Single cell transcriptomics

Analysis tools & QC

cells ->

genes ->

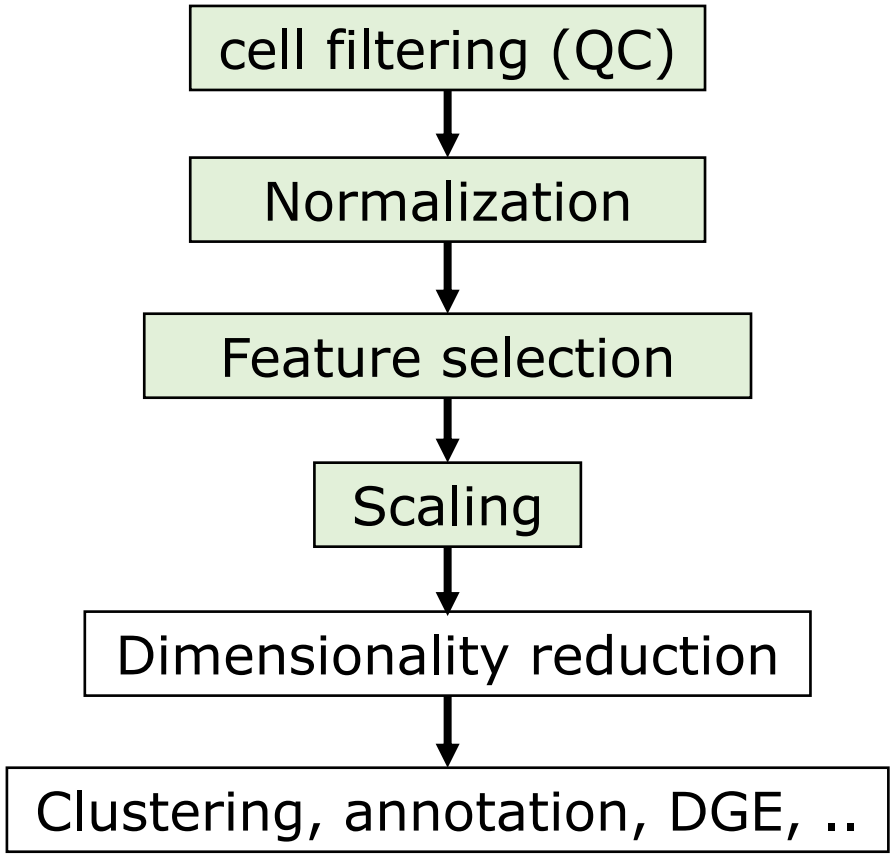
	ATAC-1	CCG-1	GCGA-1
RPL22	5	13	3
PARK7	0	9	3
ENO1	1	35	0
PLA2G2A	0	0	0
CAMK2N1	0	6	0
CDC42	0	8	1
C1QA	0	0	25
C1QC	0	0	25
C1QB	0	0	29
ID3	0	35	0
RPL11	18	29	16
CLIC4	0	4	0

Frequently used analysis tools

- Major tools perform (at least) the following:
  - QC
  - normalization & scaling
  - dimensionality reduction
- `scanpy` (python)
- `scater + scran` (R, Bioconductor)
- `monocle3` (R, beta on github)
- `Seurat` (R, CRAN)

https://nbisweden.github.io/workshop-scRNAseq/exercises

Analysis overview




```
cellranger + Seurat
```

```
Seurat::NormalizeData()
```

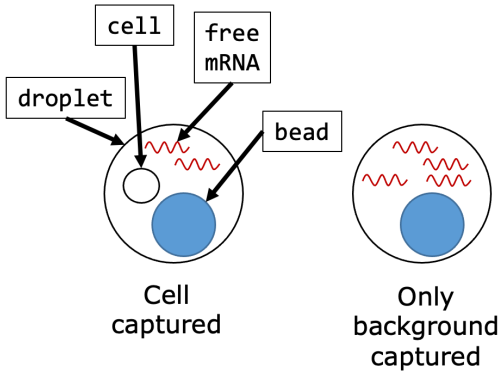
```
Seurat::FindVariableFeatures()
```

```
Seurat::ScaleData()
```

```
Seurat
```

```
Seurat, SingleR,
clusterProfiler, ..
```

