

Cell Colony Counting

We know what counts. You can count on us.

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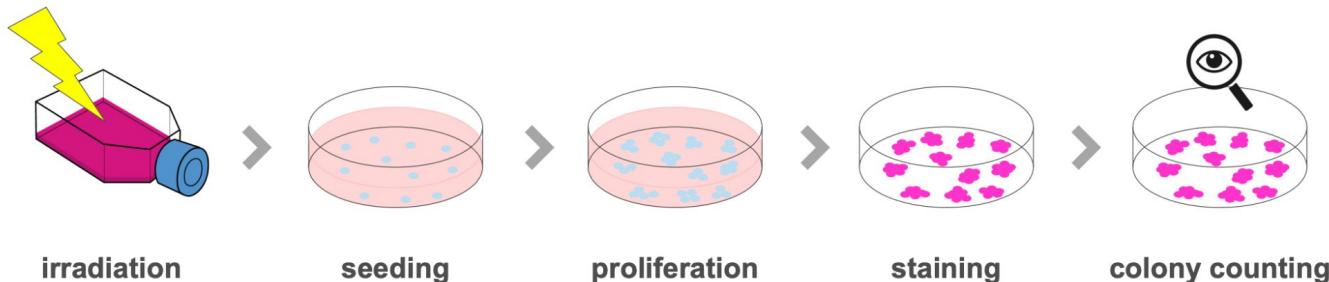
Supporting research to fight cancer



- Proton therapy targets difficult to operate tumors non-surgically
- Colony-forming cell (CFC) assays assess therapy effectiveness
- Cells are treated, seeded & grown
- Colonies are identified by counting cells
- Manual counting for CFC is slow, labor intensive & inconsistent

