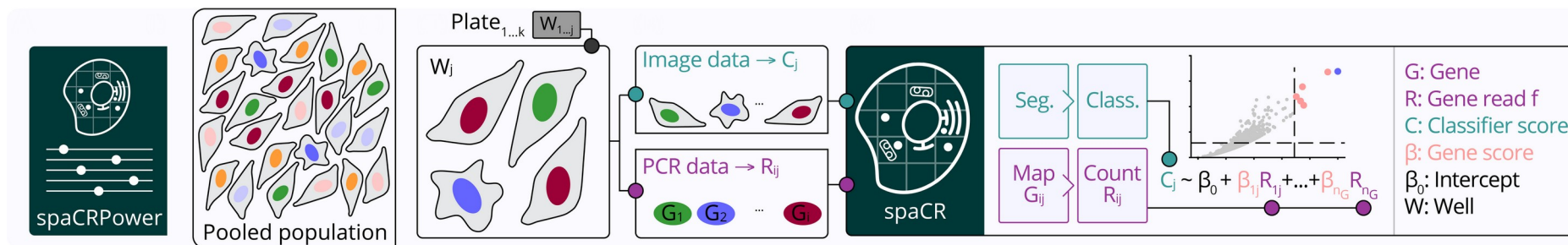


# spaCR

Spatial phenotype analysis of CRISPR  
screens



# What is spaCR?



*Pooled screen: every well holds a mix of knockouts. Image it, sequence it, and regress. (Preprint, Fig. 1A)*



## Image every well

Segment, measure and classify every cell. The phenotype becomes a number per well.



## Sequence every well

Read the gRNA barcodes to learn which knockouts are in each well, and in what proportion.



## Link the two

Regress the phenotype on guide composition. Out comes an effect per gene: a ranked hit list.

**No sequencing?** Segmentation, measurement, annotation, classification and the assays work on any high-content imaging experiment.



## INTRODUCTION

# Where spaCR fits among existing tools

- tool lacks it
- tool has it: spaCR has it too
- tool has it: spaCR does not

|                                            | CellProfiler            | Fiji / ImageJ         | napari               | Cellpose            | ilastik                  | QuPath               | DL4Mic            | MAGeCK                    | spaCR                      | Category       |
|--------------------------------------------|-------------------------|-----------------------|----------------------|---------------------|--------------------------|----------------------|-------------------|---------------------------|----------------------------|----------------|
| <i>Best for</i>                            | <i>cell measurement</i> | <i>image analysis</i> | <i>image viewing</i> | <i>segmentation</i> | <i>pixel / object ML</i> | <i>tissue slides</i> | <i>no-code DL</i> | <i>sequencing screens</i> | <i>image-based screens</i> |                |
| Point-and-click interface                  | ✓                       | ✓                     | ✓                    | ✓                   | ✓                        | ✓                    | limited           | ✗                         | ✓                          | Usability      |
| Batch and headless runs (server, cluster)  | ✓                       | limited               | ✗                    | ✓                   | ✓                        | ✓                    | limited           | ✓                         | ✓                          | Automation     |
| Reads most microscope file formats         | ✓                       | ✓                     | plugin               | limited             | limited                  | ✓                    | limited           | ✗                         | ✓                          | Input          |
| Deep-learning segmentation                 | plugin                  | plugin                | plugin               | ✓                   | limited                  | plugin               | ✓                 | ✗                         | ✓                          | Segmentation   |
| Train your own segmentation model          | ✗                       | plugin                | plugin               | ✓                   | ✓                        | limited              | ✓                 | ✗                         | ✓                          | Segmentation   |
| Live assisted segmentation                 | ✗                       | plugin                | plugin               | limited             | ✓                        | ✓                    | ✗                 | ✗                         | ✓                          | Segmentation   |
| Correct masks by hand                      | limited                 | ✓                     | ✓                    | ✓                   | ✓                        | ✓                    | ✗                 | ✗                         | ✓                          | Segmentation   |
| Per-cell measurements                      | ✓                       | ✓                     | plugin               | ✗                   | ✓                        | ✓                    | ✗                 | ✗                         | ✓                          | Measurement    |
| 3-D (z-stack) images                       | ✓                       | ✓                     | ✓                    | ✓                   | ✓                        | limited              | limited           | ✗                         | beta                       | Image types    |
| Time-lapse tracking                        | ✓                       | plugin                | plugin               | ✗                   | ✓                        | ✗                    | ✗                 | ✗                         | ✓                          | Image types    |
| Whole plates in one run                    | ✓                       | limited               | ✗                    | limited             | limited                  | limited              | limited           | ✗                         | ✓                          | Screening      |
| Plate maps, heat maps and QC               | limited                 | ✗                     | ✗                    | ✗                   | ✗                        | ✗                    | ✗                 | ✗                         | ✓                          | Screening      |
| Classify cells from images (deep learning) | ✗                       | ✗                     | ✗                    | ✗                   | ✗                        | ✗                    | ✗                 | ✗                         | ✓                          | Classification |
| Classify cells from measurements           | limited                 | ✗                     | plugin               | ✗                   | ✓                        | ✓                    | ✗                 | ✗                         | ✓                          | Classification |
| Explore cells: UMAP with images, gating    | limited                 | ✗                     | plugin               | ✗                   | ✗                        | ✗                    | ✗                 | ✗                         | ✓                          | Analysis       |
| Plots and statistics without code          | limited                 | limited               | plugin               | ✗                   | ✗                        | limited              | ✗                 | limited                   | ✓                          | Analysis       |
| GPU acceleration                           | ✗                       | plugin                | plugin               | limited             | limited                  | limited              | ✓                 | ✗                         | ✓                          | Performance    |
| Pooled CRISPR screens: images to genes     | ✗                       | ✗                     | ✗                    | ✗                   | ✗                        | ✗                    | ✗                 | limited                   | ✓                          | Screening      |
| Pixel classifier from brush strokes        | ✗                       | plugin                | plugin               | ✗                   | ✓                        | ✓                    | ✗                 | ✗                         | ✗                          | Segmentation   |
| Whole-slide / tissue images                | ✗                       | limited               | ✗                    | ✗                   | ✗                        | ✓                    | ✗                 | ✗                         | ✗                          | Image types    |
| Large plugin ecosystem                     | limited                 | ✓                     | ✓                    | ✗                   | ✗                        | limited              | ✗                 | ✗                         | ✗                          | Extensibility  |

Core features; 'plugin' = needs an add-on, 'limited' = partly. Simplified: check each tool for details.

# Alpha features: switch them on in Preferences

| Category                | Alpha feature                                       |
|-------------------------|-----------------------------------------------------|
| Segmentation            | StarDist segmentation backend                       |
|                         | InstanSeg segmentation backend                      |
|                         | Omnipose segmentation backend                       |
|                         | DINOCell segmentation backend                       |
|                         | SAMCell segmentation backend                        |
|                         | Spotiflow spot detection                            |
|                         | SpotNet spot detection                              |
|                         | Click- and box-prompted segmentation (micro-SAM)    |
|                         | Segmentation uncertainty maps                       |
|                         | Parameter-robustness report                         |
|                         | Run CellProfiler .cppipe pipelines                  |
|                         | Masks and ROIs in and out: QuPath, ImageJ, COCO     |
|                         | Virtual staining from brightfield                   |
|                         | Mask batches across several GPUs                    |
| Image preprocessing     | Use Make Masks settings in Mask Generation          |
|                         | Spectral unmixing (bleed-through correction)        |
|                         | Photobleaching correction                           |
|                         | Vendor flat-field profiles (Harmony, ZEN)           |
|                         | Intensity calibration across sessions               |
|                         | Deep-learning image QC: focus, debris, empty fields |
| Measurements and assays | Self-supervised denoising (Noise2Void)              |
|                         | Illumination, enhancement and PSF settings          |
|                         | Cell-cycle phase classification                     |
|                         | Live/dead viability and cytotoxicity index          |
|                         | Confluency for any channel                          |
|                         | Scratch / wound-closure assay                       |
|                         | Colony / CFU counting                               |
|                         | Image-based profiling: normalise, select, mAP       |
|                         | GPU measurement (cuCIM, CuPy)                       |
|                         | Estimate plaque scale and growth time               |

| Category                   | Alpha feature                                          |
|----------------------------|--------------------------------------------------------|
| Time-lapse                 | SAM2 propagation: segment once, follow each object     |
|                            | Timeflows: a temporal Cellpose                         |
|                            | Lineage trees from tracks                              |
|                            | Event detection: mitosis, egress, invasion, death      |
|                            | Time-to-event: Kaplan-Meier and Cox                    |
| Cells and machine learning | Foundation-model embeddings (SubCell, OpenPhenom, ...) |
|                            | Well labels to responding cells (multiple-instance)    |
|                            | Anomaly detection against negative controls            |
|                            | Find cells like this one (similarity search)           |
|                            | Blind scoring                                          |
|                            | Counterfactuals: morph a control cell toward a hit     |
|                            | Self-supervised DINO pretraining                       |
|                            | Judge every label from cross-channel ground truth      |
| Screens and statistics     | Arrayed-screen hits: SSMD, robust z, B-score           |
|                            | Chemistry-aware hits: SMILES, clusters, SAR            |
|                            | Sample-size planner for arrayed assays                 |
|                            | Spatial transcriptomics: Visium and Xenium             |
|                            | LIMS / plate-barcode linkage                           |
| Automation and data        | Watch a folder, analyse images as they arrive          |
|                            | Smart microscopy: send hits back for re-imaging        |
|                            | Cloud storage (S3 and others)                          |
|                            | Export a run as a Nextflow / Snakemake workflow        |
|                            | Run-finished notifications (email, Slack, ntfy)        |
|                            | Export for R (SingleCellExperiment), tidy Parquet      |
|                            | Plugin and recipe catalogue                            |
| Reproducibility            | Postgres or DuckDB / Parquet measurement store         |
|                            | Test data on seven more modules                        |
|                            | Figure-integrity check                                 |
|                            | Pre-registered analysis lock                           |
|                            | Metadata and archive packages (REMBI, IDR)             |
|                            | One-click Zenodo archive with a DOI                    |

Alpha species

 Plasmodium

 Candida

 Trypanosoma

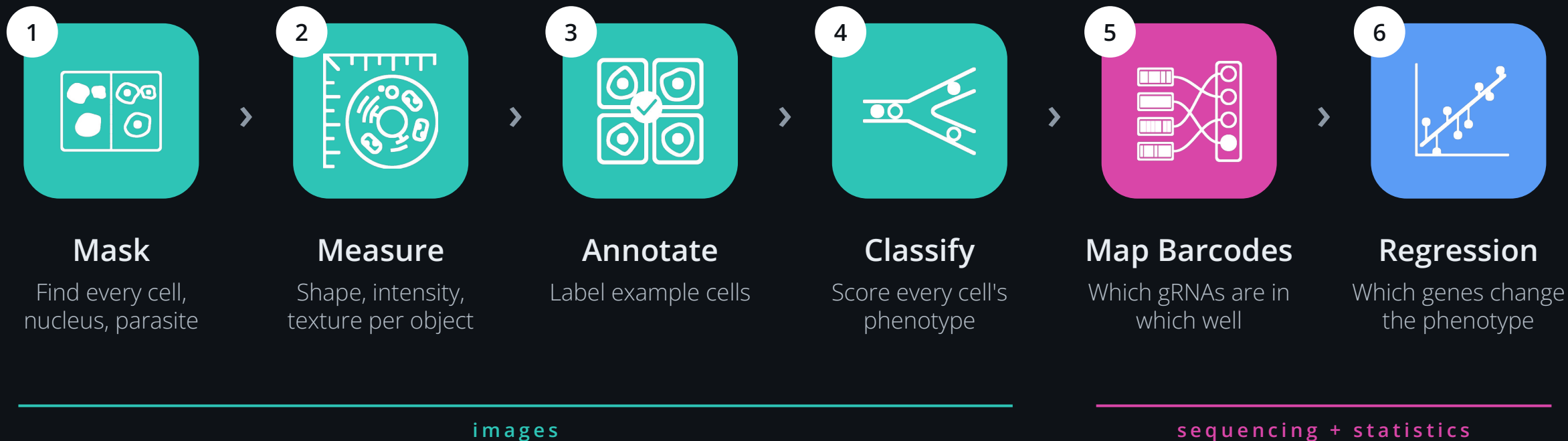
 Leishmania

 Giardia

 Virus

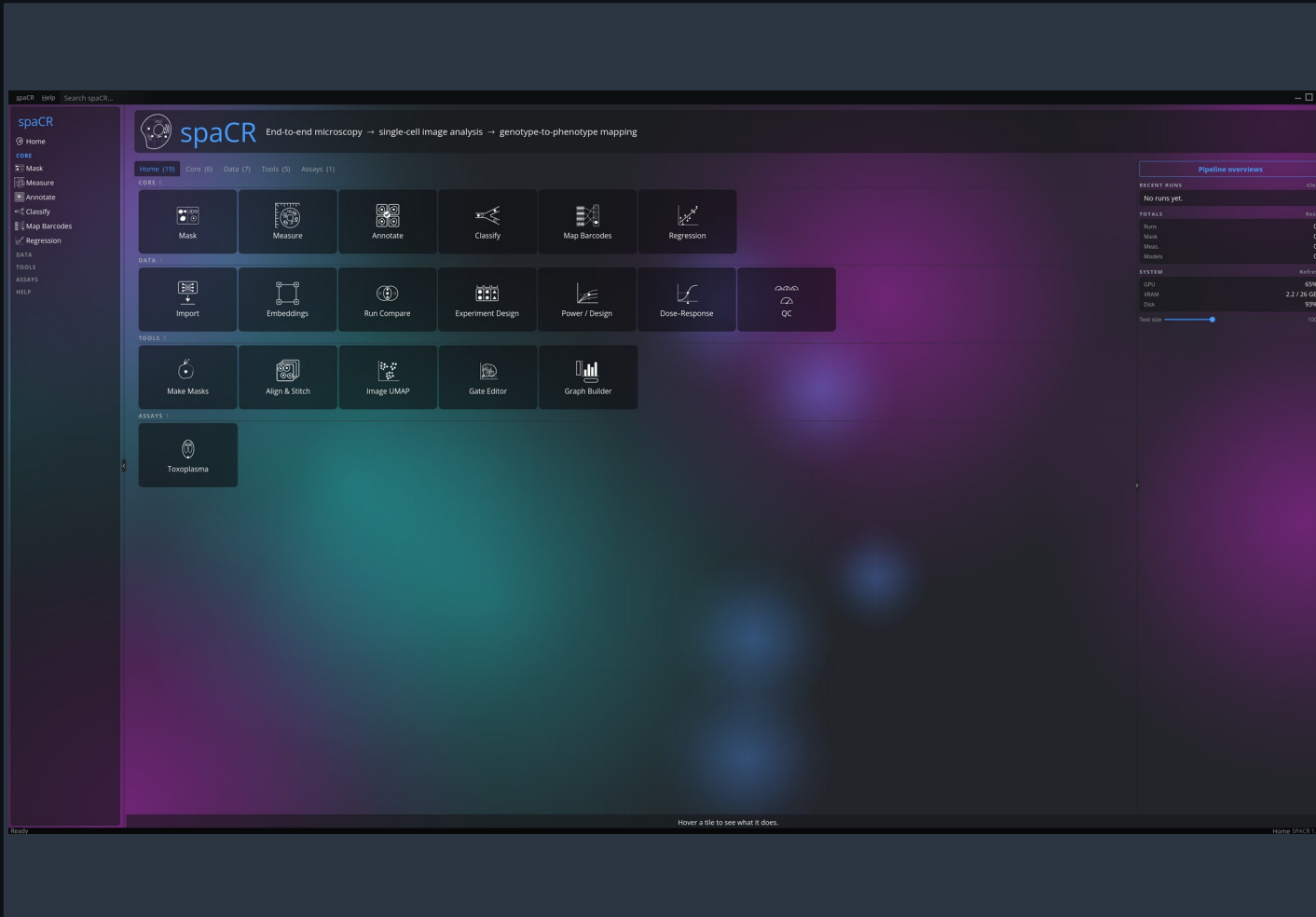
 Mammalian

# Six steps from plate to hit list



**One project, one database.** Images, masks, crops, measurements, annotations, predictions, barcodes and well IDs all live in one SQLite file (measurements.db). Every module reads from it and writes back to it, and every run saves its settings so it can be replayed.

# 19 modules in one desktop app



*The home screen: every module is a tile, grouped by what it is for*

6

## Core

Mask · Measure · Annotate · Classify ·  
Map Barcodes · Regression

7

## Data

Import · Embeddings · Run Compare ·  
Experiment Design · Power · Dose-  
Response · QC

5

## Tools

Make Masks · Align & Stitch · Image  
UMAP · Gate Editor · Graph Builder

1

## Assays

Toxoplasma compartments and assays;  
other organisms appear when alpha  
species are enabled.

Plus ~40 sub-tools inside the modules.  
Ctrl+K opens a command palette that  
reaches every one of them.

# Runs on a laptop, a workstation or a cluster

## Desktop app

Point-and-click Qt interface. Live previews, a console per module, settings search, recipes, and a Run button.

## Headless

The same modules and settings files run without the window, on a server or a cluster: or send jobs there from the app.

## Reproducible

Every run records its settings. spacerepro RUN\_DIR replays it. Run History and Run Compare show what changed.

4

## kinds of graphics card

NVIDIA, AMD, Apple and Intel: or just the CPU

10

## interface languages

switch in Preferences without restarting

77

## interactive tutorials

narrated videos from install to hit investigation

3.9–3.14

## Python versions

3.12 recommended; Linux, macOS, Windows

## INTRODUCTION

# Install spaCR

### Desktop installer

*Easiest. Bundles its own Python: no conda needed.*

```
GitHub → Releases → latest
Windows .exe
macOS .pkg (Intel + Apple silicon)
Linux  chmod +x SpaCR-*.run && ./SpaCR-*.run
```

### pip (conda env)

*Most flexible; add optional extras.*

```
conda create -n spacr python=3.12 -y
conda activate spacr
pip install "spacr[qt]"
spacr
```

### conda-forge

*One command into an existing env.*

```
conda create -n spacr python=3.12 -y
conda activate spacr
conda install conda-forge::spacr
spacr
```

### From source

*For development (nightly branch).*

```
git clone --branch nightly \
  https://github.com/EinarOlafsson/spacr
cd spacr && pip install -e .
spacr
```

**Something off?** `spacr-doctor` checks the install and says how to fix it. Headless server: `pip install spacr` (no Qt).

# 01

## Core pipeline

From raw plate images and FASTQ reads to a ranked list of genes.



