follow_SPOTlight_vignette

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R Markdown

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When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

```
##github. (2020). GitHub. Retrieved from https://github.com/MarcElosua/SPOTlight/tree/spotlight-0.1.7
#install.packages("devtools") #Install it if you need
#devtools::install_github("https://github.com/MarcElosua/SPOTlight/tree/spotlight-0.1.7") #Download the sample_data
                                                                                           #sample_data will saved in "D:WRWR-4.
2.1WlibraryWSPOTlight" by default setting
#Bring the packages
library(Matrix)
library(data.table)
library(Seurat) #Single cell data package
```

```
## Attaching SeuratObject
```

```
## Attaching sp
```

```
library(SeuratData)
```

```
## ── Installed datasets ──────────────────────────────────────────────────────── SeuratData v0.2.2 ──
```

```
## ✓ stxBrain 0.1.1
```

```
## ──────────────────────────────────────────────────────────── Key ─────────────────────────────────
────────────────────────
```

```
## ✓ Dataset loaded successfully
## ❯ Dataset built with a newer version of Seurat than installed
## ❓ Unknown version of Seurat installed
```

```
library(dplyr) #data structure package
```

```
## 
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:data.table':
## 
##     between, first, last
```

```
## The following objects are masked from 'package:stats':
## 
##     filter, lag
```

```
## The following objects are masked from 'package:base':
## 
##     intersect, setdiff, setequal, union
```

```
library(gt)
library(SPOTlight)
library(igraph)
```

```
## 
## Attaching package: 'igraph'
```

```
## The following objects are masked from 'package:dplyr':
## 
##     as_data_frame, groups, union
## The following objects are masked from 'package:stats':
##
##     decompose, spectrum
```

```
## The following object is masked from 'package:base':
##
##     union
```

```
library(RColorBrewer)
library(NMF)
```

```
## Loading required package: pkgmaker
```

```
## Loading required package: registry
```

```
## Loading required package: rngtools
```

```
## Loading required package: cluster
```

```
## NMF - BioConductor layer [OK] | Shared memory capabilities [NO: windows] | Cores 15/16
```

```
##
## Attaching package: 'NMF'
```

```
## The following objects are masked from 'package:igraph':
##
##     algorithm, compare
```

```
library(magrittr)

path_to_data <- system.file(package = "SPOTlight") #Set the path to "SPOTlight"
cortex_sc <- readRDS(glue::glue("{path_to_data}/allen_cortex_dwn.rds")) #Load the single cell sequencing data

# Download the data from SeuratData
if (!("stxBrain.SeuratData" %in% rownames(InstalledData()))) {
  InstallData("stxBrain")
}

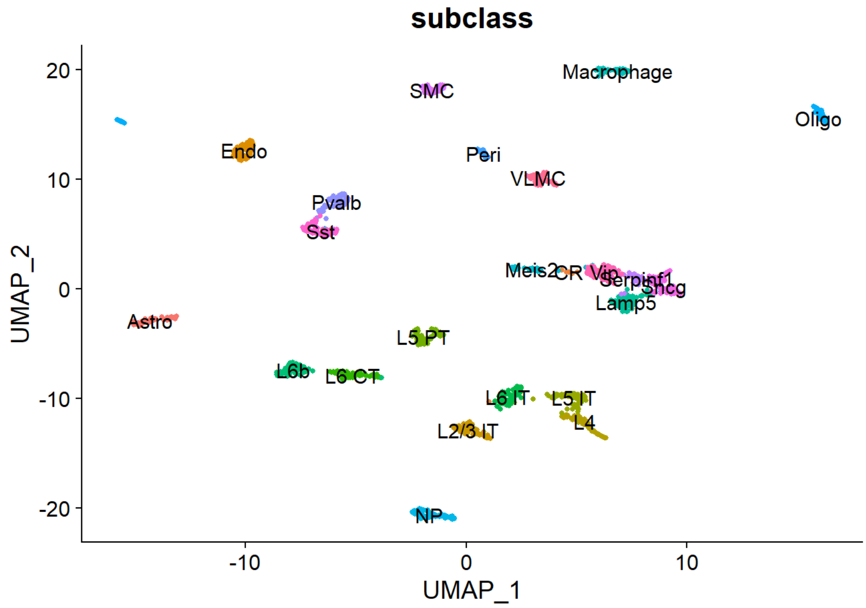
# Load SeuratData
anterior <- SeuratData::LoadData("stxBrain", type = "anterior1")

#Create seurat object
set.seed(123)
cortex_sc_se <- Seurat::SCTransform(cortex_sc, verbose = FALSE) %>%
  Seurat::RunPCA(., verbose = FALSE) %>%
  Seurat::RunUMAP(., dims = 1:30, verbose = FALSE)
```

```
## Warning: Invalid name supplied, making object name syntactically valid. New
## object name is Seurat..SCTransform.RNA; see ?make.names for more details on
## syntax validity
```

```
## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R-native UWOT using the c
osine metric
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and metric to 'correlation'
## This message will be shown once per session
```

```
#View cell type plot
Seurat::DimPlot(cortex_sc_se,
                group.by = "subclass", #Choose the criteria
                label = TRUE) + Seurat::NoLegend()
```



```
#cell type dataset
cortex_sc_se@meta.data %>%
  dplyr::count(subclass) %>%
  gt::gt(.[-1, ]) %>%
  gt::tab_header(
    title = "Cell types present in the reference dataset",
  ) %>%
  gt::cols_label(
    subclass = gt::html("Cell Type")
  )
```

```
## Warning in !is.null(rowname_col) && rowname_col %in% colnames(data_tbl):
## 'length(x) = 2 > 1' in coercion to 'logical(1)'
```

