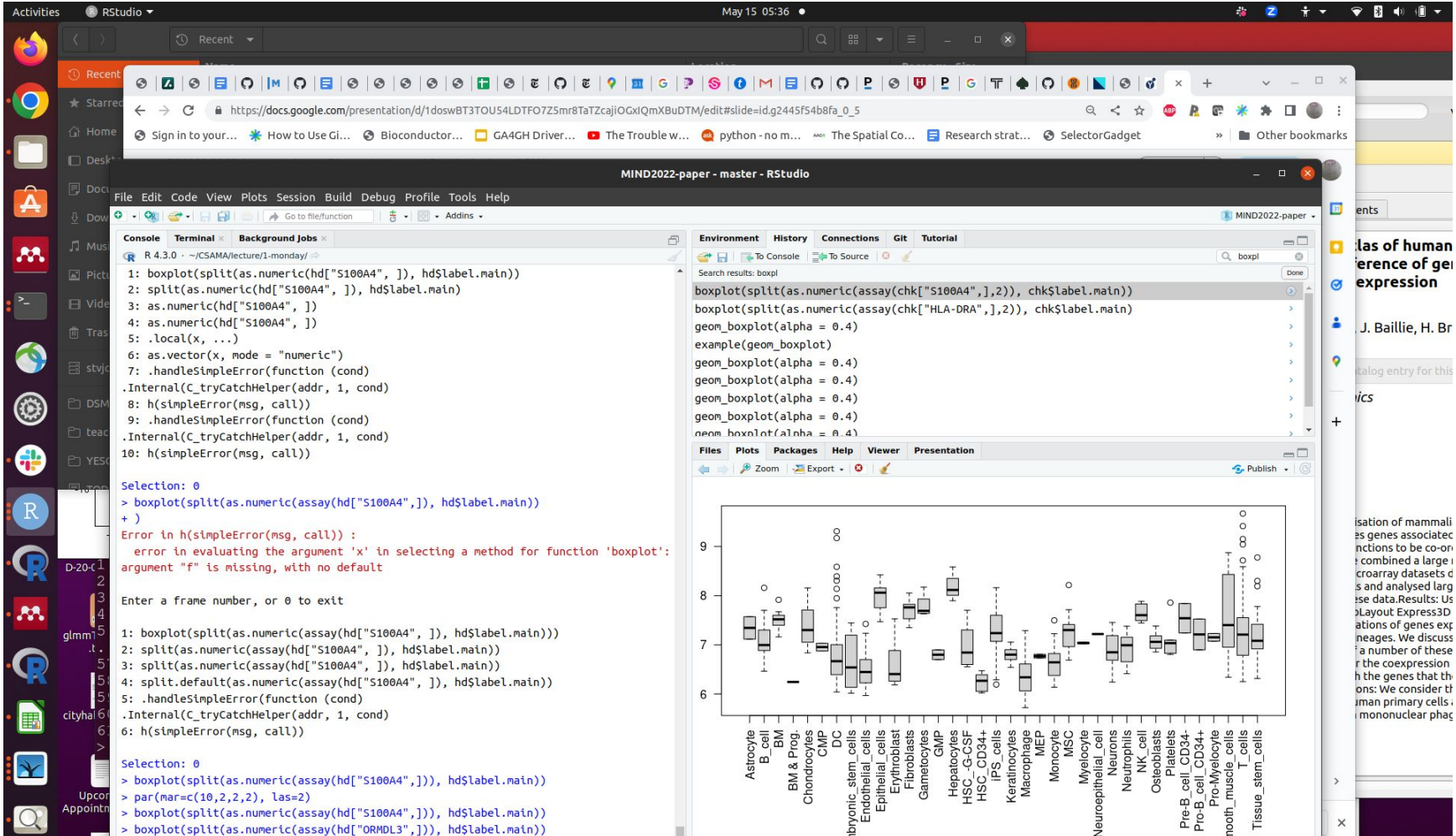
CSAMA 2023, Lecture 1a: Introduction to Bioconductor and R with single-cell RNA-seq

