Hands-on: Seurat Tutorial

Seurat v5 Standard Workflow

1. Data Loading & QC

```
# Read 10X data
data <- Read10X("filtered_feature_bc_matrix/")
seurat <- CreateSeuratObject(data, min.cells=3)
```

2. QC Filtering

```
# Calculate mitochondrial %
seurat[["percent.mt"]] <- PercentageFeatureSet(seurat, "^MT-")
seurat <- subset(seurat, nFeature_RNA > 200 & percent.mt < 10)
```

### 3. Normalization & Scaling

```
seurat <- NormalizeData(seurat)
seurat <- FindVariableFeatures(seurat, nfeatures=2000)
seurat <- ScaleData(seurat)
```

4. Dimension Reduction & Clustering

```
seurat <- RunPCA(seurat) %>% RunUMAP(dims=1:30)
seurat <- FindNeighbors(seurat) %>% FindClusters(res=0.5)
DimPlot(seurat, label=TRUE) + NoLegend()
```
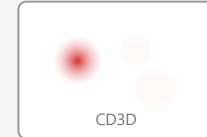
Key Visualizations

QC Metrics

nGene nUMI %MT

UMAP Clustering

0 1 2 3 4

Feature Plot

CD3D
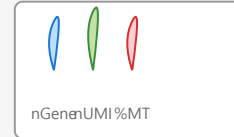
Integration with Harmony/Seurat

```
# Integration of multiple samples
seurat <- IntegrateLayers(seurat, method=HarmonyIntegration)
```

Before → After

Find Markers & Annotate

```
markers <- FindAllMarkers(seurat, only.pos=TRUE)
new.ids <- c("T cells", "B cells", "NK", "Monocytes")
```

Top Markers:

● CD3D   ● CD79A   ● NKG7   ● CD14

💡 Most widely used R package for scRNA-seq analysis