Cell types present in the reference dataset

Cell Type	n
Astro	70
CR	7
Endo	70
L2/3 IT	70
L4	70
L5 IT	70
L5 PT	70
L6 CT	70
L6 IT	70
L6b	70
Lamp5	70
Macrophage	51
Meis2	45
NP	70
Oligo	70
Peri	32
Pvalb	70

Cell types present in the reference dataset	
Serpinf1	27
SMC	55
Sncg	70
Sst	70
Vip	70
VLMC	67

```
#Compute marker genes
Seurat::Idents(object = cortex_sc_se) <- cortex_sc_se@meta.data$subclass #Change the Levels in the seurat object
cluster_markers_all <- Seurat::FindAllMarkers(object = cortex_sc_se,
                                              assay = "SCT",
                                              slot = "data",
                                              verbose = TRUE,
                                              only.pos = TRUE)
```

```
## Calculating cluster Astro
```

```
## Calculating cluster CR
```

```
## Calculating cluster Endo
```

```
## Calculating cluster L2/3 IT
```

```
## Calculating cluster L4
```

```
## Calculating cluster L5 IT
```

```
## Calculating cluster L5 PT
```

```
## Calculating cluster L6 CT
```

```
## Calculating cluster L6 IT
```

```
## Calculating cluster L6b
```

```
## Calculating cluster Lamp5
```

```
## Calculating cluster Macrophage
```

```
## Calculating cluster Meis2
```

```
## Calculating cluster NP
```

```
## Calculating cluster Oligo
```

```
## Calculating cluster Peri
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```
## Calculating cluster Pvalb
```

```
## Calculating cluster Serpinf1
```

```
## Calculating cluster SMC
```

```
## Calculating cluster Sncg
```

```
## Calculating cluster Sst
```

```
## Calculating cluster Vip
## Calculating cluster VLMC
```

```
saveRDS(object = cluster_markers_all, #Save the marker file