Vincent Carey

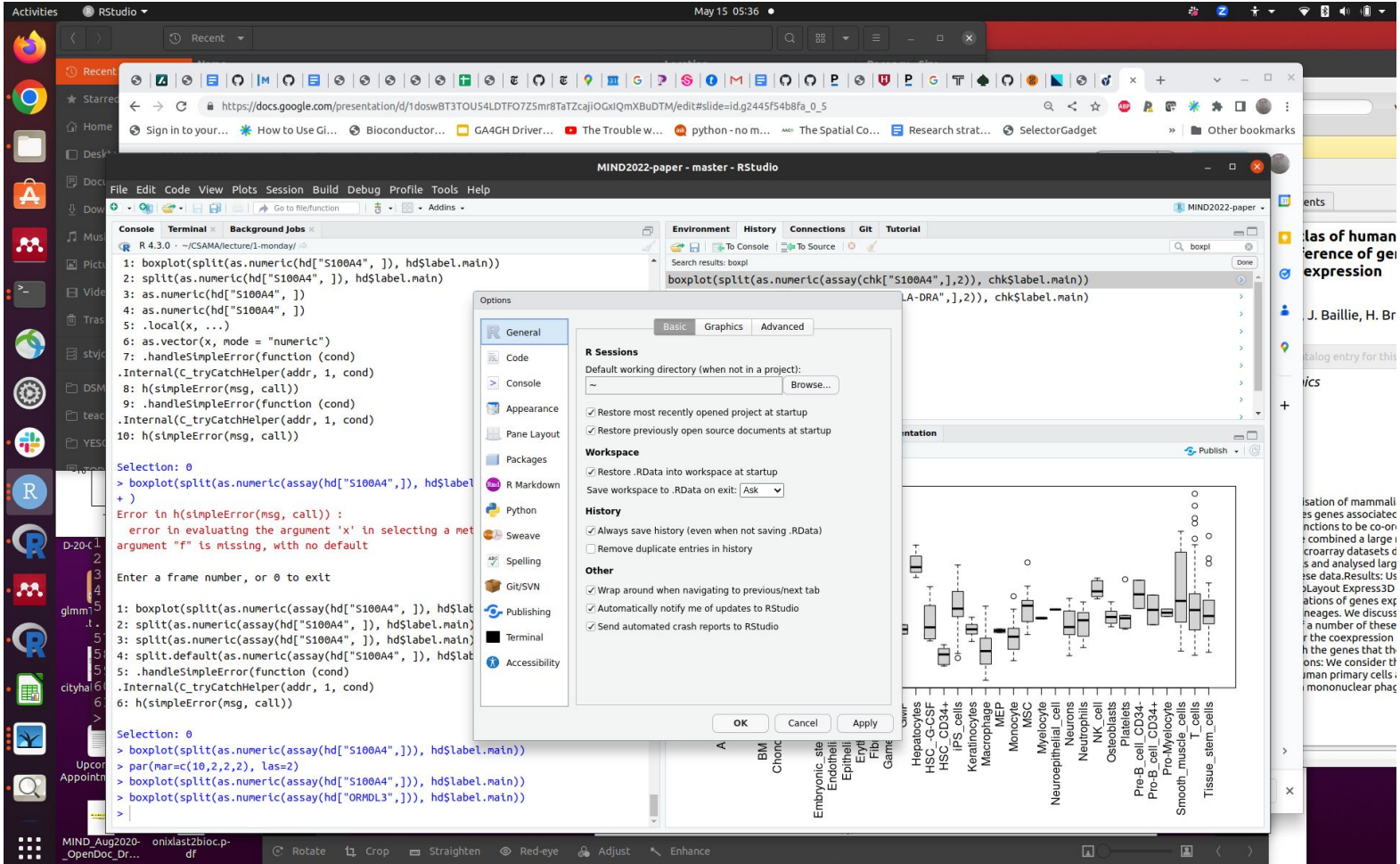
Road map

- RStudio in brief
  - panes, global settings
  - help, plots, zoom
  - history and debugging
- R in brief: vectors, lists, functions, packages
- Collecting and annotating experimental data: SummarizedExperiment
- HumanPrimaryCellAtlas
- TENxPBMCData in SingleCellExperiment
- Annotating cells with SingleR, exploring the outcome