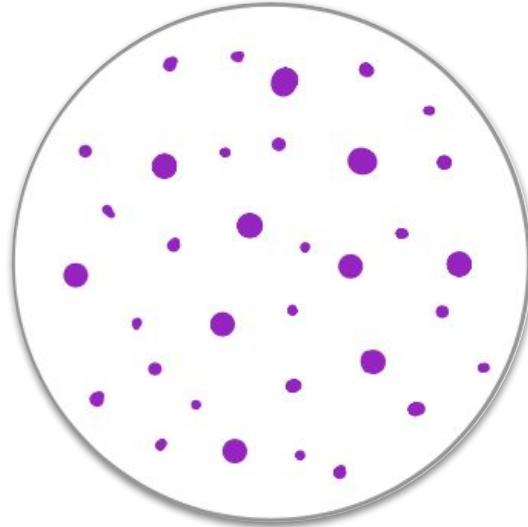


Proton Beam Therapy System



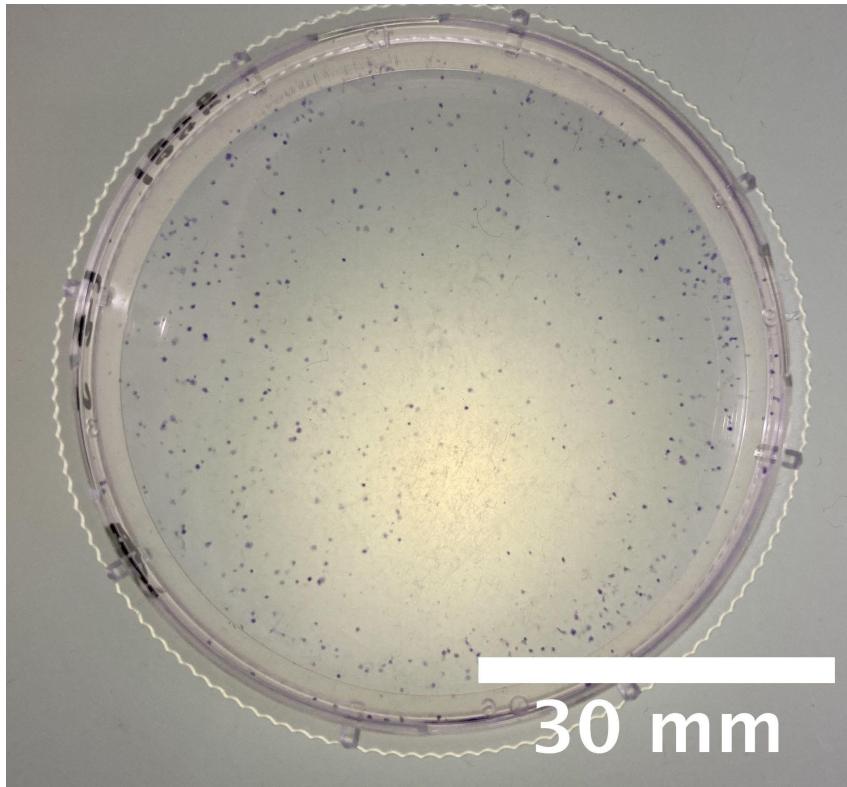
Our Mission

Create an **affordable**,
accessible, **automated** system
to **identify cell clumps** and
count colonies.