        file = here::here("D:/pankyung/intern_back_up/sub/follow_SPOTlight_vignette/markers_sc_n.RDS")) #Set the appropriate directory, use the "getwd()"

#Load the marker file
cluster_markers_all<-readRDS('D:/pankyung/intern_back_up/sub/follow_SPOTlight_vignette/markers_sc_n.RDS')

#SPOTlight deconvolution
set.seed(1234)
spotlight_ls <- spotlight_deconvolution(
  se_sc = cortex_sc_se,
  counts_spatial = anterior@assays$Spatial@counts,
  clust_vr = "subclass", # Variable in sc_seu containing the cell-type annotation
  cluster_markers = cluster_markers_all, # Dataframe with the marker genes
  cl_n = 100, # number of cells per cell type to use
  hvg = 3000, # Number of HVG to use
  ntop = NULL, # How many of the marker genes to use (by default all)
  transf = "uv", # Perform unit-variance scaling per cell and spot prior to factorzation and NLS
  method = "nsNMF", # Factorization method
  min_cont = 0 # Remove those cells contributing to a spot below a certain threshold
)
```

```
## [1] "Preparing Gene set"
## [1] "Normalizing count matrix"
## [1] "Seeding initial matrices"
## [1] "Training..."
## [1] "Time to train NMF model was 62.89mins"
## [1] "Deconvoluting spots"
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```

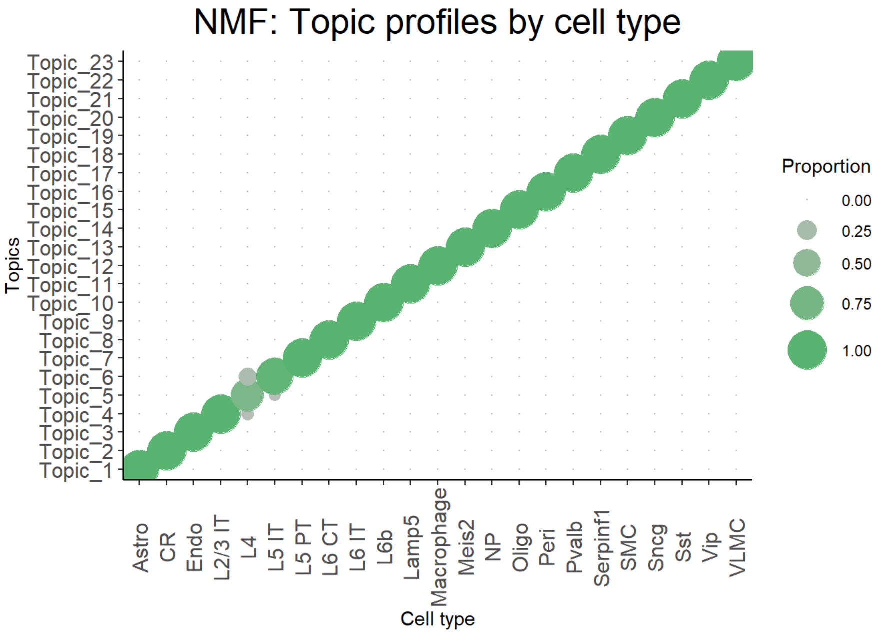
```
saveRDS(object = spotlight_ls, file = here::here("D:/pankyung/intern_back_up/sub/follow_SPOTlight_vignette/spotlight_ls_n.rds"
)) #Save the spotlight file

#Load the spotlight file
spotlight_ls <- readRDS(file = "D:/pankyung/intern_back_up/sub/follow_SPOTlight_vignette/spotlight_ls_n.rds")

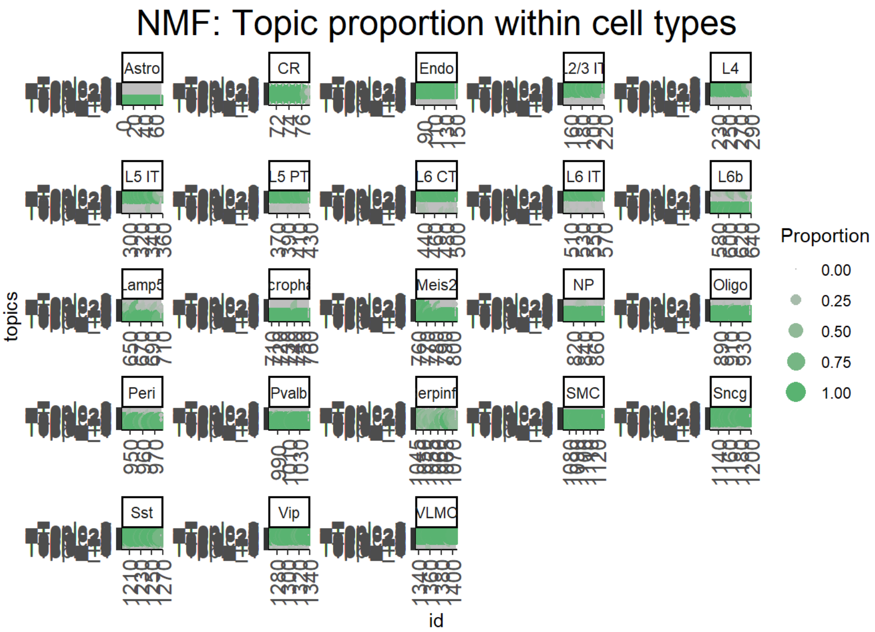
#Bring the data only we want
nmf_mod <- spotlight_ls[[1]]
decon_mtrx <- spotlight_ls[[2]]