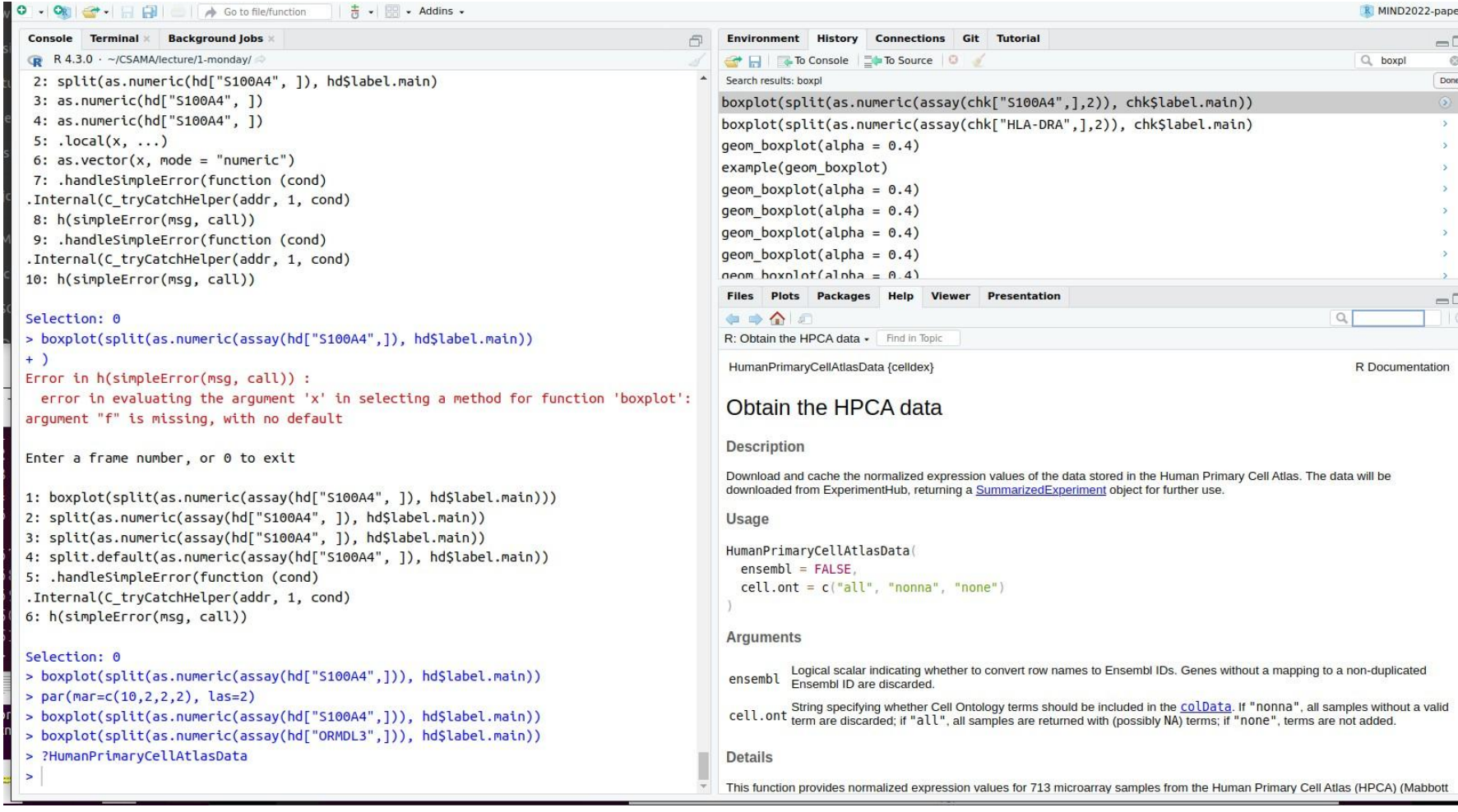
Three pane view: Console, environment/history/git, plots



Global options under "Tools"



Getting help



R in brief

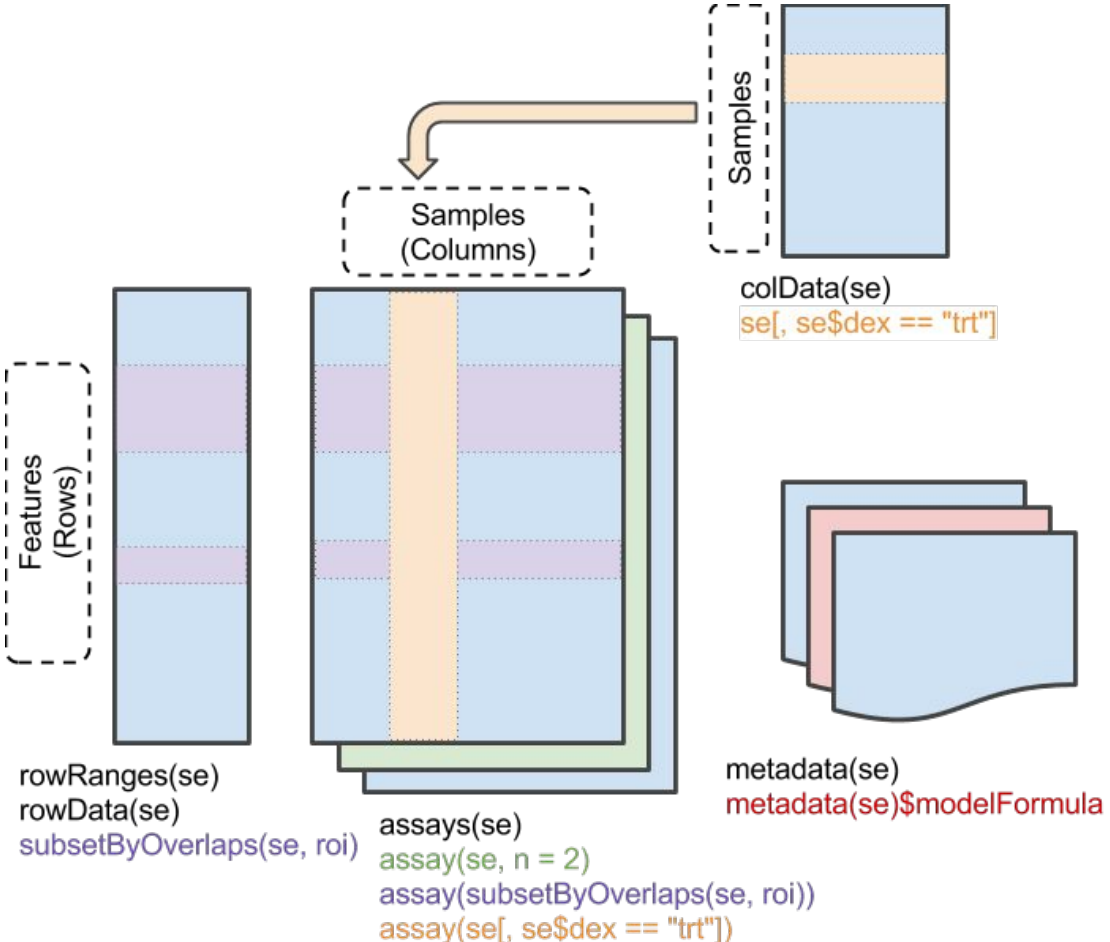
- Console: "Read-Eval-Print" loop – always asking for input
- Make your own data: `x <- c(2.3, 4, 7, 3.2, 5.2, 12)`
  - `c` is the "combine" operator that produces vectors; `"#" used for commenting`
- logic: `x[3] == 7`
- matrix, array: structure for homogeneous (typically numeric) data
  - `y <- matrix(x, ncol=2) # comment: y = matrix(x, ncol=2) also works`
  - `y[3,1]` # element
  - `y[,1]` # column; explore dimnames so that `y["c", "B"] == y[3,1]`
- `data.frame`: structure for heterogeneous tabular data
  - rows are "records", columns are "attributes"
- Import data: `read.csv, read.delim, readLines`
- Call functions: `median(x); table(cut(x,3))  # exercise: explain`
- Acquire families of functions: `library(MASS); help(package="MASS")`
- `search(); installed.packages(); install.packages()`