Cell filtering

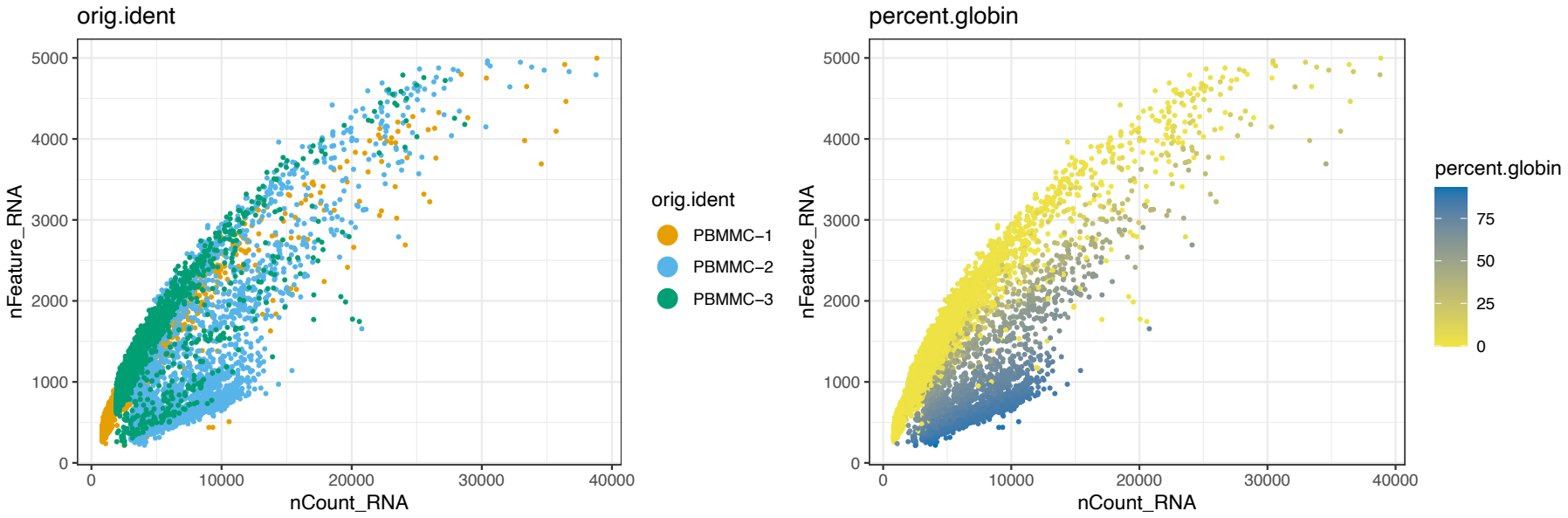


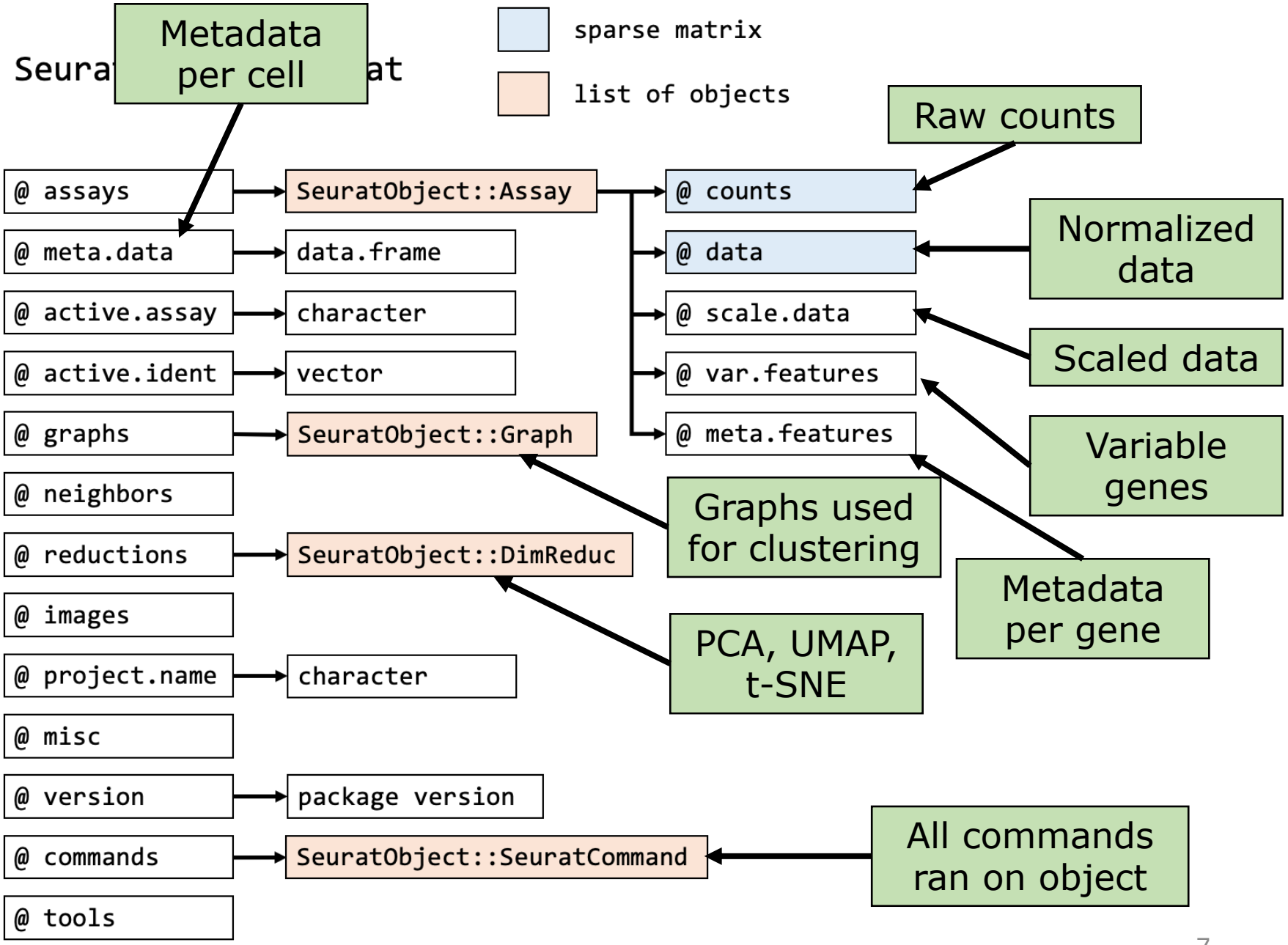
- Cellranger:
  - cell calling (filter against low #UMI)