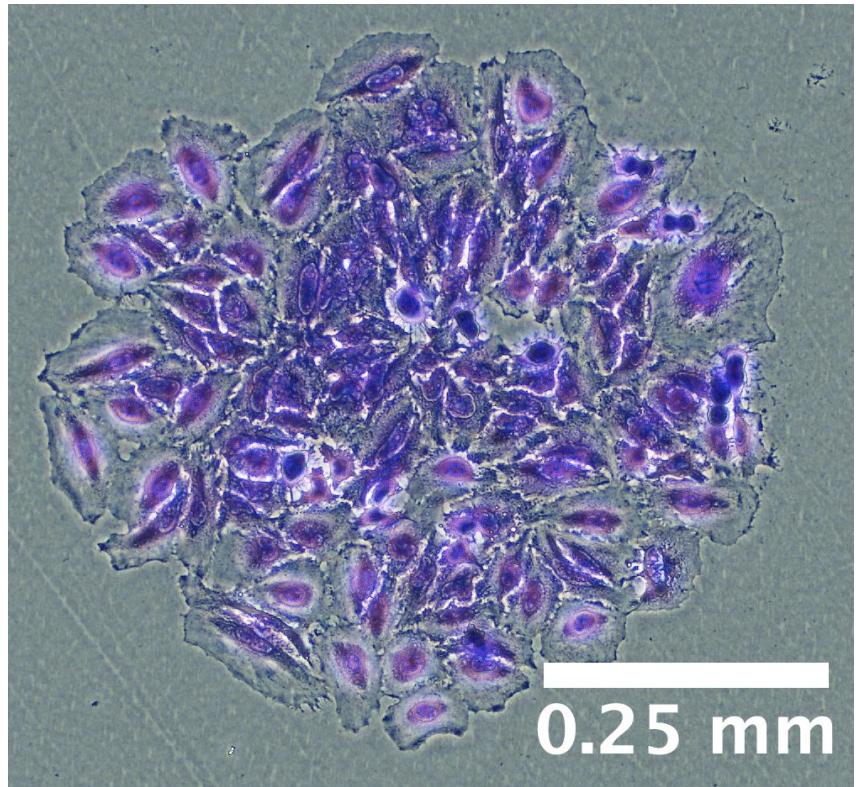


Petri Dish

1. Find clumps, 2. Numerate cells, 3. Count colonies



60 mm diameter petri dish



Cell clump

Commercial systems vary widely in cost vs. performance

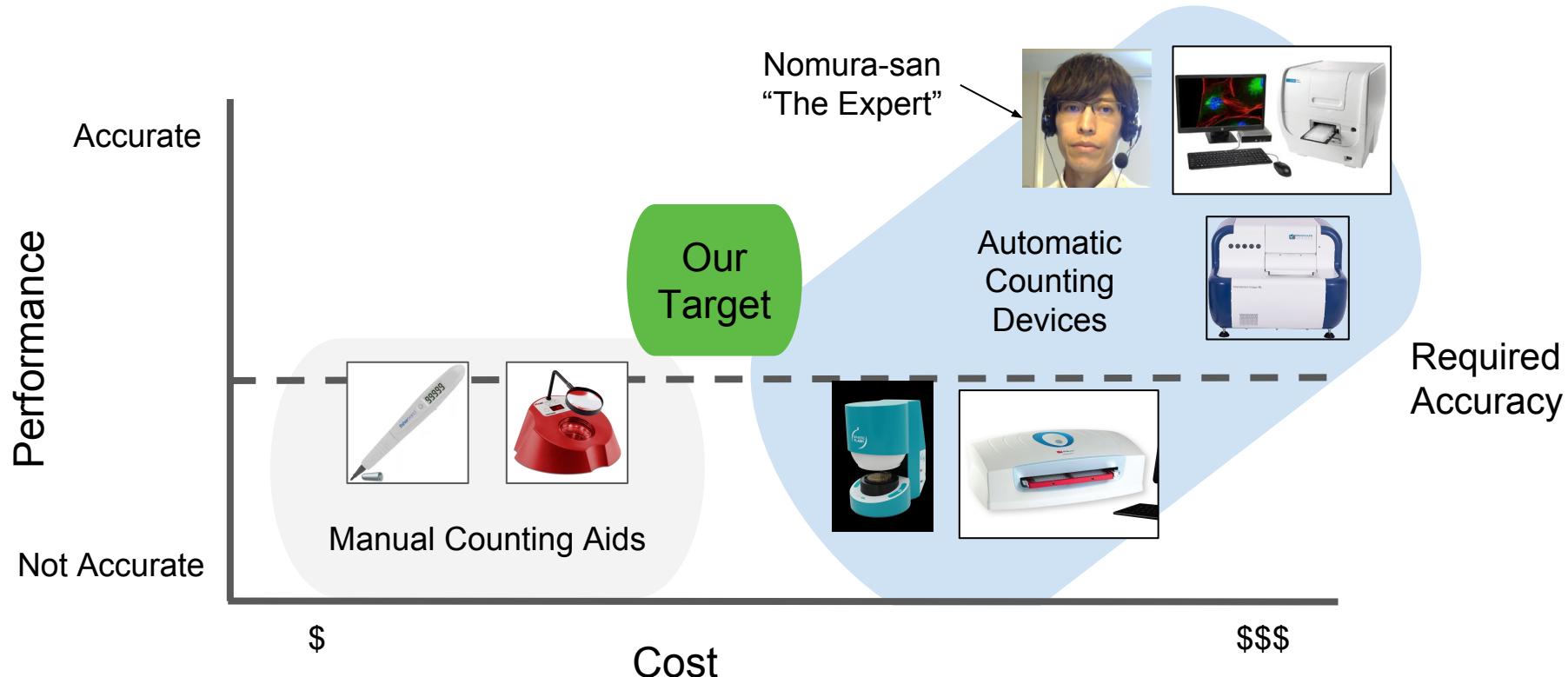


Image Sources: Sumitomo, Fisherbrand™ Counter-Pen/Colony-Counter, aCOLade 2, SphereFlash® – Automatic Colony Counter, BioTek Cytation 5 Cell Imaging Multimode Reader, GelCount™, CloneSelect Imager

Goal: Table-top system to count cells in a petri dish

The goal...	Metric	Target
affordable	cost per system	<\$50k / system
table top size	size (volume)	~ 300 x 300 x 300 mm ³
petri-dish based	compatible with 60 mm diameter petri dish	yes
fast	average time per petri dish	< 3 minutes / dish
distinguishes colony from non-colony clumps	error compared to manual colony counts	±1 colony Spatial resolution: Nyquist ≥ 2 pixels St. Venant ≥ 3X-5X (6-10 pixels per nucleus)