Mask



Measure



Annotate



Classify



Map Barcodes



Regression





CORE · 1 OF 6

STABLE

# Mask: segmentation

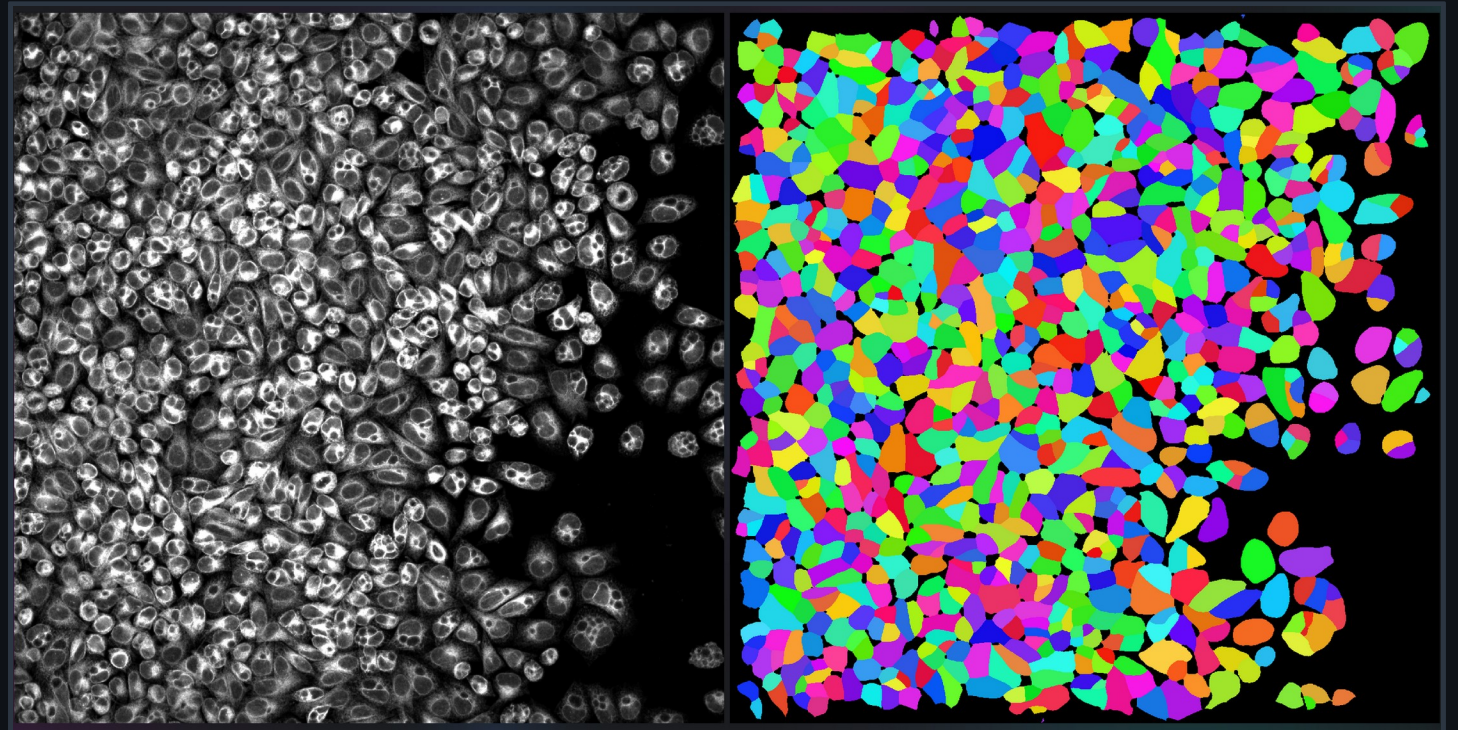
*Finds and outlines every cell, nucleus, parasite and organelle in your images.*

## WHAT YOU CAN DO

- Reads files from most microscopes (TIFF, Leica, Zeiss, Nikon)
- Cellpose-SAM by default; other segmentation models optional
- Cell, nucleus, pathogen and up to 26 organelle classes
- Plug in fine-tuned Model Zoo models (e.g. Toxoplasma vacuoles)
- 2-D, 3-D z-stacks and time-lapse; live preview before a full run

## OUTPUT

- One mask per object type, aligned to the images
- Overlay images and a quality score per field
- The settings used, saved with the run



*Live preview: host cells (left) and the label mask Cellpose-SAM returns (right)*

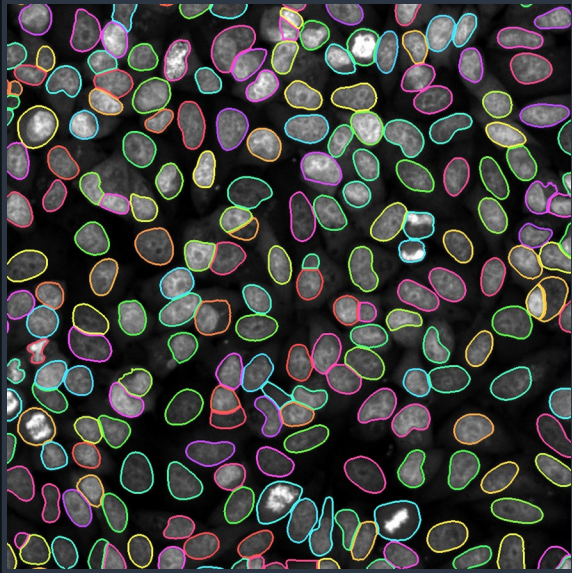




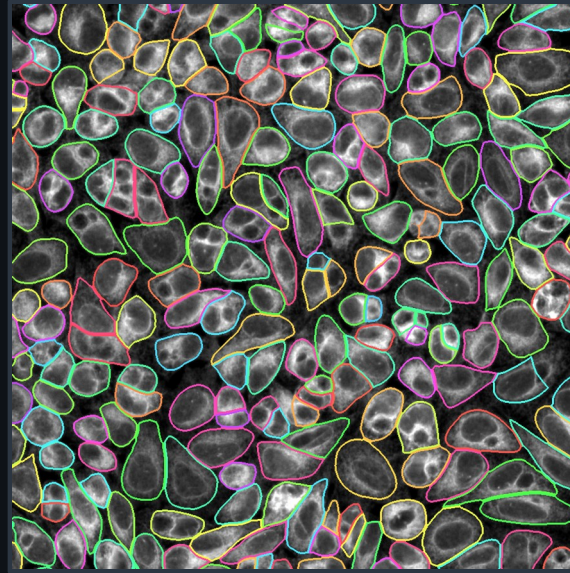
CORE · 1 OF 6

STABLE

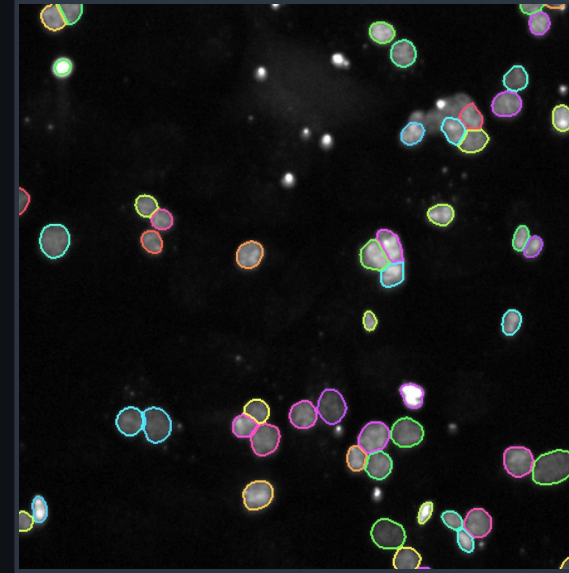
# Mask: every object class



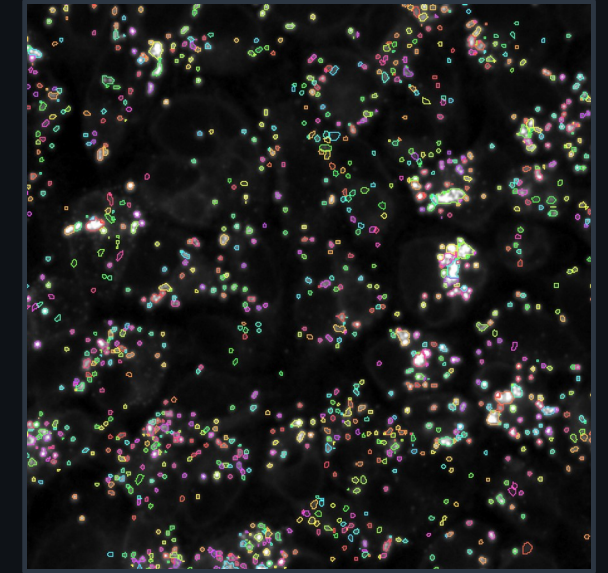
*Nuclei: Cellpose-SAM (Hoechst)*



*Cells: Cellpose-SAM (cell stain)*



*Pathogen: Toxoplasma, zoomed*



*Organelles: lipid droplets, zoomed*

## SUB-TOOLS INSIDE THIS MODULE

### Timelapse

Follow each cell and parasite from frame to frame in movies

### OPS

Optical pooled screens: read the barcodes directly in the images (alpha)

### Cellpose flows

See why two cells were merged, or one was split



# Measure: features and crops

*Turns every segmented object into a row of numbers: shape, brightness, texture and position.*

## WHAT YOU CAN DO

- Shape, size, brightness and texture of every object
- Position: neighbours, distances, touching the edge
- Channel overlap; measure inside a chosen region
- Saves object crops for Annotate and Classify
- 3-D objects measured in  $\mu\text{m}$
- Checks free RAM first and sizes its workers to fit

## OUTPUT

- One table per object type (cells, nuclei, parasites, ...)
- Per-object PNG crops

## SUB-TOOLS INSIDE THIS MODULE

### Illumination

Flat-field correction estimated from the plate itself

### AnnData export

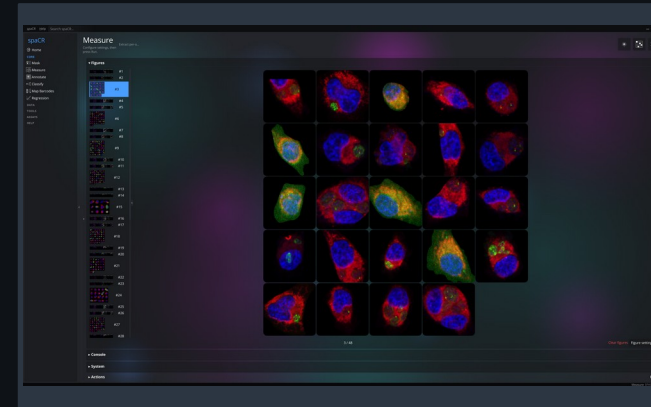
Export for single-cell analysis tools (scanpy)

### Motility assay

Track velocity and straightness, split by infection state

### Database Browser

Browse, filter and export any project table



*Object crops from a completed sixteen-field Measure run*

*The cell table in the Database Browser*



CORE · 3 OF 6

STABLE

# Annotate: label cells by hand

*Label single-cell crops so a classifier can learn what each phenotype looks like.*

## WHAT YOU CAN DO

- Pages through a grid of crops from the project
- Click, or press 1–9 with auto-advance; 0 clears, u undoes
- Several annotation columns side by side (e.g. two annotators)
- Active learning: shows the cells the model is least sure about first
- Change channels and colours for display only; files stay untouched

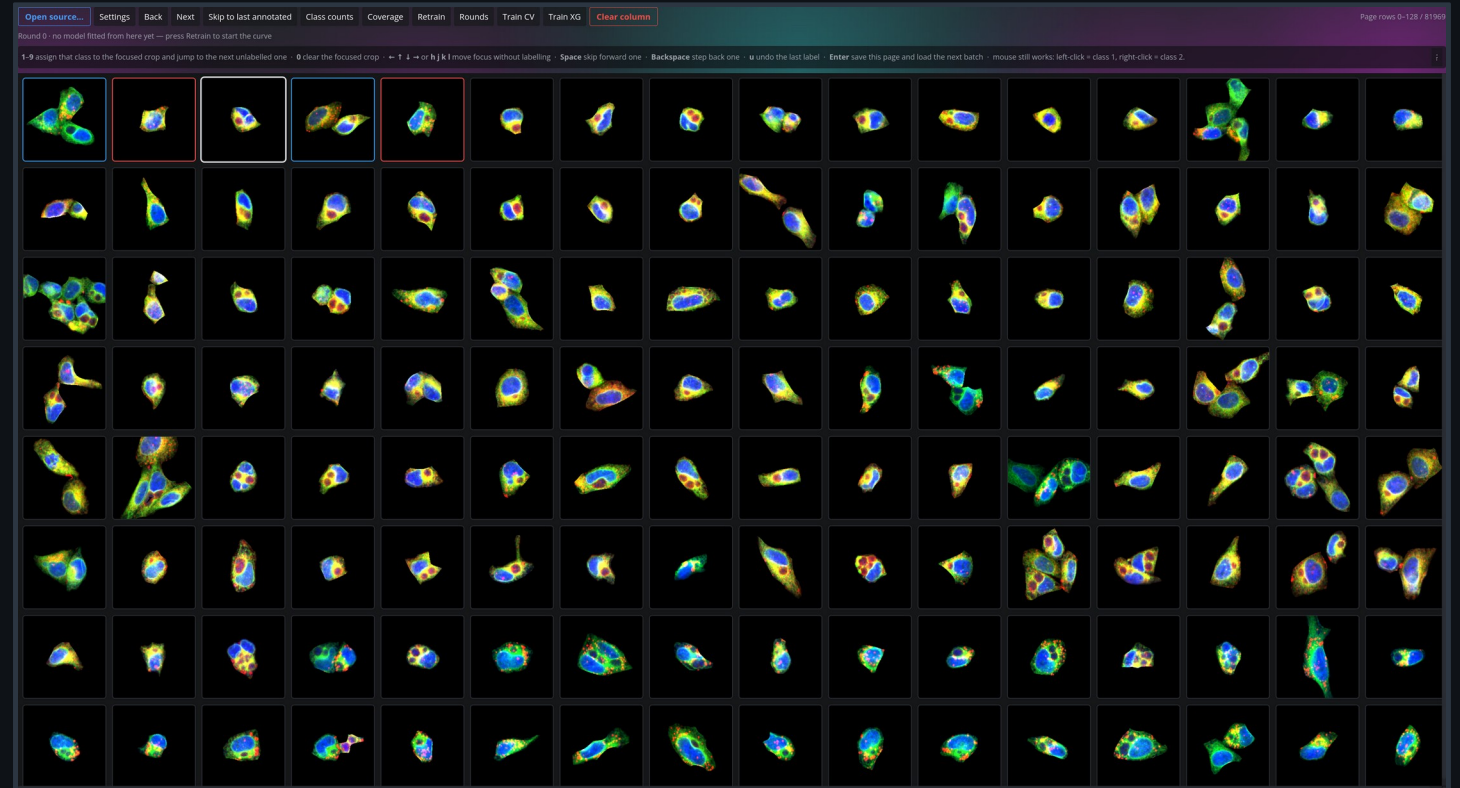
## OUTPUT

- Labels in an annotation column of the project
- Class counts and coverage per well

## SUB-TOOLS INSIDE THIS MODULE

### Annotator Agreement

How often two people label a cell the same way; and a list of the cells they disagreed on



*Annotation grid. Selected crops are outlined; the queue can be ranked by model uncertainty*





# Classify: score every cell

*Teach the computer what a phenotype looks like, then let it score every cell in the screen.*

## WHAT YOU CAN DO

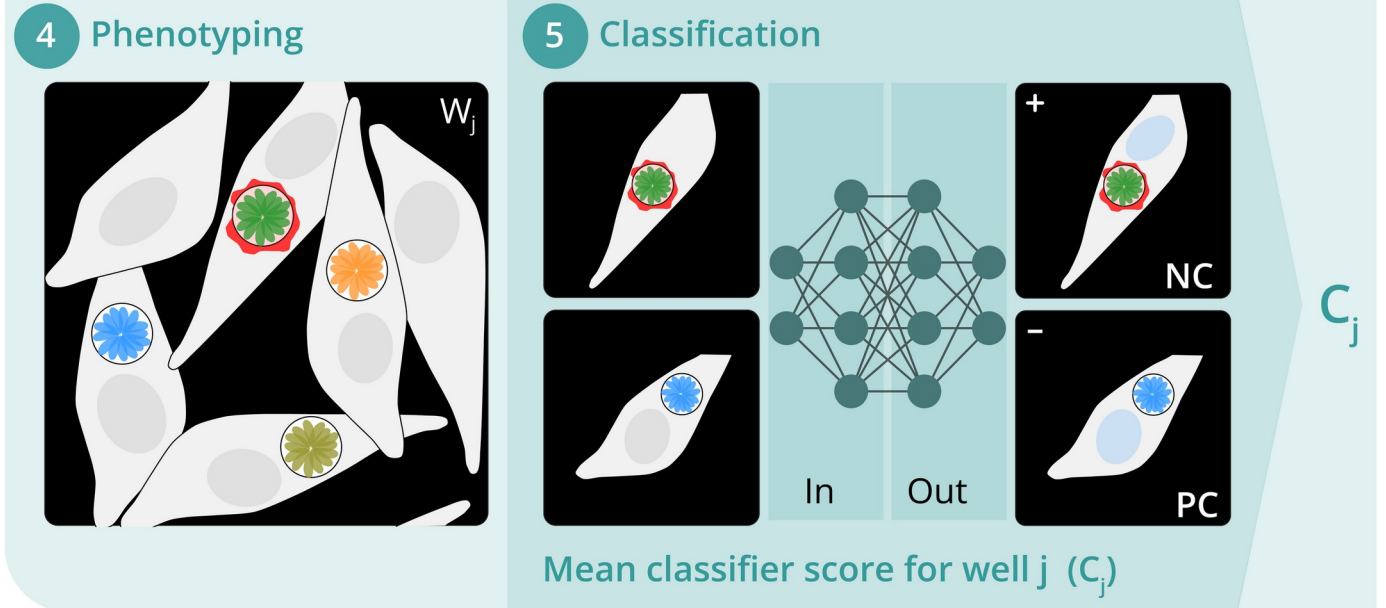
**Image model:** learns straight from the cell pictures (ResNet, EfficientNet, ViT, MaxViT, ...)

