Polyglot programming for single-cell analysis

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Introduction

1. How do you interact with a package in another language?
2. How do you make you package useable for developers in other languages?

We will be focusing on R & Python

Summary

Interoperability between languages allows analysts to take advantage of the strengths of different ecosystems

On-disk interoperability uses standard file formats to transfer data and is typically more reliable

In-memory interoperability transfers data directly between parallel sessions and is convenient for interactive analysis

While interoperability is currently possible developers continue to improve the experience

Single-cell best practices: Interoperability

How do you interact with a package in another language?

1. In-memory interoperability
2. Disk-based interoperability

How do you make your package useable for developers in other languages?

1. Package-based interoperability
2. Best practices

Package-based interoperability

or: the question of reimplementation.

Consider the pros:

1. Discoverability
2. Can your package be useful in other domains?
3. Very user friendly

Consider the cons:

1. Think twice: is it worth it?
2. **It’s a lot of work**
3. How will you keep it up to date?
4. How will you ensure parity?

Package-based interoperability

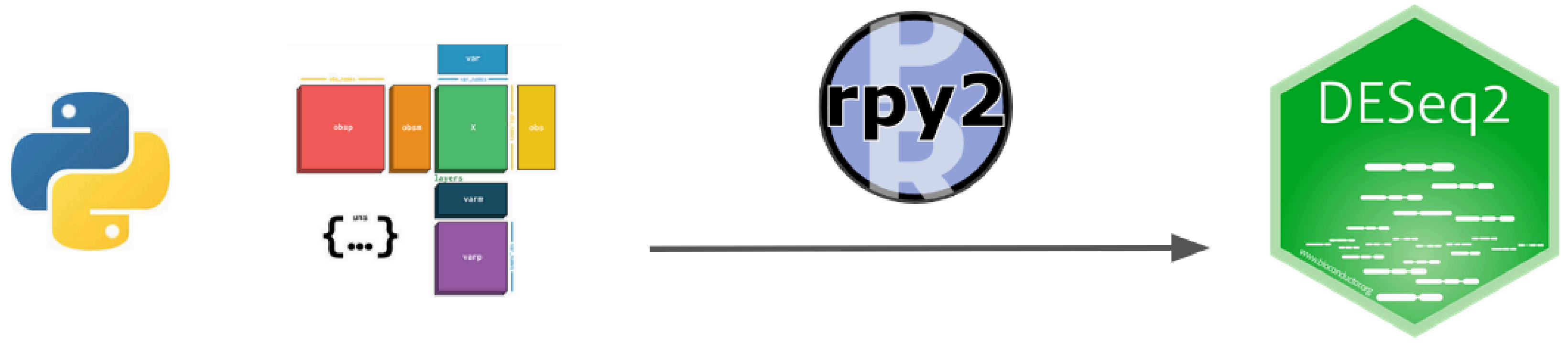
Please learn both R & Python

Best practices

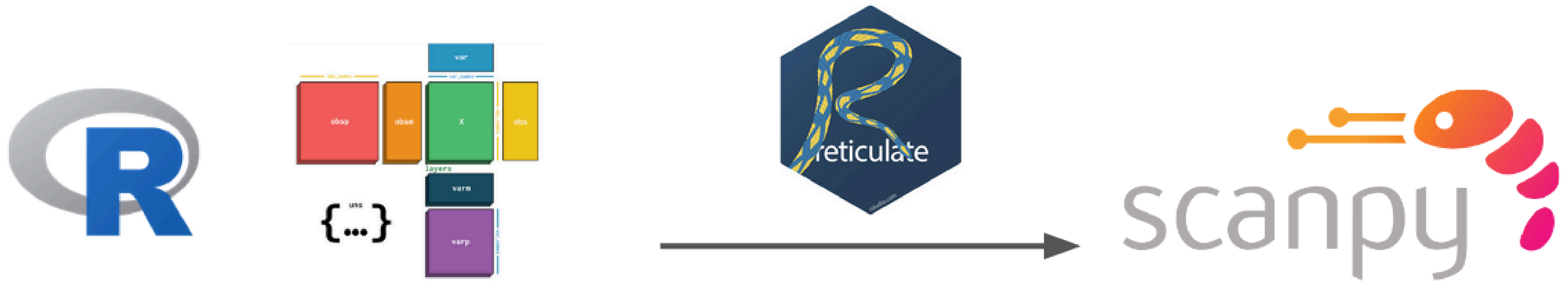
1. Work with the standards
2. Work with matrices, arrays and dataframes
3. Provide vignettes on interoperability

