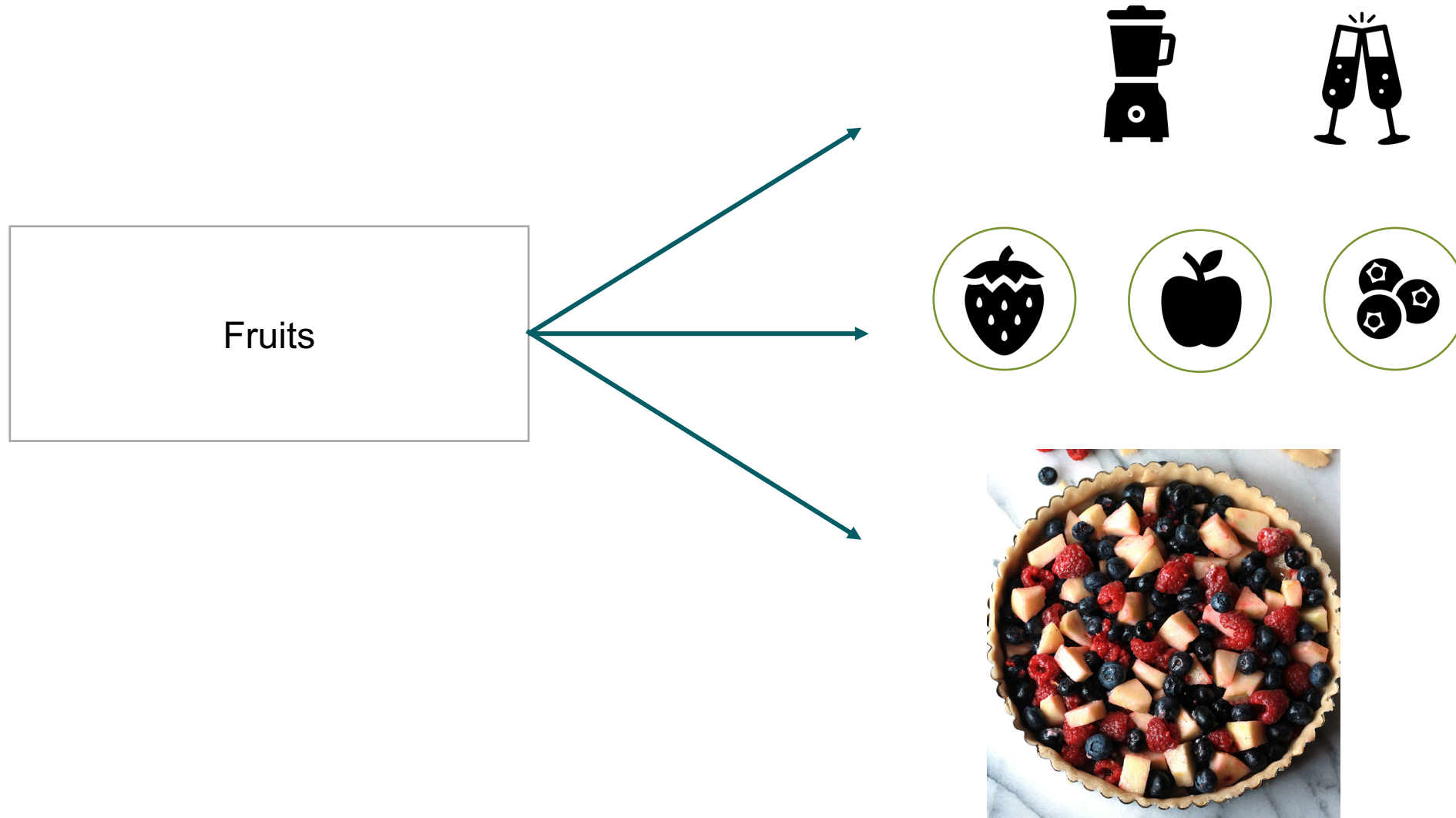# Lab 4|Spatial transcriptomics
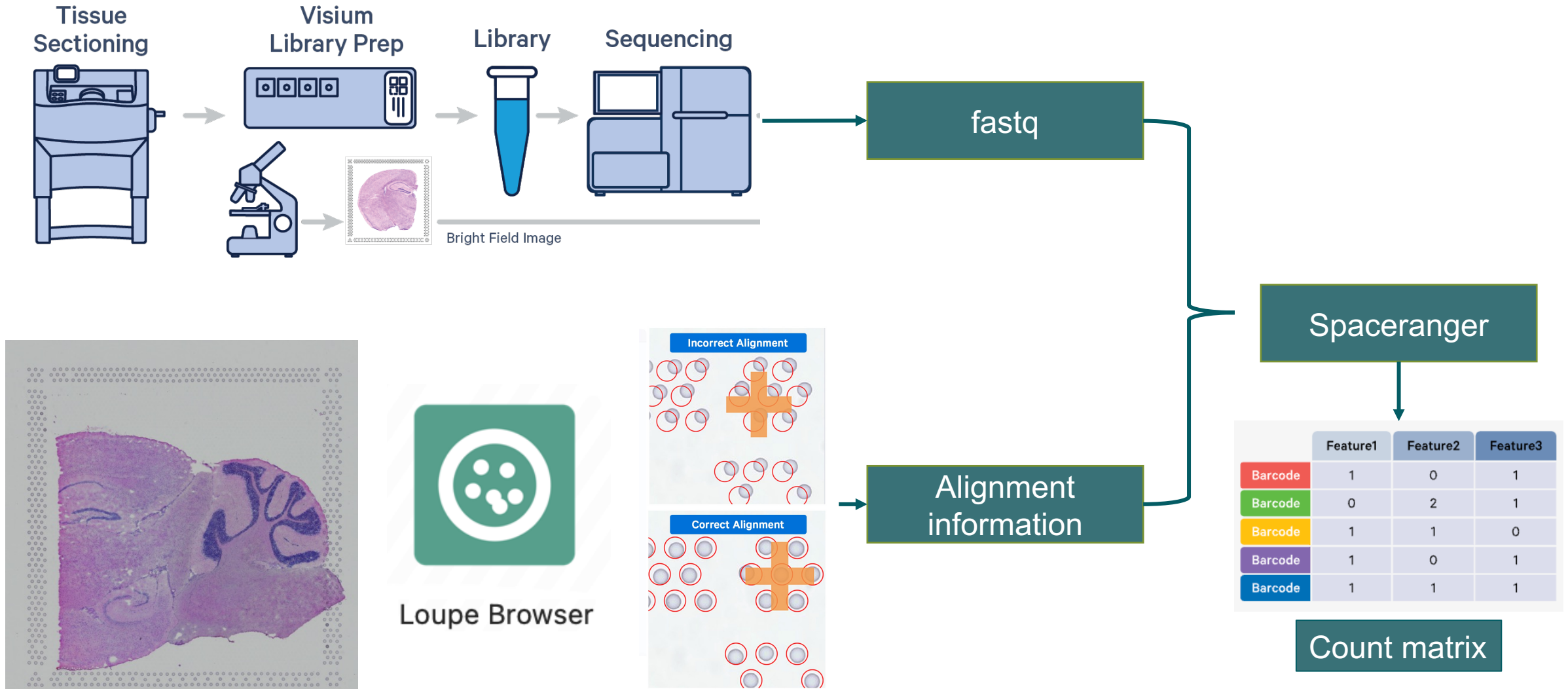
Main Responsible: Nayanika Bhalla
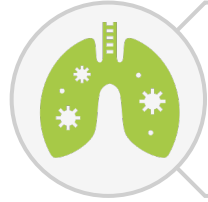
Spatial Transcriptomics: Recap