**Measurement model:** learns from the measured features (XGBoost, random forest, ...)

- Examples come from control wells or from your Annotate labels
- Tested on wells it never trained on, so the accuracy is honest
- Scores every cell and writes the result back to the project

## OUTPUT

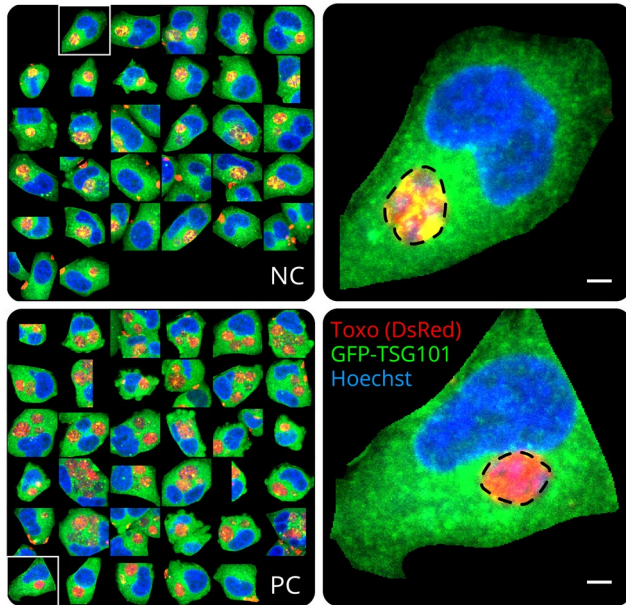
- A class and a probability for every cell
- The mean score per well → input to Regression



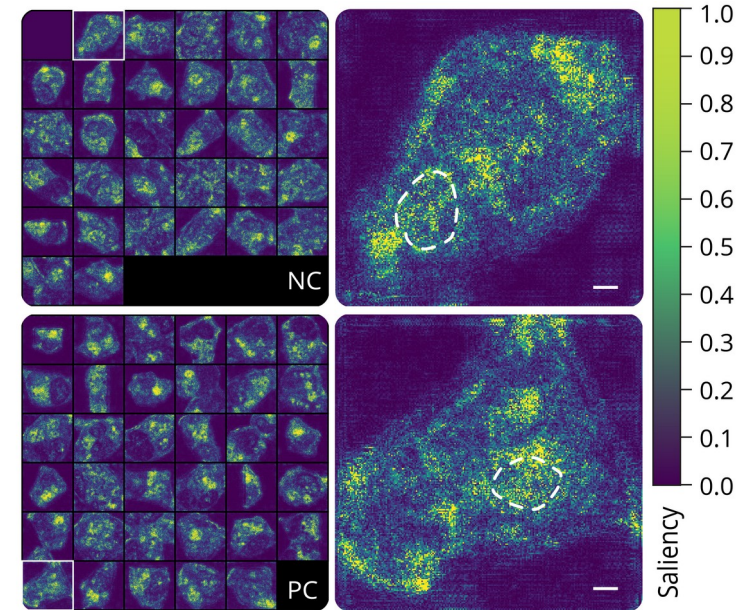
*Each cell is scored by the classifier; a well's score is the mean of its cells (preprint Fig. 2)*



# Classify: image model



Input: single-cell crops from negative (NC) and positive (PC) control wells



Saliency: where MaxViT looked: at the vacuole in PC cells

## SUB-TOOLS INSIDE THIS MODULE

### Activation Maps

Show which pixels drove each decision

### Feature Explorer

Which measurements separate the classes best

### Classifier Evaluation

Accuracy and mistakes per class and per plate, on unseen wells

### Training Runs

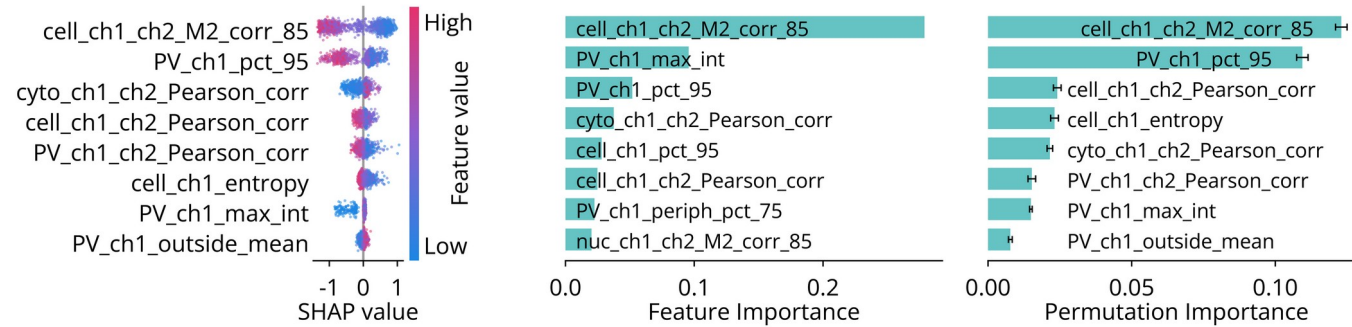
Compare training curves between runs

### Explain CV Model

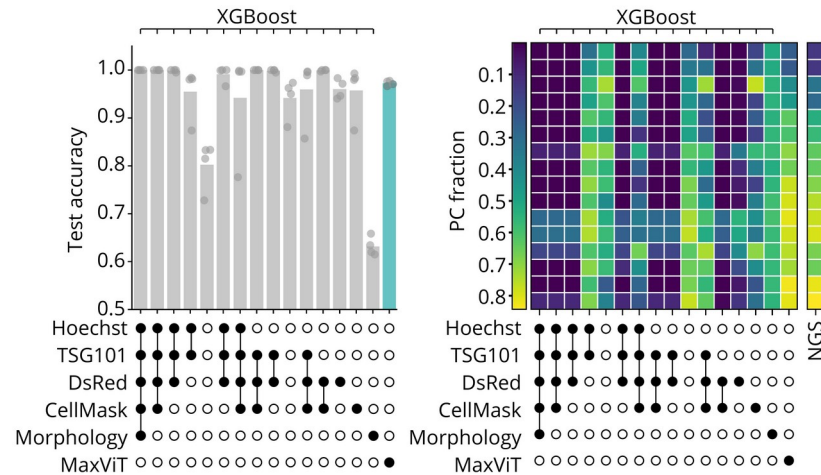
Explain an image model in terms of measurements



# Classify: measurement model



*XGBoost on the measurements: which measurements decide the class, three ways of ranking them*



*Accuracy with different channel combinations (grey, XGBoost) vs the image model (teal, MaxViT)*



CORE · 5 OF 6

STABLE

# Map Barcodes: which guides, which well

*Reads the sequencing data and works out which gRNAs are in each well, and how much of each.*

## WHAT YOU CAN DO

- Reads the row, column and gRNA barcodes from the sequencing files
- You give it the barcode lists and where they sit in the read
- Paired-end or single reads; set how many mismatches to allow
- Quick test on a small part of the file first

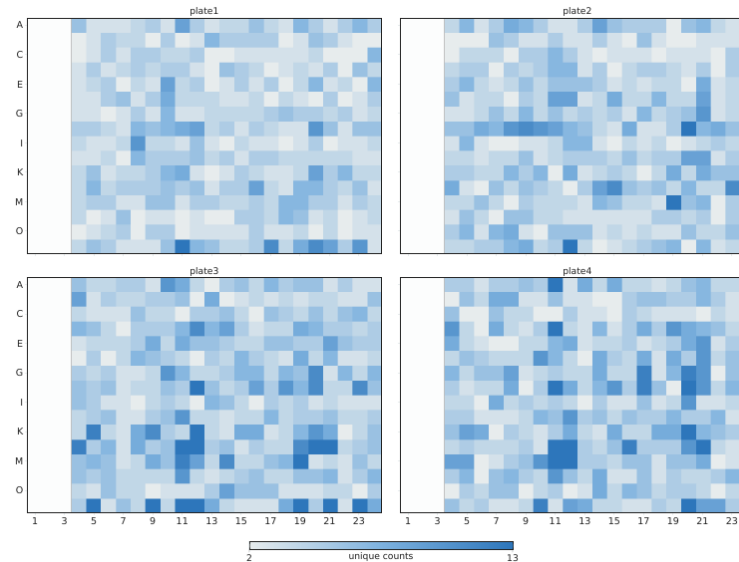
## OUTPUT

- Guide counts per well
- QC table and run manifest
- gRNA fraction per well → input to Regression

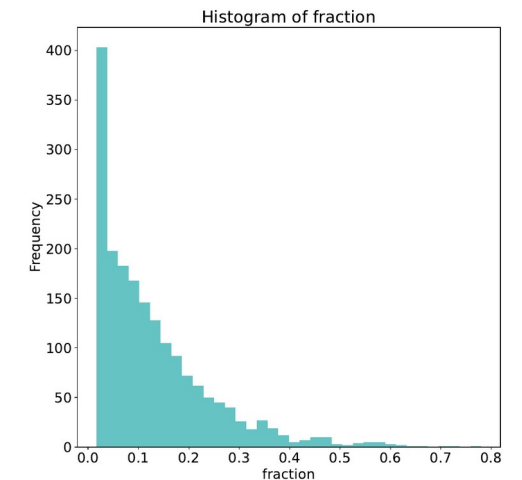
## SUB-TOOLS INSIDE THIS MODULE

### Barcode QC

Reads per well, reads that did not match, library coverage: and the cut-off that gives the expected number of gRNAs per well



*Unique gRNAs per well, four plates*



*gRNA fraction per well*





# Regression: from wells to genes

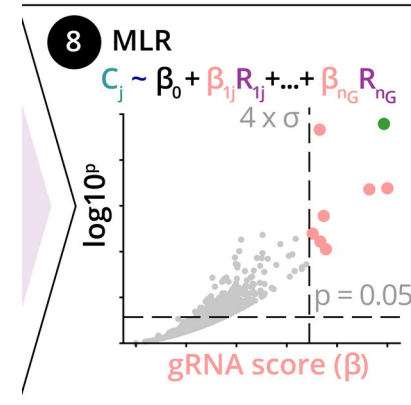
*Works out which genes change the phenotype, and ranks them.*

## WHAT YOU CAN DO

- Pairs each well's score with the fraction of each gRNA in that well
- Default: a mixed model where the gRNAs of a gene are grouped
- Other models for counts, fractions or skewed data
- Permutation test: shuffles gRNAs to check a hit is not chance

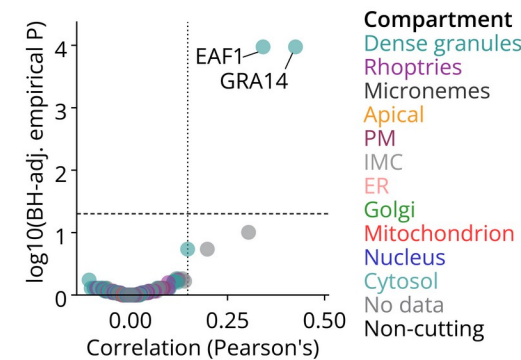
## OUTPUT

- A ranked table of genes with effect size and p-value
- Volcano, top effects and quality-check plots



G: gRNA  
R: gRNA read f  
C: Classifier score  
 $\beta$ : gRNA score  
 $\beta_0$ : Intercept  
W: Well  
i: gRNA index  
j: Well index

*The model: a well's score = its gRNA fractions × each gene's effect (preprint Fig. 2)*



*The result: EAF1 and GRA14 stand out (preprint Fig. S7)*



# Regression: choose the model

*21 model families, each explained in plain words, with what it assumes about your data.*

## WHAT YOU CAN DO

**Mixed:** the gRNAs of a gene grouped, genes allowed to vary

**OLS / WLS:** classic least squares, optionally weighted by cells per well

**GLM, Poisson, beta, logit:** for counts, fractions and yes/no readouts

**Lasso, ridge, elastic net, group lasso, horseshoe:** when there are many more genes than wells

**Robust, quantile, RRA:** when a few wells are extreme

## OUTPUT

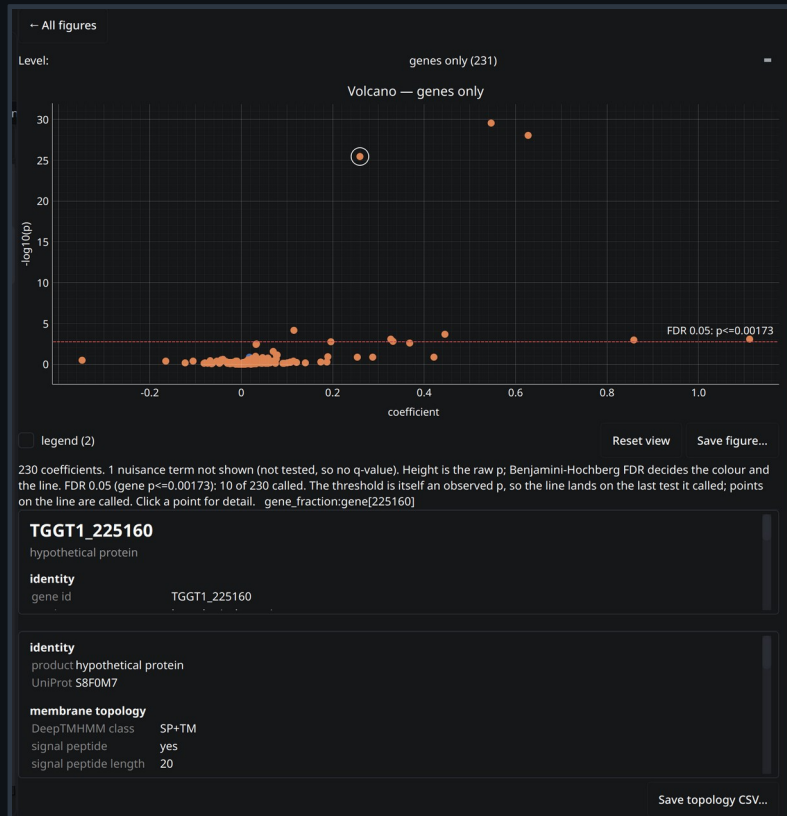
- Plate, row and column covariates; permutation test on gRNAs
- The formula and what it assumes, shown for the chosen model

```
ols — parametric: least squares: normal errors of constant variance, every well equal  
auto — chosen from the response by check_distribution  
mixed — parametric: guides nested in genes as random effects, those effects normal; guides that disagree widen their gene's interval  
beta — parametric: a fraction strictly inside 0 and 1, beta distributed; exact zeros and ones have to be moved off the boundary first  
elasticnet — parametric: both penalties mixed by l1_ratio: sparse, but correlated guides of a gene are kept or dropped together; no p value from the fit -- ranked by bootstrap selection frequency  
glm — parametric: a family chosen from the response, whose mean-variance relationship is then assumed to hold  
group_lasso — parametric: a gene's guides penalised as one block, so a gene is in or out as a whole rather than by its best guide; no p value from the fit -- ranked by bootstrap selection frequency  
hinge — parametric: a linear boundary between a positive and a negative class: the response is thresholded, so the question is which class a well is in, not by how much  
horseshoe — parametric: Bayesian shrinkage assuming most guides do nothing and a few do a lot; the interval is a posterior, not a p-value  
lasso — parametric: normal errors plus an L1 penalty: the truth is sparse and one guide of a correlated group carries the effect; no p value from the fit -- ranked by bootstrap selection frequency  
logit — parametric: a fraction as a binomial proportion, log-odds linear in the guides, weighted by the cells behind the well  
ols — parametric: least squares: normal errors of constant variance, every well equal  
poisson — parametric: counts, variance equal to the mean, log cell count as exposure so the effect is on a per-cell rate  
probit — parametric: the same binomial proportion as logit through a normal link, so the tails are lighter  
quasi_binomial — parametric: a fraction with binomial mean-variance, freely rescaled, so overdispersion is absorbed not modelled  
ridge — parametric: normal errors plus an L2 penalty: the effect is shared across correlated guides rather than assigned to one  
wls — parametric: least squares weighted by cell count: normal errors whose variance falls as a well holds more cells  
huber — robust/semiparametric: squared loss near the centre and linear in the tails, so a handful of bright wells cannot set a coefficient  
quantile — robust/semiparametric: a quantile of the response rather than its mean, which assumes nothing about the shape of the errors; tau=0.9 asks whether the guide moves the TOP of the distribution  
rlm — robust/semiparametric: a linear model fitted by an M-estimator, so extreme wells are downweighted rather than assumed absent  
spline — robust/semiparametric: least squares, but each continuous covariate is free to bend; guides keep one coefficient each, so the volcano is unchanged. Semiparametric, not fully nonparametric  
rra — rank-based: only the ORDER of the wells, so no distribution at all; its p value is a permutation p value over guide ranks
```

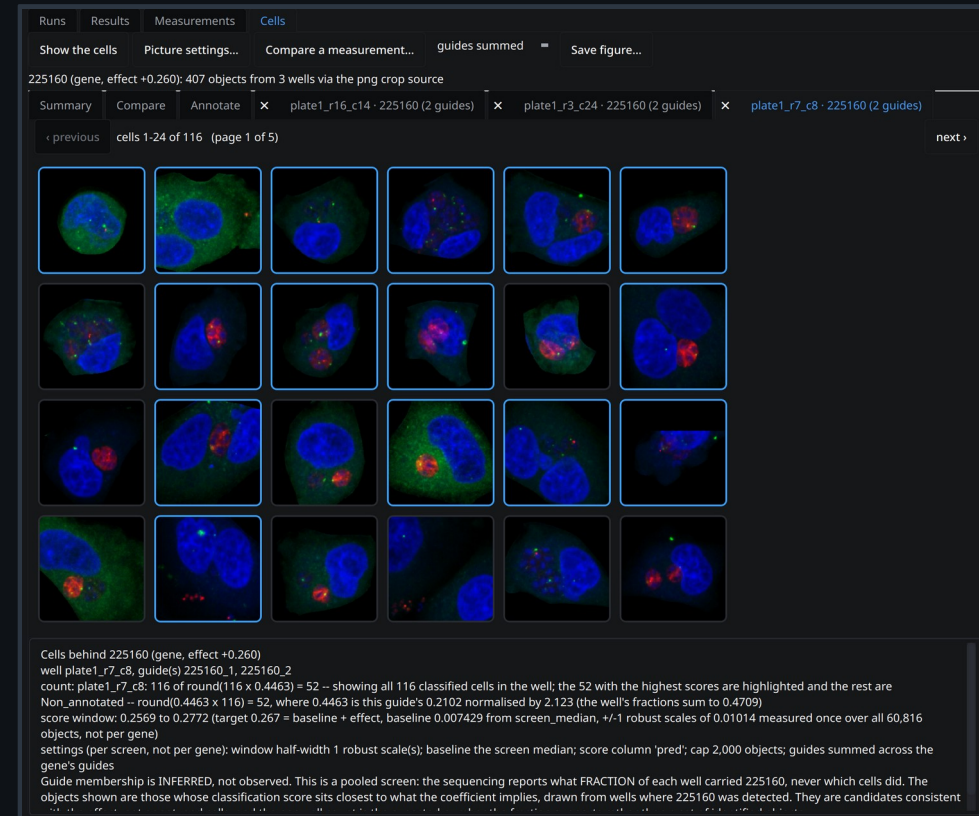
*Regression type: every model with the assumption it makes (OLS selected)*



# Regression: pick a gene, see its cells



Interactive volcano: click a point. EAF1 (TGGT1\_225160) is selected, its gene card below



Cells tab: the cells in an EAF1 well whose scores fit the effect, with why they were picked

## SUB-TOOLS INSIDE THIS MODULE

### Volcano Explorer

Publication version of the volcano;  
vector export

### Investigate Hit

Test the hit at the cell level against  
control wells

### Hit List

Ranked genes with intervals and gRNA  
agreement

### Prediction Profiler

How the prediction changes as one  
gene's fraction changes



# Regression: which assumptions hold?

*Every run checks its own assumptions and says, in red, which ones fail and what to do.*

## WHAT YOU CAN DO

**Equal variance:** Breusch-Pagan test on the residuals

**Normal residuals:** skew, kurtosis, D'Agostino test

**Independence:** Durbin-Watson; wells on one plate share handling

**Influence:** leverage and Cook's distance per well

**Collinearity:** VIF and condition number

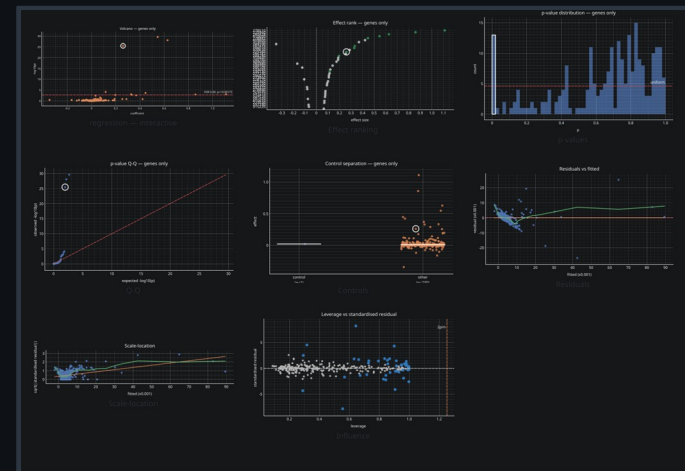
**Calibration:** p-value histogram and genomic inflation

