

pseudotime_8-4-22

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Open stroma dataset and convert to monocle object

```
####data.seurat <- readRDS("./stroma.reclustered.rds")
####data.seurat[["stroma"]] <- Idents(object = data.seurat)

##AF-TD-FAW-1 AF-TD-FAW-2 AF-TD-TED-2 AF-TTF-1
data.seurat <- readRDS("./stroma.reclustered.rds")
data.seurat[["celltype"]] <- Idents(object = data.seurat)
```

```
##data.seurat <- FindVariableFeatures(data.seurat)

#Extract data, phenotype data, and feature data from the SeuratObject
##data <- GetAssayData(object = data.seurat, slot = "counts", assay = "RNA")

##pd <- data.frame(data.seurat@meta.data)

##fData <- data.frame(gene_short_name = row.names(data), row.names = row.names(data))
##fd <- new('AnnotatedDataFrame', data = fData)

#Construct monocle cds
##data.monocle <- new_cell_data_set(data, gene_metadata = fData, cell_metadata = pd)

##data.monocle <- preprocess_cds(data.monocle, num_dim = 50)
##data.monocle <- align_cds(data.monocle, alignment_group = "orig.ident")

##data.monocle <- reduce_dimension(data.monocle)

##data.monocle <- cluster_cells(data.monocle)

# a helper function to identify the root principal points:
get_earliest_principal_node <- function(cds, time_bin="130-170"){
  cell_ids <- which(colData(cds)[, "celltype"] == time_bin)

  closest_vertex <- cds@principal_graph_aux[["UMAP"]]$pr_graph_cell_proj_closest_vertex
  closest_vertex <- as.matrix(closest_vertex[colnames(cds), ])
  root_pr_nodes <- igraph::V(principal_graph(cds)[["UMAP"]])$name[as.numeric(names(which.max(table(closest_vertex
[cell_ids,]))))]

  root_pr_nodes
}
```

Step 5: Learn a graph

```