In-memory interoperability

A Python user with an anndata object can use rpy2 to run the DESeq2 method in R



An R user with an anndata object can use reticulate to run scanpy functions in Python



Overview

1. Advantages & disadvantages
2. Pitfalls when using Python & R
3. Rpy2
4. Reticulate

in-memory interoperability advantages

- no need to write & read results
- useful when you need a limited amount of functions in another language

in-memory interoperability drawbacks

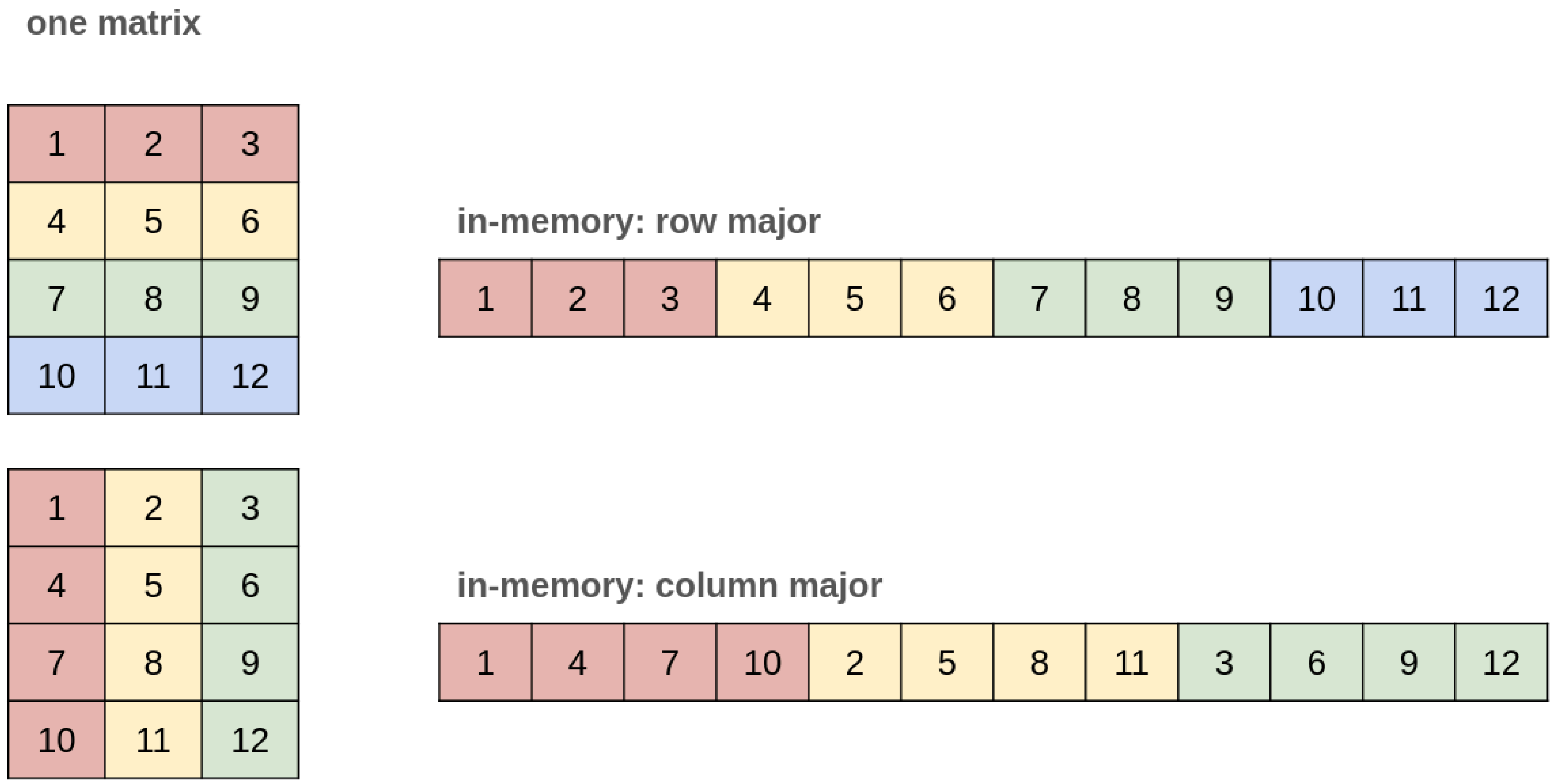
not always access to all classes

data duplication

you need to manage the environments

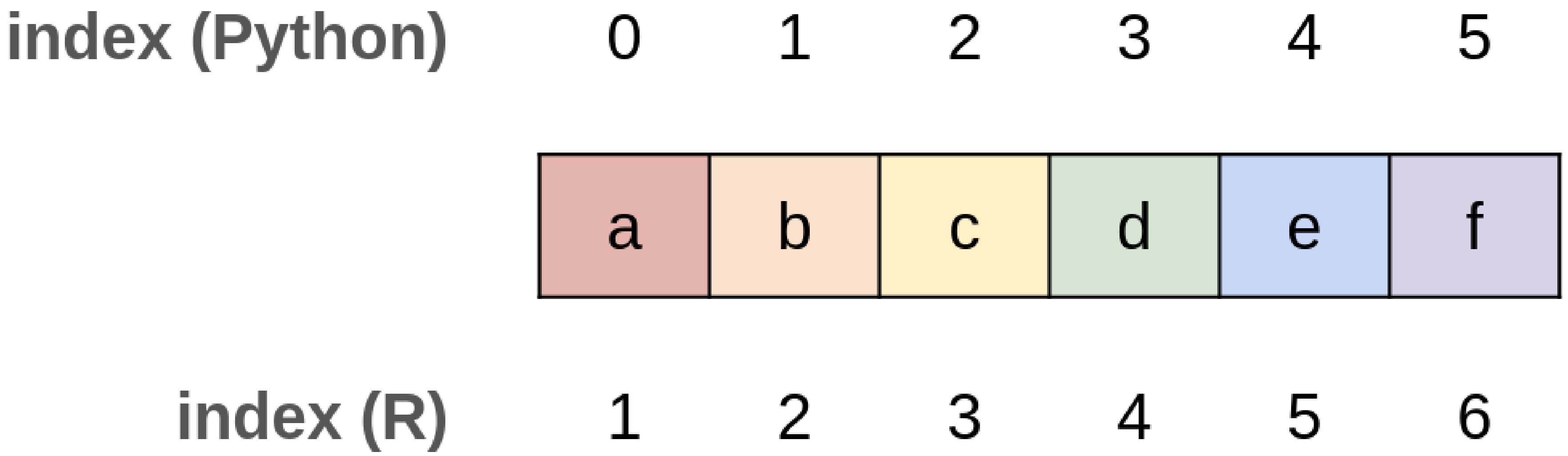
Pitfalls when using Python and R

Column major vs row major matrices In R: every dense matrix is stored as column major



Pitfalls when using Python and R

Indexing



Pitfalls when using Python and R

dots and underscores

mapping in rpy2

```
from rpy2.robjects.packages import importr

d = {'package.dependencies': 'package_dot_dependencies',
     'package_dependencies': 'package_uscore_dependencies'}
tools = importr('tools', robject_translations = d)
```

Pitfalls when using Python and R

Integers

```r
library(reticulate)
bi <- reticulate::import_builtins()

bi$list(bi$range(0, 5))
# TypeError: 'float' object cannot be interpreted as an integer
```