Key analysis: Optical parameters for resolution

Magnification, M

Pixel size, P_I

Resolution, $R = P_I / M$ - sets detail level

Size of object, S_O

in image plane: $S_I = M S_O$

in pixels: $S_P = M S_O / P_I$

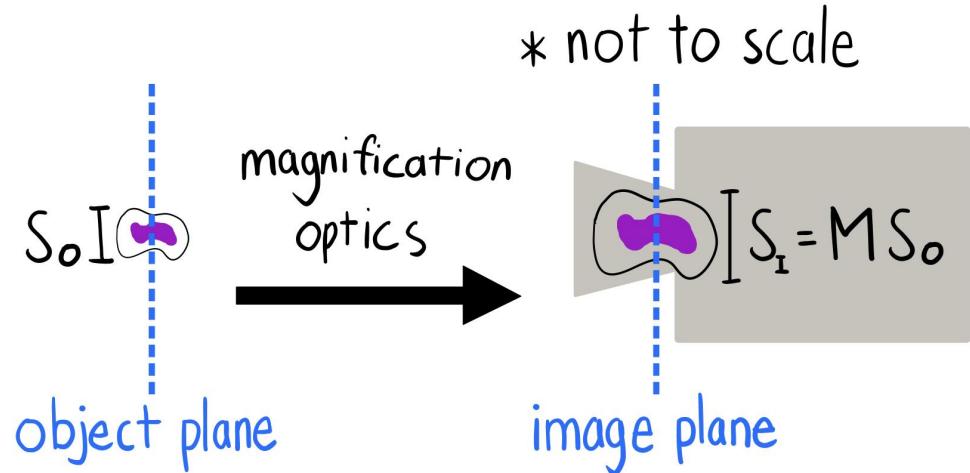
Field of view, H X W

camera sensor size

height in pixels, H

width in pixels, W

sets image size



Smallest feature size dictates our necessary resolution

$$R = S_o / S_p = P_i / M$$

$$S_p = 6-10 \text{ pixels}$$

$$S_o = \text{nucleus width} = 5 - 10 \mu\text{m}$$

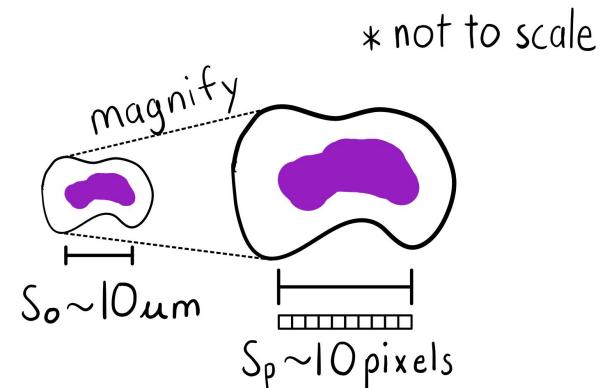
$$R \approx 1 \mu\text{m} / \text{pixel}$$

P_i and M set by camera + lens

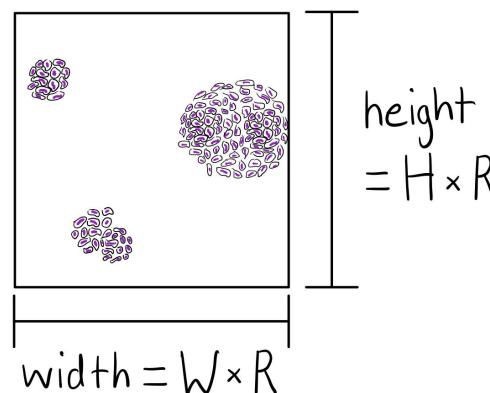
Field of view = $(H \times R) \times (W \times R)$