ST Data Production

Tissue Sectioning → Visium Library Prep → Library → Sequencing → fastq

Bright Field Image

Loupe Browser

Incorrect Alignment

Correct Alignment

Alignment information

Spaceranger

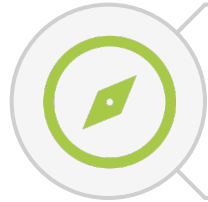|	Feature1	Feature2	Feature3
Barcode	1	0	1
Barcode	0	2	1
Barcode	1	1	0
Barcode	1	0	1
Barcode	1	1	1

Count matrix

Spaceranger output to be used

"filtered_feature_bc_matrix.h5" or "raw_feature_bc_matrix.h5"

"tissue_positions_list.csv"

"tissue_hires_image.png"

"scalefactors_json.json"

STUtility R package

- Wrapper of Seurat.

- STUtility object.

- Each 'spot' is = each 'cell' in scRNA-seq.

- Contains tutorials and walkthroughs for exploring spatial datasets.

Metadata

- Gives information about where your data is actually coming from?
  - Contains tissue and patient information
  - Also experimental information
  - Statistical information
- Why is it important?
  - Makes data 'findable'
  - Relationship between different variables
  - Can explain confounding factors.

NOTE: No tissue metadata is provided for the lab today as we will only process 1 sample.

Creating a STUtility object

- Requires a dataframe 'infoTable' containing paths to files.
- 1 tissue section of human HER2-positive breast cancer.

samples	spotfiles	imgs	json
path/to/sample_1/count_file_1.h5	path/to/sample_1/tissue_positions_list.csv	path/to/sample_1/tissue_hires_image.png	path/to/sample_1/scal
path/to/sample_2/count_file_2.h5	path/to/sample_2/tissue_positions_list.csv	path/to/sample_2/tissue_hires_image.png	path/to/sample_2/scal

```
#Creating the STUtility object (similar to a Seurat object)
se <- InputFromTable(infoTable)
```
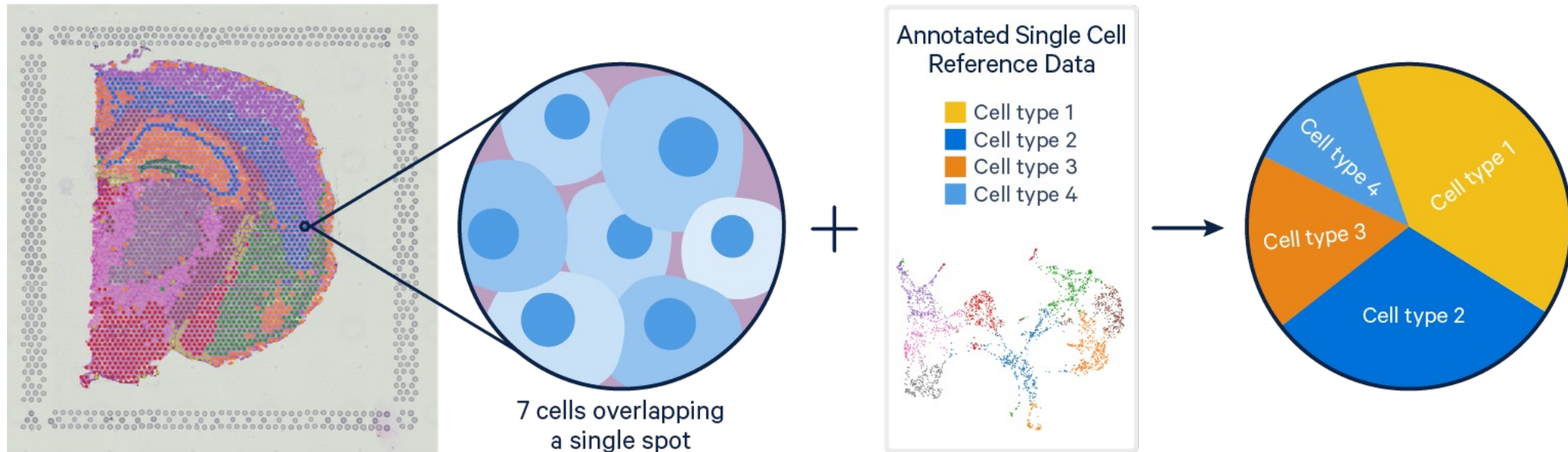
Spatial functions