## OUTPUT

- A run summary: the answer, what was fitted, each assumption with its verdict
- Residual, Q-Q, scale-location and influence plots

| ASSUMPTIONS, EACH WITH ITS TEST AND ITS VERDICT |                                                                                                                                                                                                                                                                                                                                                       |
|-------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>equal variance</b>                           | Breusch-Pagan $p = 0.000941$ (REJECTED at 0.05); White's test was not run: it squares every column pair, so it is attempted only on designs of at most 30 columns and skipped when singular — at least one test rejects equal variance, so the standard errors here are the wrong width; cov_type='HC3', or weighting wells by cell count, is the fix |
| <b>normality of residuals</b>                   | skew -0.10, excess kurtosis +27.41 over 425 residuals; D'Agostino $K^2 p = 1.22e-30$ (REJECTED at 0.05)                                                                                                                                                                                                                                               |
| <b>independence / clustering</b>                | Durbin-Watson = 1.84 (2 is none) — no first-order autocorrelation. wells within a plate share reagents, imaging and handling; this fixed-effects fit ASSUMES independent errors and models position with rowID / columnID terms rather than a plate random effect — regression_type='mixed' is the fit that relaxes it                                |
| <b>influence and leverage</b>                   | max leverage 1 against the 2p/n guide of 1.25 (0 above it); max Cook's D 8.6, 42 observation(s) above the 4/n rule of 0.00941                                                                                                                                                                                                                         |
| <b>multicollinearity</b>                        | max VIF 33.9, 15 above 10; scaled condition number 46.59 — moderate to strong collinearity — standard errors are inflated; 0 predictor pair(s) correlated at 1.0 (worst 0.35)                                                                                                                                                                         |
| THE CALL                                        |                                                                                                                                                                                                                                                                                                                                                       |
| <b>correction</b>                               | fdr_bh (Benjamini-Hochberg FDR)                                                                                                                                                                                                                                                                                                                       |
| <b>alpha</b>                                    | 0.05 (fdr_alpha, the level the correction targets)                                                                                                                                                                                                                                                                                                    |
| <b>coefficients tested</b>                      | 230 of 231 rows are hypotheses; the other 1 are covariates (the intercept and the row / column terms), which are fitted so the real effects are estimated cleanly and are not themselves tested                                                                                                                                                       |
| <b>coefficients called</b>                      | 10 — q value below 0.05                                                                                                                                                                                                                                                                                                                               |
| <b>critical raw P</b>                           | 0.001726 — every test with a raw P at or below this was called by fdr_bh at alpha=0.05. It is NOT alpha, and it is far below it                                                                                                                                                                                                                       |
| <b>genomic inflation</b>                        | 0.4601 — DEFLATED: the tests are conservative; the null is not flat and the P values are larger than the evidence warrants                                                                                                                                                                                                                            |
| <b>effect-size cut</b>                          | none — 1 control coefficient(s) is not enough to measure a spread                                                                                                                                                                                                                                                                                     |
| <b>not applicable here</b>                      | 2 field(s) — positive control rank, positive control percentile. Why each does not apply is under NOT APPLICABLE, AND WHY at the end.                                                                                                                                                                                                                 |

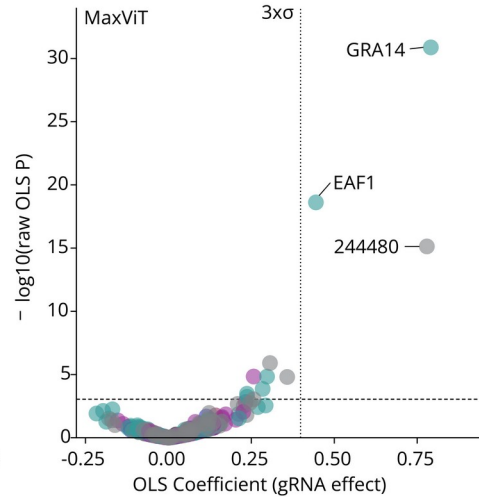
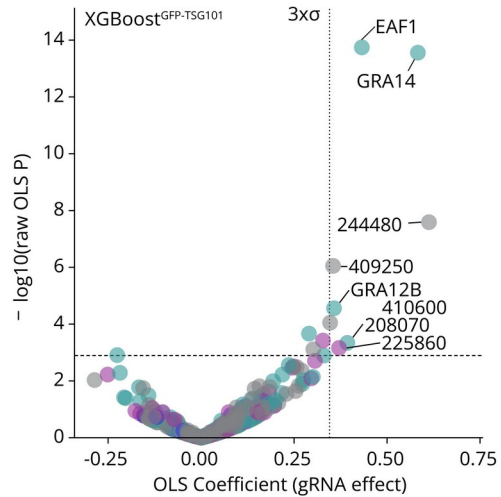
*Summary tab: each assumption with its test and verdict; equal variance and normality rejected (red)*



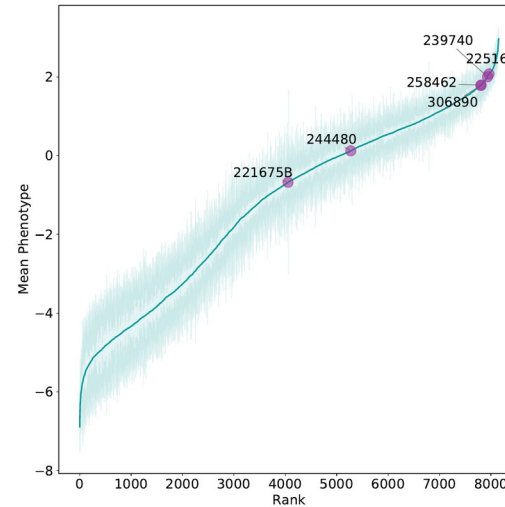
*The diagnostic plots behind the verdicts*



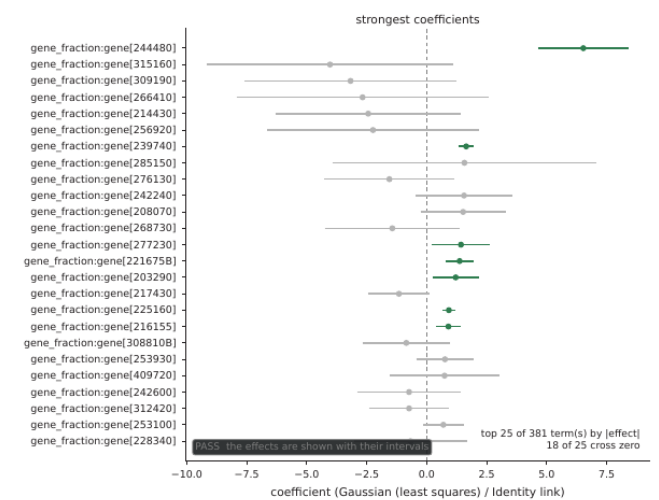
# Regression: the hit list



Volcanos from the screen: XGBoost (left) and MaxViT (right) scores both find EAF1 and GRA14 (preprint Fig. 5)



Genes ranked by phenotype



Strongest effects with 95% intervals

## SUB-TOOLS INSIDE THIS MODULE

### Volcano Explorer

Click a point to see the gene; export for publication

### Hit List

Ranked genes with confidence intervals and gRNA agreement; export as a table

### Investigate Hit

Jump from a gene to its wells and cells

### Diagnostics

Checks that the model fits the data (plate, row and column effects)

### Methods & Results

Drafts the methods text from the run itself

### Prediction Profiler

How a prediction changes across one input

### Parameter Sweep

Re-run across settings to see how stable the hits are



# 02

## Data

Bring data in, plan experiments, and check that a run can be trusted.



Import



Embeddings



Run Compare



Experiment Design



Power / Design



Dose-Response



QC



# Import: bring outside data in

*Turns images, masks and tables from other tools into a spaCR project.*

## WHAT YOU CAN DO

- Pair images, instance masks and a measurement CSV
- Match their column names to spaCR's, converting units
- Choose what happens when names collide
- Preview before anything is written; save and reuse the mapping

## OUTPUT

- A spaCR project that records where the data came from

| Their column       | spaCR column               | Transform | Unit in | Unit out | Note                                                                                                              |
|--------------------|----------------------------|-----------|---------|----------|-------------------------------------------------------------------------------------------------------------------|
| AreaShape_Area_um2 | foreign_areashape_area_um2 | area      | um^2    | px^2     | unit um^2 read from the column name; spaCR measures in px^2, so this needs a pixel size                           |
| MeanIntensity_ER   | foreign_meanintensity_er   | identity  |         |          | copied unchanged; declare unit_in/unit_out if it needs scaling                                                    |
| Metadata_Well      | foreign_metadata_well      | identity  |         |          | copied unchanged; declare unit_in/unit_out if it needs scaling                                                    |
| cell_area          | foreign_cell_area          | identity  |         |          | name suggests a area, but no unit is declared — set unit_in (and transform=area) if these are not already in px^2 |

*Column mapping: their name → spaCR column, with unit conversion*

| Source       | Target                               | Plate  | Well | Field | Channel | Z | T | From well | From field   | From channel | Z handling | Status  |
|--------------|--------------------------------------|--------|------|-------|---------|---|---|-----------|--------------|--------------|------------|---------|
| fov01_C1.tif | plate1_A01_T0001F001L01A01Z01C01.tif | plate1 | A01  | 1     | 1       | 1 | 1 | A01       | images/fov01 | C1           | keep       | planned |
| fov01_C2.tif | plate1_A01_T0001F001L01A01Z01C02.tif | plate1 | A01  | 1     | 2       | 1 | 1 | A01       | images/fov01 | C2           | keep       | planned |
| fov01_C3.tif | plate1_A01_T0001F001L01A01Z01C03.tif | plate1 | A01  | 1     | 3       | 1 | 1 | A01       | images/fov01 | C3           | keep       | planned |
| fov01_C4.tif | plate1_A01_T0001F001L01A01Z01C04.tif | plate1 | A01  | 1     | 4       | 1 | 1 | A01       | images/fov01 | C4           | keep       | planned |
| fov02_C1.tif | plate1_A01_T0001F002L01A01Z01C01.tif | plate1 | A01  | 2     | 1       | 1 | 1 | A01       | images/fov02 | C1           | keep       | planned |
| fov02_C2.tif | plate1_A01_T0001F002L01A01Z01C02.tif | plate1 | A01  | 2     | 2       | 1 | 1 | A01       | images/fov02 | C2           | keep       | planned |
| fov02_C3.tif | plate1_A01_T0001F002L01A01Z01C03.tif | plate1 | A01  | 2     | 3       | 1 | 1 | A01       | images/fov02 | C3           | keep       | planned |
| fov02_C4.tif | plate1_A01_T0001F002L01A01Z01C04.tif | plate1 | A01  | 2     | 4       | 1 | 1 | A01       | images/fov02 | C4           | keep       | planned |

*Format Converter: plan for turning vendor files into spaCR's TIFF layout*

## SUB-TOOLS INSIDE THIS MODULE

### Import Images

Reads any microscope's folder and works out the naming itself

### Format Converter

Turns Nikon, Zeiss and Leica files into spaCR's layout

### External Masks

Measure masks made elsewhere, without re-segmenting



# Embeddings: a fingerprint per cell

*Gives every cell a label-free feature vector from a pretrained image model, next to the measured features.*

## WHAT YOU CAN DO

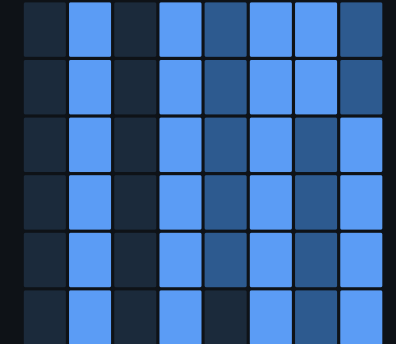
- Crops from the project (filter by object class or plate) or from a folder
- Choose the network: ResNet-18 (default), ResNet-50, ConvNeXt, ViT
- Each stain is described separately, or all together
- Runs on the graphics card when there is one



*cell crops*



**pretrained  
backbone**  
e.g. ResNet-18



*one row of numbers per cell*

## OUTPUT

- One row of numbers per cell, next to its measurements
- Which network was used is recorded

**Use it for** clustering and UMAPs without choosing features, finding cells that look like a hit, and checking whether a classifier is picking up something the measurements miss.



# Run Compare: what changed?

*Puts two runs of the same project side by side, so you know why the results moved.*

## WHAT YOU CAN DO

- Pick a project and any two of its runs
- Settings diff, grouped by settings panel
- Object, well and field counts per plate
- Hits that appeared, vanished or changed rank
- Incompatible runs are blocked unless you choose 'compare anyway'

## OUTPUT

- Side-by-side comparison tables

### Settings

|                 |                          |
|-----------------|--------------------------|
| pathogen_model  | cpsam → toxoplasma_pv_v3 |
| cell_diameter   | 90 → 110                 |
| regression_type | ols → mixed              |

### Counts per plate

|           |                   |
|-----------|-------------------|
| cells     | 182,410 → 176,902 |
| pathogens | 40,112 → 52,774   |
| wells     | 384 → 384         |

### Hits

|              |          |
|--------------|----------|
| appeared     | 3 genes  |
| vanished     | 1 gene   |
| rank changed | 12 genes |

*Illustration of the three comparison panels (values made up)*



# Experiment Design: plan the plate

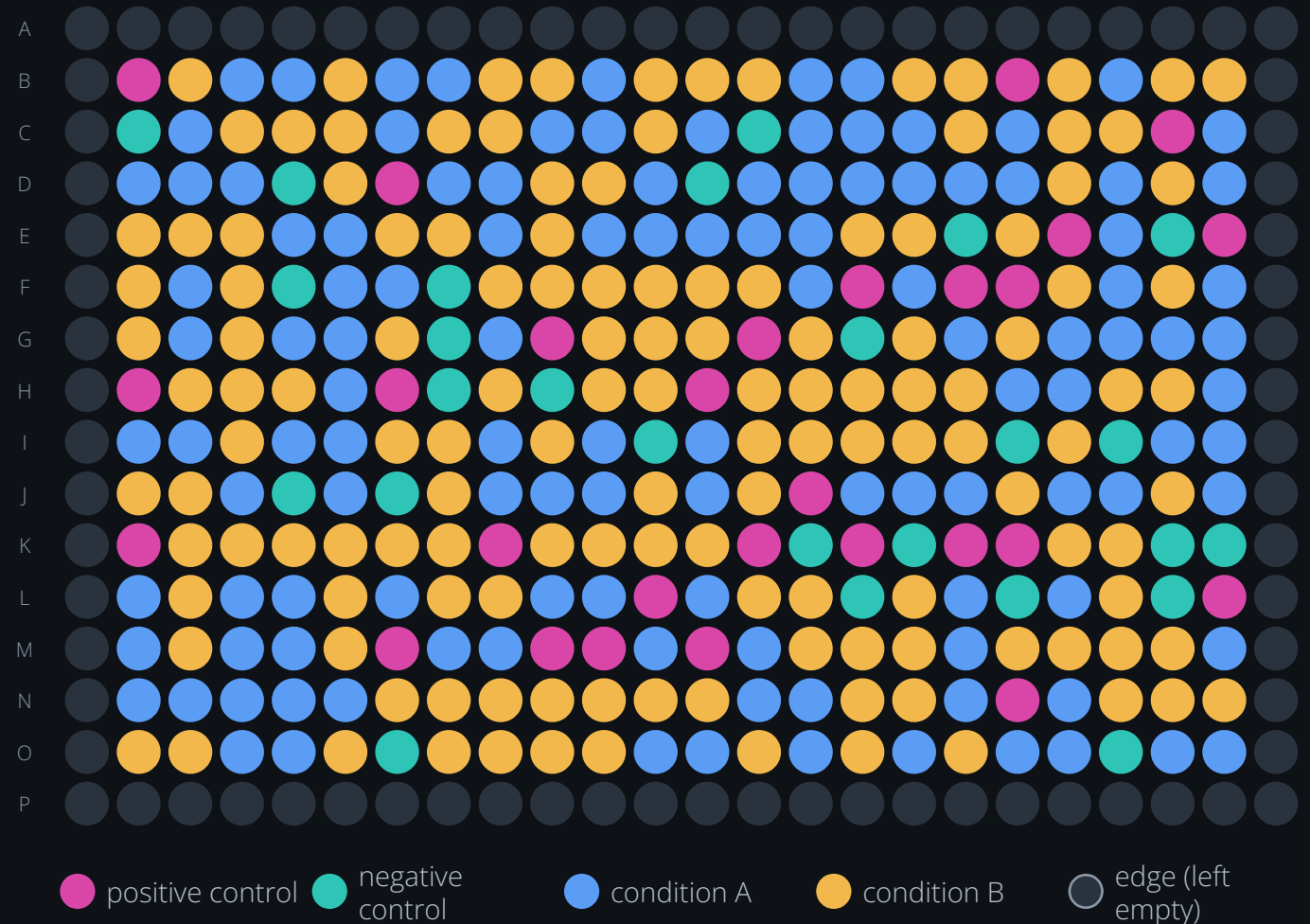
*Lay out conditions, controls and replicates before you image anything.*

## WHAT YOU CAN DO

- Assign conditions and control roles to wells
- Warnings for capacity, too few replicates, and row / column / edge confounding
- Randomise with a seed, so the layout can be rebuilt exactly

## OUTPUT

- A plate map that later spaCR steps use directly
- The settings used, so the layout can be rebuilt



*Illustration: a randomised 384-well layout with edge wells left empty*





# Power / Design: how big a screen?

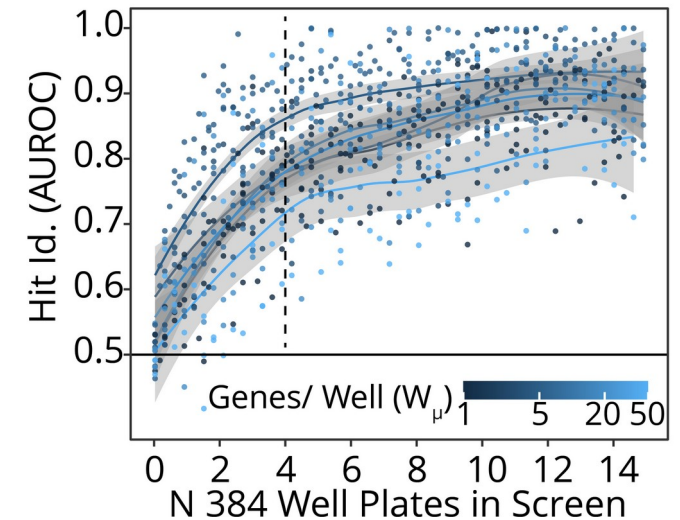
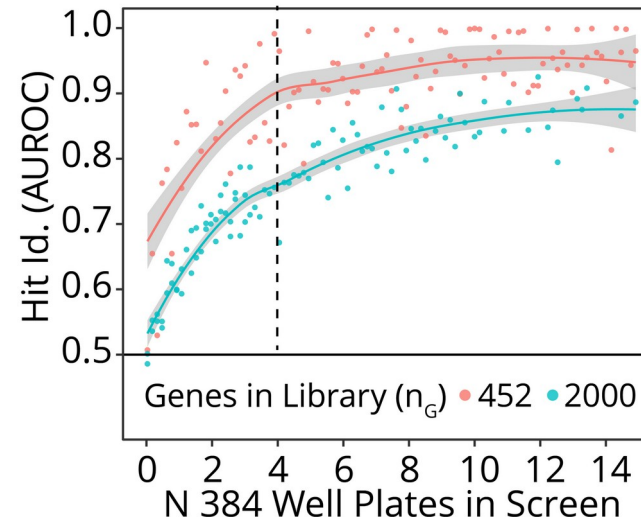
*Estimates how many cells per well and how many plates you need to find a real hit.*

## WHAT YOU CAN DO

- Enter the library: genes, gRNAs per gene, constructs per well
- Enter the plate format and number of plates
- Enter the classifier's background rate and the effect you expect
- Simulates the screen many times to see how often the real hit is found

