

Integration 10x Hive

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Load Packages

```
suppressMessages(library(dplyr))
suppressMessages(library(Seurat))
suppressMessages(library(patchwork))
suppressMessages(library(ggplot2))
```

Loading Hive Data

```
Cell_HiveAnnotate <- readRDS("../Hive-2-Annotation_cells/BAL_hive.annotated_noDB.rds")

col <- c("#E2B80C", "#998025", "#AE8052", "#e6641e",
        "red", "#0F8140", "#4F2569", "#ABD6E4", "#1CB7D5",
        "#6B7BBA", "#FFC0CB", "#BDBCBC", "#DF15AE")
DimPlot(Cell_HiveAnnotate, group.by = "Annotation",
        cols = col)
```

Loading 10X data

```
Cell_10xAnnotate <- readRDS("../10x-2-Annotation_cells/BAL_10x.annotated_noDB.rds")

col <- c("#E2B80C", "#998025", "#AE8052", "#0F8140",
        "#4F2569", "#ABD6E4", "#1CB7D5", "#6B7BBA", "#2D368B",
        "#DF15AE", "#BDBCBC")

DimPlot(Cell_10xAnnotate, group.by = "Annotation",
```

```
cols = col) + theme(legend.text = element_text(size = 15)) +
labs(title = NULL)
```

```
Cell_10xAnnotate$Annotation10x <- Cell_10xAnnotate$Annotation
Cell_HiveAnnotate$AnnotationHive <- Cell_HiveAnnotate$Annotation
```

Integration by CCA

```
list_sample <- c(Cell_10xAnnotate, Cell_HiveAnnotate)

list_sample <- lapply(list_sample, function(x) {
  x <- FindVariableFeatures(x, selection.method = "vst",
    nfeatures = 2000)
})

features <- SelectIntegrationFeatures(list_sample)

list_sample <- lapply(list_sample, function(x) {
  x <- ScaleData(x, features = features)
  x <- RunPCA(x, features = features)
})

BAL.anchors <- FindIntegrationAnchors(object.list = list_sample,
  anchor.features = features, reduction = "rpca")
BAL_integrated <- IntegrateData(anchorset = BAL.anchors)
```