```r
library(reticulate)
bi <- reticulate::import_builtins()

bi$list(bi$range(0L, 5L))
# [1] 0 1 2 3 4
```

Rpy2: basics

Accessing R from Python

- `rpy2.rinterface`, the low-level interface
- `rpy2.robjects`, the high-level interface

```python
import rpy2
import rpy2.robjects as robjects

vector = robjects.IntVector([1,2,3])
rsum = robjects.r['sum']

rsum(vector)
```

IntVector with 1 elements.

6

Rpy2: basics

```python
str_vector = robjects.StrVector(['abc', 'def', 'ghi'])
flt_vector = robjects.FloatVector([0.3, 0.8, 0.7])
int_vector = robjects.IntVector([1, 2, 3])
mtx = robjects.r.matrix(robjects.IntVector(range(10)), nrow=5)
print(mtx)
```

```
     [,1] [,2]
[1,]    0    5
[2,]    1    6
[3,]    2    7
[4,]    3    8
[5,]    4    9
```

Rpy2: numpy

```python
import numpy as np

from rpy2.robjects import numpy2ri
from rpy2.robjects import default_converter

rd_m = np.random.random((5, 4))

with (default_converter + numpy2ri.converter).context():
    mtx = robjects.r.matrix(rd_m, nrow = 5)
    print(mtx)
```

```
[[0.73294749 0.55953375 0.69944132 0.52744075]
 [0.09756794 0.39535684 0.80669803 0.10540606]
 [0.35662206 0.70148737 0.12002733 0.28026677]
 [0.19947608 0.84421019 0.82702188 0.82531633]
 [0.56938249 0.04640811 0.34178679 0.3285883 ]]
```