## OUTPUT

- Detection probability vs cells per well
- A one-sentence answer, with caveats



*spaCRPower simulations: hit detection vs plates, by library size and genes per well (preprint Fig. 1D-E)*



# Dose-Response: honest EC50s

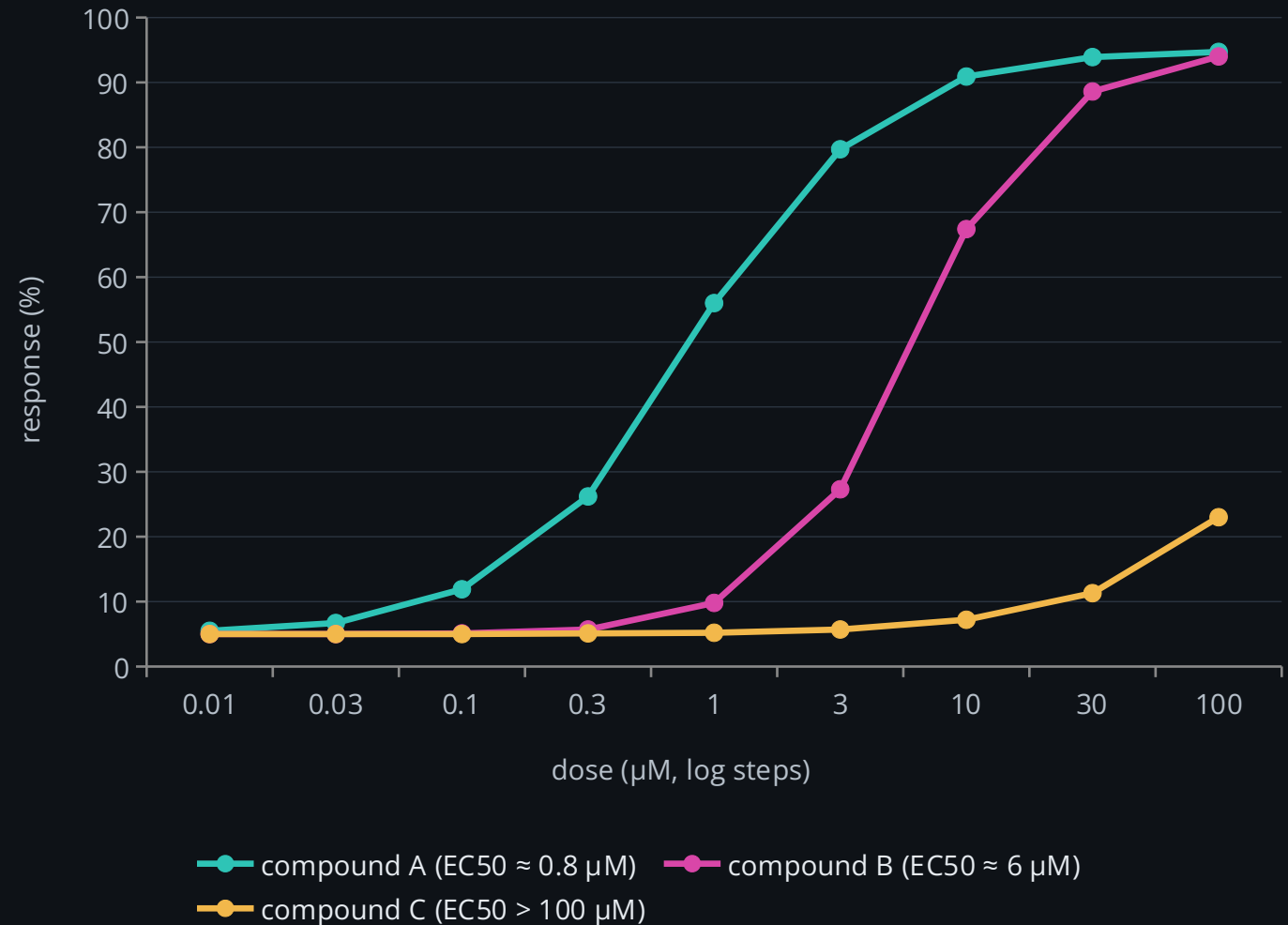
*Fits dose-response curves per gene or compound, and says so when an EC50 can't be pinned down.*

## WHAT YOU CAN DO

- Choose concentration, response and grouping columns
- Fits the standard S-shaped curve
- A confidence interval on every EC50

## OUTPUT

- Curves on a log-dose axis
- EC50 table with CI. Out-of-range → a bound ('EC50 > 30  $\mu$ M'); failed fits listed as 'refused'
- Warns when a curve does not fit well



*Schematic of the output (simulated curves)*



DATA · 7 OF 7

ALPHA

# QC: is this run usable?

*Five stored checks on one page. Reads what the run already wrote; recomputes nothing.*

## WHAT YOU CAN DO

- Segmentation QC scorecards
- Pixel size recorded
- Classifier tested on truly unseen wells
- Plate effects (row / column / edge)
- Annotator agreement

## OUTPUT

- Verdict cards: ok · warn · fail · missing · stale
- Nothing is written to disk

### Segmentation

OK

98% of fields pass

### Units

OK

0.325  $\mu\text{m}$  / pixel

### Classifier test

WARN

same well used to train and test

### Plate effects

FAIL

row gradient,  $p = 0.41$

### Annotator agreement

MISSING

no second annotator

*Illustration of the dashboard's verdict cards (values made up)*

## SUB-TOOLS INSIDE THIS MODULE

### Layer Viewer

Images and masks as layers you can switch on and off

### Control Charts

Follow a control over time and flag drift

### Outliers

Find unusual cells or wells

# 03

## Tools

Point these at a project: fix masks, stitch tiles, explore cells, gate populations, plot.



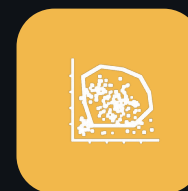
Make Masks



Align & Stitch



Image UMAP



Gate Editor



Graph Builder



TOOLS · 1 OF 5

BETA

# Make Masks: edit labels and boxes

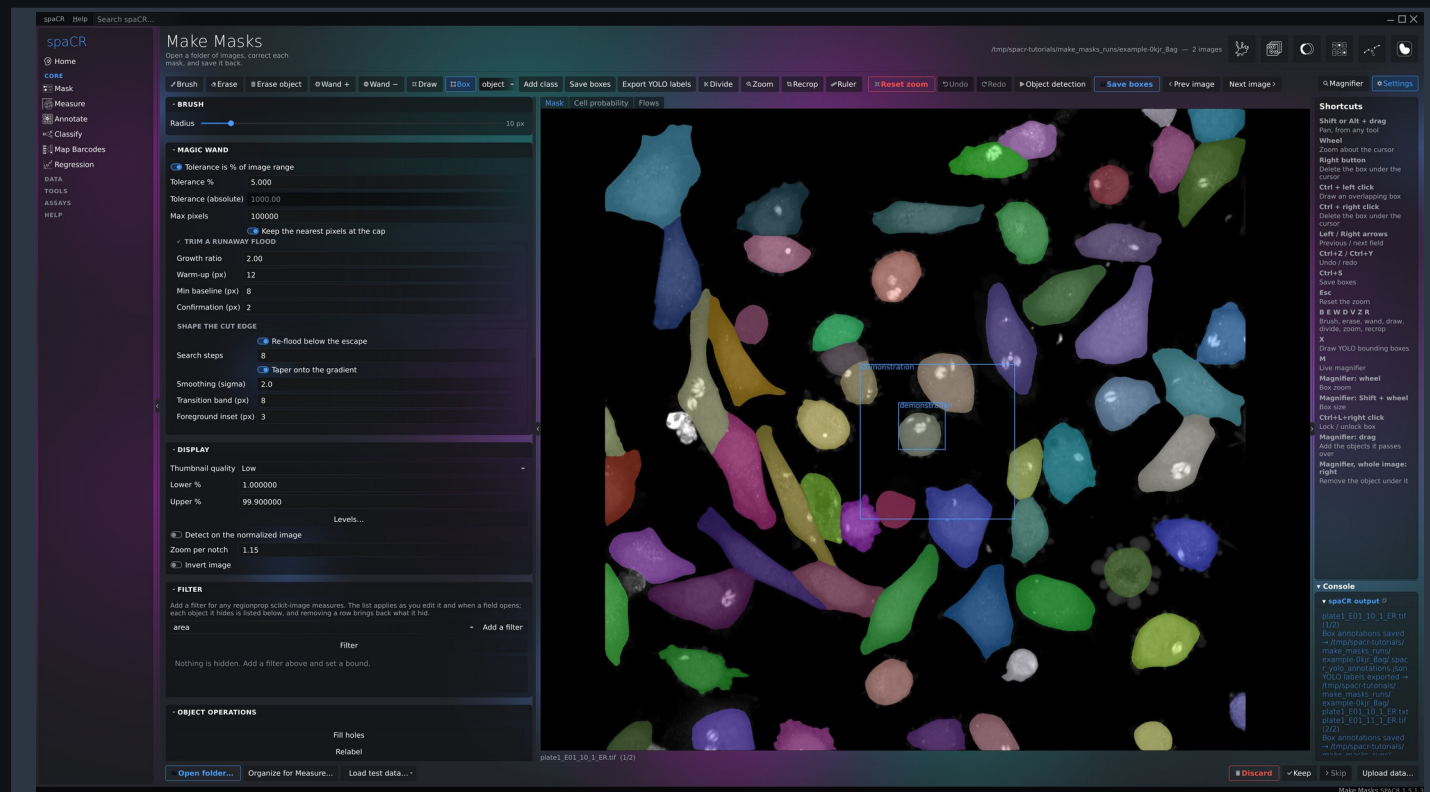
*Correct instance masks, prepare training pairs, or annotate independent YOLO boxes.*

## WHAT YOU CAN DO

- Mask tools: brush, erase, magic wand, draw, divide, zoom, recrop and ruler
- Live magnifier: a box that zooms in and outlines cells as you move
- Detect Cellpose masks, threshold objects or puncta inside parent masks
- Add classes; draw, move and resize boxes with Undo and Redo
- Open a folder, Organize for Measure or Load test data; Discard, Keep, Skip or Upload data per field

## OUTPUT

- Curated masks, ready for training or measuring
- Saved box projects and normalized YOLO labels; source images stay unchanged



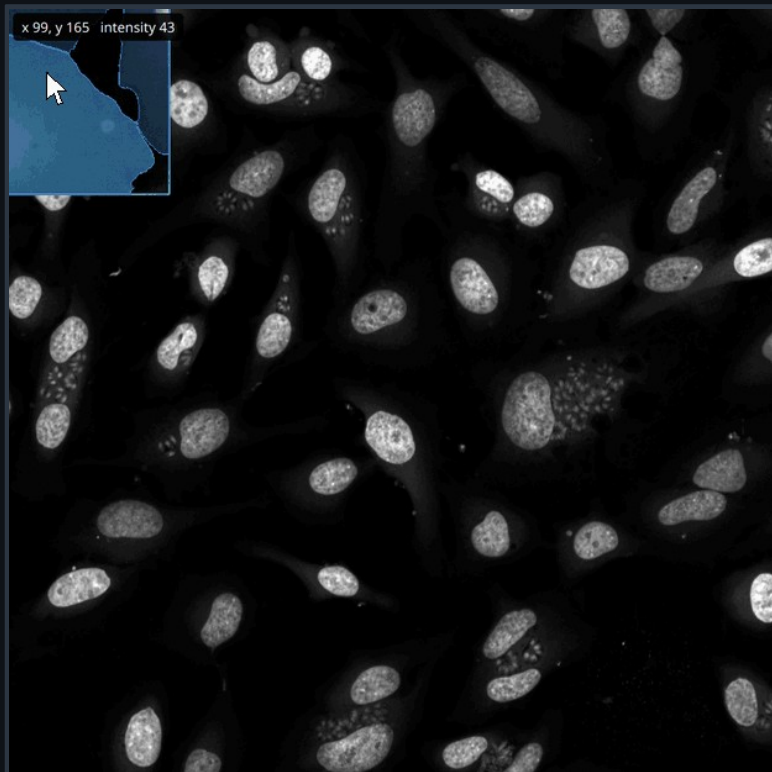
*Native Make Masks: demonstration boxes on an acquired microscopy field*



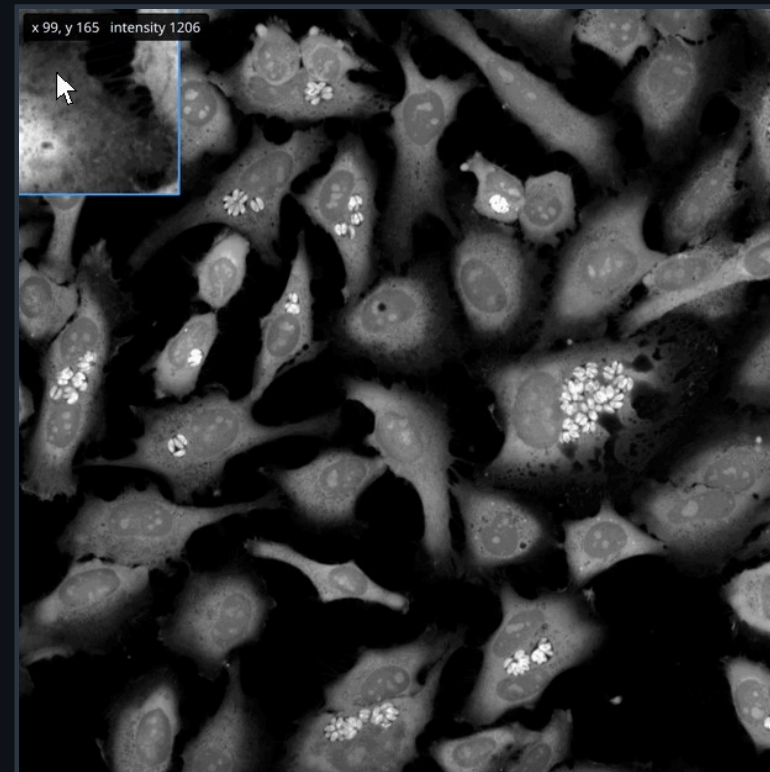
TOOLS · 1 OF 5

BETA

# Make Masks: live magnifier



*Cell from Hoechst: whole cells found from the nuclear (Hoechst) image*



*Toxoplasma from Cell Mask: vacuoles found from the cell-mask image*

## Box

follows the mouse, zoomed and contrast-adjusted

## Model

Cellpose-SAM, your own model, or a threshold, outlining live

## Click

adds what the box found; every click can be undone

## Drag

along a cell bigger than the box joins its pieces into one object





TOOLS · 1 OF 5

BETA

# Make Masks: organize for Measure

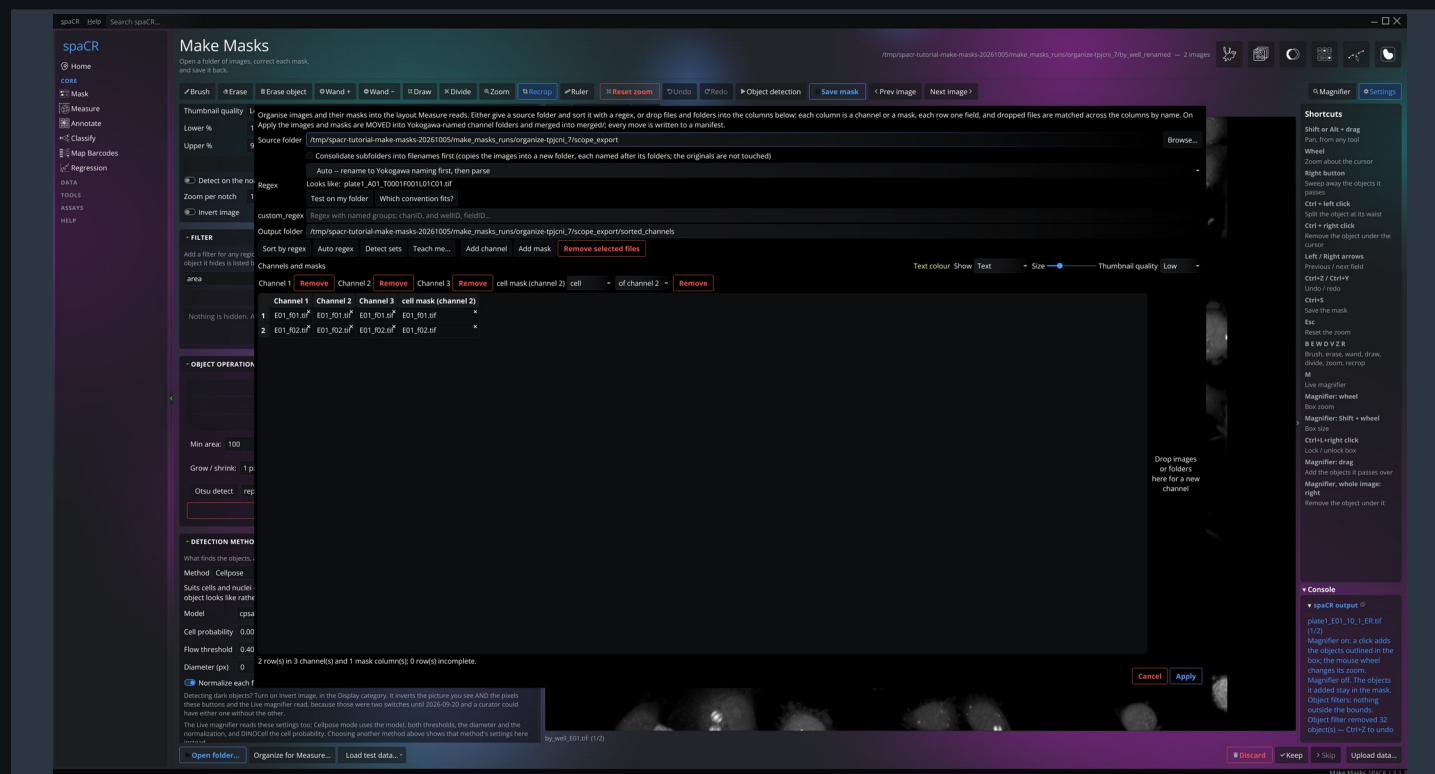
*Prepare images and corrected masks for the Measure module.*

## WHAT YOU CAN DO

- One row per field; columns are the image channels and the mask types
- Drop files, select files in the table, or detect sets from their names
- Apply the layout, then open Measure using the prepared merged arrays

## OUTPUT

- Channel-sorted images, merged arrays and an auditable move manifest



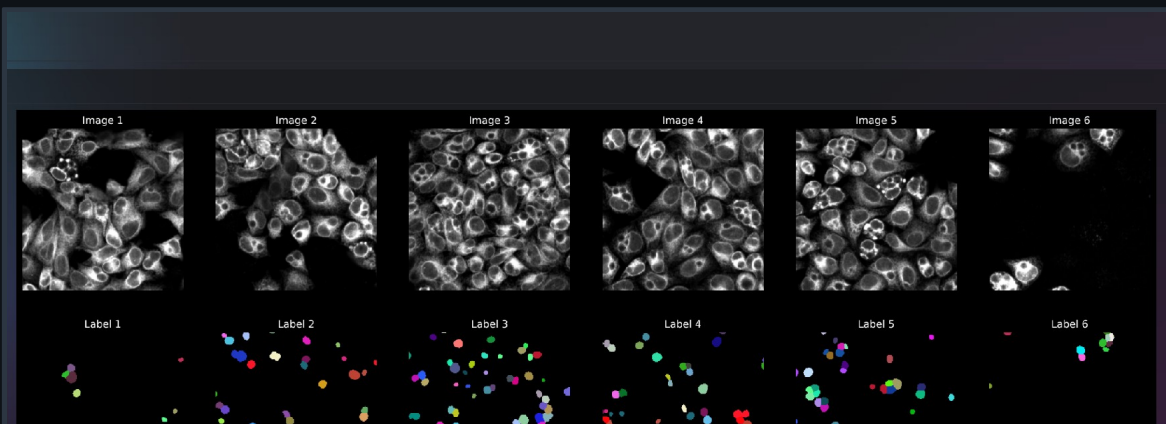
*Two acquired fields with three image channels and their cell masks*