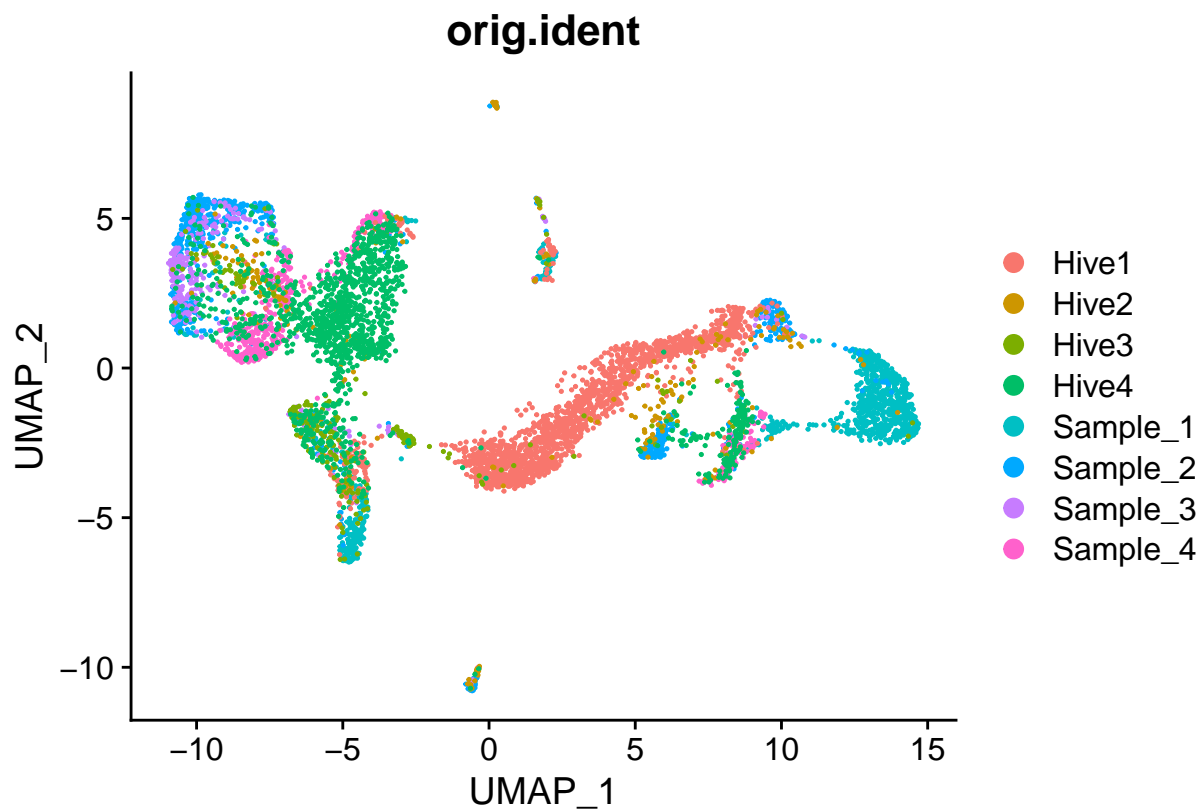
Perform an integrated analysis

```
DefaultAssay(BAL_integrated) <- "integrated"

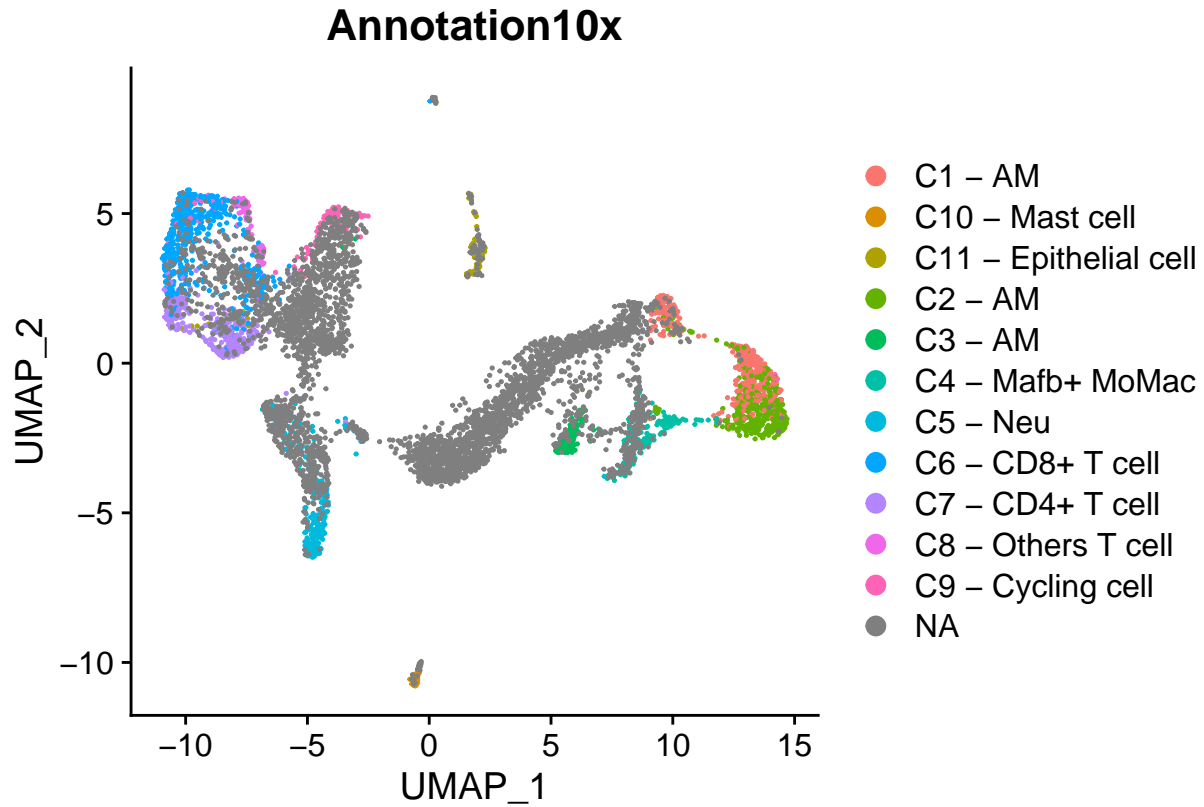
# Run the standard workflow for visualization and
# clustering
BAL_integrated <- ScaleData(BAL_integrated, verbose = FALSE)
BAL_integrated <- RunPCA(BAL_integrated, npcs = 30,
  verbose = FALSE)
BAL_integrated <- RunUMAP(BAL_integrated, reduction = "pca",
  dims = 1:15)
BAL_integrated <- FindNeighbors(BAL_integrated, reduction = "pca",
  dims = 1:15)
BAL_integrated <- FindClusters(BAL_integrated, resolution = 0.5)

BAL_integrated <- readRDS("BAL_integrated.rds")

DimPlot(BAL_integrated, reduction = "umap", group.by = "orig.ident")
```