Rpy2: pandas

```python
import pandas as pd

from rpy2.robjects import pandas2ri

pd_df = pd.DataFrame({'int_values': [1,2,3],
                      'str_values': ['abc', 'def', 'ghi']})

with (default_converter + pandas2ri.converter).context():
    pd_df_r = robjects.DataFrame(pd_df)
    print(pd_df_r)
```

```
  int_values str_values
0          1        abc
1          2        def
2          3        ghi
```

Rpy2: sparse matrices

```python
import scipy as sp

from anndata2ri import scipy2ri

sparse_matrix = sp.sparse.csc_matrix(rd_m)

with (default_converter + scipy2ri.converter).context():
    sp_r = scipy2ri.py2rpy(sparse_matrix)
    print(sp_r)
```

```
5 x 4 sparse Matrix of class "dgCMatrix"

[1,] 0.73294749 0.55953375 0.6994413 0.5274408
[2,] 0.09756794 0.39535684 0.8066980 0.1054061
[3,] 0.35662206 0.70148737 0.1200273 0.2802668
[4,] 0.19947608 0.84421019 0.8270219 0.8253163
[5,] 0.56938249 0.04640811 0.3417868 0.3285883
```

Rpy2: anndata

```python
import anndata as ad
import scanpy.datasets as scd

import anndata2ri

adata_paul = scd.paul15()

with anndata2ri.converter.context():
    sce = anndata2ri.py2rpy(adata_paul)
    ad2 = anndata2ri.rpy2py(sce)
```

Rpy2: interactivity

```
%load_ext rpy2.ipython  # line magic that loads the rpy2 ipython extension.
                        # this extension allows the use of the following cell magic

%%R -i input -o output  # this line allows to specify inputs
                        # (which will be converted to R objects) and outputs
                        # (which will be converted back to Python objects)
                        # this line is put at the start of a cell
                        # the rest of the cell will be run as R code
```

Reticulate

R	Python	Examples
Single-element vector	Scalar	`1`, `1L`, `TRUE`, `"foo"`
Multi-element vector	List	`c(1.0, 2.0, 3.0)`, `c(1L, 2L, 3L)`
List of multiple types	Tuple	`list(1L, TRUE, "foo")`
Named list	Dict	`list(a = 1L, b = 2.0)`, `dict(x = x_data)`
Matrix/Array	NumPy ndarray	`matrix(c(1,2,3,4), nrow = 2, ncol = 2)`
Data Frame	Pandas DataFrame	`data.frame(x = c(1,2,3), y = c("a", "b", "c"))`
Function	Python function	`function(x) x + 1`
Raw	Python bytearray	`as.raw(c(1:10))`
NULL, TRUE, FALSE	None, True, False	`NULL`, `TRUE`, `FALSE`

Reticulate

```r
library(reticulate)

bi <- reticulate::import_builtins()
rd <- reticulate::import("random")

example <- c(1,2,3)
bi$max(example)
# [1] 3
rd$choice(example)
# [1] 2
cat(bi$list(bi$reversed(example)))
# [1] 3 2 1
```

Reticulate numpy

```r
np <- reticulate::import("numpy")

a <- np$asarray(tuple(list(1,2), list(3, 4)))
b <- np$asarray(list(5,6))
b <- np$reshape(b, newshape = tuple(1L,2L))

np$concatenate(tuple(a, b), axis=0L)
#       [,1] [,2]
# [1,]     1    2
# [2,]     3    4
# [3,]     5    6
```

Reticulate conversion

```r
np <- reticulate::import("numpy", convert = FALSE)

a <- np$asarray(tuple(list(1,2), list(3, 4)))
b <- np$asarray(list(5,6))
b <- np$reshape(b, newshape = tuple(1L,2L))

np$concatenate(tuple(a, b), axis=0L)
# array([[1., 2.],
#        [3., 4.],
#        [5., 6.]])
```

You can explicitly convert data types:

```r
result <- np$concatenate(tuple(a, b), axis=0L)

py_to_r(result)
#      [,1] [,2]
# [1,]    1    2
# [2,]    3    4
# [3,]    5    6

result_r <- py_to_r(result)
r_to_py(result_r)
# array([[1., 2.],
#        [3., 4.],
#        [5., 6.]])
```

Reticulate scanpy

```r
library(anndata)
library(reticulate)
sc <- import("scanpy")

adata_path <- "../usecase/data/sc_counts_subset.h5ad"
adata <- anndata::read_h5ad(adata_path)
```