#Assess deconvolution
h <- NMF::coef(nmf_mod[[1]])
rownames(h) <- paste("Topic", 1:nrow(h), sep = "_")
topic_profile_plts <- SPOTlight::dot_plot_profiles_fun(
  h = h,
  train_cell_clust = nmf_mod[[2]])

#Topic profile cell type plot
topic_profile_plts[[2]] + ggplot2::theme(
  axis.text.x = ggplot2::element_text(angle = 90),
  axis.text = ggplot2::element_text(size = 12))
```



```
#Topic proportion within cell type
topic_profile_plts[[1]] + theme(axis.text.x = element_text(angle = 90),
                                axis.text = element_text(size = 12))
```



```
#Take a look at which genes are the most important for each topic
basis_spotlight <- data.frame(NMF::basis(nmf_mod[[1]]))

colnames(basis_spotlight) <- unique(stringr::str_wrap(nmf_mod[[2]], width = 30))

basis_spotlight %>%
  dplyr::arrange(desc(Astro)) %>%
  round(., 5) %>%
  DT::datatable(., filter = "top")
```

```
## Warning in instance$preRenderHook(instance): It seems your data is too big
## for client-side DataTables. You may consider server-side processing: https://
## rstudio.github.io/DT/server.html
```

Show 10 **entries** **Search:**

	Astro	CR	Endo	L2/3 IT	L4	L5 IT	L5 PT	L6 CT	L6 IT	L6b
Mt3	0.00129	0	0	0.00004	0.00001	0.00005	0.00017	0.00024	0.00015	0.00006
Gstm5	0.0012	0.00014	0	0	0	0.00002	0	0.00002	0.00003	0.00003
Prdx6	0.00119	0	0	0	0	0	0	0	0	0
Malat1	0.00116	0.00038	0.00007	0.00012	0.00037	0	0.00003	0.00006	0.00011	0
Clu	0.00114	0	0	0	0	0	0	0.00002	0.00002	0.00011
Hmgn3	0.00109	0.00042	0	0.00008	0.00018	0.00003	0.0001	0.00007	0.00006	0.00008
Ntrk2	0.00108	0	0	0.00009	0.00026	0.00001	0.00009	0.00006	0.00007	0.00005
Cspg5	0.00107	0	0	0.00004	0.00003	0.00012	0.0001	0	0.00005	0
Gm3764	0.00107	0	0	0	0.00016	0	0	0	0	0
Msmo1	0.00106	0	0	0	0	0	0	0	0.00006	0.00009

Showing 1 to 10 of 14,293 entries

Previous 1 2 3 4 5 … 1,430 Next

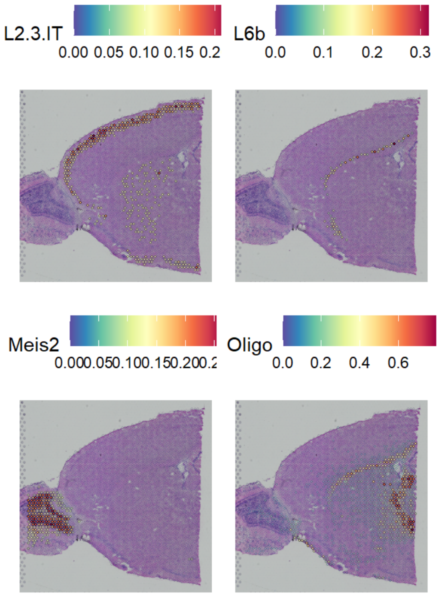
```
#Visualization
#This is the equivalent to setting min_cont to 0.04
decon_mtrx_sub <- decon_mtrx[, colnames(decon_mtrx) != "res_ss"]
decon_mtrx_sub[decon_mtrx_sub < 0.08] <- 0
decon_mtrx <- cbind(decon_mtrx_sub, "res_ss" = decon_mtrx[, "res_ss"])
rownames(decon_mtrx) <- colnames(anterior)

decon_df <- decon_mtrx %>%
  data.frame() %>%
  tibble::rownames_to_column("barcodes")

anterior@meta.data <- anterior@meta.data %>%
  tibble::rownames_to_column("barcodes") %>%
  dplyr::left_join(decon_df, by = "barcodes") %>%
  tibble::column_to_rownames("barcodes")