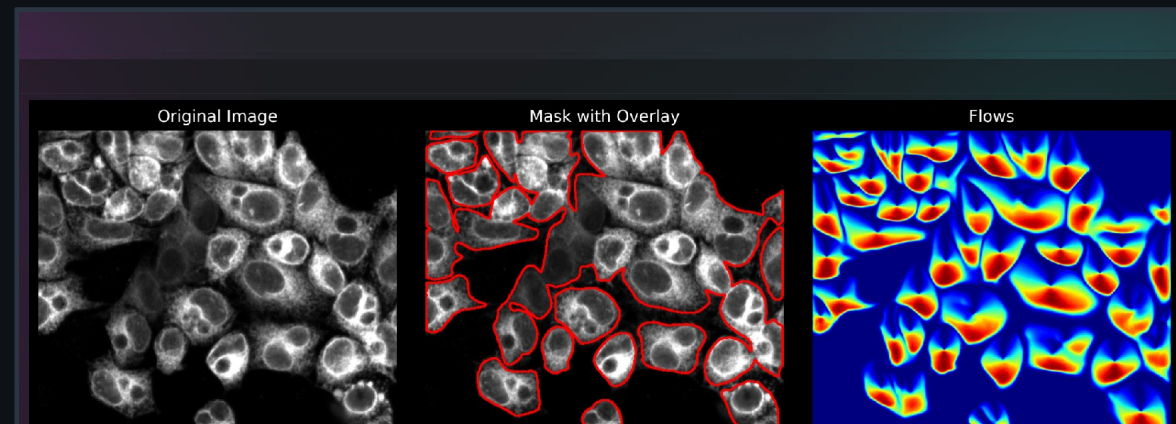
TOOLS · 1 OF 5

BETA

# Make Masks: train and compare models



*Cellpose Workbench, training: image / label pairs it will learn from*



*Cellpose Workbench, applying a model: image, mask overlay and flows*

## SUB-TOOLS INSIDE THIS MODULE

### Cellpose Workbench

Fine-tune Cellpose on your masks, then mask the whole folder

### Model Compare

Two models' masks side by side, and how much they agree

### Model Zoo

Browse, download and test trained models

### Curate

Edit masks and tracks, with an edit log

### Napari Bridge

Edit a mask in napari and bring it back



TOOLS · 2 OF 5

STABLE

# Align & Stitch: tiles to mosaic

*Stitches overlapping tiles into one large image without running out of memory.*

## WHAT YOU CAN DO

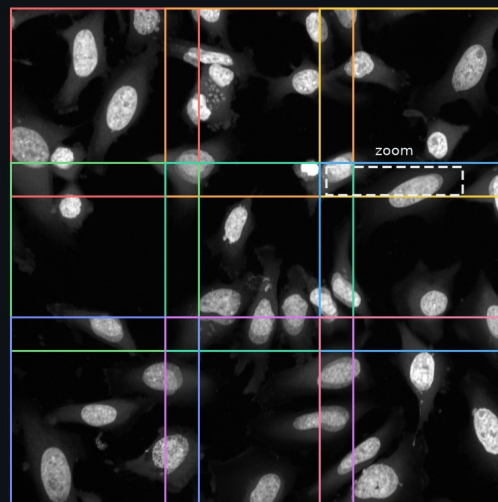
- Set grid, overlap, tile order and reference channel
- Plans first, reading only the overlaps: fast on huge plates
- Finds how far each tile is off by matching nuclei in the overlaps
- Shows how sure it is about every tile
- Tells you the final size and memory needed before it starts

## OUTPUT

- The stitched mosaic, written piece by piece so memory stays low
- Where every tile ended up, saved with the project

### 1 Tiles as acquired

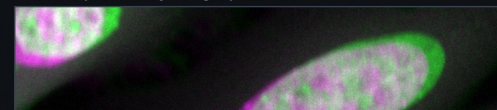
Placed where the stage said: each tile is up to 20 px off, so nuclei in the overlaps do not line up.



### 2 Overlap matched

Zoom on the strip two neighbouring tiles share. Tile A magenta, tile B green: white where they agree.

Before: placed by stage position



tile B moved 19 px to match tile A

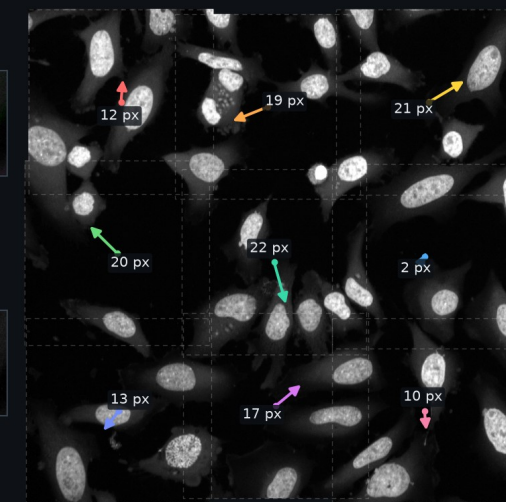
After: shifted until the nuclei line up



■ tile A ■ tile B ■ both

### 3 Stitched mosaic

Every tile moved to where its nuclei match. One seamless image; arrows show each move.



arrows drawn 8x longer · all 9 tiles placed, 20/20 overlaps matched

Real Hoechst image, cut into 9 overlapping tiles with simulated stage error; shifts found and blended by spaCR (spacr.align).

*A real Hoechst image cut into 9 overlapping tiles, each up to 20 px off (simulated); spaCR put every tile back where it came from*



TOOLS · 3 OF 5

BETA

# Image UMAP: map all cells

*Places every cell on a 2-D map where similar cells sit together: with the real images.*

## WHAT YOU CAN DO

- UMAP, t-SNE or PCA maps
- Finds groups of similar cells automatically
- Embed by controls (positive vs negative groups)
- Can choose the map settings for you
- Click a point to see that cell's crop

## OUTPUT

- Scatter coloured by cluster or condition, with thumbnails
- Table with a cluster column per cell



*5,000 real cells from TSG101 screen plate 1: they fall into groups by number of nuclei and vacuoles*

## SUB-TOOLS INSIDE THIS MODULE

### Image Scatter

Any two measured features, with the crop shown on hover

### PCA

Which measurements drive the main differences





TOOLS · 4 OF 5

# Gate Editor: gate cells like FACS

ALPHA

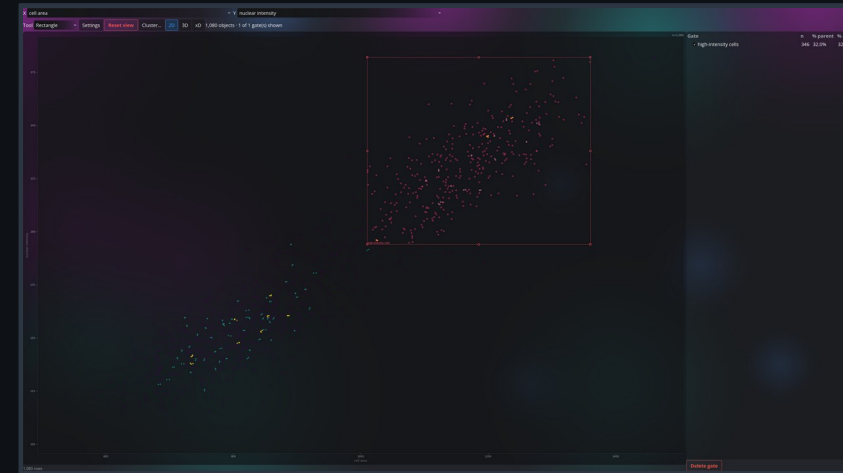
*Select cell populations the way a flow cytometrist gates: in 1, 2 or 3 dimensions.*

## WHAT YOU CAN DO

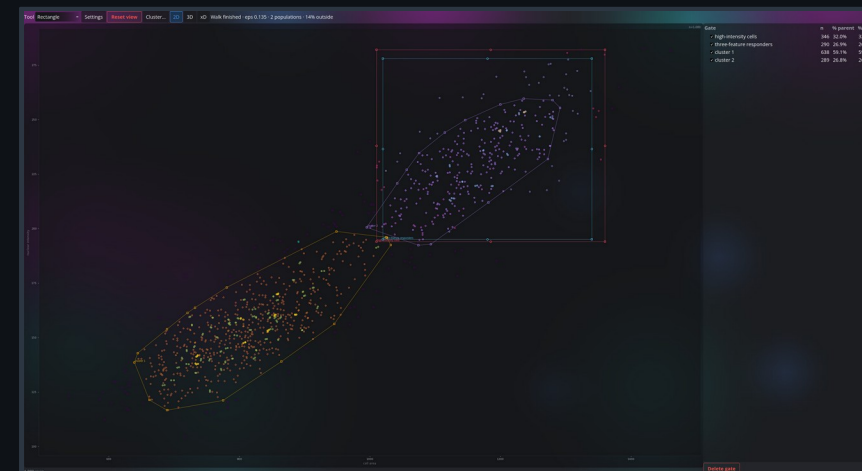
- Load tables from one or several projects, or a CSV
- Threshold, rectangle, oval, polygon, wand gates in 1-D and 2-D
- Box, cylinder and prism gates in 3-D
- Nest and combine gates; count and % of parent for each
- Publish a gate as a filter every linked view follows

## OUTPUT

- A gating strategy you can reuse on other plates
- Gate-membership columns in the project; PNG / PDF plots



*2-D rectangle gate on cell area vs nuclear intensity*



*Cluster walk: populations found and gated automatically*



TOOLS · 5 OF 5

BETA

# Graph Builder: plots without code

*Build a plot by dragging columns onto axes. The chart type follows the data.*

## WHAT YOU CAN DO

- Six drop zones: x, y, colour, size and two facets
- Histogram, bar, scatter, box or heatmap chosen for you; violin and line on request
- Brush points to select cells: linked to the UMAP and plate map
- Annotate conditions from metadata; reusable schemas
- Merge tables by name; save a chart and load it back

## OUTPUT

- Interactive charts and annotated tables, exportable

## SUB-TOOLS INSIDE THIS MODULE

### Plate Viewer

Plate heatmaps and edge-effect detection

### Small Multiples

One plot per group, shared or independent axes

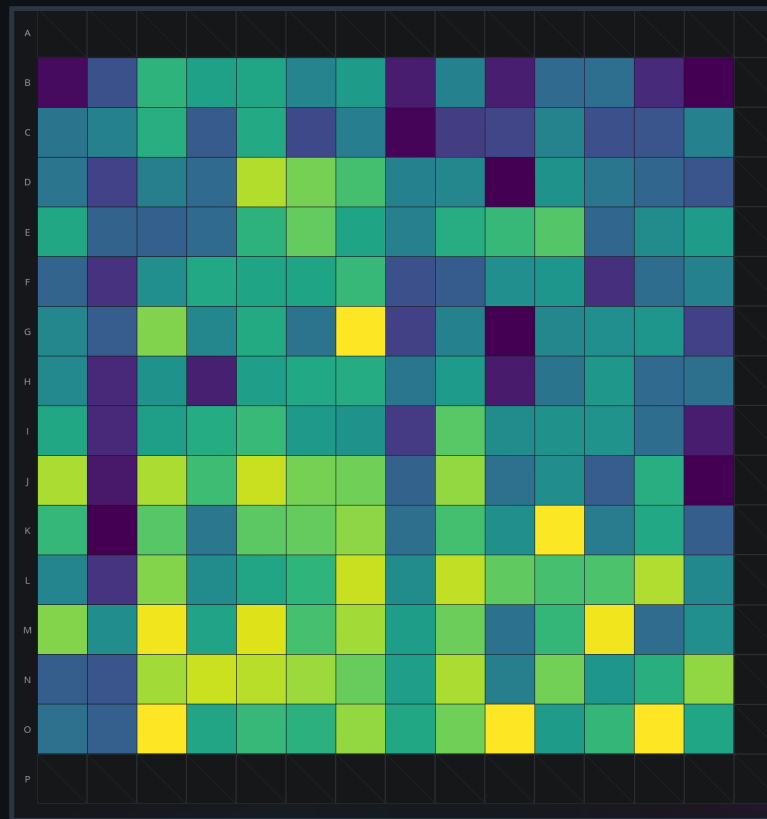
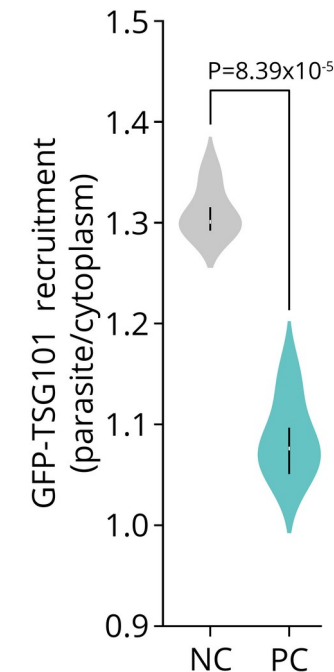


Plate Viewer: mean cell intensity per well



Violin plot of per-well values



# 04

## Assays

One module per organism, each with its own assays. Toxoplasma ships today; more organisms are alpha species.



Toxoplasma

# One module per organism

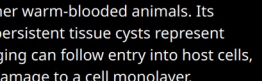
## Toxoplasma gondii

Toxoplasma gondii is an intracellular protozoan parasite that causes toxoplasmosis in humans and other warm-blooded animals. Its rapidly growing tachyzoites and persistent tissue cysts represent different stages of infection. Imaging can follow entry into host cells, replication within vacuoles, and damage to a cell monolayer.

hyperLOPIT compartment

- rhoptries 1
- rhoptries 2
- miconemes
- dense granules
- apicoplast
- IMC
- mitochondrion - soluble
- mitochondrion - membranes
- Golgi
- ER
- ER 2
- nucleus - chromatin
- nucleus - non-chromatin
- nucleolus
- cytosol

Clear components



- Starplast
- Plaque Assay
- Recruitment
- Host-Pathogen
- Invasion Assay
- Replication Assay
- Egress
- Gliding motility

- Bradyzoite conversion
- Host cell damage

Hover over the cell to identify a compartment. Check several labels to keep them highlighted.

The *Toxoplasma* module: the parasite's compartments on the left, its assays as tiles

# Toxoplasma

Plaque · Recruitment · Host-Pathogen · Invasion · Replication · Gliding motility, with Starplast's hyperLOPIT compartments

## More organisms (alpha)

Plasmodium, Candida, Trypanosoma, Leishmania, Giardia, virus and mammalian modules: Preferences > Show alpha species

*Greyed tiles are assays still being built.*



# Plaque Assay: count and size plaques

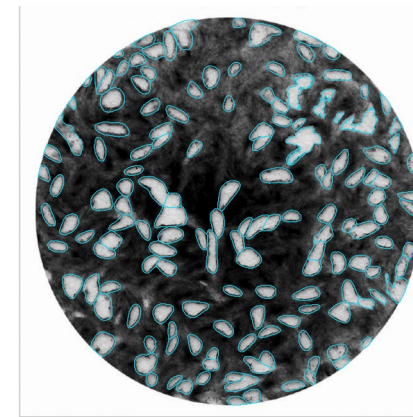
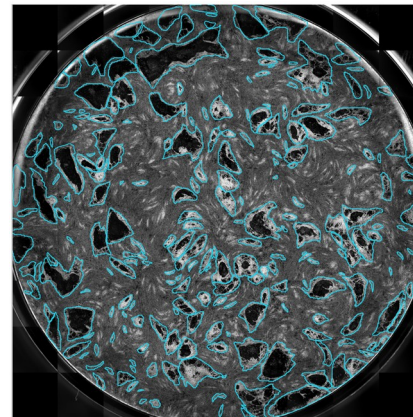
*Finds every plaque in whole-well images and measures it in mm<sup>2</sup>.*

## WHAT YOU CAN DO

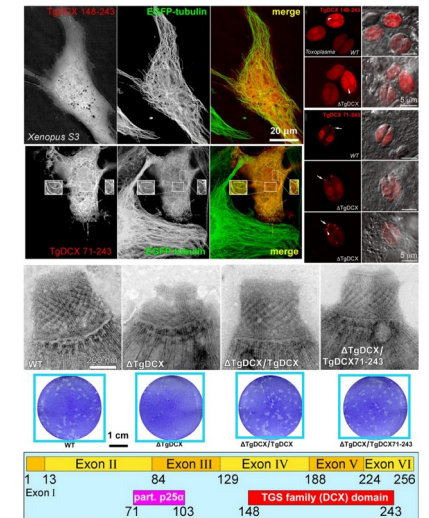
- Segments plaques with the Model Zoo plaque model (Cellpose-SAM)
- A YOLO detector finds and splits wells first
- Areas converted to mm<sup>2</sup> from the well diameter
- 'Figure' mode for plaque images taken from papers
- Can reuse existing masks

## OUTPUT

- A table of every plaque, and a summary per well
- Area, perimeter, diameter, eccentricity, solidity per plaque



*Curated plaque outlines: our microscope (left) and a published figure (right)*



*Wells found in a figure*



# Recruitment: marker at the vacuole

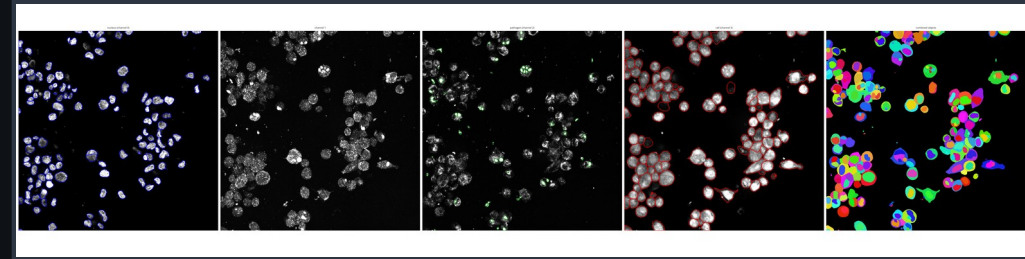
*How much does a fluorescent marker gather at the parasite vacuole compared with the host cytoplasm?*