Tradeoff between better resolution & field of view

Resolution



Field
of
View

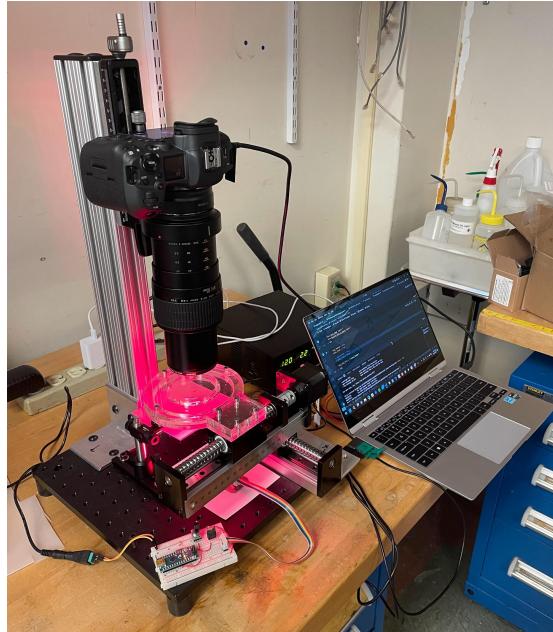


Evolution: Off-the-shelf hardware & open-source software

Initial Prototype



Proof of Concept



Full Vision

Accessible
Affordable
Automated
Cell Colony
Counting
System
(A³C³S)



EOS R5 + MP-E 65 mm macro lens + motion stage +



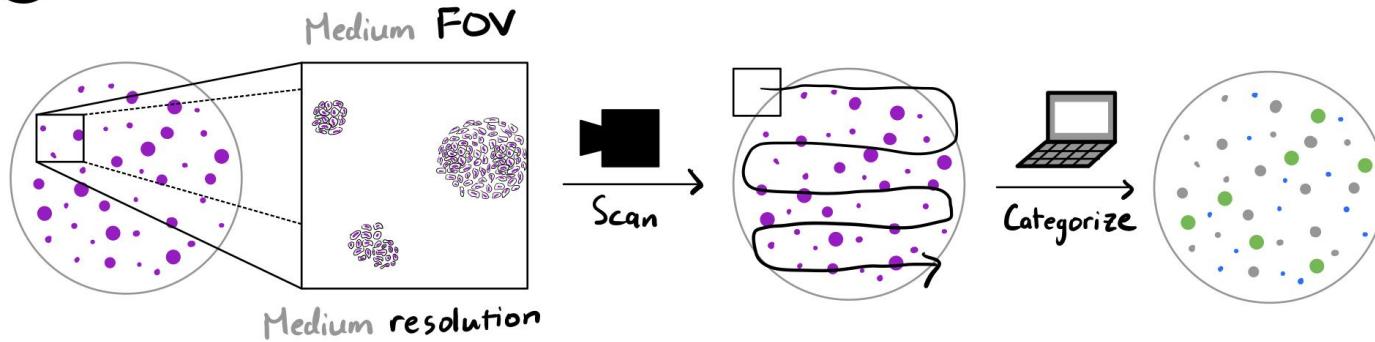
MIST



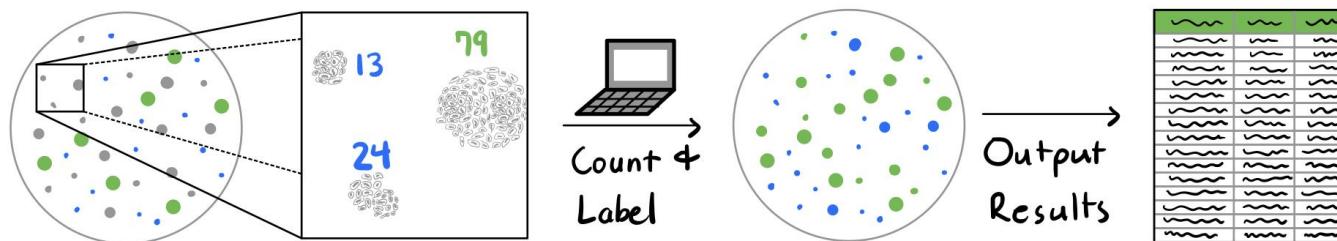
ImageJ (Fiji)

