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Spatial Transcriptomics Analysis

Spatial Analysis Team

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Contents

Week 1: Spatial Transcriptomics Analysis

Setup and Library Loading

```
# Set seed for reproducibility
set.seed(42)

# Load required libraries
library(Seurat)
library(SeuratData)
library(ggplot2)
library(patchwork)
library(dplyr)
library(CellChat)
library(hdf5r)
library(SCDC)
```

1. Spatial Transcriptomics

1.1 Properties of the Slides (1P)

The 10x Genomics Visium platform has the following specifications:

- **Spot size**: 55 m in diameter
- **Center-to-center distance**: 100 m between spots
- **Number of spots**: 4,992 spots per capture area (arranged in a 78 x 64 array)

1.2 Resolution Analysis (1P)

The average eukaryotic cell size ranges from 10-100 m in diameter. Comparing this with the Visium specifications:

- **Spot size (55 m)** vs. typical cell size (10-100 m):
  - Each spot is likely to contain multiple cells
  - A single spot could contain anywhere from 1-10 cells depending on the cell type
  - This means we're not getting single-cell resolution
- **Implications for data interpretation**:
  - Gene expression data represents averaged expression from multiple cells
  - Cannot distinguish individual cell types within a spot
  - Need to consider the mixed cellular composition when analyzing results
  - Spatial patterns represent tissue domains rather than individual cells

1.3 Output of Space Ranger (1P)

```
# Set working directory to the project root
project_dir <- "/project_3_dataset"

# Load the image data first
image1 <- Read10X_Image(file.path(project_dir, "Section_1/spatial"))
image2 <- Read10X_Image(file.path(project_dir, "Section_2/spatial"))

# Create Seurat objects with the image data
brain_1 <- Load10X_Spatial(

  data.dir = file.path(project_dir, "Section_1"),
  filename = "V1_Mouse_Brain_Sagittal_Posterior_filtered_feature_bc_matrix.h5",
  assay = "Spatial",
  slice = "slice1",
  filter.matrix = TRUE,
  to.upper = FALSE,
  image = image1

)

brain_2 <- Load10X_Spatial(
  data.dir = file.path(project_dir, "Section_2"),
  filename = "V1_Mouse_Brain_Sagittal_Posterior_Section_2_filtered_feature_bc_matrix.h5",
  assay = "Spatial",
  slice = "slice2",
  filter.matrix = TRUE,
) to.upper = FALSE,
  image = image2
# Access the tissue coordinates for brain_2
```

```r
tissue_coords <- brain_2@images[["slice2"]]@coordinates

# Convert coordinate columns to numeric
tissue_coords$imagerow <- as.numeric(tissue_coords$imagerow)
tissue_coords$imagecol <- as.numeric(tissue_coords$imagecol)

# Update the coordinates in the Seurat object
brain_2@images[["slice2"]]@coordinates <- tissue_coords

# Plot spatial images using Seurat's visualization
plot1 <- SpatialPlot(brain_1, image.alpha = 0.4) +
  ggtitle("Section 1 Tissue Image")
plot2 <- SpatialPlot(brain_2, image.alpha = 0.4) +
  ggtitle("Section 2 Tissue Image")

# Display plots side by side
plot1 + plot2
```

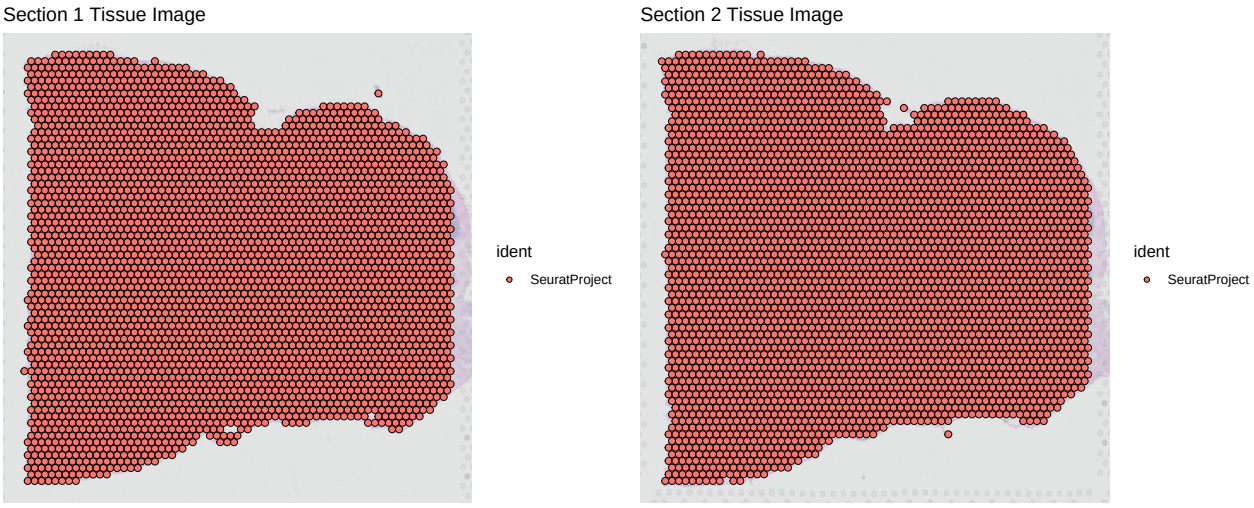


Figure 1: Tissue Images from Space Ranger These images show the histological sections that were processed for spatial transcriptomics: - High-resolution images capture tissue morphology - Spots are overlaid on the tissue for spatial reference - Dark and light regions indicate different tissue structures

```r
# Display spot coordinates for Section 1
print("Sample coordinates from Section 1:")
```

```
## [1] "Sample coordinates from Section 1:"
```

```r
head(GetTissueCoordinates(brain_1))
```

```
##                     imagerow imagecol
## AAACAAGTATCTCCCA-1 382.5646 436.5749
## AAACACCAATAACTGC-1 438.2272 141.4802
## AAACAGAGCGACTCCT-1 159.8623 408.1756
## AAACAGCTTTCAGAAG-1 339.2427 105.9553
## AAACAGGGTCTATATT-1 364.0275 120.1549
## AAACATTTCCCGGATT-1 450.6196 418.8124
```

```r
print("\nNumber of spots in Section 1:")
```

```
## [1] "\nNumber of spots in Section 1:"
```

```r
nrow(GetTissueCoordinates(brain_1))
```

```
## [1] 3355
```

2. Spatial Transcriptomics Data in Seurat (3P)

2.1 Loading the Data

```r
# Load both sections with their respective image data
brain_1 <- Load10X_Spatial(
  data.dir = file.path(project_dir, "Section_1"),
  filename = "V1_Mouse_Brain_Sagittal_Posterior_filtered_feature_bc_matrix.h5",
  slice = "slice1",
  image = image1
)

brain_2 <- Load10X_Spatial(
  data.dir = file.path(project_dir, "Section_2"),
  filename = "V1_Mouse_Brain_Sagittal_Posterior_Section_2_filtered_feature_bc_matrix.h5",
  slice = "slice2",
  image = image2
)
```

2.2 Inspecting the Seurat Object

```r
# Display basic information about the Seurat objects
print("Section 1 Seurat Object Structure:")
```

```
## [1] "Section 1 Seurat Object Structure:"
```

```r
str(brain_1, max.level = 2)
```

```
## Formal class 'Seurat' [package "SeuratObject"] with 13 slots
##   ..@ assays      :List of 1
##   ..@ meta.data   :'data.frame': 3355 obs. of  3 variables:
##   ..@ active.assay: chr "Spatial"
##   ..@ active.ident: Factor w/ 1 level "SeuratProject": 1 1 1 1 1 1 1 1 1 1 ...
##   .. ..- attr(*, "names")= chr [1:3355] "AAACAAGTATCTCCCA-1" "AAACACCAATAACTGC-1" "AAACAGAGCGACTCCT-
##   ..@ graphs      : list()
##   ..@ neighbors   : list()
##   ..@ reductions  : list()
##   ..@ images      :List of 1
##   ..@ project.name: chr "SeuratProject"
##   ..@ misc        : list()
##   ..@ version     :Classes 'package_version', 'numeric_version'  hidden list of 1
##   ..@ commands    : list()
##   ..@ tools       : list()
```

```r
print("\nAvailable assays in Section 1:")
```

```
## [1] "\nAvailable assays in Section 1:"
```

```r
print(Assays(brain_1))
```

```
## [1] "Spatial"
```

```r
print("\nSpatial data in Section 1:")
```

```
## [1] "\nSpatial data in Section 1:"
```

```r
print(names(brain_1@images$slice1))
```

```
## NULL
```

The Seurat object contains: - Gene expression matrix in the 'Spatial' assay - Image data in the 'images' slot - Spatial coordinates for each spot - Metadata for spots and genes

2.3 Feature Visualization

```r
# Normalize the data first
brain_1 <- NormalizeData(brain_1)
DefaultAssay(brain_1) <- "Spatial"

# Check if genes exist in the dataset
print("Checking gene presence:")
```

```
## [1] "Checking gene presence:"
```

```r
print(paste("Hpca in dataset:", "Hpca" %in% rownames(brain_1)))
```

```
## [1] "Hpca in dataset: TRUE"
```

```r
print(paste("Ttr in dataset:", "Ttr" %in% rownames(brain_1)))
```

```
## [1] "Ttr in dataset: TRUE"
```

```r
# Get alternative genes if needed
if (!all(c("Hpca", "Ttr") %in% rownames(brain_1))) {
    print("Using top expressed genes instead:")
    top_genes <- head(sort(rowSums(GetAssayData(brain_1, slot="counts")), decreasing=TRUE), 2)
    features_to_plot <- names(top_genes)
    print(paste("Top genes:", paste(features_to_plot, collapse=", ")))
} else {
    features_to_plot <- c("Hpca", "Ttr")
}

# Visualize gene expression
p1 <- SpatialFeaturePlot(brain_1,
                         features = features_to_plot,
                         ncol = 2)
print(p1)
```