## WHAT YOU CAN DO

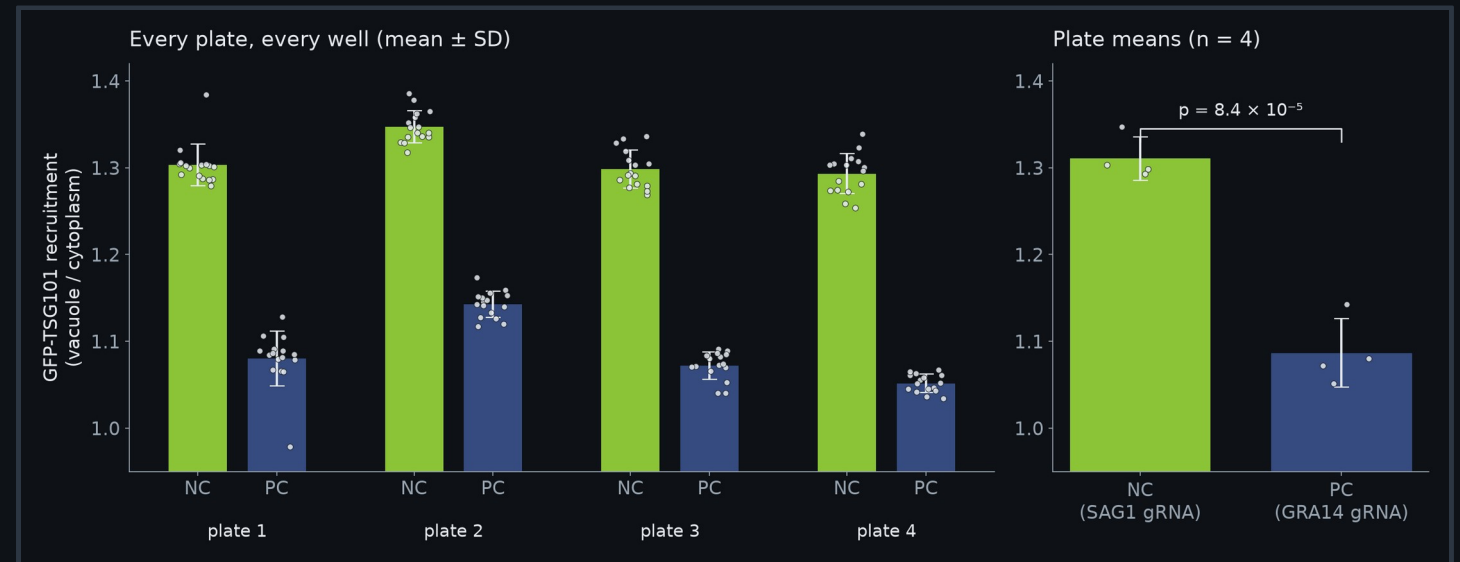
- Map wells to cell type, pathogen and treatment
- Size and intensity filters per object class
- Choose the channel of interest
- Minimum number of cells per well

## OUTPUT

- Tables per cell and per well
- Bar plots of recruitment by condition, with per-well points



*One field: channels and the cell / nucleus / parasite masks*



*GFP-TSG101 recruitment to the vacuole, NC vs PC, 4 plates (preprint data)*



# Invasion: attached or invaded?

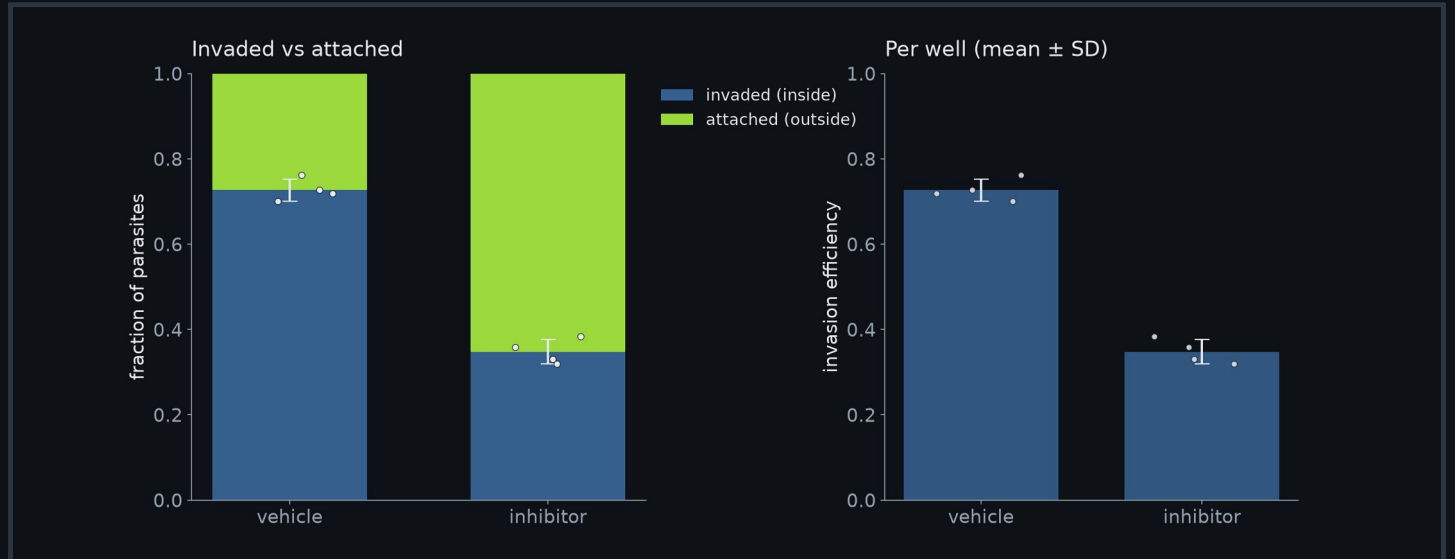
*Scores each parasite as outside (attached) or inside (invaded) from two-colour differential staining.*

## WHAT YOU CAN DO

- Set the outside-stain and total-stain channels
- Thresholds per field, or from control wells
- Flags fields where inside and outside cannot be told apart
- Checks the answer holds if the threshold moves

## OUTPUT

- Per-parasite calls, per-field thresholds, per-well invasion
- Statistics comparing conditions, per well
- Plots per well, by condition, and of the thresholds





# Replication: parasites per vacuole

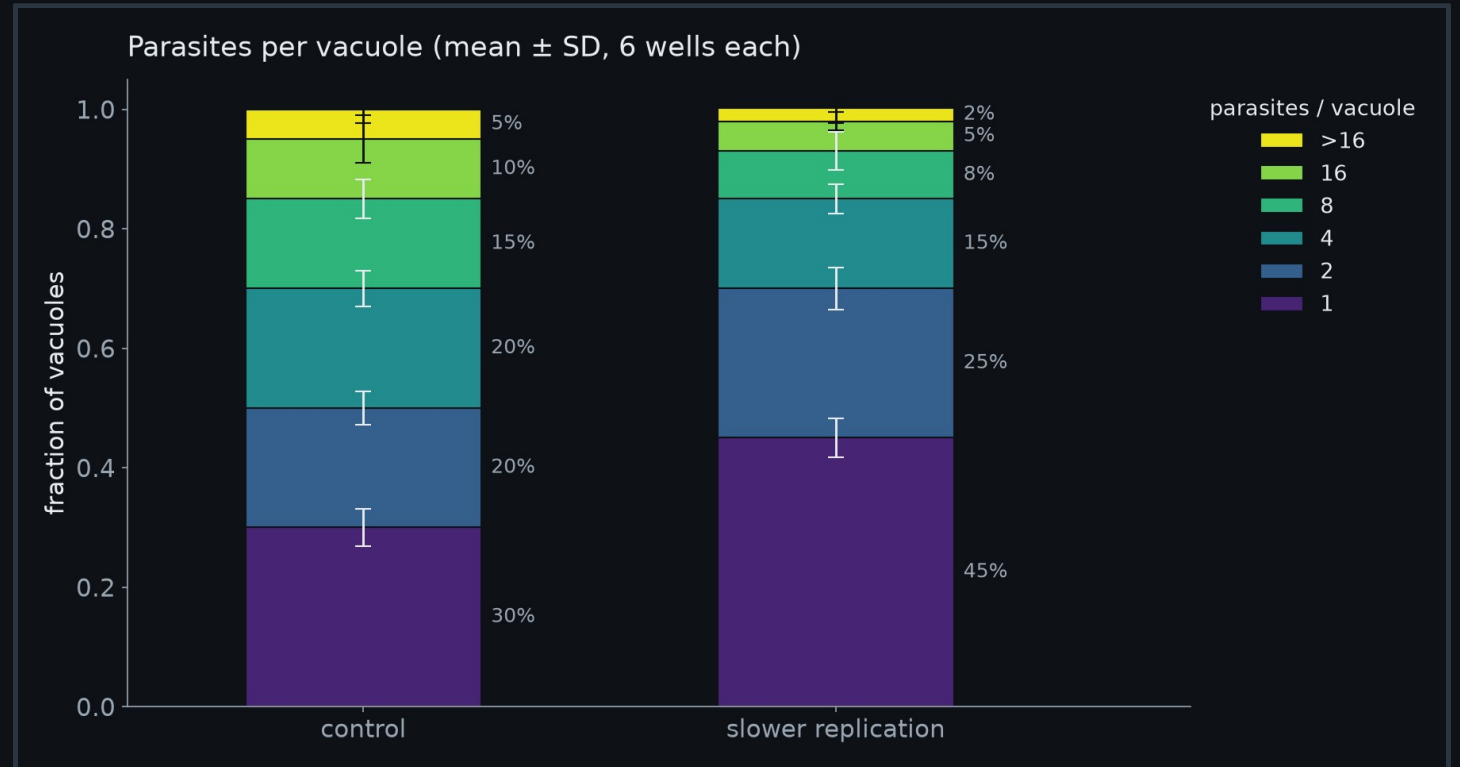
*Counts parasites per vacuole (1, 2, 4, 8, 16 ...) and compares the distributions between conditions.*

## WHAT YOU CAN DO

- Groups parasites into vacuoles: auto, spatial, by cell, or by object
- Area filters for parasites and vacuoles
- Warns when too many vacuoles have a non-power-of-two count

## OUTPUT

- Vacuole counts, per-well distributions, condition summary
- Statistics comparing conditions
- Stacked-bar plots per well and by condition



*Example output on synthetic data: 6 wells per condition, mean  $\pm$  SD*

**In progress:** a classification model that finds and counts Toxoplasma from the Toxoplasma stain, from the nuclear stain, or from the cell mask, so parasites per vacuole can be scored without a dedicated parasite channel.



# Project tools in the Help menu



## Report

HTML / PDF report of a run: figures, statistics, settings



## Pipeline Graph

Which run produced which file, as a graph



## Batch Runner

Queue modules with dependencies; validate, then run unattended



## Plate Queue

Line up plates for Mask / Measure and let them run overnight



## Run History

Every run, its settings and its outputs



## Distributed Jobs

Send work to SSH hosts, Slurm or the cloud



## Database Browser

Browse, search, filter and export project tables



## Data Manager

What is on disk, how big, and what can go



## Project Browser

All projects on this machine



## Feature Dictionary

What every measured column means, and its unit

# 05

## Trained models

Models trained for Toxoplasma and host-cell images, shipped through the spaCR Model Zoo. Newest version of each.



PV



Plaque



Wells



Cross-channel



MODEL ZOO

# Seven models, one line in a settings file

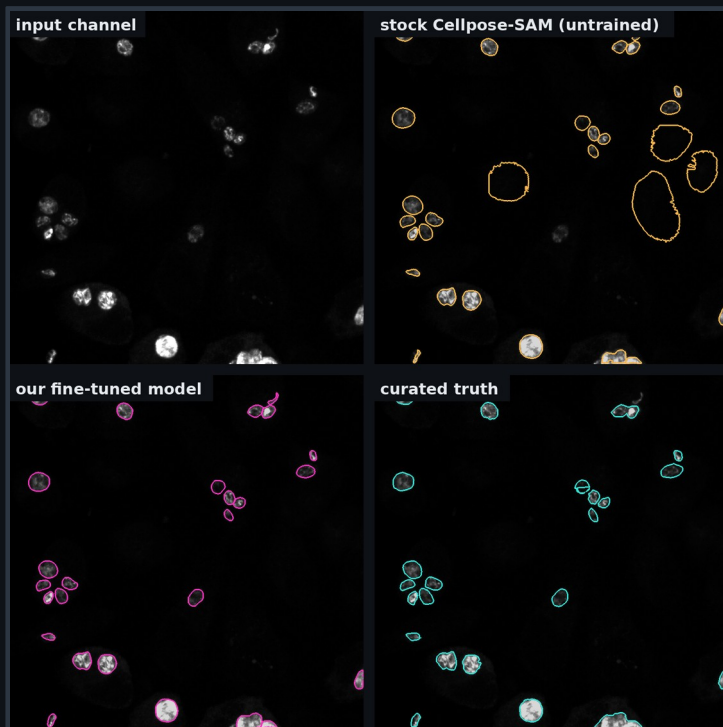
| Model                     | Settings key                | Finds                          | Architecture | Score on unseen images      |
|---------------------------|-----------------------------|--------------------------------|--------------|-----------------------------|
| Toxoplasma PV v3          | toxoplasma_pv_v3            | Parasite vacuoles              | Cellpose-SAM | F1 0.860 (stock 0.765)      |
| Toxoplasma Plaque v2      | toxoplasma_plaque_v2        | Plaques in wells               | Cellpose-SAM | F1 0.819 on published wells |
| Plaque Well Detector v2   | toxoplasma_well_detector_v2 | Wells in plates / figures      | YOLO26n      | mAP50 0.946                 |
| Toxoplasma from Cell Mask | toxoplasma_from_cellmask_v1 | PVs from the cell channel      | Cellpose-SAM | F1 0.606 (stock 0.021)      |
| Nuclei from Cell Mask     | nuclei_from_cellmask_v1     | Nuclei from the cell channel   | Cellpose-SAM | F1 0.888 (stock 0.201)      |
| Cell from Hoechst         | cell_from_hoechst_v1        | Cells from the nuclear channel | Cellpose-SAM | F1 0.870 (stock 0.301)      |
| Toxoplasma from Hoechst   | toxoplasma_from_hoechst_v1  | PVs from the nuclear channel   | Cellpose-SAM | F1 0.569 (stock 0.002)      |

**How it works** Name a key in a settings file: `pathogen_model: toxoplasma_pv_v3:` and spaCR downloads it the first time it is needed. Anyone can contribute a trained model: it is uploaded to the spaCR Hugging Face and then appears in every user's Model Zoo.



MODEL · TOXOPLASMA\_PV\_V3

# Toxoplasma PV v3



Unseen test field. Orange = vanilla Cellpose-SAM (cpsam\_v2, never trained on our images), magenta = PV v3, cyan = curated truth

## WHAT THE SCORES MEAN

**F1** share of objects found correctly; lower if objects are missed or invented (1 = perfect). Found = outlines overlap by at least half.

**AJI** how well each outline matches; lower if a cell is split in two or two cells merged.

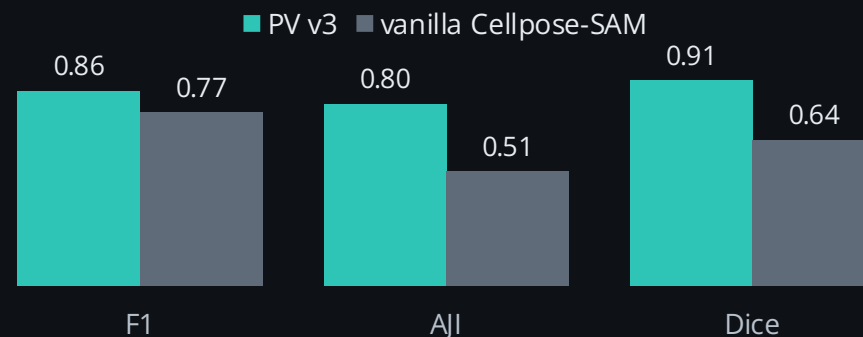
**Dice** pixel overlap between the model's mask and the true mask.

**stock** vanilla Cellpose-SAM (cpsam\_v2), never trained on our images.

## Cellpose-SAM (cpsam\_v2), round 6

Outlines Toxoplasma parasitophorous vacuoles in anti-Toxoplasma or DsRed images (RH and ME49).

### 11 wells never used for training



## TRAINED ON

556 hand-curated fields from five rounds of curation: 15,550 vacuoles. 11 wells have never been used for training.

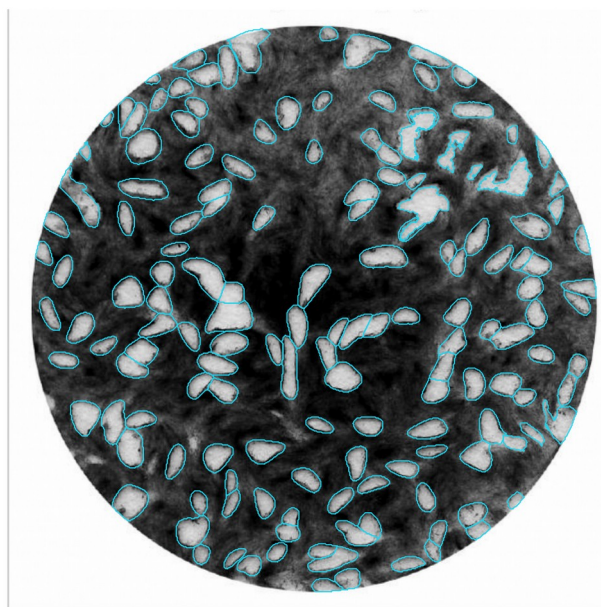
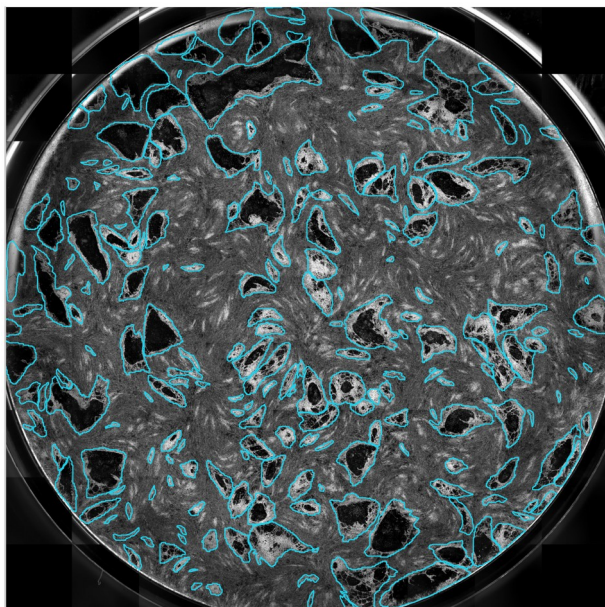
## GOOD TO KNOW

- Compared with vanilla Cellpose-SAM, not with PV v2
- Across five different train / test splits: F1 0.82
- Best for counting and size; less exact on fine outline detail
- Scores the same as round 2 on these wells (0.860 vs 0.865)



MODEL · TOXOPLASMA\_PLAQUE\_V2

# Toxoplasma Plaque v2



*Curated plaque outlines (cyan): our microscope (left) and a published figure (right)*

## WHAT THE SCORES MEAN

**F1** share of objects found correctly; lower if objects are missed or invented (1 = perfect). Found = outlines overlap by at least half.

**AJI** how well each outline matches; lower if a cell is split in two or two cells merged.

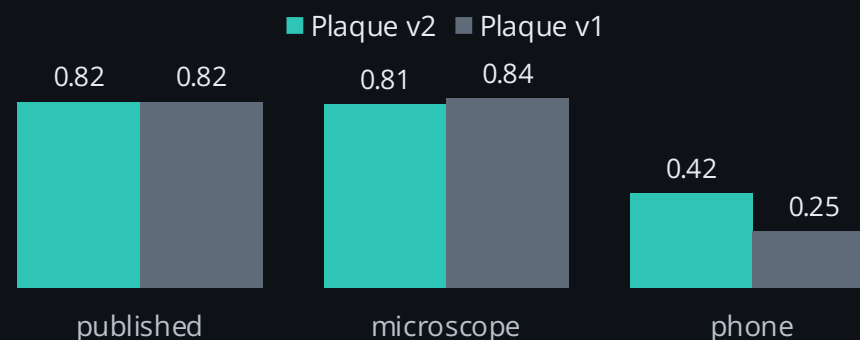
**Dice** pixel overlap between the model's mask and the true mask.