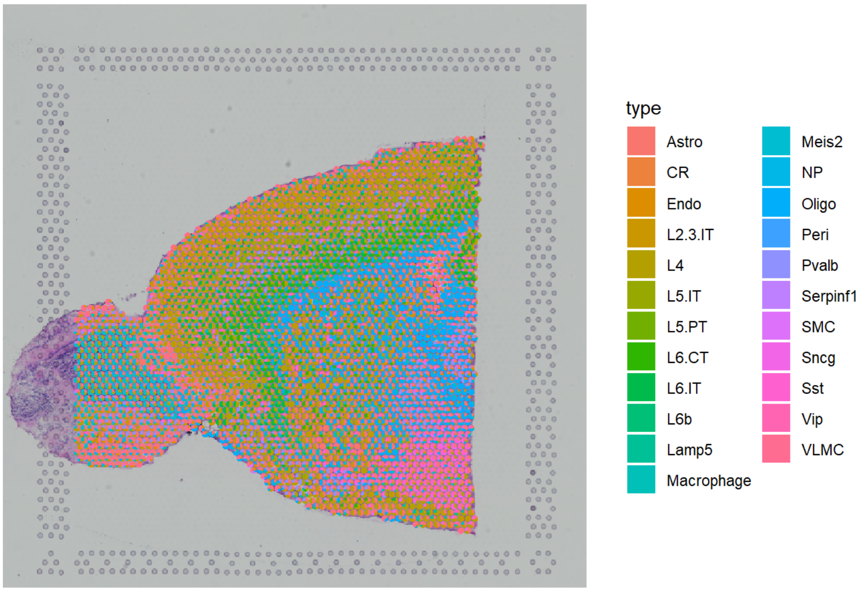
#Spatial plot by specific cell-types
Seurat::SpatialFeaturePlot(
  object = anterior,
  features = c("L2.3.IT", "L6b", "Meis2", "Oligo"), #Set the target cell type
  alpha = c(0.1, 1))
```

```
## Warning: The following variables were found in both object metadata and the default assay: Meis2
## Returning metadata; if you want the feature, please use the assay's key (eg. spatial_Meis2)
```



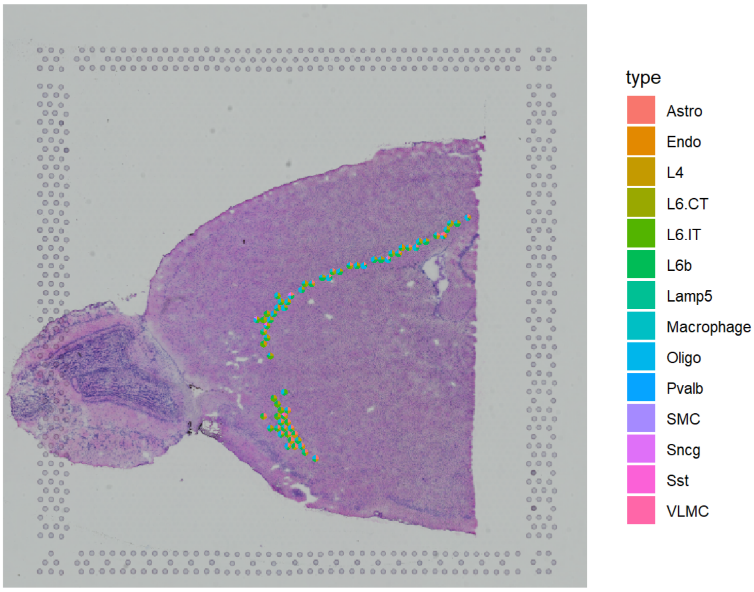
```
#Spatial plot by all cell-types
cell_types_all <- colnames(decon_mtrx)[which(colnames(decon_mtrx) != "res_ss")]
#Integration of cell type image
SPOTlight::spatial_scatterpie(se_obj = anterior,
                              cell_types_all = cell_types_all,
                              img_path = "D:/pankyung/intern_back_up/sub/follow_SPOTlight_vignette/tissue_lowres_image.png", #T
his image should have been downloaded with the SeuratData
                              pie_scale = 0.4)
```