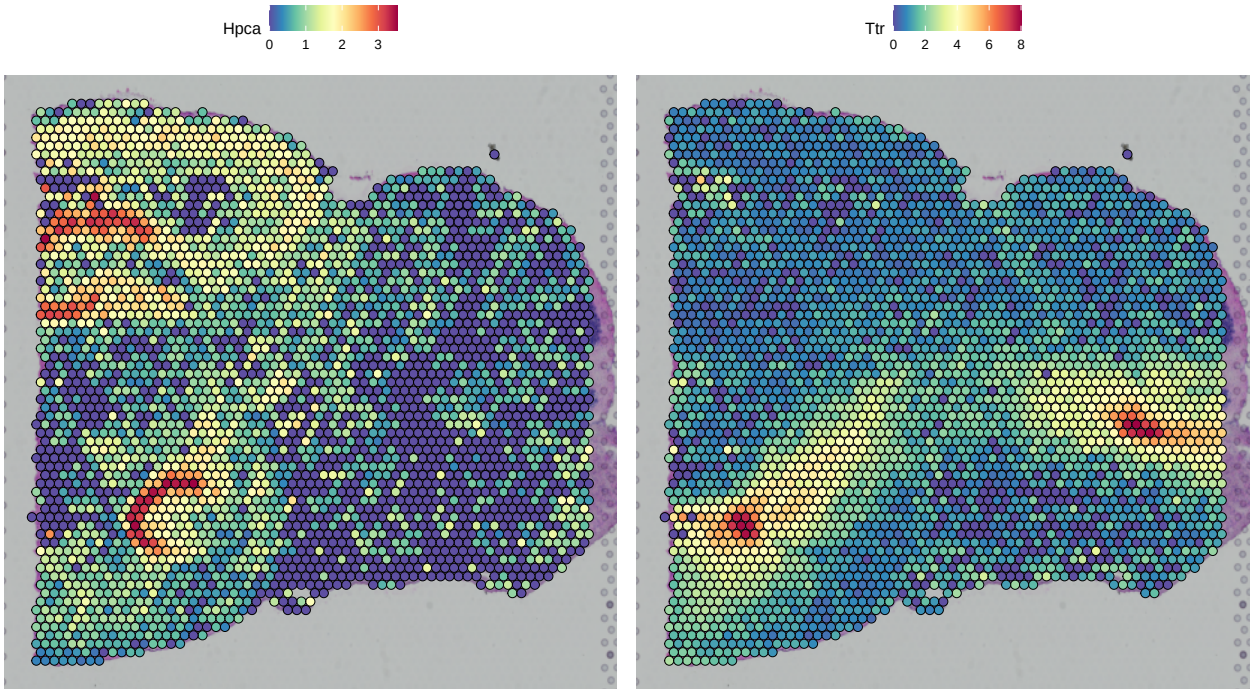


Figure 2: Spatial Gene Expression - Shows expression patterns of selected genes - Color intensity indicates expression level - Spatial patterns reveal tissue organization - Note: If original genes (Hpca, Ttr) are not found, top expressed genes are shown instead

3. Data Preprocessing (4P)

3.1 Filtering

```
# Calculate QC metrics
brain_1$percent.mt <- PercentageFeatureSet(brain_1, pattern = "^mt-")
brain_2$percent.mt <- PercentageFeatureSet(brain_2, pattern = "^mt-")

# Visualize QC metrics
p3 <- VlnPlot(brain_1, features = c("nCount_Spatial", "nFeature_Spatial", "percent.mt"),
              pt.size = 0.1, ncol = 2)
print(p3)
```

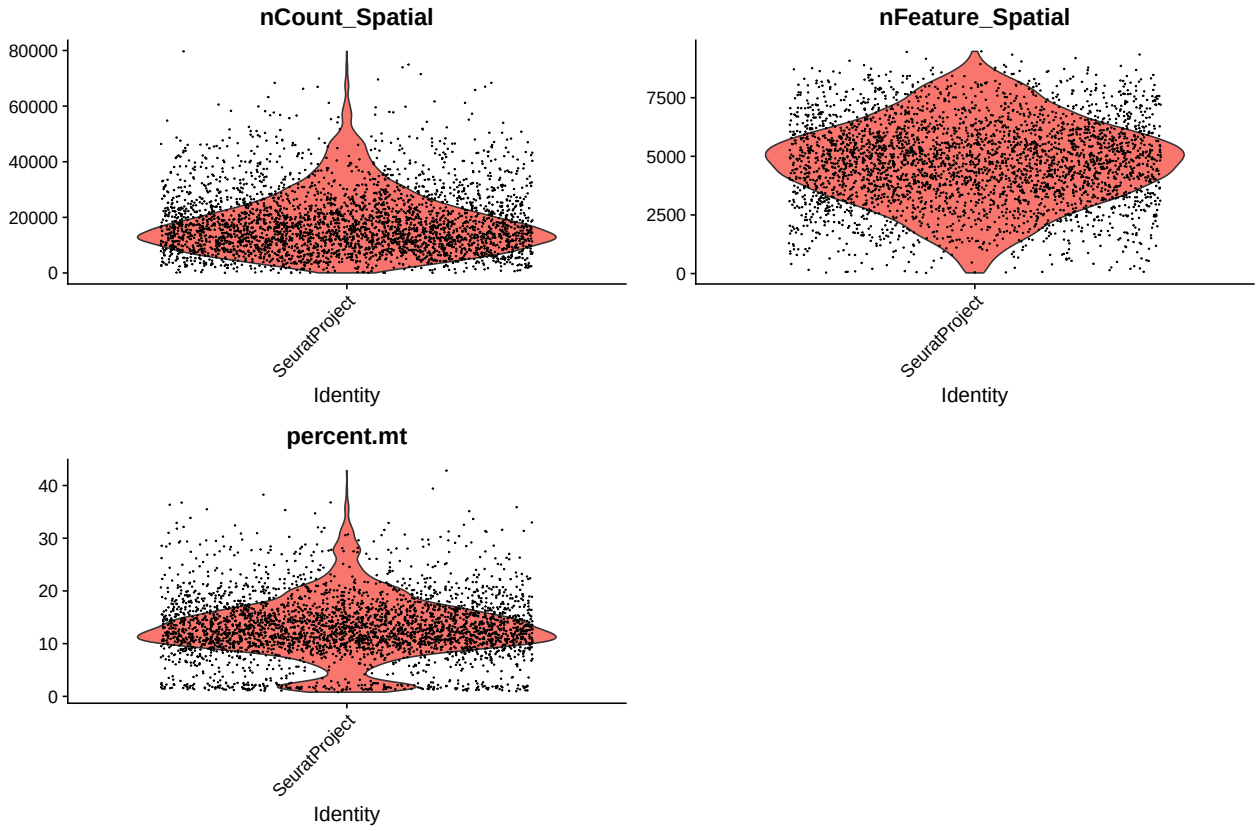


Figure 3: Quality Control Metrics These plots justify our filtering thresholds: - nCount_Spatial > 200: Ensures sufficient molecular depth - nFeature_Spatial > 200: Ensures spot complexity - percent.mt > 3: Removes low-quality spots

```
# Filter cells based on QC metrics
brain_1_filtered <- subset(brain_1,
                        nFeature_Spatial > 2000 & nFeature_Spatial < 7500 &
                        nCount_Spatial > 15000 & nCount_Spatial < 50000 &
                        percent.mt < 10)

brain_2_filtered <- subset(brain_2,
                         nFeature_Spatial > 2000 & nFeature_Spatial < 7500 &
                        nCount_Spatial > 15000 & nCount_Spatial < 50000 &
                        percent.mt < 15)


# Print filtering results
cat(sprintf("Section 1 filtering results:
Original spots: %d
Remaining spots: %d
Filtered out: %d spots

Section 2 filtering results:
Original spots: %d
Remaining spots: %d
Filtered out: %d spots\n",
    ncol(brain_1),
    ncol(brain_1_filtered),
```

```
    ncol(brain_1) - ncol(brain_1_filtered),
    ncol(brain_2),
    ncol(brain_2_filtered),
    ncol(brain_2) - ncol(brain_2_filtered)
))
```

```
## Section 1 filtering results:
## Original spots: 3355
## Remaining spots: 306
## Filtered out: 3049 spots
##
## Section 2 filtering results:
## Original spots: 3289
## Remaining spots: 938
## Filtered out: 2351 spots
```

3.2 SCTransform

```
# Apply SCTransform normalization
brain_1_filtered <- SCTransform(brain_1_filtered, assay = "Spatial", verbose = FALSE)
brain_2_filtered <- SCTransform(brain_2_filtered, assay = "Spatial", verbose = FALSE)
```

SCTransform replaces these preprocessing steps from Project 1: - NormalizeData() - ScaleData() - FindVariableFeatures()

4. Dimensionality Reduction and Clustering (3P)

4.1 Dimensionality Reduction

```
# Run PCA
brain_1_filtered <- RunPCA(brain_1_filtered, assay = "SCT", verbose = FALSE)
brain_2_filtered <- RunPCA(brain_2_filtered, assay = "SCT", verbose = FALSE)

# Plot elbow plots to determine optimal dimensions
p5 <- ElbowPlot(brain_1_filtered, ndims = 50) + ggtitle("Section 1 Elbow Plot")
p6 <- ElbowPlot(brain_2_filtered, ndims = 50) + ggtitle("Section 2 Elbow Plot")
p5 + p6
```