Our strategy: Digital two-step

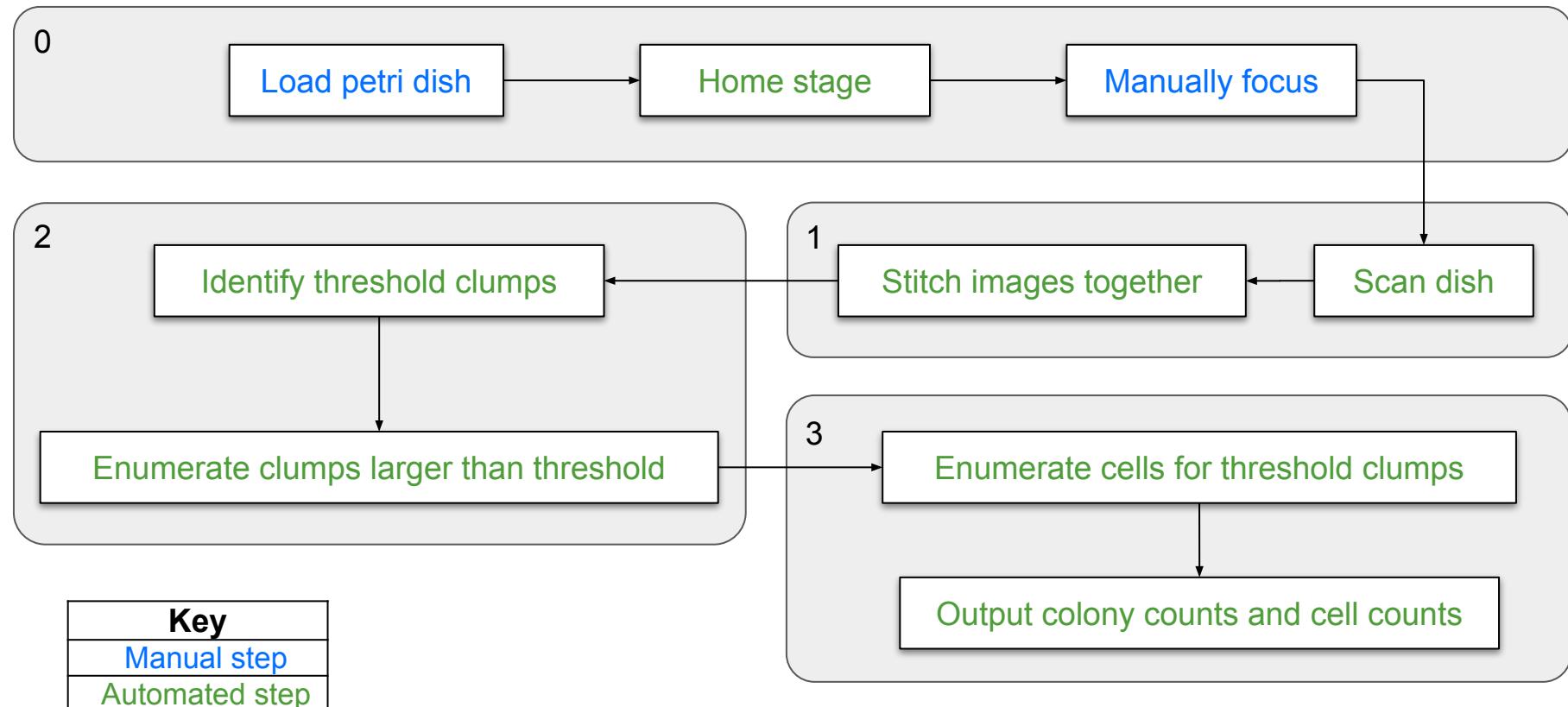
① Find large colonies and threshold cell clumps



