#Seurat New version of the tutorial: analysing spatial transcriptome data.

https://www.jianshu.com/p/f6da86489784

# devtools::install\_github('satijalab/seurat-data')

setwd("D:\\ Sangshin Project Paper\\ R Language Related Modules\\ Spatial Transcriptome Analysis\\ Colorectal")

library(Seurat)

library(SeuratData)

library(ggplot2)

library(patchwork)

library(dplyr)

library(cowplot)

Colorectal <- Load10X\_Spatial(data.dir =".",

filename = "Parent\_Visium\_Human\_ColorectalCancer\_filtered\_feature\_bc\_matrix.h5",

slice ="Colorectal", assay = "Spatial"

)

# Observational data

plot1 <- VlnPlot(Colorectal, features = "nCount\_Spatial", pt.size = 0.1) + NoLegend()

plot2 <- SpatialFeaturePlot(Colorectal, features = "nCount\_Spatial") + theme(legend.position = "right")

plot\_grid(plot1, plot2)

#Expansion - demonstrating gene expression at spatial locations

plot <- SpatialFeaturePlot(Colorectal, features = "CDKN2A");

plot

plot <- SpatialFeaturePlot(Colorectal, features = "SLC7A11");

plot

plot <- SpatialFeaturePlot(Colorectal, features = "GNG12");

plot

plot <- SpatialFeaturePlot(Colorectal, features = "CCT7");

plot

plot <- SpatialFeaturePlot(Colorectal, features = "CTNNB1");

plot

plot <- SpatialFeaturePlot(Colorectal, features = "HSPA1A");

plot

#The previous visualisation just gives us a brief look at the data, later on we have to preprocess the data in the same way as the single cell

#Data preprocessing (refer to single-cell process)

##The data were normalised using SCTransform(), while highly variable genes were detected and the output was stored in the SCT assay;

Colorectal <- SCTransform(Colorectal, assay = "Spatial", verbose = FALSE)# Standardised data

Colorectal <- RunPCA(Colorectal, assay = "SCT", verbose = FALSE) # Reduced dimensionality

plot1 <- DimPlot(Colorectal, reduction = "pca", group.by="orig.ident")# Determine the number of PCs

plot2 <- ElbowPlot(Colorectal, ndims=20, reduction="pca") # Determine the number of PCs

plot1+plot2

pc.num=1:20#Setting the number of PCs

Colorectal <- FindNeighbors(Colorectal, reduction = "pca", dims = pc.num)

Colorectal <- FindClusters(Colorectal, verbose = FALSE)

# UMAP Reduced dimensional visualisation

Colorectal <- RunUMAP(Colorectal, reduction = "pca", dims = pc.num)

p1 <- DimPlot(Colorectal, reduction = "umap", label = TRUE)

p1

# Visualisation using the SpatialDimPlot function

p2 <- SpatialDimPlot(Colorectal, label = TRUE, label.size = 3)

p1 + p2

#Identify spatially highly variable genes

#Seurat provides two workflows to identify molecular signatures associated with the spatial location of tissues

#The first is to differentially express based on pre-annotated anatomical regions within the tissue, which can be identified by unsupervised clustering or a priori knowledge

#But clearly we prefer an automated approach, which is the second approach looking for genes that are not pre-annotated in the spatial pattern

#

Colorectal <- FindSpatiallyVariableFeatures(Colorectal, assay = "SCT", features = VariableFeatures(Colorectal)[1:100],

selection.method = "markvariogram")

#This one step is very slow (so when debugging it myself, if it's 1000, it takes about 45min (the demo here only uses the first 100))

#Visualisation of the distribution of the first 2 difference markers in spatial position

top.features <- head(SpatiallyVariableFeatures(Colorectal, selection.method = "markvariogram"), 2)

SpatialFeaturePlot(Colorectal, features = "CDKN2A", ncol = 1, alpha = c(0.1, 1))

SpatialFeaturePlot(Colorectal, features = "SLC7A11", ncol = 1, alpha = c(0.1, 1))

SpatialFeaturePlot(Colorectal, features = "GNG12", ncol = 1, alpha = c(0.1, 1))

SpatialFeaturePlot(Colorectal, features = "CCT7", ncol = 1, alpha = c(0.1, 1))

SpatialFeaturePlot(Colorectal, features = "CTNNB1", ncol = 1, alpha = c(0.1, 1))

SpatialFeaturePlot(Colorectal, features = "HSPA1A", ncol = 1, alpha = c(0.1, 1))

#Dimensionality reduction, clustering and visualisation

Colorectal <- RunPCA(Colorectal, assay = "SCT", verbose = FALSE)

Colorectal <- FindNeighbors(Colorectal, reduction = "pca", dims = 1:30)

Colorectal <- FindClusters(Colorectal, verbose = FALSE)

Colorectal <- RunUMAP(Colorectal, reduction = "pca", dims = 1:30)

p1 <- DimPlot(Colorectal, reduction = "umap", label = TRUE)

p2 <- SpatialDimPlot(Colorectal, label = TRUE, label.size = 3)

plot\_grid(p1, p2)

SpatialDimPlot(Colorectal)

SpatialDimPlot(Colorectal, cells.highlight = CellsByIdentities(object = Colorectal,

idents = c(1, 2, 5, 3, 4, 8)), facet.highlight = TRUE, ncol = 3)

LinkedDimPlot(Colorectal)

#Identification of spatially variable features

de\_markers <- FindMarkers(Colorectal, ident.1 = 4, ident.2 = 6)

SpatialFeaturePlot(object = Colorectal,

features = rownames(de\_markers)[1:6],

alpha = c(0.1, 1), ncol = 3)

#We visualised the expression of the first 6 features identifying this measure

Colorectal <- FindSpatiallyVariableFeatures(Colorectal, assay = "SCT",

features = VariableFeatures(Colorectal)[1:100],

selection.method = "markvariogram")

top.features <- head(SpatiallyVariableFeatures(Colorectal, selection.method = "markvariogram"), 6)

SpatialFeaturePlot(Colorectal, features = top.features, ncol = 3, alpha = c(0.1, 1))