- Manually (e.g. with Seurat):
  - #UMI: high -> possible doublet
  - #detected genes
  - % mitochondrial UMI: dying cells
  - % ribosomal UMI
  - % globin UMI
  - Relationships between variables

Cell filtering

Often it makes sense to look at relationships




Seura at
Metadata per cell
sparse matrix
list of objects
Raw counts
@ assays
SeuratObject::Assay
@ counts
@ meta.data
data.frame
@ data
Normalized data
@ active.assay
character
@ scale.data
Scaled data
@ active.ident
vector
@ var.features
Variable genes
@ graphs
SeuratObject::Graph
@ meta.features
Graphs used for clustering
Metadata per gene
@ neighbors
@ reductions
SeuratObject::DimReduc
PCA, UMAP, t-SNE
@ images
@ project.name
character
@ misc
@ version
package version
@ commands
SeuratObject::SeuratCommand
All commands ran on object
@ tools

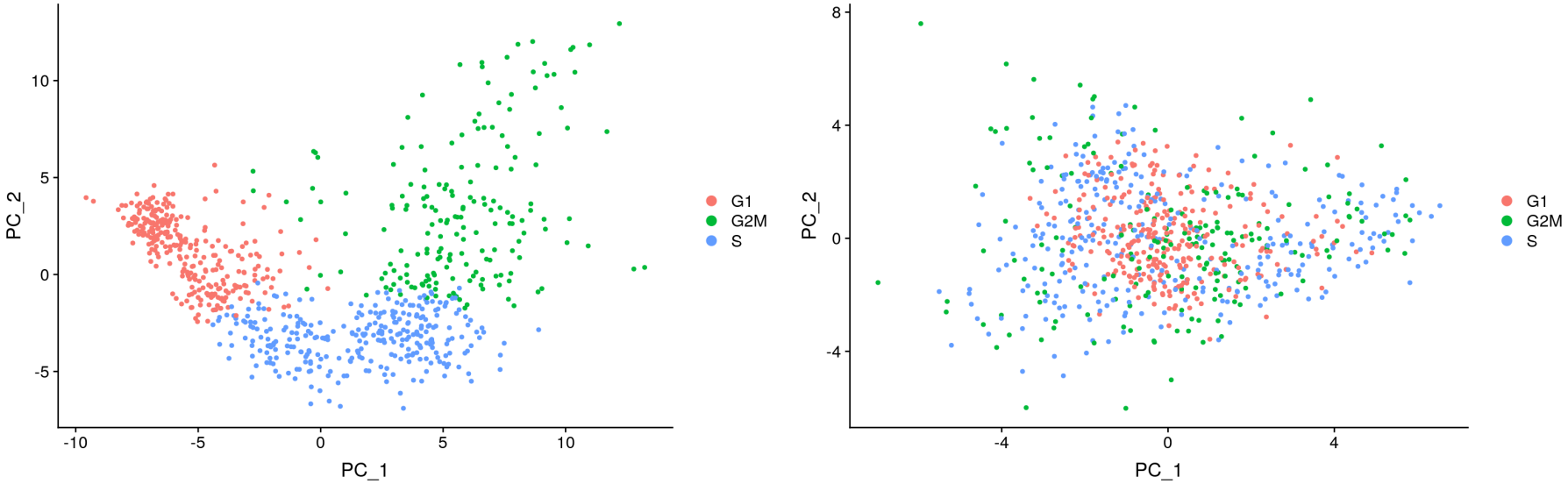
Normalization & scaling

- **Normalization** (per cell): remove technical effects (i.e. library size)
- **Scaling** (per gene): standardize range, mean and variance

Both are mainly for the purpose of dimensionality reduction

Regress out variables

- Typically done after visualization with PCA/UMAP/t-SNE
- Embedding might be according to e.g. cell cycle phase -> but you want cell type



https://satijalab.org/seurat/articles/cell_cycle_vignette.html