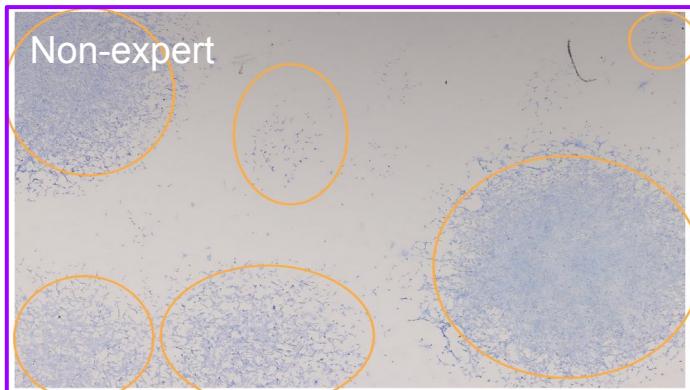
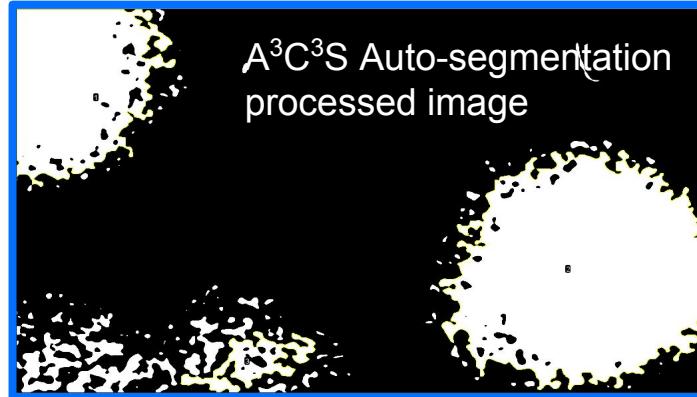
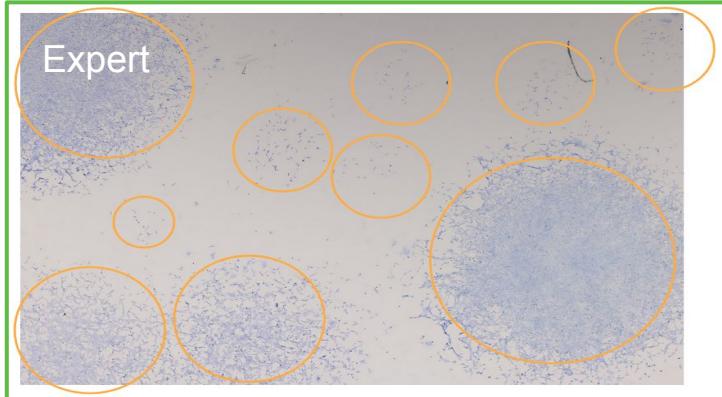
② Count cells in threshold cell clumps