```
## Warning in SPOTlight::spatial_scatterpie(se_obj = anterior, cell_types_all =
## cell_types_all, : Using slice anterior1
```



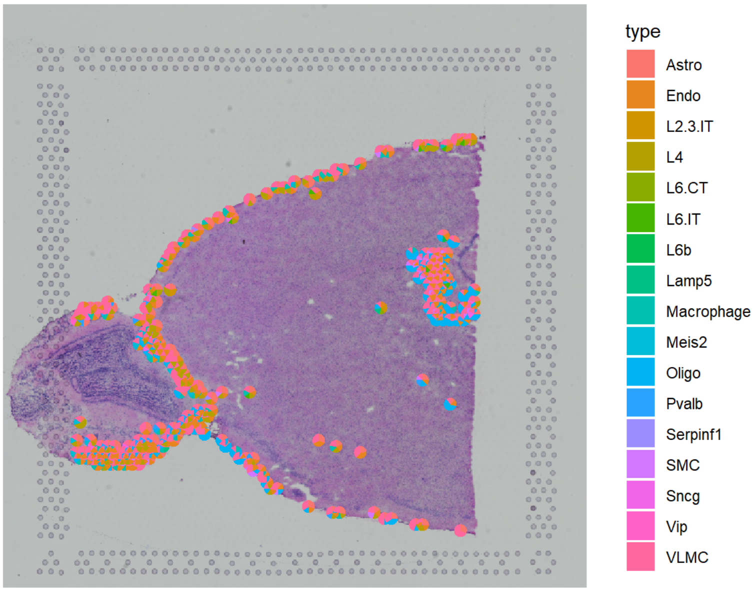
```
#Plot spot composition of spots containing cell-types of interest
SPOTlight::spatial_scatterpie(se_obj = anterior,
                              cell_types_all = cell_types_all,
                              img_path = "D:/pankyung/intern_back_up/sub/follow_SPOTlight_vignette/tissue_lowres_image.png",
                              cell_types_interest = "L6b",
                              pie_scale = 0.8)
```

```
## Warning in SPOTlight::spatial_scatterpie(se_obj = anterior, cell_types_all =
## cell_types_all, : Using slice anterior1
```



```
#composition of spots containing another cell-type
SPOTlight::spatial_scatterpie(se_obj = anterior,
                              cell_types_all = cell_types_all,
                              img_path = "D:/pankyung/intern_back_up/sub/follow_SPOTlight_vignette/tissue_lowres_image.png",
                              cell_types_interest = "VLMC",
                              pie_scale = 0.8)
```

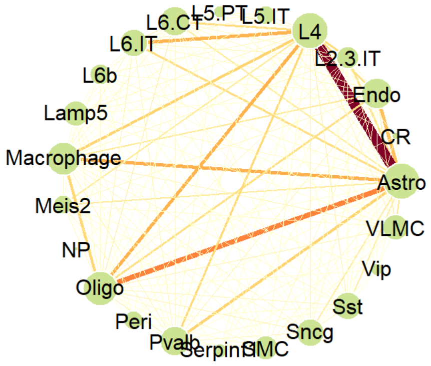
```
## Warning in SPOTlight::spatial_scatterpie(se_obj = anterior, cell_types_all =
## cell_types_all, : Using slice anterior1
```



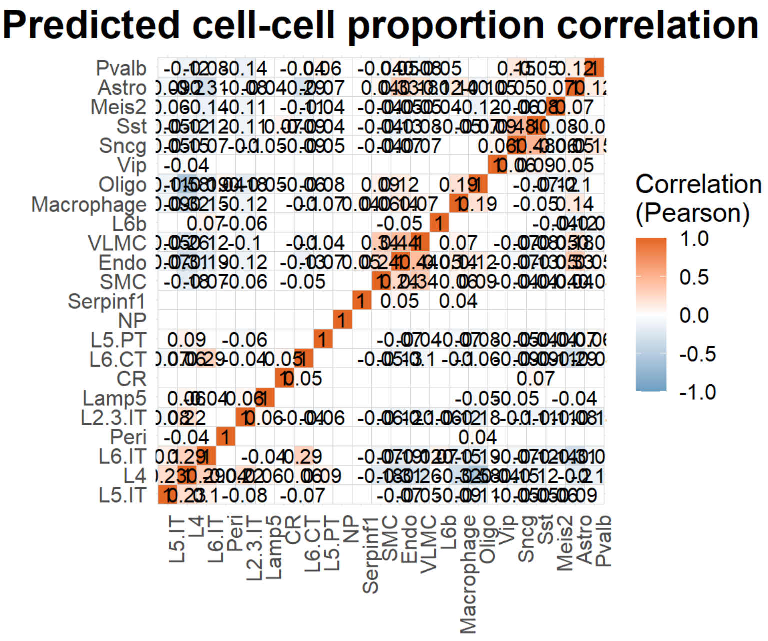
```
#Spatial interaction graph
#bring the function for interaction graph
graph_ntw <- SPOTlight::get_spatial_interaction_graph(decon_mtrx = decon_mtrx[, cell_types_all])