Bioconductor in brief: SummarizedExperiment

Used to organize information on multiple samples.

If `SE` is a SummarizedExperiment `g` is a vector of feature identifiers and `s` is a vector of sample identifiers then `SE[g,s]` is a new SummarizedExperiment restricted to genes in g and samples in s; quantifications via `assay()`

If `cv` is a variable in `colData(SE)`, then `SE$cv` is a vector with its values



An SE based on a paper

Mabbott *et al. BMC Genomics* 2013, **14**:632
http://www.biomedcentral.com/1471-2164/14/632

BMC Genomics

RESEARCH ARTICLE Open Access

An expression atlas of human primary cells: inference of gene function from coexpression networks

Neil A Mabbott*†, J Kenneth Baillie, Helen Brown, Tom C Freeman*† and David A Hume*†

Abstract

Background: The specialisation of mammalian cells in time and space requires genes associated with specific pathways and functions to be co-ordinately expressed. Here we have combined a large number of publically available microarray datasets derived from human primary cells and analysed large correlation graphs of these data.

Results: Using the network analysis tool BioLayout *Express*^{3D} we identify robust co-associations of genes expressed in a wide variety of cell lineages. We discuss the biological significance of a number of these associations, in particular the coexpression of key transcription factors with the genes that they are likely to control.

Conclusions: We consider the regulation of genes in human primary cells and specifically in the human mononuclear phagocyte system. Of particular note is the fact that these data do not support the identity of putative markers of antigen-presenting dendritic cells, nor classification of M1 and M2 activation states, a current subject of debate within immunological field. We have provided this data resource on the BioGPS web site (http://biogps.org/dataset/2429/primary-cell-atlas/) and on macrophages.com (http://www.macrophages.com/hu-cell-atlas).

Keywords: Clustering, Meta-analysis, Human, Primary cells, Dendritic cell, Macrophage, Microarray, Transcriptomics