Four-phase workflow



Clump categorization works, parameters need tuning

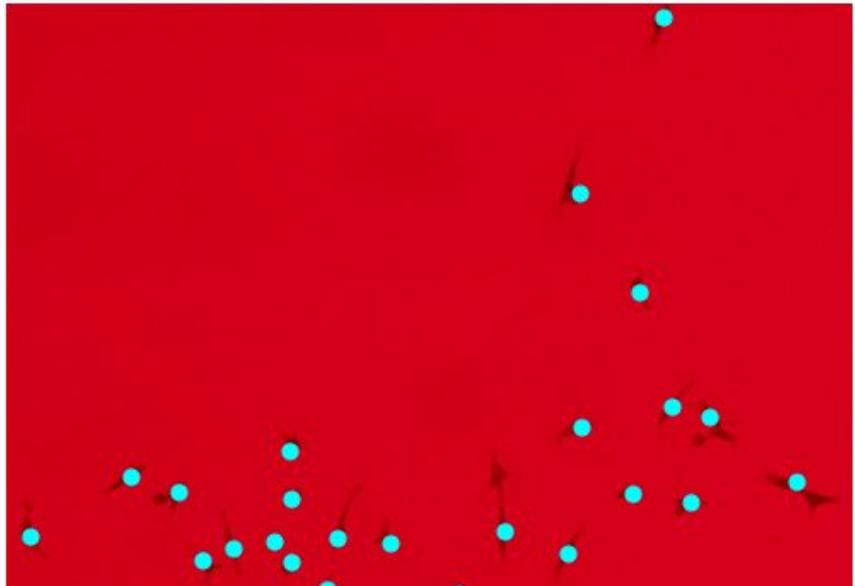


Mouse 3T3 fibroblasts, Giemsa stain

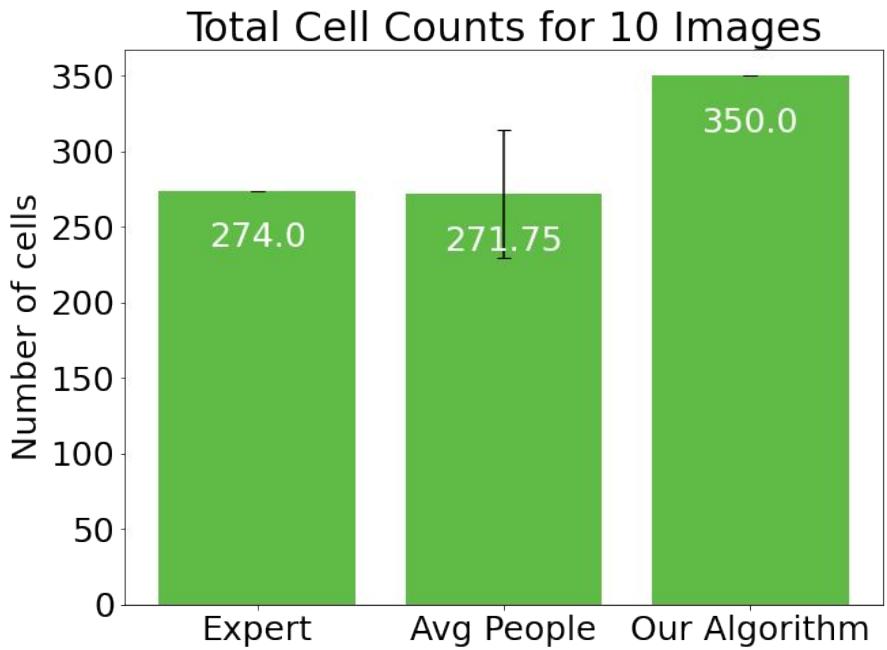
	Colonies Detected
Expert (Nomura-san)	5
Non-experts ($n=4$)	4 - 7*
A^3C^3S	3

* wide human variability

Cell counting image analysis works, needs tuning



Example of A³C³S cell counting



Progress to date

Key	
Green	Successfully met
Blue	In progress
Orange	Work in progress

The goal...	Target	Realized
affordable	<\$50k / system	~\$5.6k / system
petri-dish based	yes	yes
table top size	~300 x 300 x 300 mm ³	305 x 343 x 540 mm ³
fast	< 3 minutes / dish	> 8 minutes / dish
distinguishes colony from non-colony clumps	±1 colony Spatial resolution: Nyquist ≥ 2 pixels St. Venant ≥ 3X-5X (6-10 pixels per nucleus)	Successful clump identification Successful cell counting Need to optimize thresholds

Summary & Future Work

Current features

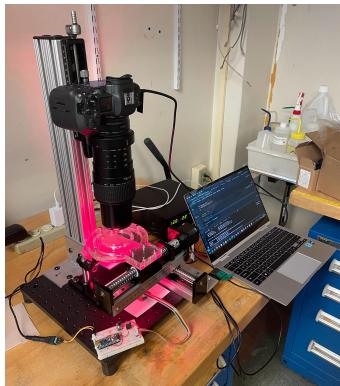
Semi-automatic image acquisition

Image analysis

 Image stitching

 Clump identification

 Cell counting



Essential next steps

Fully integrate software & hardware

Tune image analysis parameters

Evaluate image analysis process

 Repeatability

 Sensitivity to parameters

Evaluate cell assay process

 Determine cell growth distributions

 Evaluate requisite accuracy

 Establish trust for a reduced computation evaluation task



Thank you for your attention!
Questions & Feedback