#graph drawing protocol
deg <- degree(graph_ntw, mode="all")
# Get color palette for difusion
edge_importance <- E(graph_ntw)$importance
# Select a continuous palette
qual_col_pals <- brewer.pal.info[brewer.pal.info$category == 'seq',]
# Create a color palette
getPalette <- colorRampPalette(brewer.pal(9, "YlOrRd"))
# Get how many values we need
grad_edge <- seq(0, max(edge_importance), 0.1)
# Generate extended gradient palette dataframe
graph_col_df <- data.frame(value = as.character(grad_edge),
                           color = getPalette(length(grad_edge)),
                           stringsAsFactors = FALSE)
# Assign color to each edge
color_edge <- data.frame(value = as.character(round(edge_importance, 1)), stringsAsFactors = FALSE) %>%
  dplyr::left_join(graph_col_df, by = "value") %>%
  dplyr::pull(color)
# Open a pdf file
plot(graph_ntw,
     # Size of the edge
     edge.width = edge_importance,
     edge.color = color_edge,
     # Size of the buble
     vertex.size = deg,
     vertex.color = "#cde394",
     vertex.frame.color = "white",
     vertex.label.color = "black",
     vertex.label.family = "Ubuntu", # Font family of the label (e.g.??Times??, ??Helvetica??)
     layout = layout.circle)
```

```
## Warning in text.default(x, y, labels = labels, col = label.color, family =
## label.family, : font family not found in Windows font database
```



```
#compute the correlation
#Remove cell types not predicted to be on the tissue
decon_mtrx_sub <- decon_mtrx[, cell_types_all]
decon_mtrx_sub <- decon_mtrx_sub[, colSums(decon_mtrx_sub) > 0]
# Compute correlation
decon_cor <- cor(decon_mtrx_sub)
# Compute correlation P-value
p.mat <- corrplot::cor.mtest(mat = decon_mtrx_sub, conf.level = 0.95)
# Visualize
#install.packages('ggcorrplot')
ggcorrplot::ggcorrplot(
  corr = decon_cor,
  p.mat = p.mat[[1]],
  hc.order = TRUE,
  type = "full",
  insig = "blank",
  lab = TRUE,
  outline.col = "lightgrey",
  method = "square",
  # colors = c("#4477AA", "white", "#BB4444"))
  colors = c("#6D9EC1", "white", "#E46726"),
  title = "Predicted cell-cell proportion correlation",
  legend.title = "Correlation₩n(Pearson)") +
  ggplot2::theme(
    plot.title = ggplot2::element_text(size = 22, hjust = 0.5, face = "bold"),
     legend.text = ggplot2::element_text(size = 12),
     legend.title = ggplot2::element_text(size = 15),
    axis.text.x = ggplot2::element_text(angle = 90),
    axis.text = ggplot2::element_text(size = 18, vjust = 0.5))
```


Predicted cell-cell proportion correlation
Pvalb
Astro
Meis2
Sst
Sncg
Vip
Oligo
Macrophage
L6b
VLMC
Endo
SMC
Serpinf1
NP
L5.PT
L6.CT
CR
Lamp5
L2.3.IT
Peri
L6.IT
L4
L5.IT
Correlation
(Pearson)