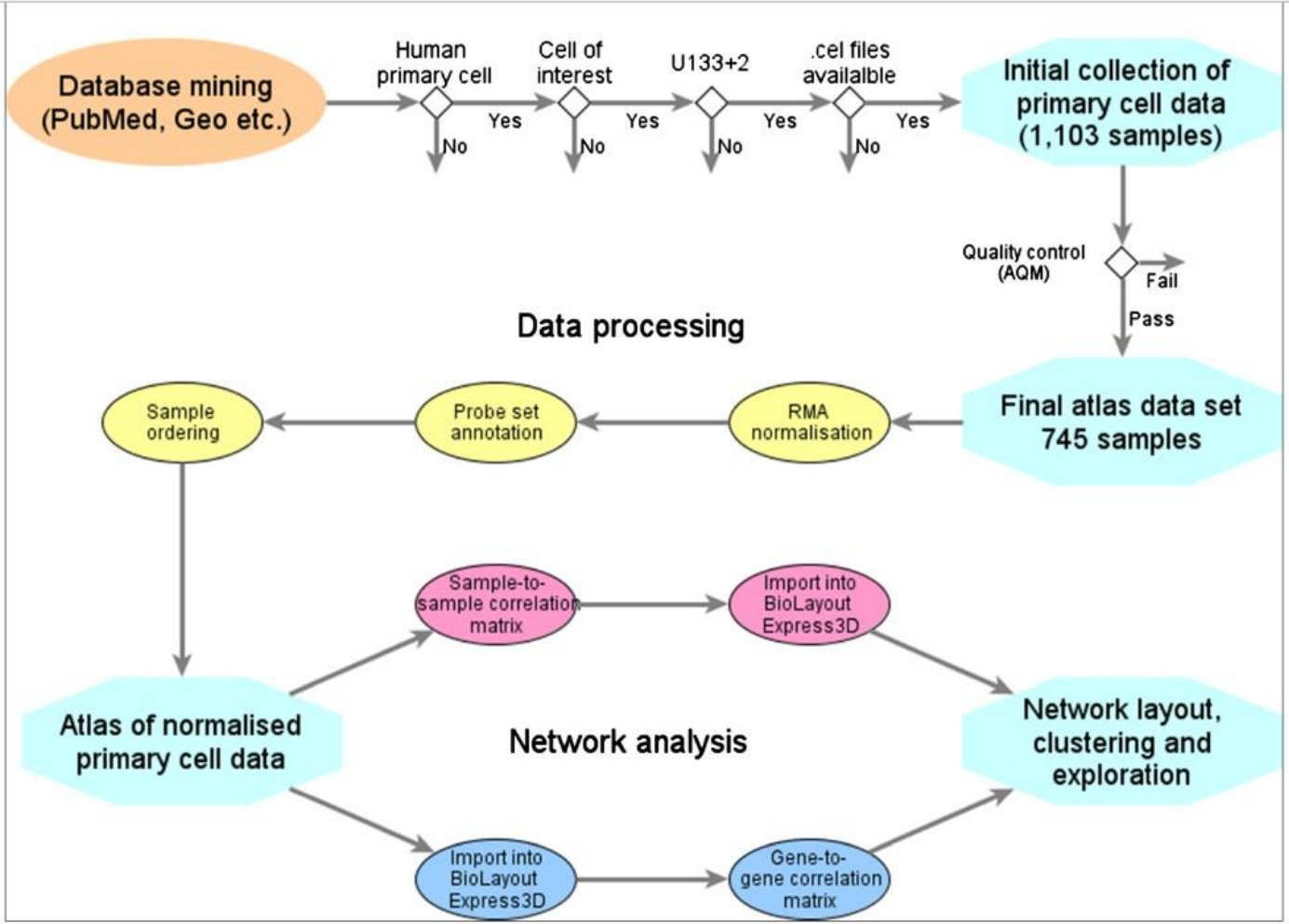


Figure 1 Data analysis workflow. Data analysis pipeline, from the selection of microarray data, through to normalisation, annotation and network analysis.

File Edit Code View Plots Session Build Debug Profile Tools Help

Three steps to data:

- library
- help or ls [optional]
- call a function and assign the result

```
> library(celldex)
0/0 packages newly attached/loaded, see sessionInfo() for details.
> ?HumanPrimaryCellAtlasData
> hd = HumanPrimaryCellAtlasData()
snapshotDate(): 2023-04-24
see ?celldex and browseVignettes('celldex') for documentation
loading from cache
see ?celldex and browseVignettes('celldex') for documentation
loading from cache
> hd
class: SummarizedExperiment
dim: 19363 713
metadata(0):
assays(1): logcounts
rownames(19363): A1BG A1BG-AS1 ... ZZEF1 ZZZ3
rowData names(0):
colnames(713): GSM112490 GSM112491 ... GSM92233 GSM92234
colData names(3): label.main label.fine label.ont
>
```

Search results: boxpl

```
boxplot(split(as.numeric(assay(chk["S100A4",],2)), chk$label.main))
boxplot(split(as.numeric(assay(chk["HLA-DRA",],2)), chk$label.main)
geom_boxplot(alpha = 0.4)
example(geom_boxplot)
geom_boxplot(alpha = 0.4)
geom_boxplot(alpha = 0.4)
geom_boxplot(alpha = 0.4)
```

R: Obtain the HPCA data

HumanPrimaryCellAtlasData {celldex} R Documentation

Obtain the HPCA data

Description

Download and cache the normalized expression values of the data stored in the Human Primary Cell Atlas. The data will be downloaded from ExperimentHub, returning a SummarizedExperiment object for further use.

Usage

```
HumanPrimaryCellAtlasData(
  ensembl = FALSE,
  cell.ont = c("all", "nonna", "none")
)
```

Arguments

Console | Terminal | Background Jobs

R 4.3.0 · ~/CSAMA/lecture/1-monday/

```
> assay(hd[c("S100A4", "LYZ"), 1:4])
       GSM112490 GSM112491 GSM112540 GSM112541
S100A4   13.0099   12.9919   12.9057   11.9467
LYZ      11.6434   11.4303   12.9031   11.5178
> table(hd$label.main)
```