- FeaturePlot() → ST.FeaturePlot()
- Subset() → SubsetSTData()
- FeatureOverlay()
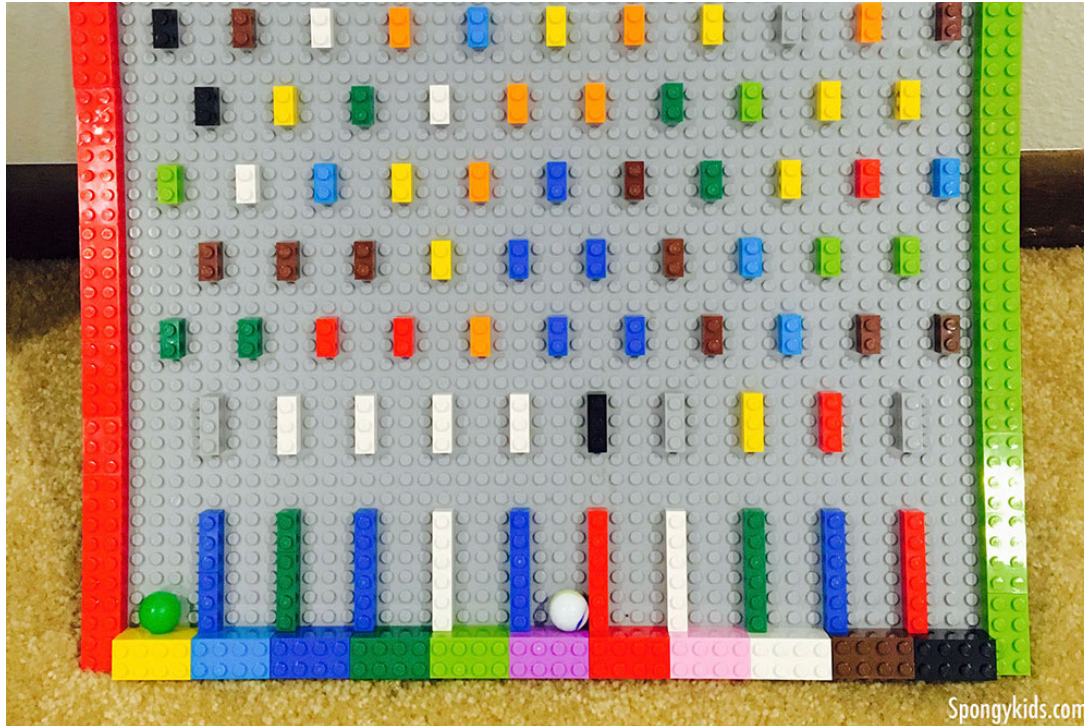- DimPlot() → ST.DimPlot()

Stereoscope

- Visium lacks single cell resolution.

- Probabilistic method that uses single cell data to deconvolve the cell mixtures in spatial data.

7 cells overlapping a single spot

Annotated Single Cell Reference Data

Cell type 1
Cell type 2
Cell type 3
Cell type 4

Reproducibility

- When you generate anything involving randomisation in R, it is pseudorandom (simulate randomness).
  - If you know the "seed" and the generator, you would end up with the same results every time.
  - The seed acts as an initiator.
- "Seed value"
  - Ensures that we get the same results for randomisation.

Reports

- Complete all questions.
- Hand in a knitted .html file.
- Hand in your reports by 20 Oct (23:59).

GOODLUCK!