**stock** vanilla Cellpose-SAM (cpsam\_v2), never trained on our images.

## Cellpose-SAM (cpsam\_v2), round 5

Outlines plaques in crystal-violet wells: from our microscopes, phone cameras and published figures.

### F1 on 81 unseen fields, by image source



## TRAINED ON

488 curated fields in four domains: 298 published-figure wells, 96 phone-camera wells, 94 microscope scans: 27,582 plaques.

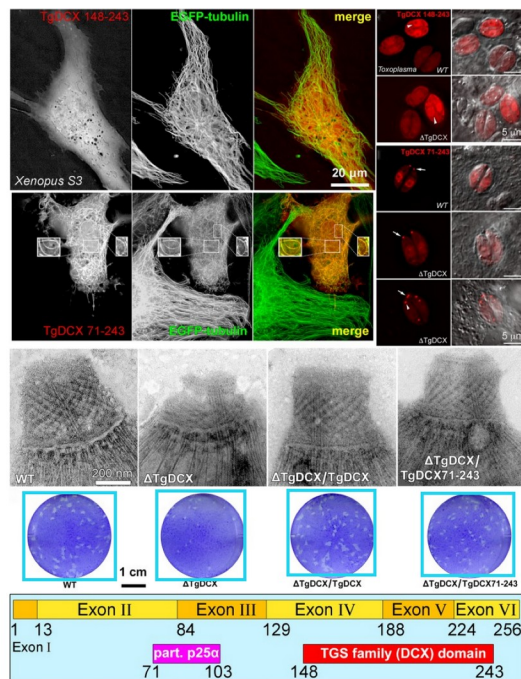
## GOOD TO KNOW

- Only plaque model trained on phone images: still misses most plaques there (recall 0.30)
- Balanced precision / recall (0.81 / 0.83); v1 undercounts
- 2 false plaques in 6 blank wells (v1: 19)
- For microscope-only work v1 is still slightly better



MODEL · TOXOPLASMA\_WELL\_DETECTOR\_V2

# Plaque Well Detector v2



*Detected wells (cyan) in a published figure; fluorescence and EM panels ignored. Shown with the previous detector*

## WHAT THE SCORES MEAN

**precision** of the boxes it draws, the share that really are wells.

**recall** of the real wells, the share it finds.

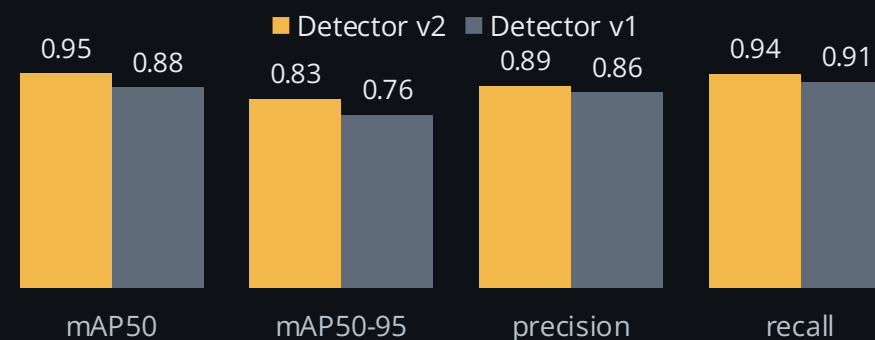
**mAP50** overall detection score; a box counts when it overlaps the true well by at least half.

**mAP50-95** the same, but also rewards boxes drawn tightly around the well.

## YOLO26n object detector

Draws a box around every plaque well in a plate photo or a published figure: the first step before plaque segmentation.

129 unseen images, 84 of them with no well



## TRAINED ON

1,070 training images: our plates plus 939 figures from published papers. No paper appears in both training and testing.

## GOOD TO KNOW

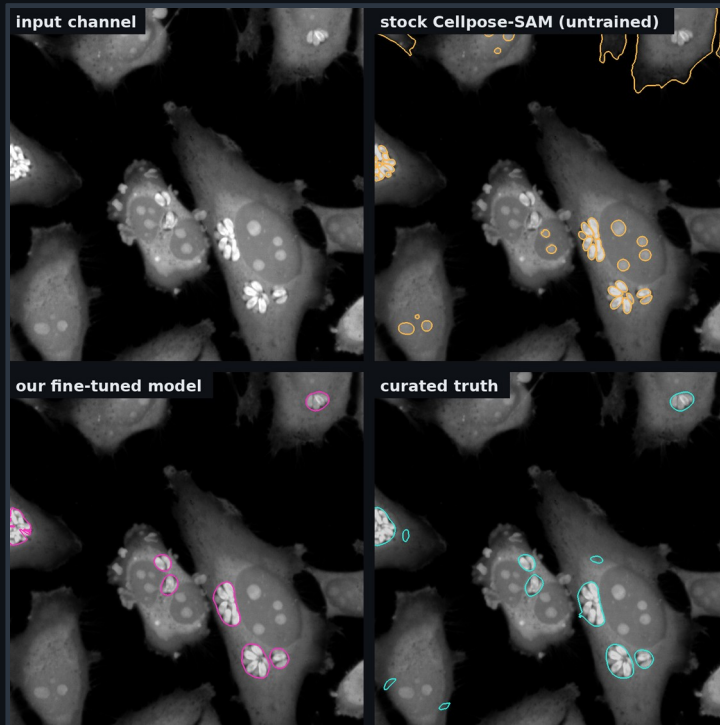
- Better than v1 on every score
- False wells on figures with no well: 152 → 49
- Finds wells, not plaques; the well size also gives the mm<sup>2</sup> scale





MODEL · TOXOPLASMA\_FROM\_CELLMASK\_V1

# Toxoplasma from Cell Mask



Unseen test field: orange = vanilla Cellpose-SAM (untrained), magenta = our fine-tuned model, cyan = curated truth

## WHAT THE SCORES MEAN

**F1** share of objects found correctly; lower if objects are missed or invented (1 = perfect). Found = outlines overlap by at least half.

**AJI** how well each outline matches; lower if a cell is split in two or two cells merged.

**Dice** pixel overlap between the model's mask and the true mask.

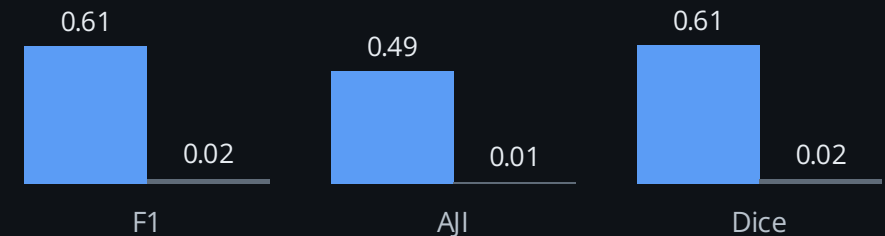
**stock** vanilla Cellpose-SAM (cpsam\_v2), never trained on our images.

## Cellpose-SAM (cpsam\_v2), cross-channel

Finds Toxoplasma vacuoles from the host cell-mask channel alone: no parasite stain needed.

463 unseen fields

■ fine-tuned ■ vanilla Cellpose-SAM



## TRAINED ON

2,567 training fields; tested on 463 fields from other wells. HFF, HeLa and THP-1 hosts.

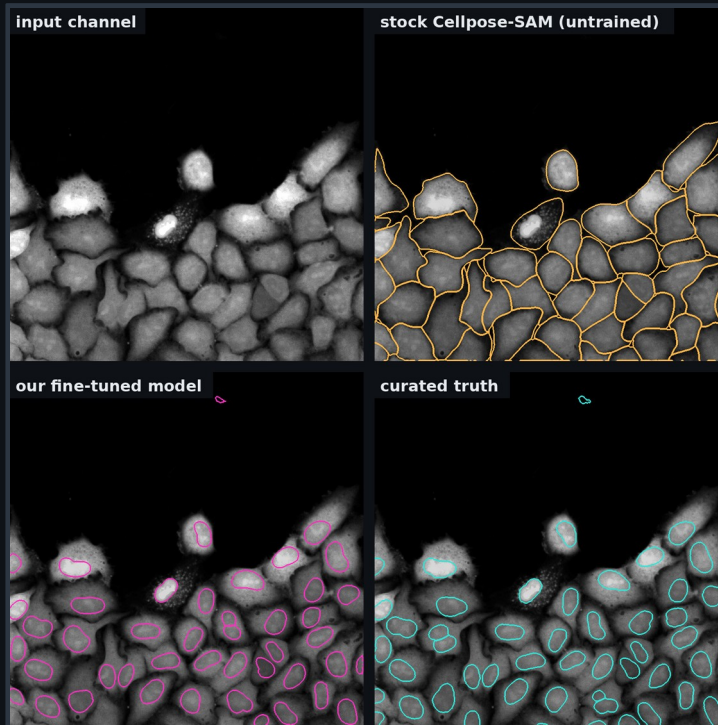
## GOOD TO KNOW

- Stock Cellpose cannot do this at all
- F1 by host: HeLa 0.71 · HFF 0.56 · THP-1 0.47
- For counting, occupancy and area: not fine morphology



MODEL · NUCLEI\_FROM\_CELLMASK\_V1

# Nuclei from Cell Mask



Unseen test field: orange = vanilla Cellpose-SAM (untrained), magenta = our fine-tuned model, cyan = curated truth

## WHAT THE SCORES MEAN

**F1** share of objects found correctly; lower if objects are missed or invented (1 = perfect). Found = outlines overlap by at least half.

**AJI** how well each outline matches; lower if a cell is split in two or two cells merged.

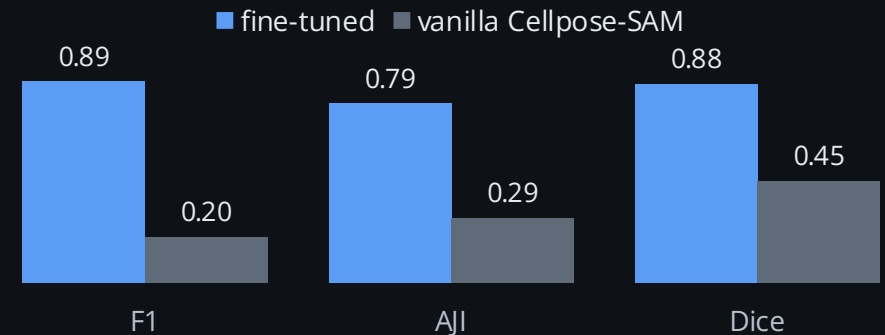
**Dice** pixel overlap between the model's mask and the true mask.

**stock** vanilla Cellpose-SAM (cpsam\_v2), never trained on our images.

## Cellpose-SAM (cpsam\_v2), cross-channel

Finds nuclei from the cell-mask channel alone: frees the DAPI / Hoechst channel for another marker.

### 453 unseen fields



## TRAINED ON

Cell-mask images paired with curated nuclear masks; tested on 453 fields from wells never used for training. HFF, HeLa and THP-1.

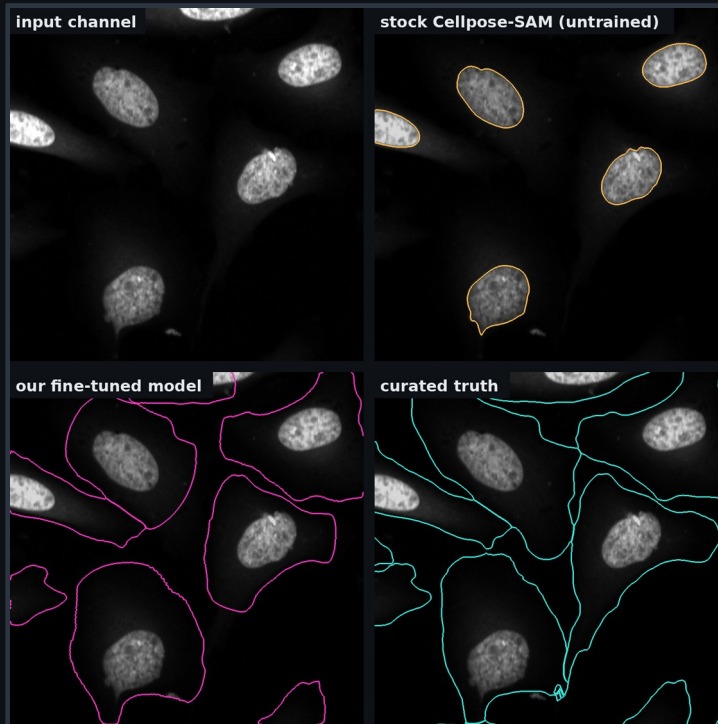
## GOOD TO KNOW

- F1 by host: HFF 0.93 · HeLa 0.86 · THP-1 0.86
- Learns nuclei from cell shape: expect worse on unusual or very confluent cells



MODEL · CELL\_FROM\_HOECHST\_V1

# Cell from Hoechst



Unseen test field: orange = vanilla Cellpose-SAM (untrained), magenta = our fine-tuned model, cyan = curated truth

## WHAT THE SCORES MEAN

**F1** share of objects found correctly; lower if objects are missed or invented (1 = perfect). Found = outlines overlap by at least half.

**AJI** how well each outline matches; lower if a cell is split in two or two cells merged.

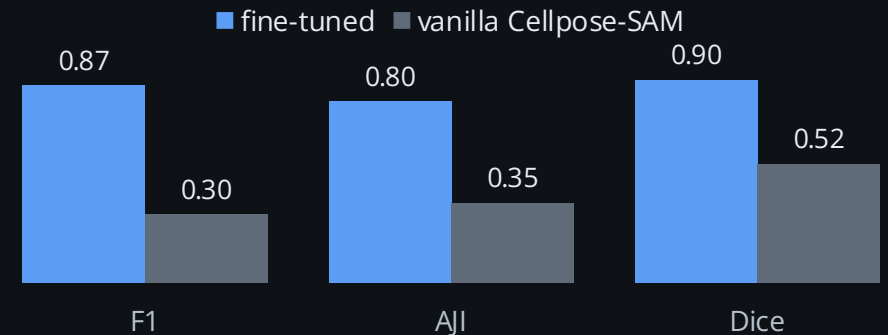
**Dice** pixel overlap between the model's mask and the true mask.

**stock** vanilla Cellpose-SAM (cpsam\_v2), never trained on our images.

## Cellpose-SAM (cpsam\_v2), cross-channel

Finds whole host cells from the Hoechst (nuclear) channel alone: the counterpart of nuclei-from-cell-mask.

### 451 unseen fields



## TRAINED ON

2,578 training fields (237,957 cells); tested on 451 fields (45,098 cells) from other wells.

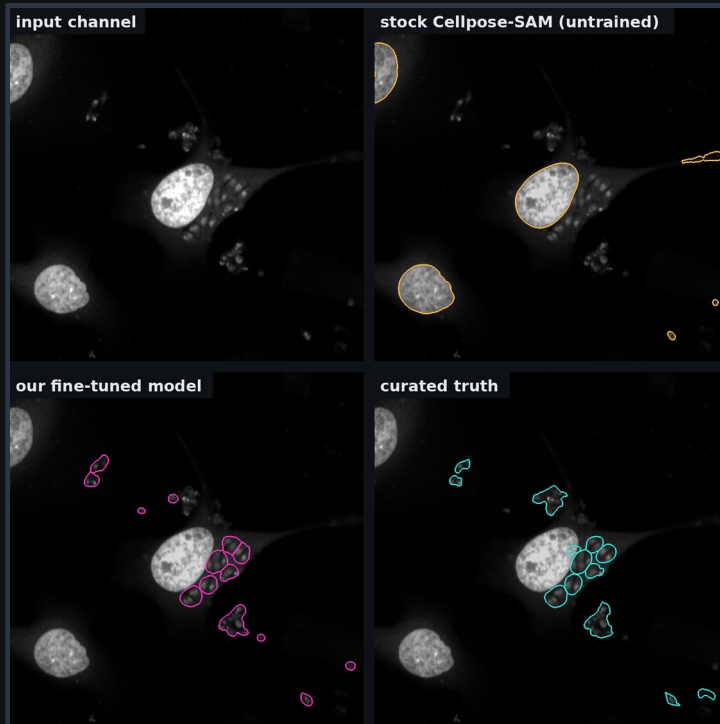
## GOOD TO KNOW

- Misses some cells but rarely invents one (precision 0.94, recall 0.81)
- F1 0.57 higher than stock



MODEL · TOXOPLASMA\_FROM\_HOECHST\_V1

# Toxoplasma from Hoechst



Unseen test field: orange = vanilla Cellpose-SAM (untrained), magenta = our fine-tuned model, cyan = curated truth

## WHAT THE SCORES MEAN

**F1** share of objects found correctly; lower if objects are missed or invented (1 = perfect). Found = outlines overlap by at least half.

**AJI** how well each outline matches; lower if a cell is split in two or two cells merged.

**Dice** pixel overlap between the model's mask and the true mask.

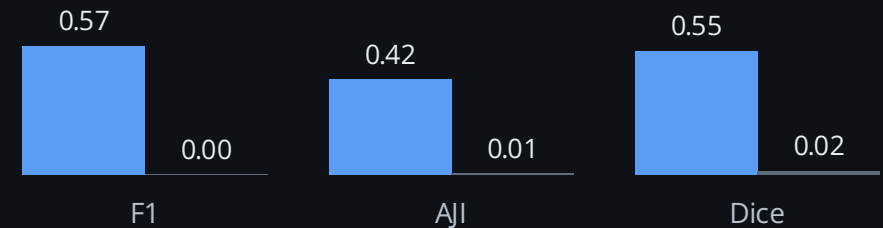
**stock** vanilla Cellpose-SAM (cpsam\_v2), never trained on our images.

## Cellpose-SAM (cpsam\_v2), cross-channel

Finds Toxoplasma vacuoles from the Hoechst channel alone: no parasite stain needed.

463 unseen fields

■ fine-tuned ■ vanilla Cellpose-SAM



## TRAINED ON

2,567 training fields; tested on 463 fields from other wells. HFF, HeLa and THP-1 hosts.

## GOOD TO KNOW

- Harder than the cell-mask route (F1 0.57 vs 0.61)
- Stock Cellpose finds essentially nothing
- For counting, occupancy and area



MODEL ZOO

# Other models available in spaCR

## Segmentation

- Cellpose 4: Cellpose-SAM (default) and Cellpose-DINO
- Cellpose 3 models (cyto3, nuclei, ...)
- SAMCell and DINOCell for label-free / live-cell images
- Cellpose models from bioimage.io
- Models other users have contributed

## Image classifiers (Classify)

- ResNet (default ResNet-50), ResNeXt, Wide ResNet
- EfficientNet, MobileNet, RegNet
- ConvNeXt, VGG, DenseNet
- Vision transformers: ViT, Swin, MaxViT
- All start from networks pre-trained on everyday photos

## Feature classifiers (Classify)

- XGBoost (default), LightGBM, CatBoost
- Random forest and other tree models
- Logistic regression, support vector machine, small neural network

## Embeddings & detection

- Embeddings: ResNet-18 (default), ResNet-50, ConvNeXt, ViT
- YOLO object detection (behind the well detector)

# Get started

```
pip install "spacr[qt]" && spacr
```

## Docs & tutorials

[einarolafsson.github.io/spacr](https://einarolafsson.github.io/spacr)

## Source & installers

[github.com/EinarOlafsson/spacr](https://github.com/EinarOlafsson/spacr)

## Preprint

[bioRxiv 10.64898/2026.07.08.737057](https://doi.org/10.64898/2026.07.08.737057)

## Models

[huggingface.co/einarolafsson](https://huggingface.co/einarolafsson)

## Example data

`spacr-download --list`