Informal notations …

```
          Astrocyte              B_cell                  BM
                  2                  26                   7
        BM & Prog.         Chondrocytes                 CMP
                  1                   8                   2
                 DC Embryonic_stem_cells    Endothelial_cells
                 88                  17                  64
   Epithelial_cells         Erythroblast         Fibroblasts
                 16                   8                  10
       Gametocytes                  GMP         Hepatocytes
                  5                   2                   3
        HSC_-G-CSF             HSC_CD34+             iPS_cells
                 10                   6                  42
      Keratinocytes           Macrophage                 MEP
                 25                  90                   2
          Monocyte                  MSC           Myelocyte
                 60                   9                   2
Neuroepithelial_cell             Neurons         Neutrophils
                  1                  16                  21
           NK_cell          Osteoblasts           Platelets
                  5                  15                   5
   Pre-B_cell_CD34-     Pro-B_cell_CD34+        Pro-Myelocyte
                  2                   2                   2
```

Environment | History | Connections | Git | Tutorial

To Console | To Source | boxpl

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Files | Plots | Packages | Help | Viewer | Presentation

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Usage

… consider label.ont

```
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)
```

Arguments

Logical scalar indicating whether to convert row names to Ensembl IDs. Genes

Paradigms of syntax: classic R and tidyverse

- data.frame x[,], x$
- R 4.3 introduces a "pipe" operator, |>
- tidyverse/dplyr: `x |> select(...) |> filter(...)`
- a matter of taste? efficiencies (human and mechanical)?
- `table(hd$label.main)` gave us the frequencies of different cell types in the SummarizedExperiment `hd`
- Consider how to get frequencies of *subtypes* of "Macrophage" in the primary cell atlas, found in `hd$label.fine`
    - What functions come to mind to help carry this out?

Console | Terminal | Background Jobs

R 4.3.0 · ~/CSAMA/lecture/1-monday/

```
> table(hd[,hd$label.main=="Macrophage"]$label.fine)

                                  Macrophage:Alveolar
                                                    4
               Macrophage:Alveolar:B._anthacis_spores
                                                    3
                         Macrophage:monocyte-derived
                                                   26
                    Macrophage:monocyte-derived:IFNa
                                                    9
               Macrophage:monocyte-derived:IL-4/cntrl
                                                    5
            Macrophage:monocyte-derived:IL-4/Dex/cntrl
                                                    5
             Macrophage:monocyte-derived:IL-4/Dex/TGFb
                                                   10
               Macrophage:monocyte-derived:IL-4/TGFb
                                                    5
                   Macrophage:monocyte-derived:M-CSF
                                                    2
               Macrophage:monocyte-derived:M-CSF/IFNg
                                                    2
Macrophage:monocyte-derived:M-CSF/IFNg/Pam3Cys
                                                    2
             Macrophage:monocyte-derived:M-CSF/Pam3Cys
                                                    2
               Macrophage:monocyte-derived:S._aureus
                                                   15
>
```

Environment | History | Connections | Git | Tutorial

To Console | To Source | boxpl

Search results: boxpl | Done

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R 4.3.0 · ~/CSAMA/lecture/1-monday/

```
            Macrophage:monocyte-derived:M-CSF
                                            2
       Macrophage:monocyte-derived:M-CSF/IFNg
                                            2
Macrophage:monocyte-derived:M-CSF/IFNg/Pam3Cys
                                            2
     Macrophage:monocyte-derived:M-CSF/Pam3Cys
                                            2
         Macrophage:monocyte-derived:S._aureus
                                           15
> as.data.frame(colData(hd)) |> filter(label.main=="Macrophage") |> group_by(label.fine) |> summ
arise(n=n())
# A tibble: 13 × 2
   label.fine                                n
   <chr>                                 <int>
 1 Macrophage:Alveolar                       4
 2 Macrophage:Alveolar:B._anthacis_spores    3
 3 Macrophage:monocyte-derived              26
 4 Macrophage:monocyte-derived:IFNa          9
 5 Macrophage:monocyte-derived:IL-4/Dex/TGFb 10
 6 Macrophage:monocyte-derived:IL-4/Dex/cntrl 5
 7 Macrophage:monocyte-derived:IL-4/TGFb     5
 8 Macrophage:monocyte-derived:IL-4/cntrl    5
 9 Macrophage:monocyte-derived:M-CSF         2
10 Macrophage:monocyte-derived:M-CSF/IFNg    2
11 Macrophage:monocyte-derived:M-CSF/IFNg/Pam3Cys 2
12 Macrophage:monocyte-derived:M-CSF/Pam3Cys 2
13 Macrophage:monocyte-derived:S._aureus    15
>
```