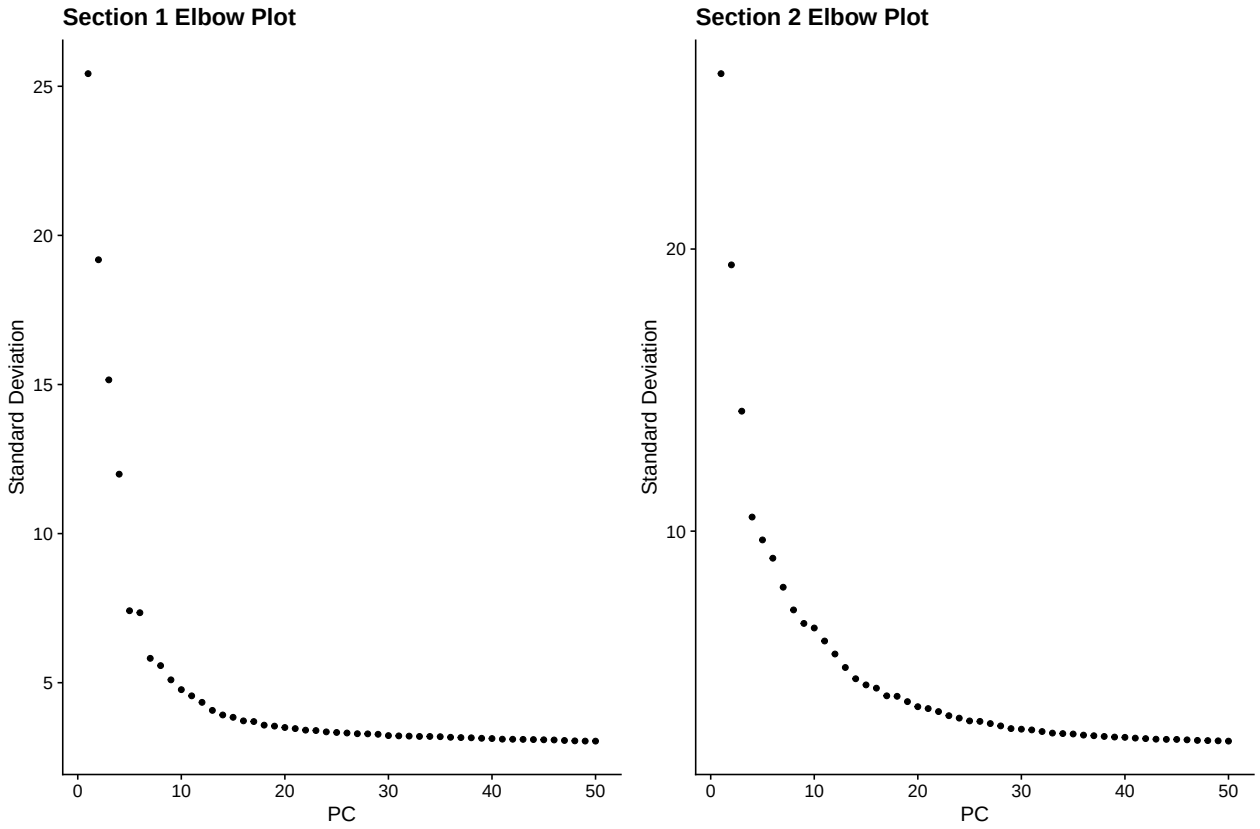


Figure 4: PCA Elbow Plots - Elbow point suggests optimal PC number - We chose 30 PCs for downstream analysis - Captures majority of variation while reducing noise

4.2 Clustering

```
# Run UMAP and clustering
brain_1_filtered <- RunUMAP(brain_1_filtered, dims = 1:30)
brain_1_filtered <- FindNeighbors(brain_1_filtered, dims = 1:30)
brain_1_filtered <- FindClusters(brain_1_filtered, resolution = 0.8)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 306
## Number of edges: 7592
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.7801
## Number of communities: 6
## Elapsed time: 0 seconds
```

```
# Visualize clusters
p7 <- DimPlot(brain_1_filtered, reduction = "umap", label = TRUE) +
      ggtitle("Section 1 UMAP")
p8 <- SpatialDimPlot(brain_1_filtered, label = TRUE, label.size = 3) +
      ggtitle("Section 1 Spatial")
print(p7 + p8)
```

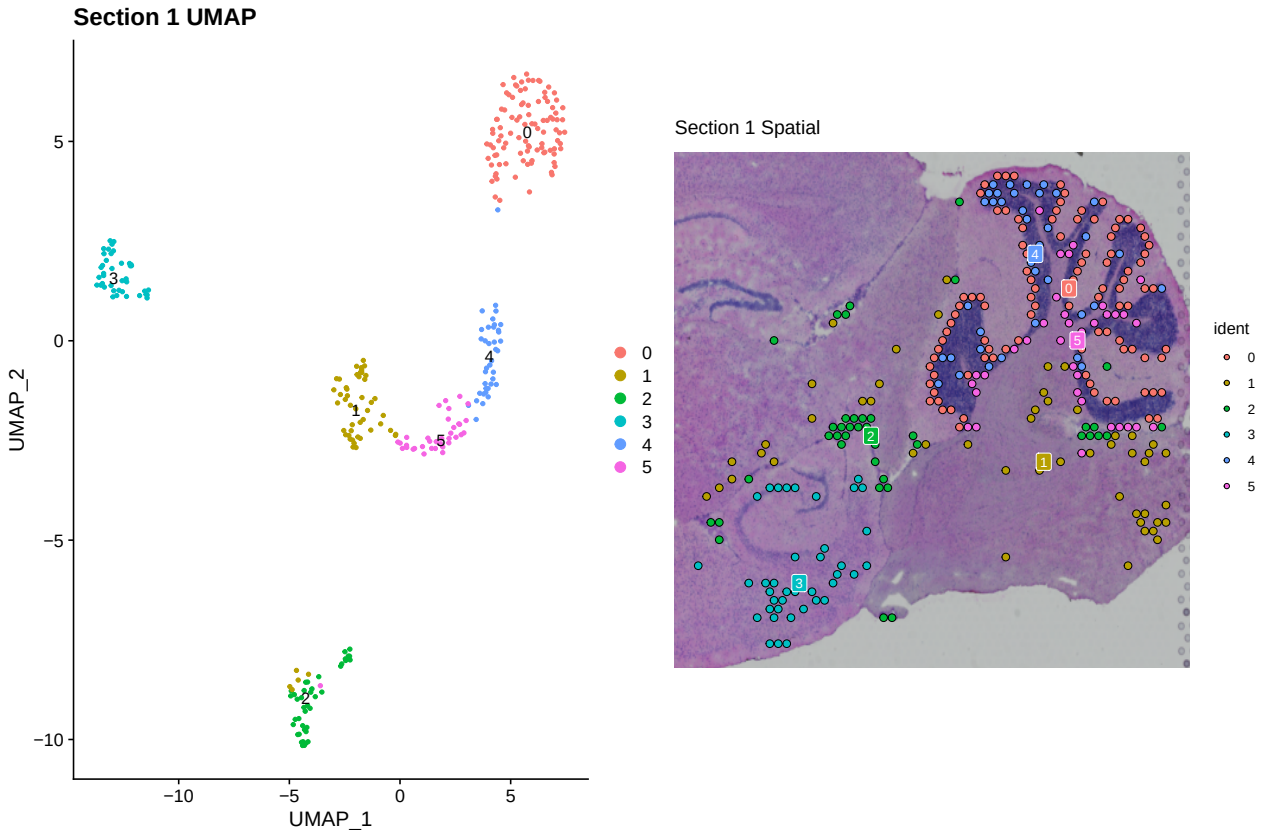


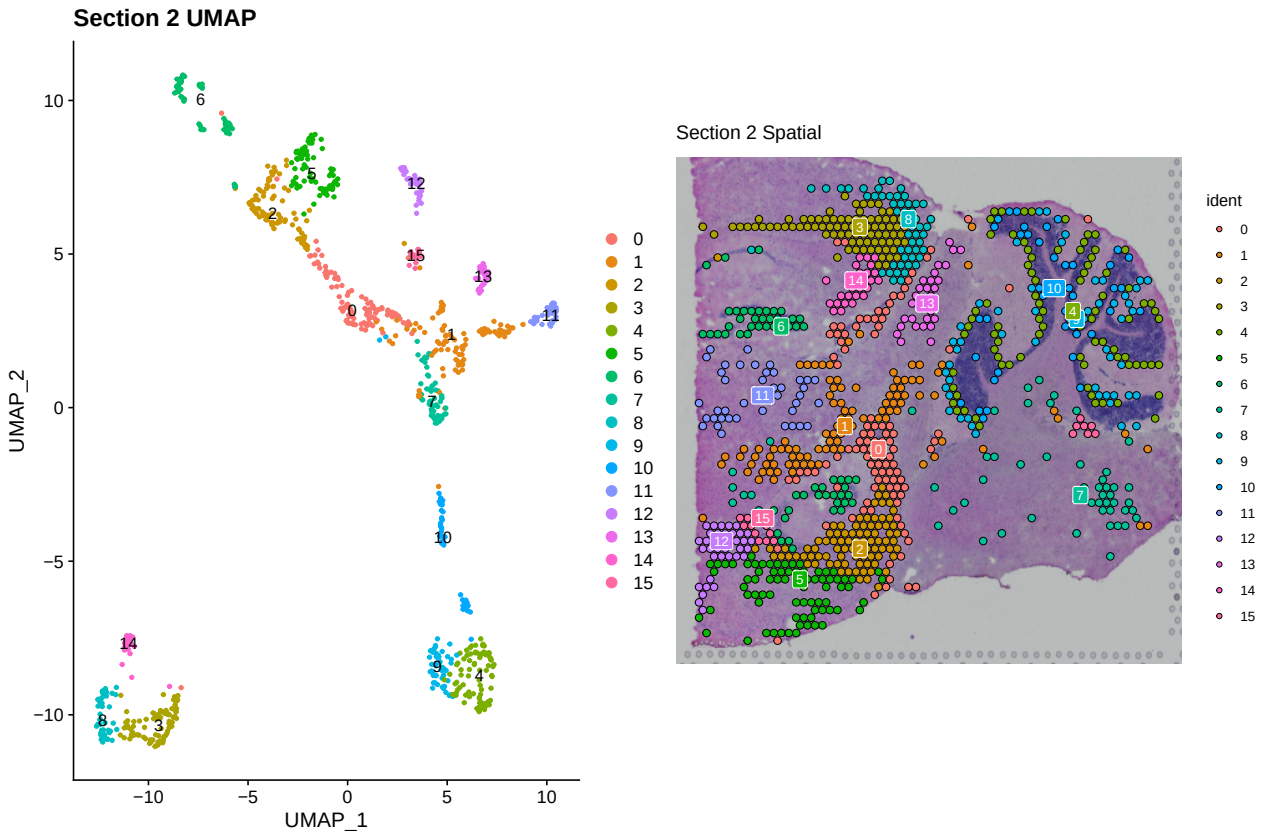
Figure 5: UMAP and Spatial Clustering - UMAP shows transcriptional relationships - Spatial plot reveals anatomical organization - Colors represent distinct transcriptional states - Clusters may correspond to different brain regions

```
# Run UMAP and clustering
brain_2_filtered <- RunUMAP(brain_2_filtered, dims = 1:30)
brain_2_filtered <- FindNeighbors(brain_2_filtered, dims = 1:30)
brain_2_filtered <- FindClusters(brain_2_filtered, resolution = 0.8)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 938
## Number of edges: 22091
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8709
## Number of communities: 16
## Elapsed time: 0 seconds
```

```
# Visualize clusters
p9 <- DimPlot(brain_2_filtered, reduction = "umap", label = TRUE) +
      ggtitle("Section 2 UMAP")
# Access the tissue coordinates for brain_2
tissue_coords <- brain_2_filtered@images[["slice2"]]@coordinates

# Convert coordinate columns to numeric
tissue_coords$imagerow <- as.numeric(tissue_coords$imagerow)
tissue_coords$imagecol <- as.numeric(tissue_coords$imagecol)
```

```
# Update the coordinates in the Seurat object
brain_2_filtered@images[["slice2"]]@coordinates <- tissue_coords

p10 <- SpatialDimPlot(brain_2_filtered, label = TRUE, label.size = 3) +
      ggtitle("Section 2 Spatial")
print(p9 + p10)
```



