metadata

tissue/culture prep
isolation
fixation
embedding
sectioning
coordinates
clearing

probe design and construction
genes
mRNA targeting oligos
detection method
fluorophores
amplification
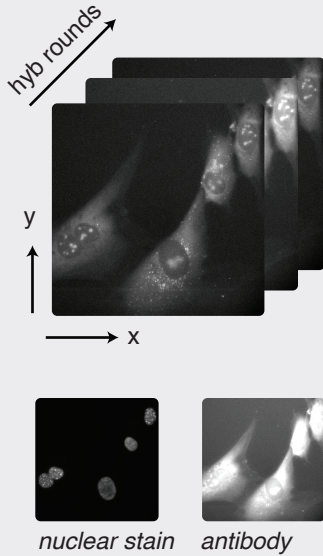hybridization rounds
extinguish method
error correction code

imaging
objective
exposure time
fields of view
laser lines to excite fluorophores
beads (alignment, chromatic, psf)
nuclear marker

raw data

hyb rounds

y

x

nuclear stain *antibody*

registration

reference

transformed

preprocessing

filtering

before *after*

segmentation & detection

decoding

gene 1 *gene 2* *gene 3*

quality control

fpkm (bulk seq)

r = 0.95

copy #/cell

---

per field of view
raw_stack.tif
dapi.tif, antibody.tif

codebook schemas
hyb | bit position
gene | bit-string
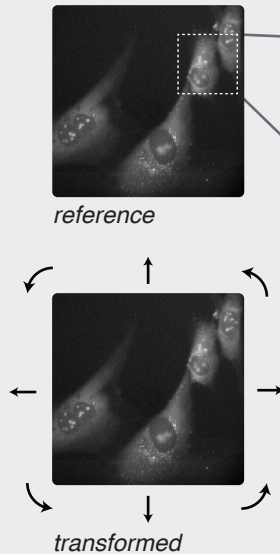
per field of view
reg_stack.tif
affine_transform.mat

global
chromatic_correc.mat
references.csv
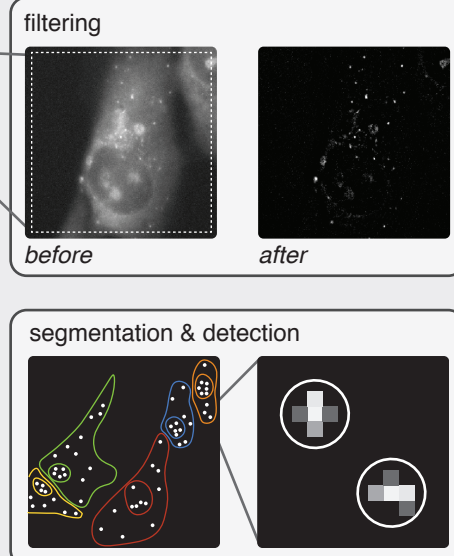
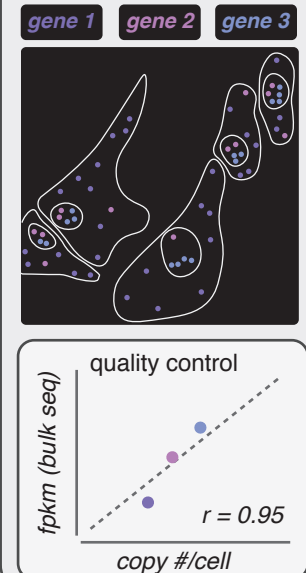**per field of view**
proc_stack.tif
cells_geo.json
spots_geo.json

encoder table schema
cell_id | spot_id | x | y | z | hyb | val

qualiy metrics
assay_comp.json
control_barcodes.json
metrics.json

decoder table schema
spot_id | gene