data.monocle <- as.cell_data_set(data.seurat)
```

```
## Warning: Monocle 3 trajectories require cluster partitions, which Seurat does
## not calculate. Please run 'cluster_cells' on your cell_data_set object
```

```
data.monocle <- cluster_cells(data.monocle)
data.monocle <- learn_graph(data.monocle)
```

```
##
|
|
|
|=====| 100%
```

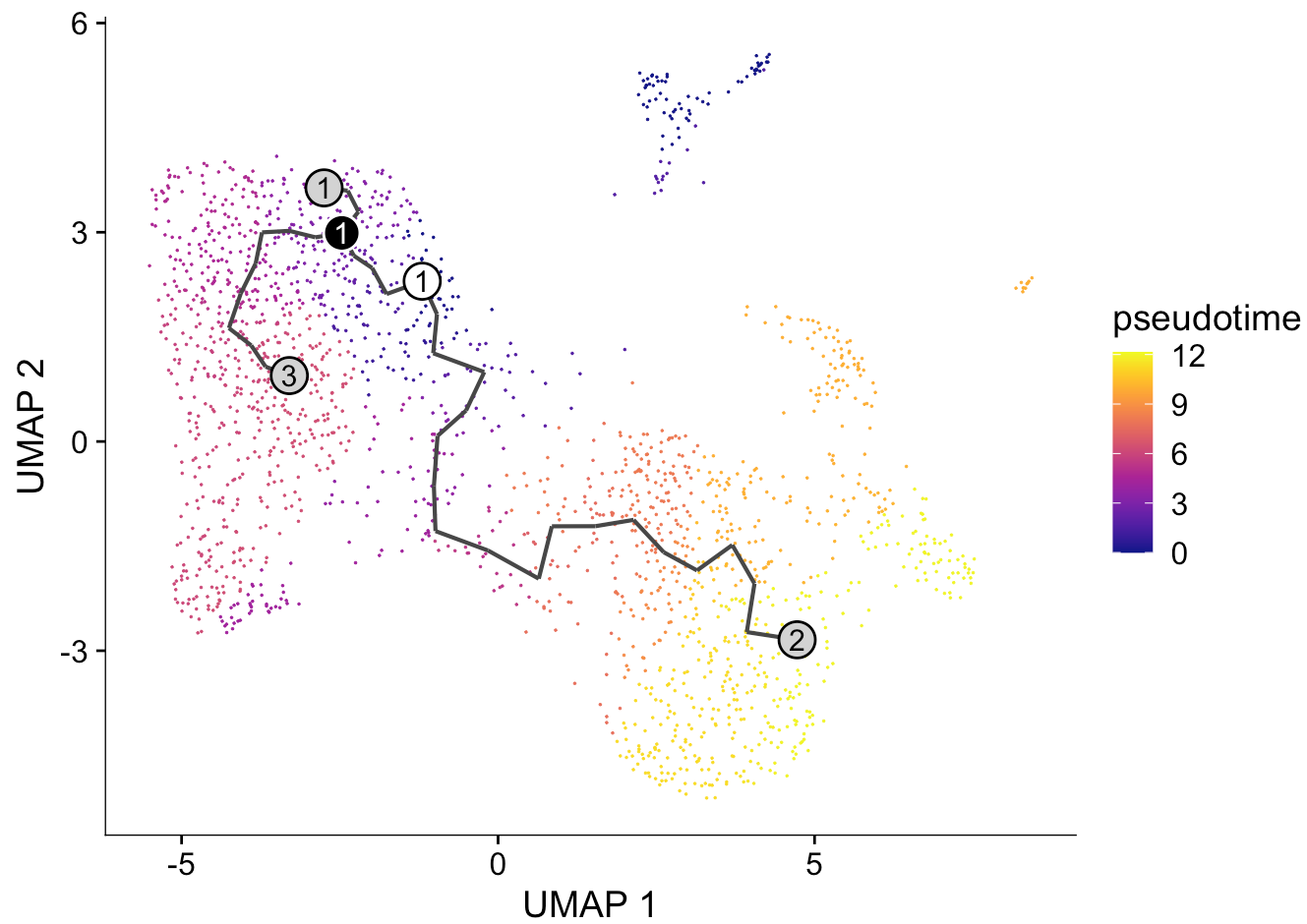
```
## Warning in igraph::graph.dfs(stree_ori, root = root_cell, neimode = "all", :
## Argument `neimode' is deprecated; use `mode' instead
```

```
## Step 6: Order cells -- change time_bin to preferred root node!!
data.monocle <- order_cells(data.monocle, root_pr_nodes=get_earliest_principal_node(data.monocle, time_bin = "Str
oma_1"))

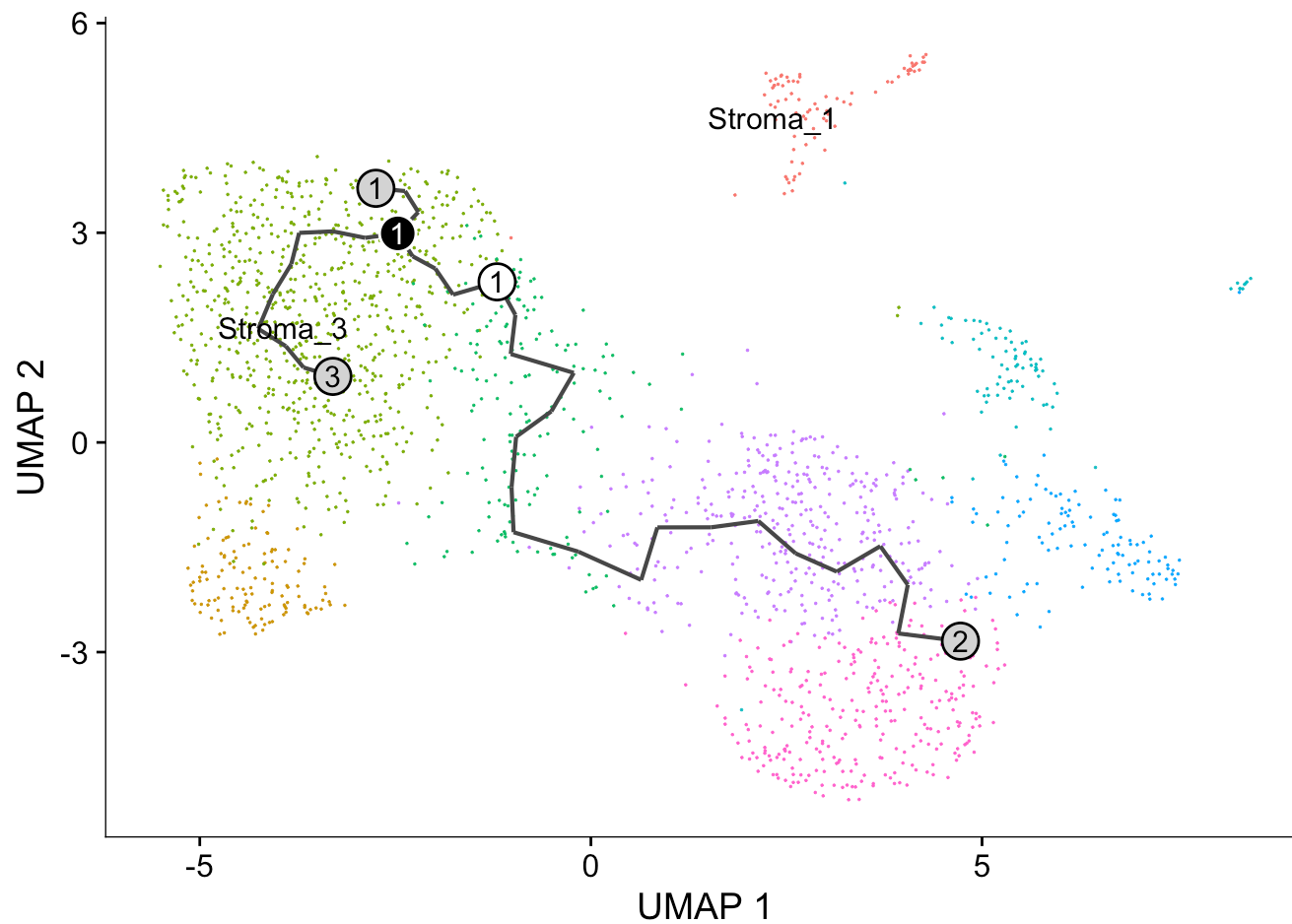
plot_cells(data.monocle, color_cells_by = "pseudotime", graph_label_size = 4, group_label_size = 4)
```

```
## Warning: `select_()` was deprecated in dplyr 0.7.0.
## i Please use `select()` instead.
## i The deprecated feature was likely used in the monocle3 package.
## Please report the issue to the authors.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
## Cells aren't colored in a way that allows them to be grouped.
```



```
plot_cells(data.monocle, color_cells_by = "celltype", label_groups_by_cluster = T, graph_label_size = 4, group_label_size = 4)
```



```
##pr_deg_ids <- row.names(subset(test.res, q_value < 0.05))

##gene_module_df <- find_gene_modules(data.monocle[pr_deg_ids,], resolution=c(10^seq(-6,-1)))

##ell_group_df <- tibble::tibble(cell=row.names(colData(data.monocle)),
##                               cell_group=colData(data.monocle)$celltype)
##agg_mat <- aggregate_gene_expression(data.monocle, gene_module_df, cell_group_df)