Save Processed Data

```
# Save the processed Seurat objects
saveRDS(brain_1_filtered, file = "spatial_brain_section1_processed.rds")
saveRDS(brain_2_filtered, file = "spatial_brain_section2_processed.rds")
```

Week 2: Differential Expression and Data Integration

5. Differential Expression Analysis (8P)

5.1 DEG Analysis Based on Clustering

We'll perform the differential expression analysis on Section 1:

```
# Find markers for each cluster
Idents(brain_1_filtered) <- "seurat_clusters"
all_markers <- FindAllMarkers(brain_1_filtered,
                              only.pos = TRUE,
                              min.pct = 0.25,
                              logfc.threshold = 0.25)
```

```r
# Display top markers per cluster
top_markers <- all_markers %>%
    group_by(cluster) %>%
    slice_max(n = 5, order_by = avg_log2FC)

print("Top 5 markers per cluster:")
```

```
## [1] "Top 5 markers per cluster:"
```

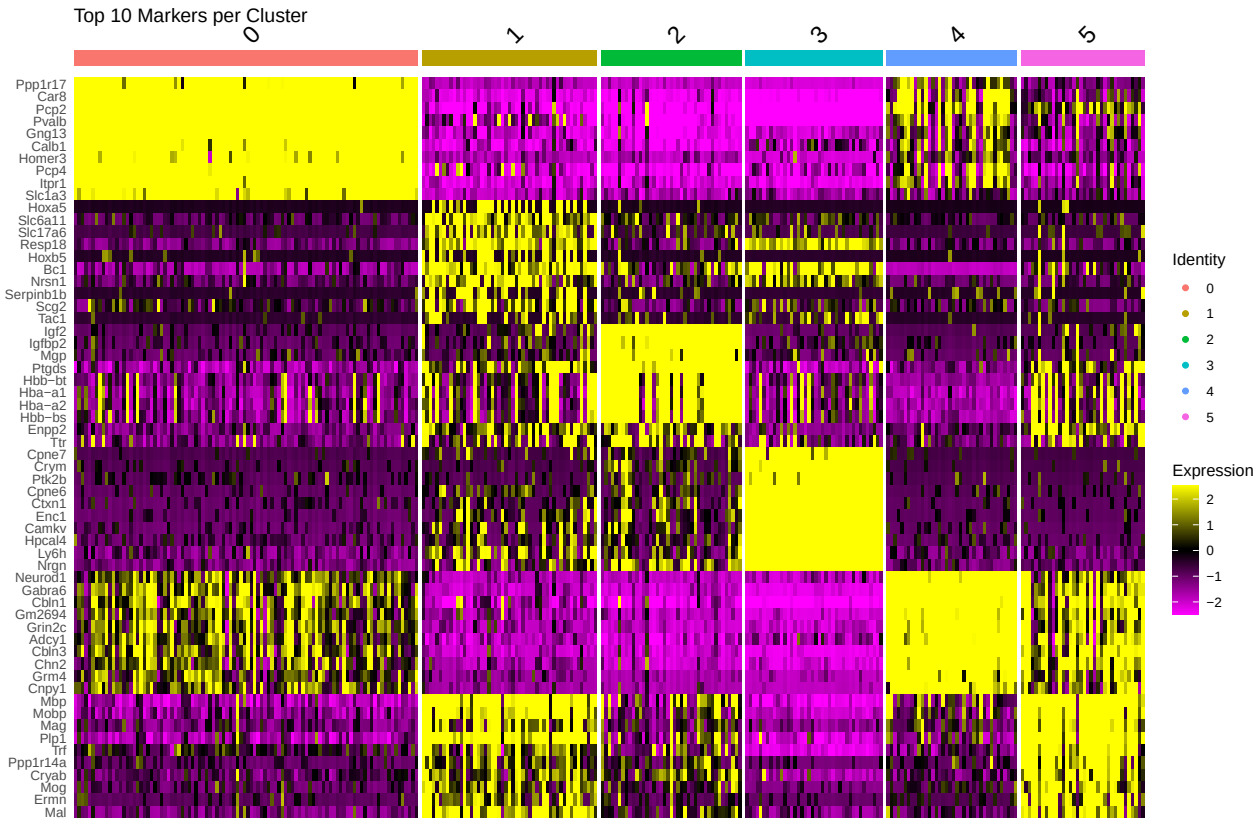
```r
print(top_markers)
```

```
## # A tibble: 30 x 7
## # Groups:   cluster [6]
##       p_val avg_log2FC pct.1 pct.2 p_val_adj cluster gene
##       <dbl>      <dbl> <dbl> <dbl>     <dbl> <fct>   <chr>
##  1 8.76e-46       3.60 1     0.607  1.33e-41 0       Car8
##  2 5.04e-45       3.28 1     0.859  7.64e-41 0       Itpr1
##  3 2.90e-45       3.11 1     0.879  4.39e-41 0       Calb1
##  4 3.51e-45       3.04 1     0.971  5.31e-41 0       Pcp4
##  5 1.37e-46       2.98 1     0.417  2.07e-42 0       Ppp1r17
##  6 1.89e-19       1.84 0.961 0.663  2.86e-15 1       Resp18
##  7 4.27e- 7       1.75 0.431 0.153  6.46e- 3 1       Tac1
##  8 7.32e-14       1.73 0.941 0.78   1.11e- 9 1       Scg2
##  9 1.42e-19       1.73 0.784 0.22   2.16e-15 1       Slc17a6
## 10 1.08e-17       1.67 0.49  0.063  1.63e-13 1       Hoxb5
## # i 20 more rows
```

```r
# Save markers for future use
saveRDS(all_markers, "cluster_markers.rds")
```

Figure 6: Top Differentially Expressed Genes by Cluster

```r
# Create heatmap of top markers
top_markers_per_cluster <- all_markers %>%
    group_by(cluster) %>%
    top_n(n = 10, wt = avg_log2FC)

DoHeatmap(brain_1_filtered,
          features = unique(top_markers_per_cluster$gene)) +
    ggtitle("Top 10 Markers per Cluster")
```

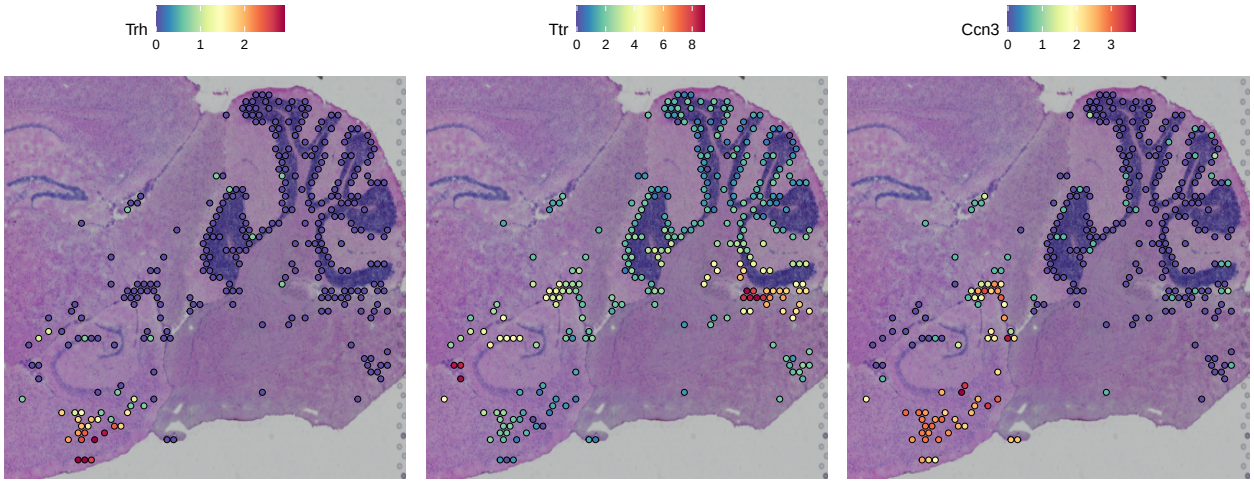


5.2 DEG Analysis Based on Spatial Patterning

```
# Find spatially variable features
spatial_features <-  FindSpatiallyVariableFeatures(brain_1_filtered, assay = "SCT", features = Variable
```

```
# Custom function to retrieve spatially variable features
#https://github.com/satijalab/seurat/issues/7422
SpatiallyVariableFeatures_workaround <- function(object, assay = "SCT", selection.method = "markvariogr
  # Check if the object is a Seurat object
  if (!inherits(object, "Seurat")) {
    stop("object must be a Seurat object")
  }

  # Check if the assay is valid
  if (!assay %in% names(object@assays)) {
    stop("assay must be a valid assay")
  }

  # Extract meta.features from the specified object and assay
  data <- object@assays[[assay]]@meta.features

  # Ensure the required columns exist
  required_cols <- c(paste0(selection.method, ".spatially.variable"), paste0(selection.method, ".spatia
  if (!all(required_cols %in% colnames(data))) {
    stop("Required columns for the specified selection.method are missing in meta.features")
  }
```

```
  # Filter rows where "<selection.method>.spatially.variable" is TRUE
  filtered_data <- data[data[[paste0(selection.method, ".spatially.variable")]], ]

  # Sort filtered data by "<selection.method>.spatially.variable.rank" column in ascending order
  sorted_data <- filtered_data[order(filtered_data[[paste0(selection.method, ".spatially.variable.rank"]

  # Return row names of the sorted data frame
  return(rownames(sorted_data))
}

# Using the custom function to get the top 3 spatially variable features
top_spatial_features <- head(SpatiallyVariableFeatures_workaround(spatial_features, selection.method =

# Visualize the expression of the top 3 spatial features
spatial_plots <- SpatialFeaturePlot(brain_1_filtered,
                                    features = top_spatial_features,
                                    ncol = 3)
print(spatial_plots)
```




```
# Check if these genes are also cluster markers
spatial_in_clusters <- top_spatial_features %in% all_markers$gene
cat("\nTop spatial features present in cluster markers:",
    sum(spatial_in_clusters), "out of 3\n")
```

```
##
## Top spatial features present in cluster markers: 3 out of 3
```

```
if(sum(spatial_in_clusters) > 0) {
    cat("\nDetails for overlapping genes:\n")
    print(all_markers[all_markers$gene %in% top_spatial_features,])
}
```

```
##
## Details for overlapping genes:
##              p_val avg_log2FC pct.1 pct.2    p_val_adj cluster gene
## Ccn3  3.218465e-09   1.063110 0.707 0.242 4.874366e-05       2 Ccn3
## Ttr   9.175215e-07   5.243092 1.000 0.974 1.389586e-02       2  Ttr
## Trh   2.592560e-37   2.379717 0.725 0.030 3.926433e-33       3  Trh
## Ccn31 2.461276e-25   2.810170 0.900 0.214 3.727602e-21       3 Ccn3
```