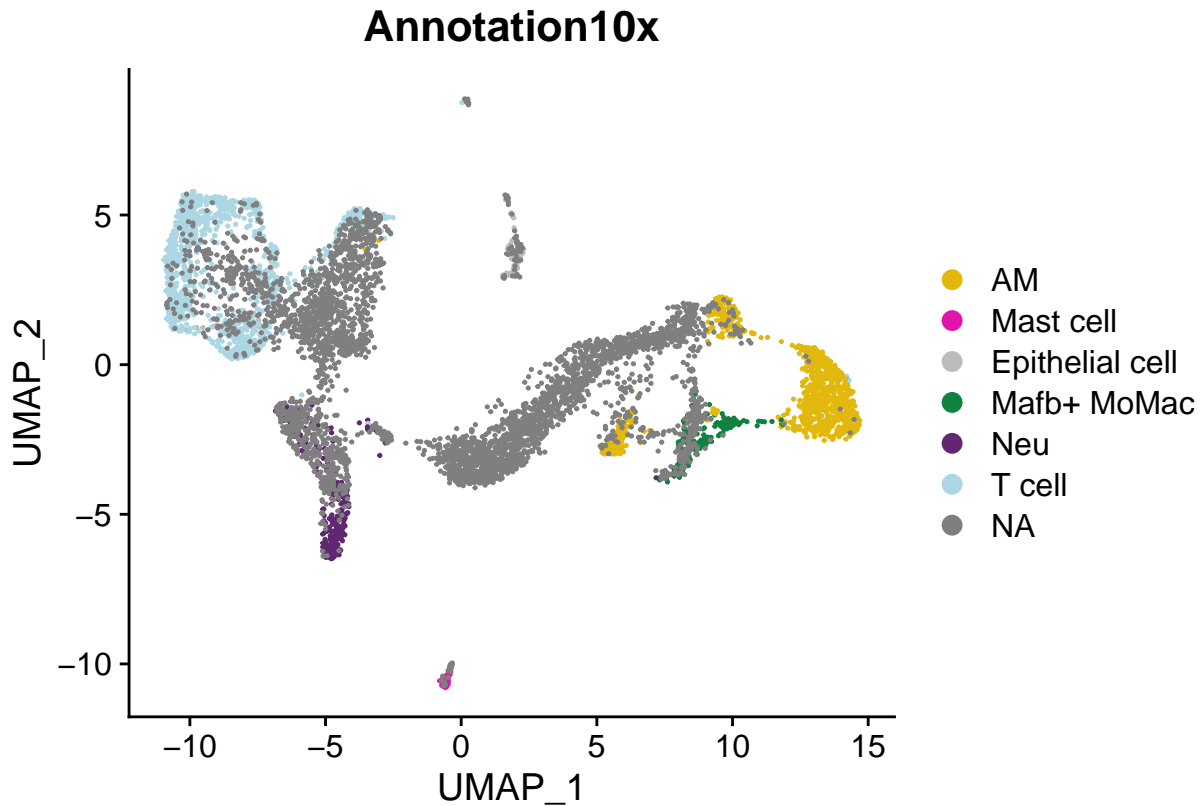


```
DimPlot(BAL_integrated, reduction = "umap", group.by = "Annotation10x")
```



```
# DimPlot(BAL_integrated, reduction = 'umap',
# group.by = 'Annotation10x')
BAL_integrated$Annotation10x <- as.factor(BAL_integrated$Annotation10x)
levels(BAL_integrated$Annotation10x) <- c("AM", "Mast cell",
      "Epithelial cell", "AM", "AM", "Mafb+ MoMac", "Neu",
      "T cell", "T cell", "T cell", "T cell")

colors <- c("#E2B80C", "#DF15AE", "#BDBCBC", "#0F8140",
      "#612773", "#ABD6E4")
DimPlot(BAL_integrated, reduction = "umap", group.by = "Annotation10x",
      cols = colors)
```