##row.names(agg_mat) <- stringr::str_c("Module ", row.names(agg_mat))
##pheatmap::pheatmap(agg_mat,
##                    scale="column", clustering_method="ward.D2")
```

```
sessionInfo()
```

```
## R version 4.1.2 (2021-11-01)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4      stats      graphics  grDevices  utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] data.table_1.14.8      gridExtra_2.3
## [3] ggplot2_3.4.3          cowplot_1.1.1
## [5] sctransform_0.3.5      gdata_2.19.0
## [7] dplyr_1.1.2            monocle3_1.0.0
## [9] SingleCellExperiment_1.16.0 SummarizedExperiment_1.24.0
## [11] GenomicRanges_1.46.1   GenomeInfoDb_1.30.1
## [13] IRanges_2.28.0         S4Vectors_0.32.4
## [15] MatrixGenerics_1.6.0   matrixStats_0.63.0
## [17] Biobase_2.54.0         BiocGenerics_0.40.0
## [19] SeuratWrappers_0.3.0   SeuratObject_4.1.3
## [21] Seurat_4.3.0
##
## loaded via a namespace (and not attached):
## [1] plyr_1.8.8            igraph_1.4.2          lazyeval_0.2.2
## [4] sp_1.6-0              splines_4.1.2         listenv_0.9.0
## [7] scattermore_1.1       digest_0.6.33         htmltools_0.5.6
## [10] viridis_0.6.3         fansi_1.0.4           magrittr_2.0.3
## [13] tensor_1.5            cluster_2.1.4         ROCR_1.0-11
## [16] remotes_2.4.2         globals_0.16.2        R.utils_2.12.2
## [19] spatstat.sparse_3.0-1 colorspace_2.1-0      ggrepel_0.9.3
## [22] xfun_0.40             crayon_1.5.2          RCurl_1.98-1.12
## [25] jsonlite_1.8.7        progressr_0.13.0      spatstat.data_3.0-1
```