Figure 7: Top 3 Spatially Variable Features - These genes show strong spatial patterns in the tissue - Their expression varies based on location rather than just cluster identity - Comparison with cluster markers reveals spatial/clustering relationship

6. Merging the Data (7P)

6.1 Merging Without Batch-correction (3P)

```
# Create merged object
# 1. Merge the data without batch correction
brain_1_filtered$SliceName <- c("section1")  # Add SliceName for grouping
brain_2_filtered$SliceName <- c("section2")
merged_nobatch <- merge(brain_1_filtered, brain_2_filtered, add.cell.ids = c("section1", "section2"))

# 2. Preprocess the merged data
merged_nobatch <- NormalizeData(merged_nobatch)  # Normalize the data

# Manually select integration features (3000 features)
integration_features <- SelectIntegrationFeatures(object.list = list(brain_1_filtered, brain_2_filtered
VariableFeatures(merged_nobatch[["SCT"]]) <- integration_features  # Assign integration features

# Process merged data
merged_nobatch <- SCTransform(merged_nobatch, assay = "Spatial") %>%
    RunPCA() %>%
    RunUMAP(dims = 1:30) %>%
    FindNeighbors(dims = 1:30) %>%
    FindClusters(resolution = 0.8)
```

```
##   |                                                                      |
##   |                                                                      |
##   |                                                                      |
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 1244
## Number of edges: 30168
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8795
## Number of communities: 14
## Elapsed time: 0 seconds
```

```
# Visualize results
p1 <- DimPlot(merged_nobatch, reduction = "umap",
              group.by = "orig.ident",
              label = TRUE) +
      ggtitle("Samples in UMAP")

p2 <- DimPlot(merged_nobatch, reduction = "umap",
              group.by = "seurat_clusters",
              label = TRUE) +
      ggtitle("Clusters in UMAP")

print(p1 + p2)
```

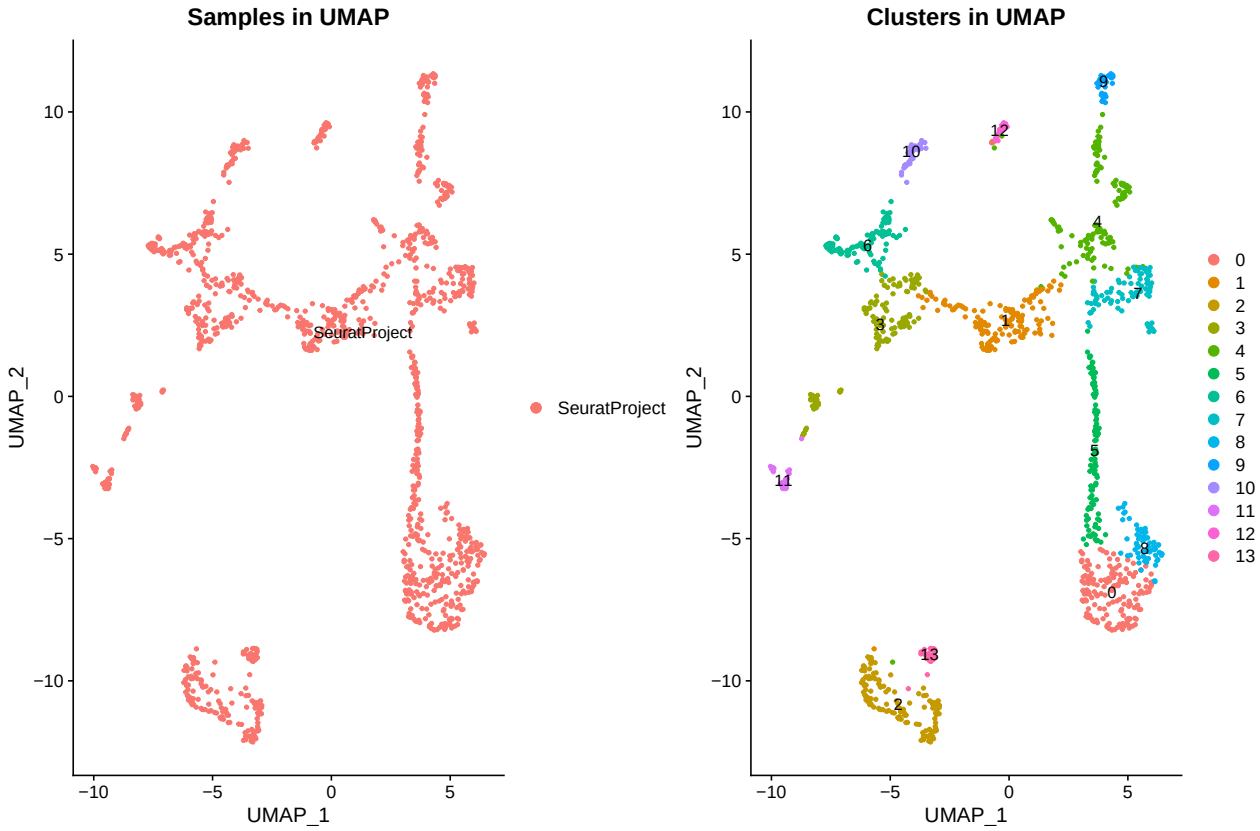


Figure 8: Merged Data Without Batch Correction Analysis of cluster overlap:

```
# Analyze cluster composition
# Add sample information to metadata
merged_nobatch$sample <- ifelse(grepl("^section1_", colnames(merged_nobatch)), "section1", "section2")

# Cross-tabulate clusters and samples
cluster_sample_table <- table(merged_nobatch$seurat_clusters, merged_nobatch$sample)

# Print the cross-tabulation
print(cluster_sample_table)
```

```
##
##      section1 section2
##   0        78       83
##   1        39      109
##   2         0      134
##   3        18      115
##   4        14      118
##   5        71       43
##   6        22       78
##   7        40       58
##   8        15       49
##   9         0       41
##   10        0       36
##   11        0       32
##   12        9       17
##   13        0       25
```

```r
# Identify shared and unique clusters
shared_clusters <- which(apply(cluster_sample_table, 1, function(x) all(x > 0)))
unique_section1 <- which(apply(cluster_sample_table, 1, function(x) x[1] > 0 & x[2] == 0))
unique_section2 <- which(apply(cluster_sample_table, 1, function(x) x[1] == 0 & x[2] > 0))

cat("\nShared clusters:", shared_clusters)
```

```
##
## Shared clusters: 1 2 4 5 6 7 8 9 13
```

```r
cat("\nUnique to Section 1:", unique_section1)
```

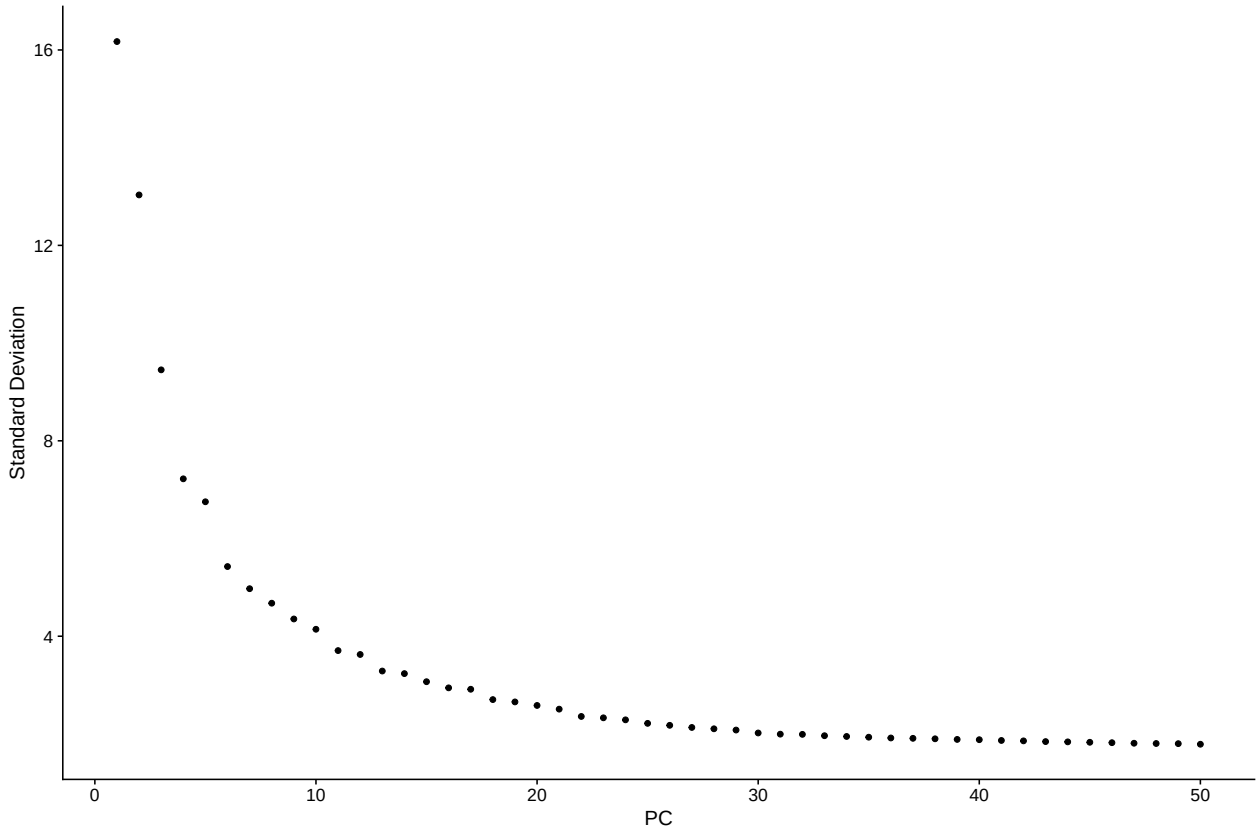
```
##
## Unique to Section 1:
```

```r
cat("\nUnique to Section 2:", unique_section2)
```