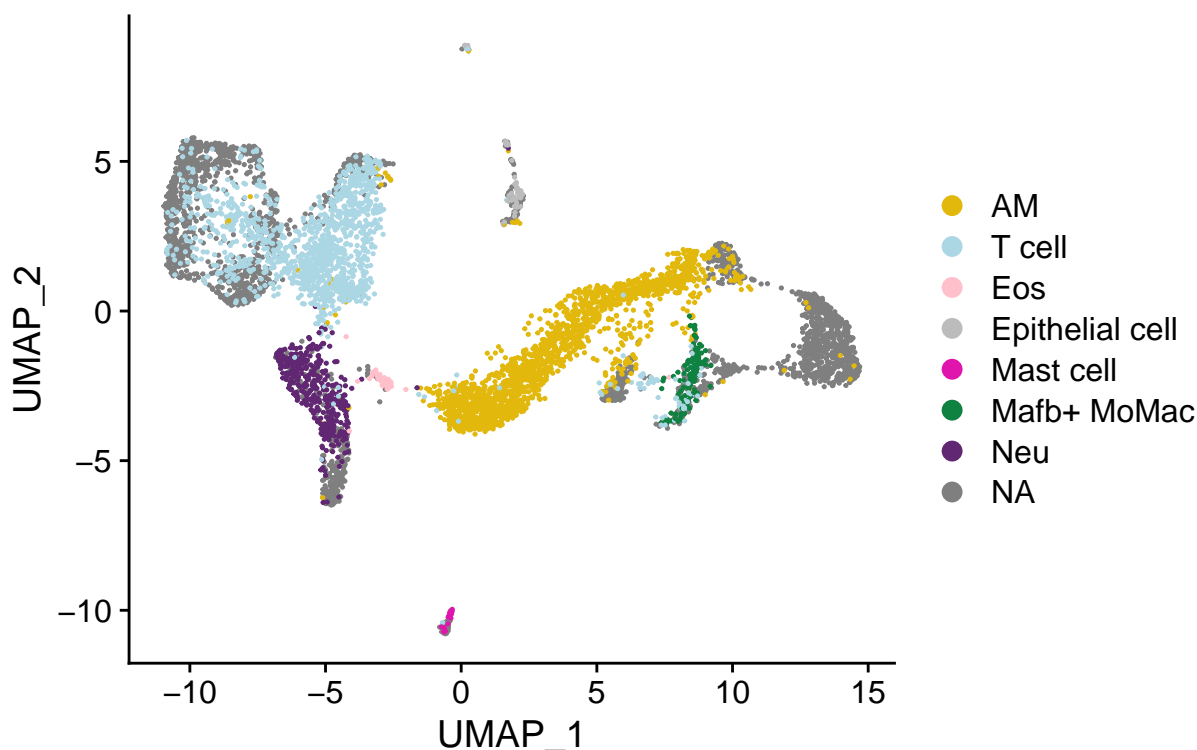
```
# ggsave('umap_Annotation10x.pdf', width = 8,
# height = 4)

# DimPlot(BAL_integrated, reduction = 'umap',
# group.by = 'AnnotationHive')
BAL_integrated$AnnotationHive <- as.factor(BAL_integrated$AnnotationHive)
levels(BAL_integrated$AnnotationHive) <- c("AM", "T cell",
      "Eos", "Epithelial cell", "Mast cell", "AM", "AM",
      "AM", "AM", "Mafb+ MoMac", "Neu", "T cell", "T cell")

colors <- c("#E2B80C", "#ABD6E4", "#FFC0CB", "#BDBCBC",
      "#DF15AE", "#0F8140", "#612773")

DimPlot(BAL_integrated, reduction = "umap", group.by = "AnnotationHive",
      cols = colors)
```

