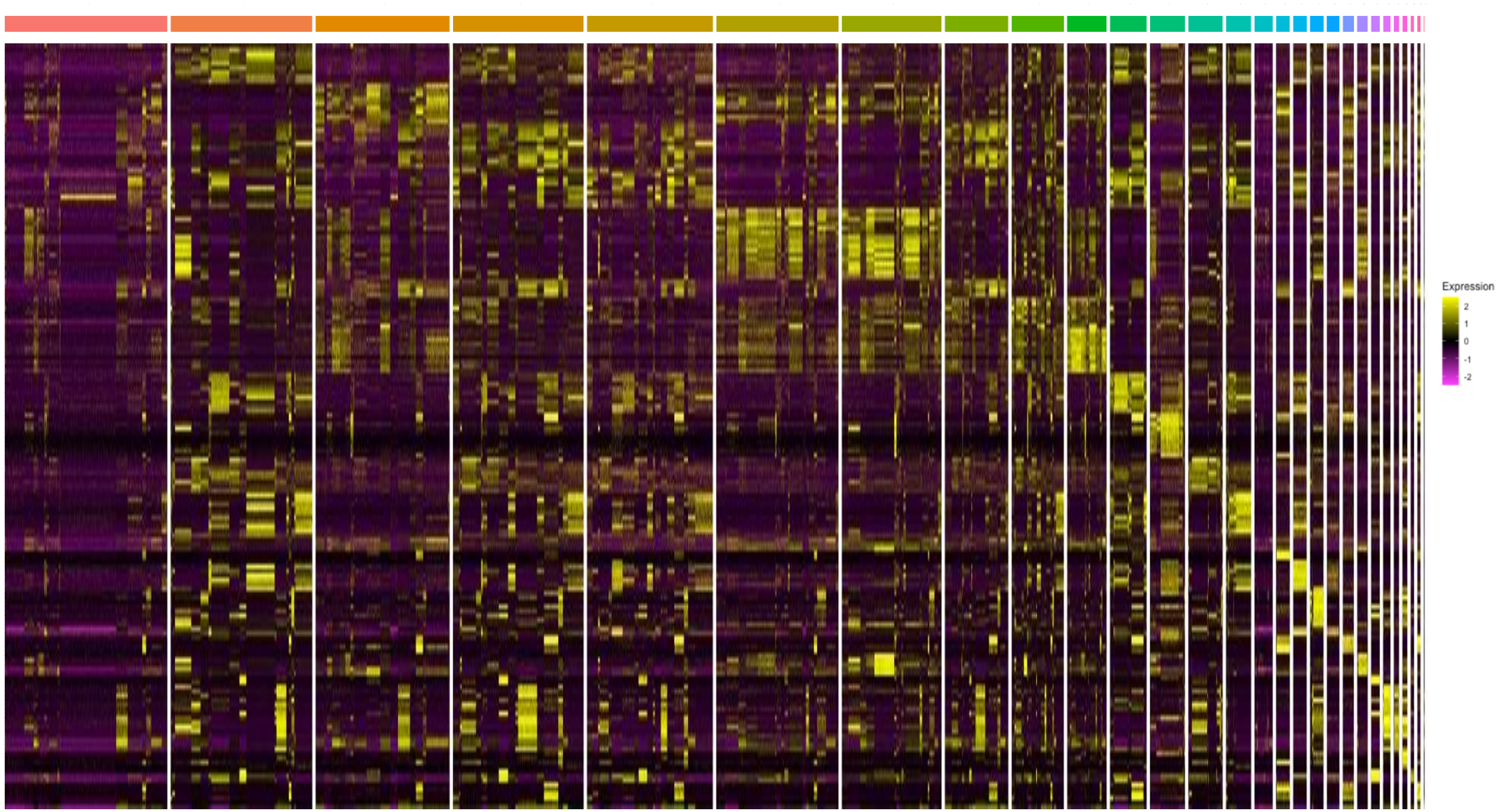


# Marker Gene Analysis

Identified top 10 marker genes for each cluster.

Visualizing expression via marker heatmap.

# Top 10 Marker Heatmap per Cluster



# Conclusion & Future Directions

**Key Findings:**

There is a marked difference between gene expression in high-stress and low-stress samples.

**Future Work:**

Further validation of key marker genes.

Manually annotating the clusters.

Overlaying key marker genes on spatial plot.

Integration with other omics data.

**Thank you** for giving me a chance to interview for this exciting PhD project.

## I would like to thank

- Dr Chrysoula Vraka, CRUK Scotland Institute and Medical University of Vienna, for offering work on this interesting project, and
- Mr. John Cole, University of Glasgow, for his invaluable bioinformatic guidance.

## Software used

- 10x Visium
- Seurat Pipeline
- Loupe Browser

## References

- Dr Chrysoula Vraka
- Dr Clemens Spielvogel
- Dr Stefan Gruenert
- And everyone else who is working or have worked on this project.