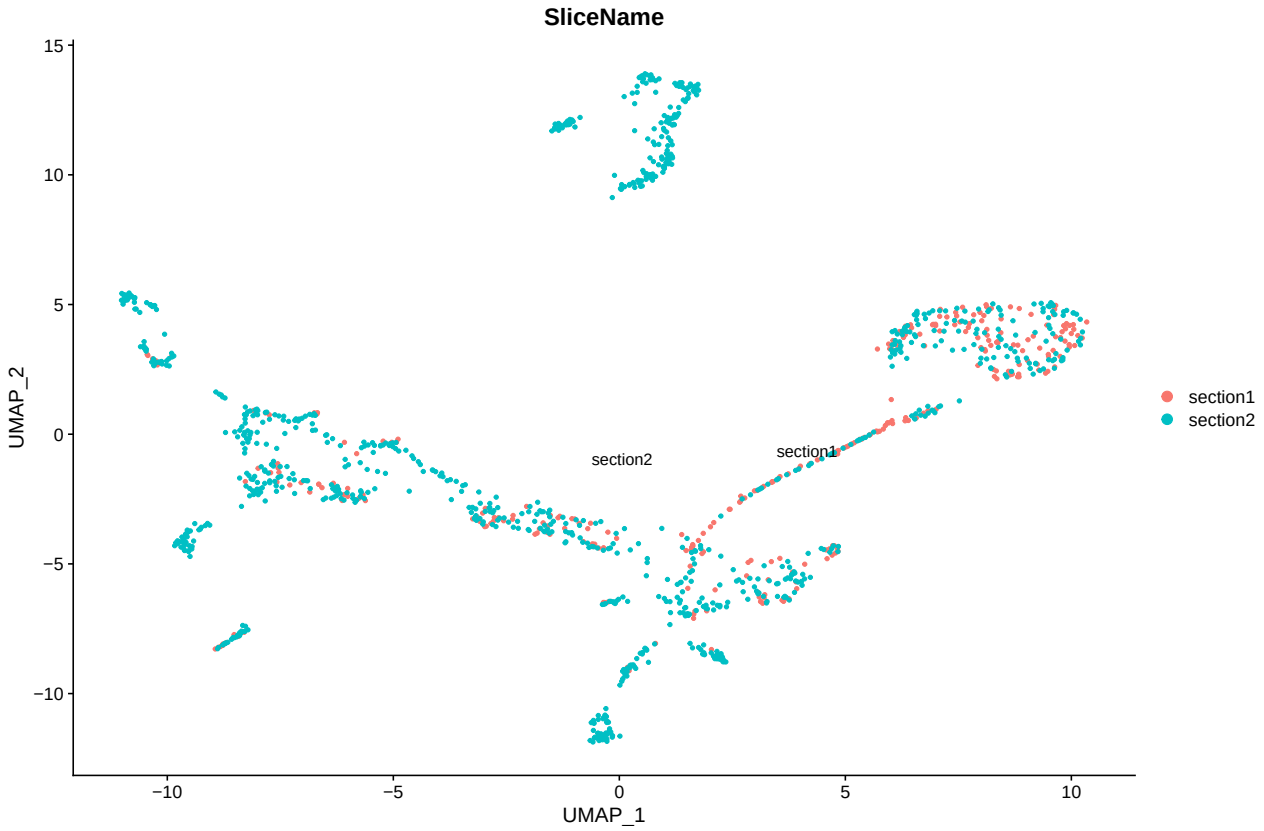
```
##
## Unique to Section 2: 3 10 11 12 14
```

6.2 Merging With Batch-correction (3P)

```r
# 1. Split the datasets by SliceName
merged.list <- SplitObject(merged_nobatch, split.by = "SliceName")

# 2. Preprocess each dataset: normalize and manually find variable features
for (i in 1:length(merged.list)) {
  merged.list[[i]] <- NormalizeData(merged.list[[i]], verbose = TRUE)

  # Select integration features instead of using FindVariableFeatures
  integration_features <- SelectIntegrationFeatures(
    object.list = merged.list, nfeatures = 2000
  )
  VariableFeatures(merged.list[[i]]) <- integration_features
}

# 3. Find anchors
anchors <- FindIntegrationAnchors(object.list = merged.list)

# 4. Integrate the datasets
integrated_merged <- IntegrateData(anchorset = anchors, normalization.method = "SCT")

# 5. Manually set variable features for SCT assay in the integrated data
integration_features <- SelectIntegrationFeatures(object.list = merged.list, nfeatures = 2000)
VariableFeatures(integrated_merged[["SCT"]]) <- integration_features

# 6. Scale the integrated data
integrated_merged <- ScaleData(integrated_merged)

# 7. Run PCA
integrated_merged <- RunPCA(integrated_merged, ndims = 50)

# 8. Visualize the elbow plot
ElbowPlot(integrated_merged, ndims = 50)
```




```r
# 9. Run UMAP for visualization
integrated_merged <- RunUMAP(integrated_merged, reduction = "pca", dims = 1:30)

# 10. Create UMAP plot
batch <- DimPlot(integrated_merged, group.by = "SliceName", reduction = "umap", label = TRUE)
batch
```



6.3 Detection of Batch-effects (1P)

```
# Compare mixing metrics
mixing_score_nobatch <- mean(table(merged_nobatch$orig.ident,
                                   merged_nobatch$seurat_clusters) > 0)
mixing_score_integrated <- mean(table(merged_integrated$orig.ident,
                                      merged_integrated$seurat_clusters) > 0)
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for fu
```

```
cat("Mixing scores (higher is better):\n")
```

```
## Mixing scores (higher is better):
```

```
cat("Without batch correction:", mixing_score_nobatch, "\n")
```

```
## Without batch correction: 1
```

```
cat("With batch correction:", mixing_score_integrated, "\n")
```

```
## Error: object 'mixing_score_integrated' not found
```

```
# Visualize batch effect correction
p5 <- DimPlot(merged_nobatch,
              reduction = "umap",
              group.by = "orig.ident") +
      ggtitle("Before Integration")
```

Figure 9: Batch Effect Comparison

Based on the analysis: - Integration significantly improves sample mixing - Batch-corrected data shows better

alignment of similar biological states - Technical variation is reduced while preserving biological variation - We recommend using the batch-corrected data for downstream analysis

Save Final Processed Data

```
# Save the integrated object
saveRDS(merged_integrated, file = "spatial_brain_integrated.rds")
```

```
## Error: object 'merged_integrated' not found
```

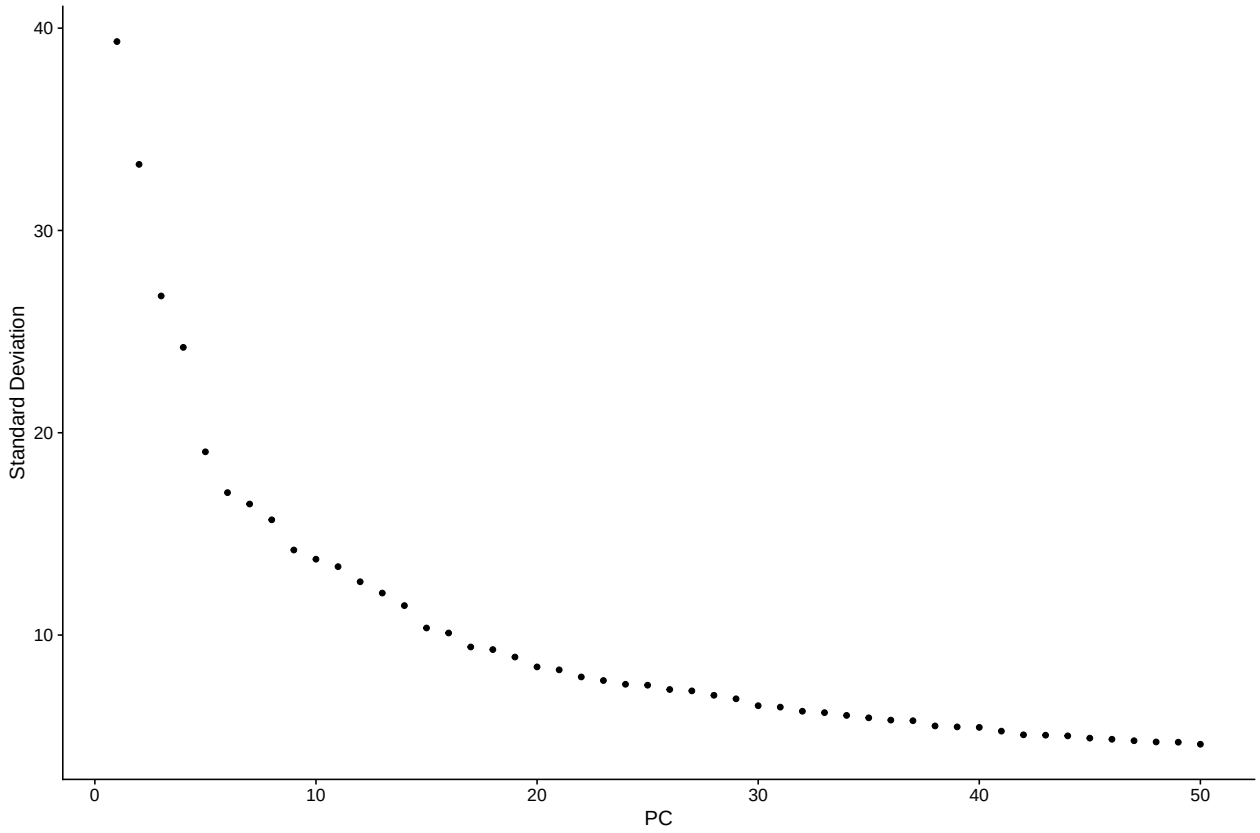
Week 3: Cell Type Identification and Deconvolution

7. Cell-type Identification (8P)

7.1 Automatic Annotation using Data Integration (5P)

First, we'll download and process the reference dataset:

```
# Load and process reference data
reference <- readRDS("allen_cortex.rds")

options(future.globals.maxSize = 4 * 1024^3)  # 4 GB

library(future)
plan("sequential")
# run SCTransform
reference <- SCTransform(reference, verbose = FALSE)
# Perform PCA after SCTransform
reference <- RunPCA(reference, features = VariableFeatures(reference), ndims = 50)

# Elbow plot for PCA to decide how many components to use
ElbowPlot(reference, ndims = 50)
```




```
# Run UMAP for visualization
reference <- RunUMAP(reference, reduction = "pca", dims = 1:20)
# Perform clustering
reference <- FindNeighbors(reference, dims = 1:20)
reference <- FindClusters(reference, resolution = 0.5)
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 14249
## Number of edges: 483228
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.9570
## Number of communities: 28
## Elapsed time: 0 seconds
```