We can preprocess & analyse the data:

```r
sc$pp$filter_cells(adata, min_genes = 200)
sc$pp$filter_genes(adata, min_cells = 3)
sc$pp$pca(adata)
sc$pp$neighbors(adata)
sc$tl$umap(adata)

adata
# AnnData object with n_obs × n_vars = 32727 × 20542
#     obs: 'dose_uM', 'timepoint_hr', 'well', 'row', 'col', 'plate_name', 'cell_id', 'cell_type', 'split', '
#     var: 'highly_variable', 'means', 'dispersions', 'dispersions_norm', 'n_cells'
#     uns: 'cell_type_colors', 'celltypist_celltype_colors', 'donor_id_colors', 'hvg', 'leiden_res1_colors',
#     obsm: 'HTO_clr', 'X_pca', 'X_umap', 'protein_counts'
#     varm: 'PCs'
#     obsp: 'connectivities', 'distances'
```

Disk-based interoperability

Disk-based interoperability is a strategy for achieving interoperability between tools written in different programming languages by **storing intermediate results in standardized, language-agnostic file formats**.

Upside:

- Simple, just add reading and witing lines
- Modular scripts

Downside:

- increased disk usage
- less direct interaction, debugging…

Important features of interoperable file formats

- Compression
- Sparse matrix support
- Large images
- Lazy chunk loading
- Remote storage

General single cell file formats of interest for Python and R

File Format	Python	R	Sparse matrix	Large images	Lazy chunk loading	Remote storage
RDS	○	●	○	◐	○	○
Pickle	●	○	○	◐	○	○
CSV	●	●	○	○	○	○
JSON	●	●	○	○	○	○
TIFF	●	●	○	◐	●	◐
.npy	●	○	○	●	○	○
Parquet	●	●	○	○	●	●
Feather	●	●	●	○	●	●
Lance	●	○	●	○	●	●
HDF5	●	●	○	●	●	◐
Zarr	●	●	○	●	●	●
TileDB	●	●	●	●	●	●

Specialized single cell file formats of interest for Python and R

File Format	Python	R	Sparse matrix	Large images	Lazy chunk loading	Remote storage
Seurat RDS	○	●	○	◐	○	○
Indexed OME-TIFF	●	●	○	●	●	●
h5Seurat	●	●	○	◐	●	◐
Loom HDF5	●	●	●	○	●	◐
AnnData h5ad	●	●	●	◐	●	◐
AnnData Zarr	●	●	●	◐	●	●
TileDB-SOMA	●	●	●	◐	●	●
TileDB-BioImaging	●	●	◐	●	●	●
SpatialData Zarr	●	●	●	●	●	◐

Disk-based pipelines

Script pipeline:

```bash
#!/bin/bash

bash scripts/1_load_data.sh
python scripts/2_compute_pseudobulk.py
Rscript scripts/3_analysis_de.R
```

Notebook pipeline:

```bash
# Every step can be a new notebook execution with inspectable output
jupyter nbconvert --to notebook --execute my_notebook.ipynb --allow-errors --output-dir outputs/
```

Just stay in your language and call scripts

```python
import subprocess

subprocess.run("bash scripts/1_load_data.sh", shell=True)
# Alternatively you can run Python code here instead of calling a Python script
subprocess.run("python scripts/2_compute_pseudobulk.py", shell=True)
subprocess.run("Rscript scripts/3_analysis_de.R", shell=True)
```

Pipelines with different environments

1. interleave with environment (de)activation functions
2. use rvenv
3. use Pixi

Pixi to manage different environments

```
pixi run -e bash scripts/1_load_data.sh
pixi run -e scverse scripts/2_compute_pseudobulk.py
pixi run -e rverse scripts/3_analysis_de.R
```

Define tasks in Pixi

```
...
[feature.bash.tasks]
load_data = "bash book/disk_based/scripts/1_load_data.sh"
...
[feature.scverse.tasks]
compute_pseudobulk = "python book/disk_based/scripts/2_compute_pseudobulk.py"
...
[feature.rverse.tasks]
analysis_de = "Rscript --no-init-file book/disk_based/scripts/3_analysis_de.R"
...
[tasks]
pipeline = { depends-on = ["load_data", "compute_pseudobulk", "analysis_de"] }
```

```
pixi run pipeline
```

Also possible to use containers

```
docker pull berombau/polygloty-docker:latest
docker run -it -v $(pwd)/usecase:/app/usecase -v $(pwd)/book:/app/book berombau/polygloty-docker:latest pixi
```

Another approach is to use multi-package containers to create custom combinations of packages. - Multi-Package BioContainers - Seqera Containers

Workflows

You can go a long way with a folder of notebooks or scripts and the right tools. But as your project grows more bespoke, it can be worth the effort to use a **workflow framework** like Viash, Nextflow or Snakemake to manage the pipeline for you.

See https://saeyslab.github.io/polygloty/book/workflow_frameworks/

Takeaways