Conversion from DataFrame, use dplyr

Environment | History | Connections

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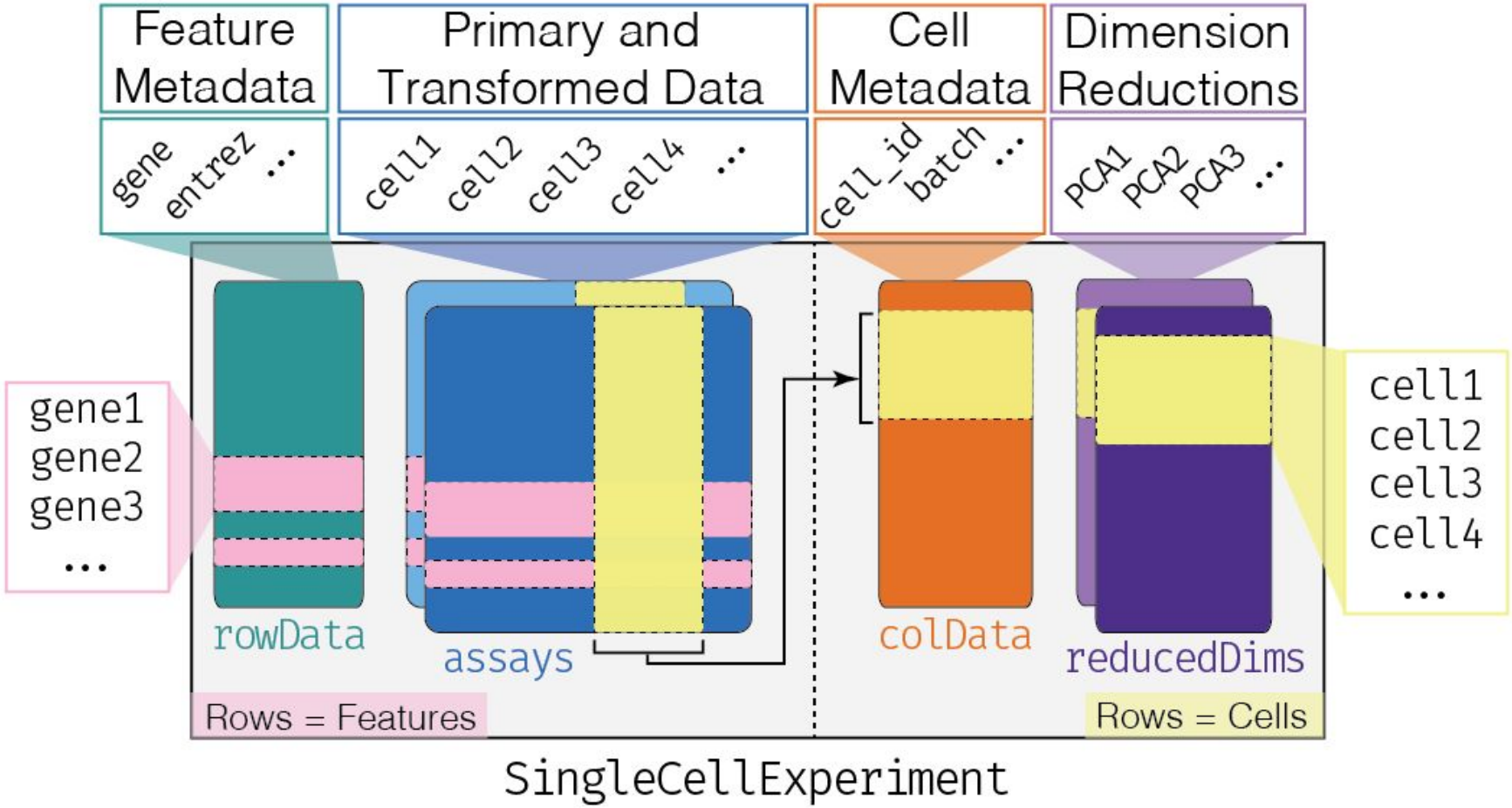
Arguments

Finale

- We are going to present code that uses HumanPrimaryCellAtlasData to "annotate" a collection of PBMCs assayed by TENx
- The relevant package is SingleR – which "trains" on the atlas data to form criteria for cell labeling on a test set
- We will project the data using principal components and produce interactive visualization of cells suggesting clustering and genes relevant to differentiation