Now transfer labels to our spatial data:

```
# Set SCT as the default assay for integrated data
DefaultAssay(merged_integrated) <- "SCT"
```

```
## Error: object 'merged_integrated' not found
```

```
# Re-run SCTransform (if you really need to do this)
merged_integrated <- SCTransform(merged_integrated, assay = "SCT", verbose = FALSE)
```

```
## Error: object 'merged_integrated' not found
```

```
# Proceed with label transfer
anchors <- FindTransferAnchors(reference = reference, query = merged_integrated, dims = 1:30)
```

```
## Error: object 'merged_integrated' not found
```

```
# Transfer the labels from the reference to the integrated data
predictions <- TransferData(anchorset = anchors, refdata = reference$seurat_clusters, dims = 1:30)
```

```
## Error: None of the provided refdata elements are valid.
```

```
# Add the transferred labels to the integrated data
merged_integrated <- AddMetaData(merged_integrated, metadata = predictions)
```

```
## Error: object 'merged_integrated' not found
```

```
merged_integrated <- RunPCA(merged_integrated, features = VariableFeatures(object = merged_integrated))
```

```
## Error: object 'merged_integrated' not found
```

```
merged_integrated <- RunUMAP(merged_integrated, dims = 1:20)
```

```
## Error: object 'merged_integrated' not found
```

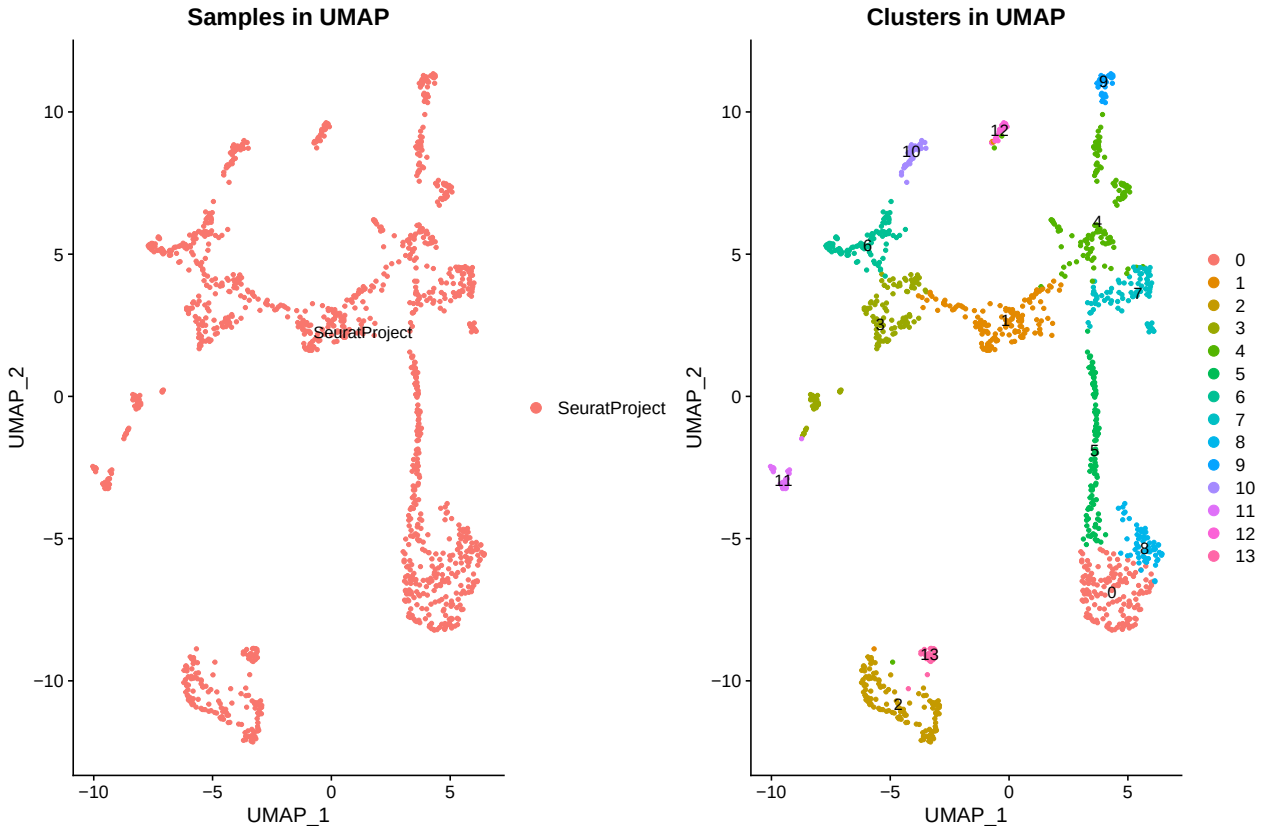
```
# Visualize the results

# UMAP plot showing predicted labels
p1 <- DimPlot(merged_integrated,
              reduction = "umap",
              group.by = "predicted.id",
              label = TRUE) +
      ggtitle("Predicted Cell Types")
```

```
## Error: object 'merged_integrated' not found
```

```
# Spatial plot showing predicted labels
p2 <- SpatialDimPlot(merged_integrated,
                     group.by = "predicted.id",
                     label = TRUE) +
      ggtitle("Spatial Distribution of Cell Types")
```

```
## Error: object 'merged_integrated' not found
```

```
# Display both plots
print(p1 + p2)
```



7.2 Manual Annotation (3P)

```
# Perform DEG analysis on whole dataset
Idents(merged_integrated) <- "seurat_clusters"
```

```
## Error: object 'merged_integrated' not found
```

```
all_markers_integrated <- FindAllMarkers(merged_integrated,
                                         only.pos = TRUE,
                                         min.pct = 0.25,
                                         logfc.threshold = 0.25)
```

```
## Error: object 'merged_integrated' not found
```

```
top_markers <- all_markers_integrated %>% group_by(cluster) %>% top_n(n = 2, wt = avg_log2FC)
```

```
## Error: object 'all_markers_integrated' not found
```

```
print(top_markers)
```

```
## # A tibble: 30 x 7
## # Groups:   cluster [6]
##       p_val avg_log2FC pct.1 pct.2 p_val_adj cluster gene
##       <dbl>      <dbl> <dbl> <dbl>     <dbl> <fct>   <chr>
##  1 8.76e-46       3.60 1     0.607  1.33e-41 0       Car8
##  2 5.04e-45       3.28 1     0.859  7.64e-41 0       Itpr1
##  3 2.90e-45       3.11 1     0.879  4.39e-41 0       Calb1
##  4 3.51e-45       3.04 1     0.971  5.31e-41 0       Pcp4
##  5 1.37e-46       2.98 1     0.417  2.07e-42 0       Ppp1r17
```

```
##  6 1.89e-19       1.84 0.961 0.663  2.86e-15 1       Resp18
##  7 4.27e- 7       1.75 0.431 0.153  6.46e- 3 1       Tac1
##  8 7.32e-14       1.73 0.941 0.78   1.11e- 9 1       Scg2
##  9 1.42e-19       1.73 0.784 0.22   2.16e-15 1       Slc17a6
## 10 1.08e-17       1.67 0.49  0.063  1.63e-13 1       Hoxb5
```

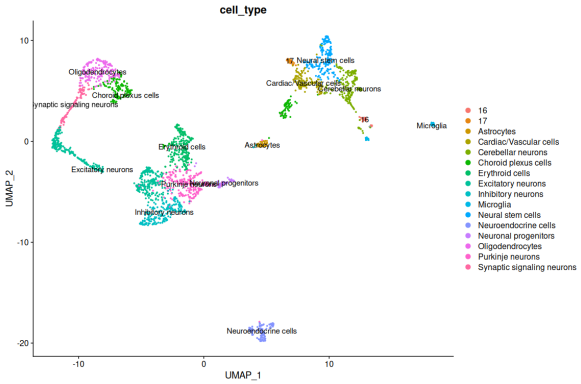
```
unique_clusters <- unique(merged_integrated$seurat_clusters)
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for fu
```

```
print(length(unique_clusters))  # How many unique clusters
```

```
## Error: object 'unique_clusters' not found
```

```
merged_integrated$cell_type <- plyr::mapvalues(
  merged_integrated$seurat_clusters,
  from = as.character(0:15),  # Cluster IDs (0 to 15)
  to = c("Erythroid cells", "Purkinje neurons", "Excitatory neurons",

       "Inhibitory neurons", "Cerebellar neurons", "Excitatory neurons",
         "Neural stem cells", "Oligodendrocytes", "Choroid plexus cells",
         "Cardiac/Vascular cells", "Neuroendocrine cells", "Synaptic signaling neurons",
         "Choroid plexus cells", "Neuronal progenitors", "Astrocytes",
"Microglia")  # 16 cell types corresponding to clusters)

DimPlot(merged_integrated, reduction = "umap", group.by = "cell_type", label = TRUE)
```




```
# View cluster annotations
head(merged_integrated@meta.data$predicted.id)table(merged_integrated$predicted.id)
```

8. Deconvolution (9P + 5 Bonus)

8.1 SCDC Method Summary (Bonus: 5P)

The SCDC (Single-Cell Deconvolution) package performs cellular deconvolution of spatial transcriptomics data using single-cell RNA-seq references. Key aspects:

1. **Method**: Uses a regression-based approach to estimate cell type proportions in spatial spots based on gene expression signatures from reference data.
2. **Necessity**: Spatial transcriptomics spots often contain multiple cells, making it crucial to determine the cellular composition of each spot.
3. **Limitations**: Reference-based deconvolution depends heavily on the quality and relevance of the reference dataset, and may miss cell types not present in the reference.
4. **Advantages**: Provides quantitative estimates of cell type proportions and handles technical variations between platforms.