AnnotationHive



```
# ggsave('umap_AnnotationHive.pdf', width = 8,  
# height = 4)
```

```
sessionInfo()
```

```
## R version 4.3.3 (2024-02-29)  
## Platform: x86_64-pc-linux-gnu (64-bit)  
## Running under: Ubuntu 22.04.4 LTS  
##  
## Matrix products: default  
## BLAS: /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3  
## LAPACK: /usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblas-r0.3.20.so; LAPACK version 3.10.0  
##  
## locale:  
## [1] LC_CTYPE=en_US.UTF-8 LC_NUMERIC=C  
## [3] LC_TIME=fr_BE.UTF-8 LC_COLLATE=en_US.UTF-8  
## [5] LC_MONETARY=fr_BE.UTF-8 LC_MESSAGES=en_US.UTF-8  
## [7] LC_PAPER=fr_BE.UTF-8 LC_NAME=C  
## [9] LC_ADDRESS=C LC_TELEPHONE=C  
## [11] LC_MEASUREMENT=fr_BE.UTF-8 LC_IDENTIFICATION=C  
##  
## time zone: Europe/Brussels  
## tzcode source: system (glibc)  
##  
## attached base packages:  
## [1] stats graphics grDevices utils datasets methods base  
##  
## other attached packages:  
## [1] ggplot2_3.4.2 patchwork_1.1.2 SeuratObject_4.1.3 Seurat_4.3.0
```

```

## [5] dplyr_1.1.2
##
## loaded via a namespace (and not attached):
##   [1] deldir_1.0-9           pbapply_1.7-2          gridExtra_2.3
##   [4] formatR_1.14          rlang_1.1.1            magrittr_2.0.3
##   [7] RcppAnnoy_0.0.21      spatstat.geom_3.2-4    matrixStats_1.0.0
##  [10] gggridges_0.5.4       compiler_4.3.3         png_0.1-8
##  [13] vctrs_0.6.3           reshape2_1.4.4         stringr_1.5.0
##  [16] pkgconfig_2.0.3       fastmap_1.1.1          ellipsis_0.3.2
##  [19] labeling_0.4.2        utf8_1.2.3             promises_1.2.0.1
##  [22] rmarkdown_2.23        purrr_1.0.1            xfun_0.39
##  [25] jsonlite_1.8.7        goftest_1.2-3          highr_0.10
##  [28] later_1.3.1           spatstat.utils_3.0-3   irlba_2.3.5.1
##  [31] parallel_4.3.3        cluster_2.1.6          R6_2.5.1
##  [34] ica_1.0-3             spatstat.data_3.0-1    stringi_1.7.12
##  [37] RColorBrewer_1.1-3    reticulate_1.30        parallelly_1.36.0
##  [40] lmtest_0.9-40         scattermore_1.2        Rcpp_1.0.11
##  [43] knitr_1.43            tensor_1.5             future.apply_1.11.0
##  [46] zoo_1.8-12           sctransform_0.3.5     httpuv_1.6.11
##  [49] Matrix_1.6-1          splines_4.3.3          igraph_1.5.0.1
##  [52] tidyselect_1.2.0      abind_1.4-5            rstudioapi_0.14
##  [55] yaml_2.3.7            spatstat.random_3.1-5  codetools_0.2-19
##  [58] miniUI_0.1.1.1        spatstat.explore_3.2-1 listenv_0.9.0
##  [61] lattice_0.22-5        tibble_3.2.1           plyr_1.8.8
##  [64] withr_2.5.0           shiny_1.7.4.1          ROCR_1.0-11
##  [67] evaluate_0.21         Rtsne_0.16            future_1.33.0
##  [70] survival_3.5-8        polyclip_1.10-4        fitdistrplus_1.1-11
##  [73] pillar_1.9.0          KernSmooth_2.23-22     plotly_4.10.2
##  [76] generics_0.1.3        sp_2.0-0              munsell_0.5.0
##  [79] scales_1.2.1          globals_0.16.2         xtable_1.8-4
##  [82] glue_1.6.2            lazyeval_0.2.2         tools_4.3.3
##  [85] data.table_1.14.8     RANN_2.6.1            dotCall64_1.0-2
##  [88] leiden_0.4.3          cowplot_1.1.1          grid_4.3.3
##  [91] tidyr_1.3.0           colorspace_2.1-0       nlme_3.1-164
##  [94] cli_3.6.1            spatstat.sparse_3.0-2  spam_2.9-1
##  [97] fansi_1.0.4           viridisLite_0.4.2      uwot_0.1.16
## [100] gtable_0.3.3          digest_0.6.33          progressr_0.13.0
## [103] ggrepel_0.9.3         farver_2.1.1           htmlwidgets_1.6.2
## [106] htmltools_0.5.5       lifecycle_1.0.3        httr_1.4.6
## [109] mime_0.12             MASS_7.3-60.0.1

```