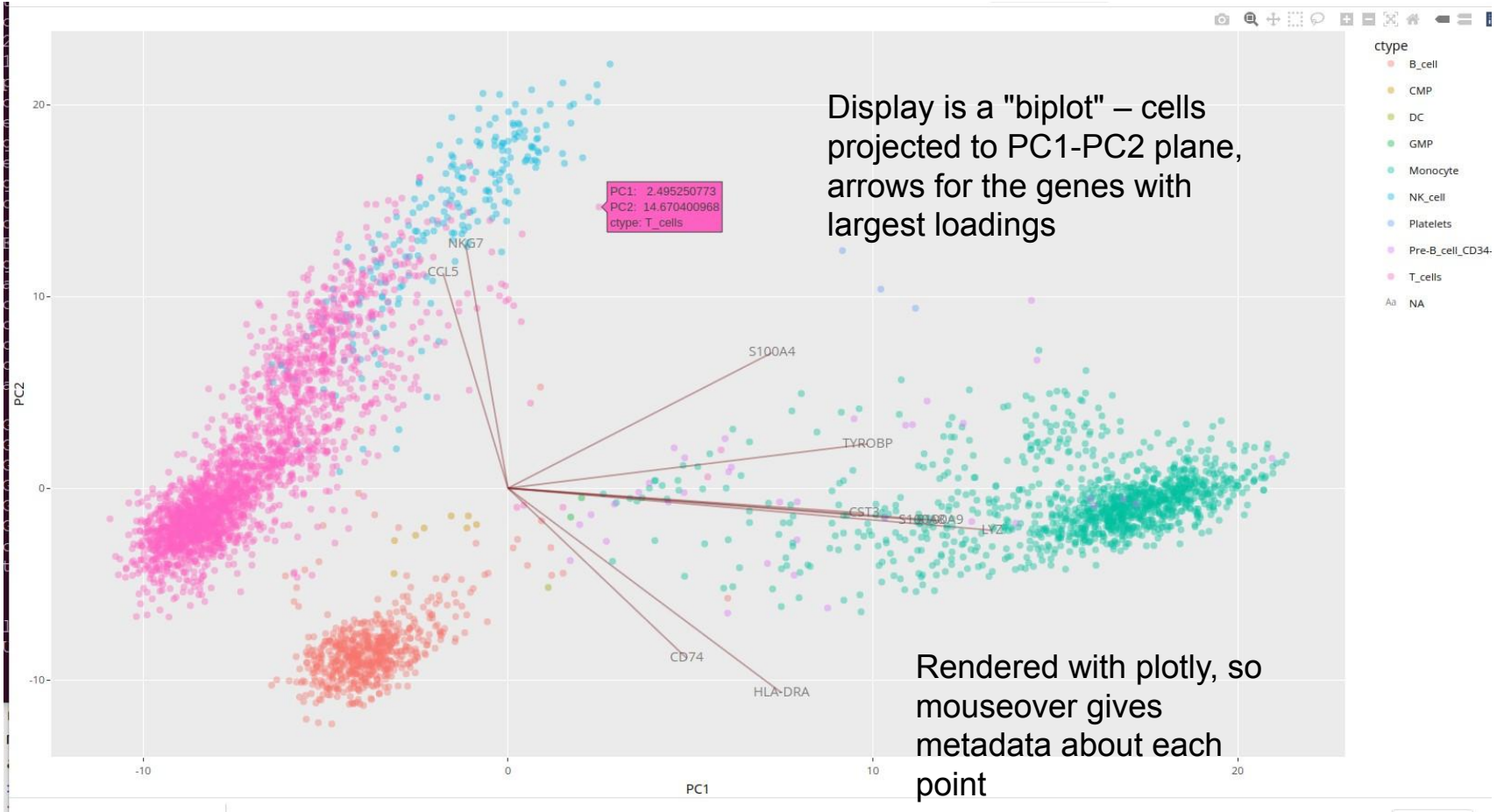
SingleCellExperiment extends SummarizedExperiment



"Desk check"

```
library(TENxPBMCData)                          # data package
vp = TENxPBMCData("pbmc4k")                    # 4340 cells SCE
vp
hassym = which(!is.na(rowData(vp)$Symbol))     # some genes have name NA
vps = vp[hassym,]                              # remove them
rownames(vps) = rowData(vps)$Symbol            # compatible with 'hd'
library(scater)                                # analysis tools
vps = logNormCounts(vps)
vps                                            # adds an assay, normalized
library(SingleR)
vsing = SingleR(vps, hd, hd$label.main)        # scores 'similarity'
                                               # between atlas and PBMC
vps$label.main = vsing$label.main
vpssds = rowSds(assay(vps))
kp = which(vpssds > quantile(vpssds, .8))      # what does it do?
vpslim = vps[kp,]
mat = t(as.matrix(assay(vpslim,2)))
library(irlba)                                 # approximate PCA
apca = prcomp_irlba(mat, 4)
```

The 4340 PBMCs were labeled using HPCA reference



Summary

- Briefly examined RStudio and basic R syntax
- Reviewed SummarizedExperiment and SingleCellExperiment
- Acquired the HumanPrimaryCellAtlasData SummarizedExperiment from celldex
- Acquired the 4K PBMC dataset from TENxPBMCData
- Used HPCA to label 4K PBMCs and showed how expression patterns lead to coherent groupings in PCA space
- Used plotly to make an interactive display of the biplot which embellishes the projective rendering with directional information about important genes
- Many questions arise: How good is the labeling? Can cell subtypes be discriminated? Can we use more modern reference data? Can other omics datatypes be used to improve cell type inference?
    - if you followed most of this talk, you can tackle all these questions directly

Gists

to produce the interactive biplot,

- use R 4.3, BiocManager version 3.17
- be sure devtools is installed
- devtools::source_url("https://gist.githubusercontent.com/vjcitn/b7a44c748c193663525ec227812f0af3/raw/fba74d7d2df667082a11ddc6626aa793f6da1704/pbmcDemo.R", sha1="fb630f3197f60fc89cecfac53644f92e51db9937")$value
- depending on how much software and data need to be installed in your R, the plot will be produced in a few minutes
- biplot code here
- overall script here