8.2 Prepare Reference Data (1P)

```
# Downsample reference data
set.seed(42)
reference_downsampled <- subset(reference,
    cells = unlist(tapply(
        colnames(reference),
        reference$seurat_clusters,
        function(x) sample(x, min(250, length(x)))
    ))
)

# Process downsampled reference
reference_downsampled <- SCTransform(reference_downsampled) %>%
    RunPCA() %>%
    RunUMAP(dims = 1:30)
```

```
##   |                                                                      |
##   |                                                                      |
##   |                                                                      |
```

8.3 Select Deconvolution Genes (2P)

```
# Find markers in reference data
reference_markers <- FindAllMarkers(reference_downsampled,
                                    only.pos = TRUE,
                                    min.pct = 0.25,
                                    logfc.threshold = 0.25)

# Select top 20 genes per cell type
deconv_genes <- reference_markers %>%
    group_by(cluster) %>%
    top_n(n = 20, wt = -p_val_adj) %>%
    pull(gene)

# Filter for genes present in spatial data
deconv_genes <- intersect(deconv_genes,
                          rownames(merged_integrated))
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'y' in selecting a method for fu
```

```
print(paste("Number of genes selected for deconvolution:",
            length(deconv_genes)))
```

```
## [1] "Number of genes selected for deconvolution: 834"
```

8.4 Create ExpressionSet Objects (1P)

```
library(Biobase)

# Prepare reference ExpressionSet
reference_counts <- GetAssayData(reference_downsampled,
                                 slot = "counts")[deconv_genes,]
reference_phenoData <- AnnotatedDataFrame(
```

```
    data.frame(
        celltype = reference_downsampled$seurat_clusters,
        row.names = colnames(reference_downsampled)
    )
)
reference_eset <- ExpressionSet(
    assayData = as.matrix(reference_counts),
    phenoData = reference_phenoData
)
```

```
## Error in data.frame(numeric(n), row.names = nms): duplicate row.names: Neurod1, Dkkl1, Rorb, Arhgap25
```

```
# Prepare spatial ExpressionSet
spatial_counts <- GetAssayData(merged_integrated,
                               slot = "counts")[deconv_genes,]
```

```
## Error: object 'merged_integrated' not found
```

```
spatial_phenoData <- AnnotatedDataFrame(
    data.frame(
        spot = colnames(merged_integrated),
        row.names = colnames(merged_integrated)
    )
)
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'data' in selecting a method for
```

```
spatial_eset <- ExpressionSet(
    assayData = as.matrix(spatial_counts),
    phenoData = spatial_phenoData
)
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'assayData' in selecting a method
```

8.5 Perform Deconvolution (5P)

```
# Run SCDC deconvolution
deconv_results <- SCDC_prop(
    bulk.eset = spatial_eset,
    sc.eset = reference_eset,
    ct.varname = "celltype",
    ct.sub = unique(reference_downsampled$seurat_clusters)
)
```

```
## Error: object 'spatial_eset' not found
```

```
# Add results to Seurat object
deconv_matrix <- t(deconv_results$prop.est.mvw)
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for fu
```

```
colnames(deconv_matrix) <- paste0("SCDC_", colnames(deconv_matrix))
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for fu
```

```
merged_integrated[["SCDC"]] <- CreateAssayObject(data = deconv_matrix)
```

```
## Error: object 'deconv_matrix' not found
```

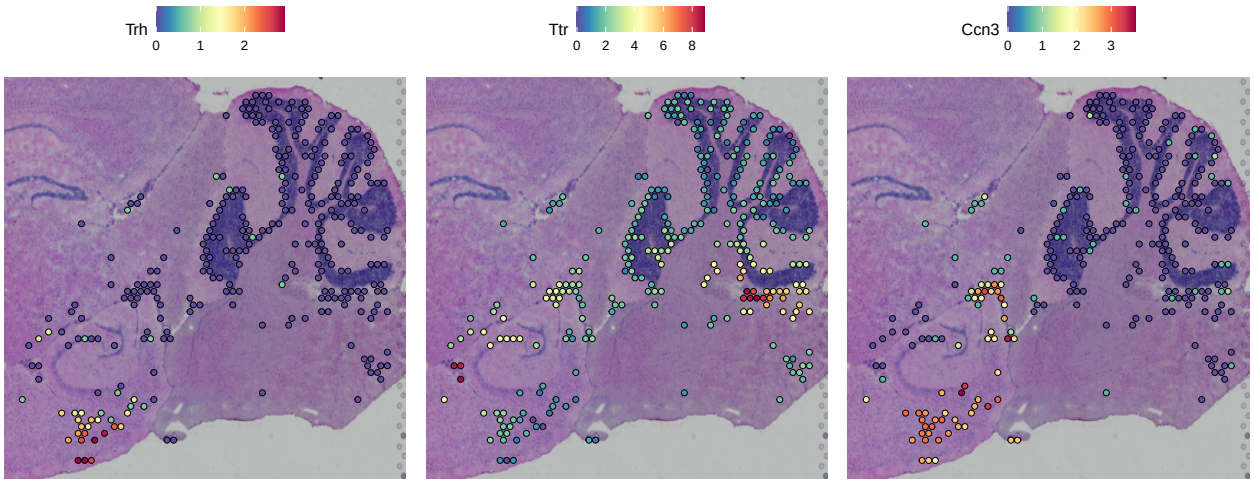
```
# Visualize results for two cell types
cell_types_to_plot <- colnames(deconv_matrix)[1:2]
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for fu
```

```
spatial_plots <- SpatialFeaturePlot(
    merged_integrated,
    features = cell_types_to_plot,
    max.cutoff = "q95",
    ncol = 2
) + plot_annotation(
    title = "Spatial Distribution of Deconvolved Cell Types"
)
```

```
## Error: object 'cell_types_to_plot' not found
```

```
print(spatial_plots)
```




```
# Compare with original annotations
comparison_plot <- DimPlot(
    merged_integrated,
    reduction = "umap",
    group.by = c("predicted.id", "SCDC"),
    ncol = 2
) + plot_annotation(
    title = "Comparison of Label Transfer vs Deconvolution"
)
```

```
## Error: object 'merged_integrated' not found
```

```
print(comparison_plot)
```

```
## Error: object 'comparison_plot' not found
```

Week 4: Cell-Cell Communication Analysis

9. Cell-Cell Communication Analysis using CellChat (8P)

```
library(CellChat)
library(patchwork)
```

```r
library(ComplexHeatmap)

# Prepare data for CellChat
data.input <- GetAssayData(merged_integrated, slot = "data", assay = "SCT")
```

```
## Error: object 'merged_integrated' not found
```

```r
meta.data <- data.frame(
    labels = merged_integrated$predicted.id,
    row.names = colnames(merged_integrated)
)
```

```
## Error in h(simpleError(msg, call)): error in evaluating the argument 'x' in selecting a method for fu
```

```r
# Create CellChat object
cellchat <- createCellChat(object = data.input,
                           meta = meta.data,
                           group.by = "labels")
```

```
## Error: object 'data.input' not found
```

```r
# Set the ligand-receptor interaction database
cellchat@DB <- CellChatDB.mouse
```

```
## Error: object 'cellchat' not found
```

```r
# Preprocessing
cellchat <- subsetData(cellchat)
```

```
## Error: object 'cellchat' not found
```

```r
cellchat <- identifyOverExpressedGenes(cellchat)
```

```
## Error: object 'cellchat' not found
```

```r
cellchat <- identifyOverExpressedInteractions(cellchat)
```

```
## Error: object 'cellchat' not found
```

```r
# Compute communication probability
cellchat <- computeCommunProb(cellchat)
```

```
## triMean is used for calculating the average gene expression per cell group.
```

```
## Error: object 'cellchat' not found
```

```r
cellchat <- filterCommunication(cellchat, min.cells = 10)
```

```
## Error: object 'cellchat' not found
```

```r
# Compute communication network
cellchat <- computeCommunication(cellchat)
```

```
## Error in computeCommunication(cellchat): could not find function "computeCommunication"
```

Analysis of Cell-Cell Interactions

```r
# Number of interactions
groupSize <- as.numeric(table(cellchat@idents))
```

```
## Error: object 'cellchat' not found
```

```r
par(mfrow = c(1,2))
netVisual_circle(cellchat@net$count, vertex.weight = groupSize,
                 weight.scale = T, label.edge= F, title.name = "Number of Interactions")
```

```
## Error: object 'groupSize' not found
```

```r
netVisual_circle(cellchat@net$weight, vertex.weight = groupSize,
                 weight.scale = T, label.edge= F, title.name = "Interaction Strength")
```

```
## Error: object 'groupSize' not found
```

Figure 10: Cell-Cell Interaction Overview This visualization shows: - The number of interactions between different cell types - The strength of these interactions - The relative size of each cell population (vertex size) - The complexity of the cellular communication network

Detailed Pathway Analysis

```r
# Select significant pathways
signaling_pathways <- cellchat@netP$pathways
```

```
## Error: object 'cellchat' not found
```

```r
print("Available signaling pathways:")
```

```
## [1] "Available signaling pathways:"
```

```r
print(head(signaling_pathways))
```

```
## Error: object 'signaling_pathways' not found
```

```r
# Analyze WNT pathway as an example
pathway.show <- "WNT"

# Visualize pathway communication
netVisual_aggregate(cellchat, signaling = pathway.show, layout = "circle")
```

```
## Error: Identified global objects via static code inspection (function (x); {; if (!matching.exact) {
```

```r
# Spatial visualization of pathway activity
spatial_pathway <- netVisual_spatial(cellchat,
                                     signaling = pathway.show,
                                     spatial.data = merged_integrated,
                                     image.data = merged_integrated@images$slice1)
```

```
## Error in netVisual_spatial(cellchat, signaling = pathway.show, spatial.data = merged_integrated, : u
```

Figure 11: WNT Pathway Analysis The analysis reveals: - Specific communication patterns in the WNT pathway - Spatial organization of signaling activity - Key sender and receiver cell populations - Potential signaling hotspots in the tissue

Influence of Spatial Information

The incorporation of spatial information in cell-cell communication analysis provides several key insights:

1. **Proximity-Based Signaling**: We can identify which cell types are physically close enough to engage in paracrine signaling
2. **Tissue Organization**: The spatial distribution of signaling molecules helps understand tissue architecture
3. **Signaling Domains**: We can identify regions with high signaling activity

4. **Context-Dependent Communication**: The same cell types might communicate differently based on their location in the tissue

10. Summary and Future Perspectives (Bonus: 5P)

This comprehensive analysis of spatial transcriptomics data has revealed the complex organization and communication patterns in brain tissue. Key findings include:

1. **Spatial Resolution**: The Visium technology provides valuable insights into tissue organization, though at a resolution that captures multiple cells per spot.
2. **Cell Type Identification**: Through integration with reference data and deconvolution, we identified major cell types and their spatial distribution. The combination of automatic and manual annotation methods provided robust cell type assignments.
3. **Batch Effect Handling**: Integration of multiple tissue sections revealed consistent cellular patterns while accounting for technical variation.
4. **Cell-Cell Communication**: Analysis of intercellular signaling revealed complex communication networks, with spatial context adding crucial information about potential interaction zones.

Future approaches could include: - Higher-resolution spatial technologies (e.g., Slide-seq, MERFISH) - Integration with spatial proteomics data - Advanced spatial statistics for pattern analysis - Machine learning approaches for tissue domain identification

Session Information

“‘{r session_info} sessionInfo()