## [28] survival_3.5-5	zoo_1.8-12	glue_1.6.2
## [31] polyclip_1.10-4	gtable_0.3.3	zlibbioc_1.40.0
## [34] XVector_0.34.0	leiden_0.4.3	DelayedArray_0.20.0
## [37] future.apply_1.10.0	abind_1.4-5	scales_1.2.1
## [40] DBI_1.1.3	spatstat.random_3.1-5	miniUI_0.1.1.1
## [43] Rcpp_1.0.11	viridisLite_0.4.2	xtable_1.8-4
## [46] reticulate_1.28	proxy_0.4-27	rsvd_1.0.5
## [49] htmlwidgets_1.6.2	httr_1.4.6	RColorBrewer_1.1-3
## [52] ellipsis_0.3.2	ica_1.0-3	farver_2.1.1
## [55] pkgconfig_2.0.3	R.methodsS3_1.8.2	sass_0.4.7
## [58] uwot_0.1.14	deldir_1.0-9	utf8_1.2.3
## [61] labeling_0.4.2	tidyselect_1.2.0	rlang_1.1.1
## [64] reshape2_1.4.4	later_1.3.1	munsell_0.5.0
## [67] tools_4.1.2	cachem_1.0.8	cli_3.6.1
## [70] generics_0.1.3	ggridges_0.5.4	evaluate_0.21
## [73] stringr_1.5.0	fastmap_1.1.1	yaml_2.3.7
## [76] goftest_1.2-3	knitr_1.43	fitdistrplus_1.1-11
## [79] purrr_1.0.2	RANN_2.6.1	pbapply_1.7-0
## [82] future_1.32.0	nlme_3.1-162	mime_0.12
## [85] R.oo_1.25.0	compiler_4.1.2	rstudioapi_0.14
## [88] plotly_4.10.2	png_0.1-8	spatstat.utils_3.0-3
## [91] tibble_3.2.1	bslib_0.5.1	stringi_1.7.12
## [94] highr_0.10	lattice_0.21-8	Matrix_1.5-4.1
## [97] vctrs_0.6.3	pillar_1.9.0	lifecycle_1.0.3
## [100] BiocManager_1.30.20	spatstat.geom_3.2-1	lmtest_0.9-40
## [103] jquerylib_0.1.4	RcppAnnoy_0.0.20	bitops_1.0-7
## [106] irlba_2.3.5.1	httpuv_1.6.11	patchwork_1.1.2
## [109] R6_2.5.1	promises_1.2.1	KernSmooth_2.23-21
## [112] parallelly_1.35.0	codetools_0.2-19	assertthat_0.2.1
## [115] gtools_3.9.4	MASS_7.3-60	leidenbase_0.1.18
## [118] withr_2.5.0	GenomeInfoDbData_1.2.7	parallel_4.1.2
## [121] grid_4.1.2	tidyr_1.3.0	rmarkdown_2.24
## [124] Rtsne_0.16	spatstat.explore_3.2-1	shiny_1.7.4