Poster 1101-B

Automated Ultra-Fast High Content Imaging Enables High Throughput Live Cell Assays with Submicron Resolution

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Introduction

The advantage of live cell assays
Live cell assays provide kinetic insight into dynamic cellular processes, following the same cells over time. End-point assays might overlook robust effects or come to erroneous conclusions because cells were fixed too early or late. Further, fixation distorts cell morphology and induces artifacts. Therefore, a live cell assay should be the gold standard for looking at biological processes (e.g. protein translocation, cell killing, and drug responses).

The challenges of scaling up live cell assays
Live cell imaging with good sample coverage and resolution is limited by acquisition speed and instrument availability: With 1-2 hours to acquire a plate, is the first well imaged in the same state as the last? Time constraints mean compromises, limiting time points and number of plates acquired on traditional instruments.

Summary

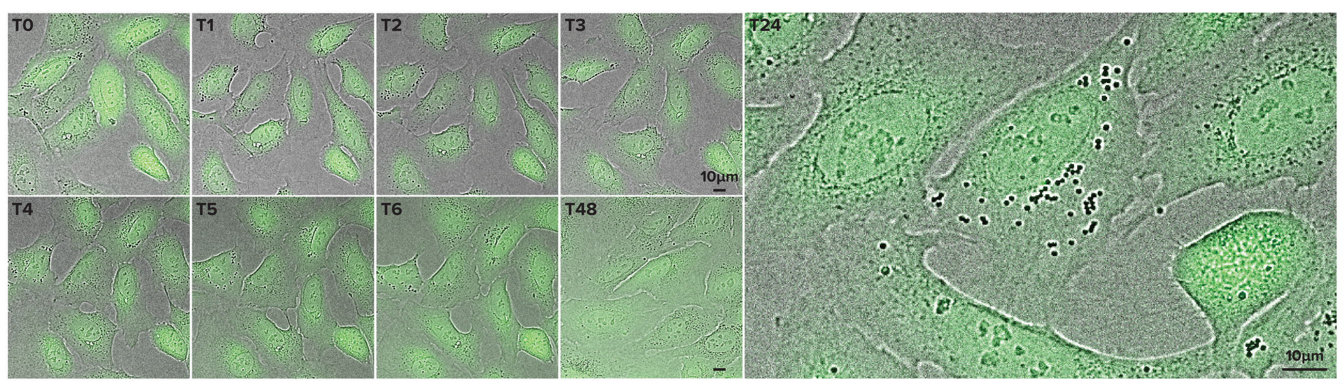
Here we demonstrate **how to achieve scale with high resolution live cell imaging:** The speed of the Araceli Endeavor Pro allows timepoint-based high content imaging at scale, with hourly and daily data. These hourly-imaged cells look less perturbed than if they were imaged with traditional high content imaging (HCI). Expanding on these capabilities, we show that live cell assays are possible with pooled screens, repetitively imaging a one-well plate with 100% well coverage in <20 minutes with single cell analysis of pooled cells. Automation can expand these capabilities further, imaging nearly **500 million cells in 24 hours at submicron resolution.**

High speed imaging enables timepoint-based live cell imaging

Plate Format	1 FOV (1.2 x 1.2mm) 5 Channels	TL Alone	Maximized Coverage 5 Channels	TL Alone
96	0:22	0:10	5:59	2:21
384	1:17	0:38	5:57	2:17
1536	5:47	2:54	5:47	2:54

Tab.1: Imaging speeds (min) for the Araceli Endeavor Pro. 5ch is 4 fluorescent channels plus transmitted light (TL). All imaging at 0.27µm/pixel. 1 FOV yields **~1,000** confluent endothelial cells. 'Maximized well coverage' represents the majority of the well area.

Fig.1: A single ROI from a live imaged 96-well plate showing cellular changes over time (hours).



- Epithelial cells were imaged hourly for 6 hours (T0-6), then again at 24 and 48 hours, using a nontoxic fluorescent cell proliferation dye and brightfield
- Fast imaging times (Tab.1) enable live cell imaging, with cells loaded in and out of an incubator between timepoints, never spending more than 10 minutes outside
  - This means >10 96- 384- or 1536- well plates can be imaged hourly at submicron resolution, with a majority of cells imaged in every plate
  - These fast imaged cells are compared against cells imaged at traditional HCI speeds (Figures 2 and 3), showing less perturbance
- Machine learning reveals fast imaged cells look like they were undisturbed

High speed live imaging perturbs cells less than traditional HCI

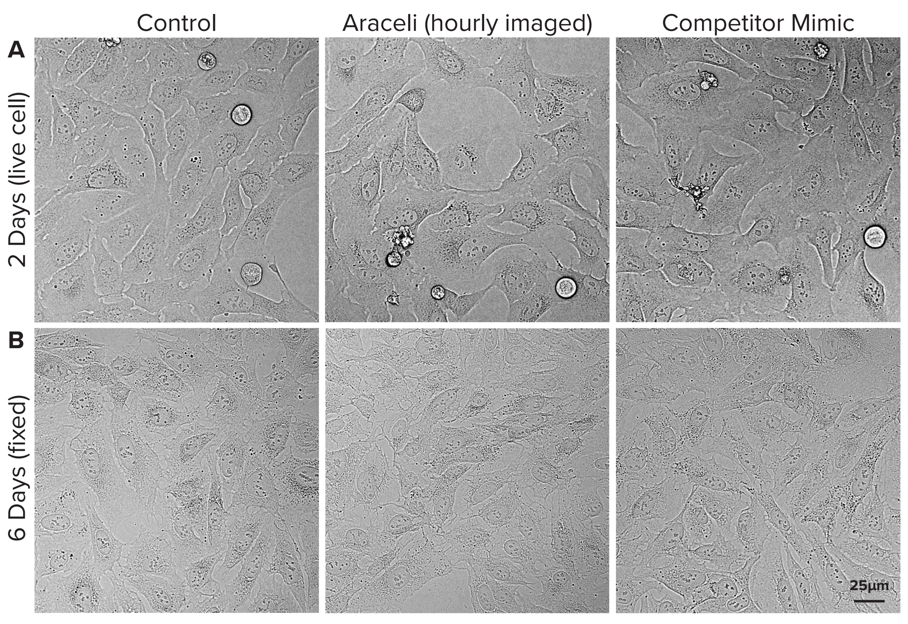


Fig.2: Brightfield imaging of cells imaged at day 2 and 6:
- Control (kept in incubator)
- Imaged hourly 7x at high speed on day 1
- Competitor imaging mimicking traditional HCI times (ambient temperature for 4 h on day 1)

Note effects of fixation (lower panel) on cellular morphology: Membrane retraction and nuclear morphology.

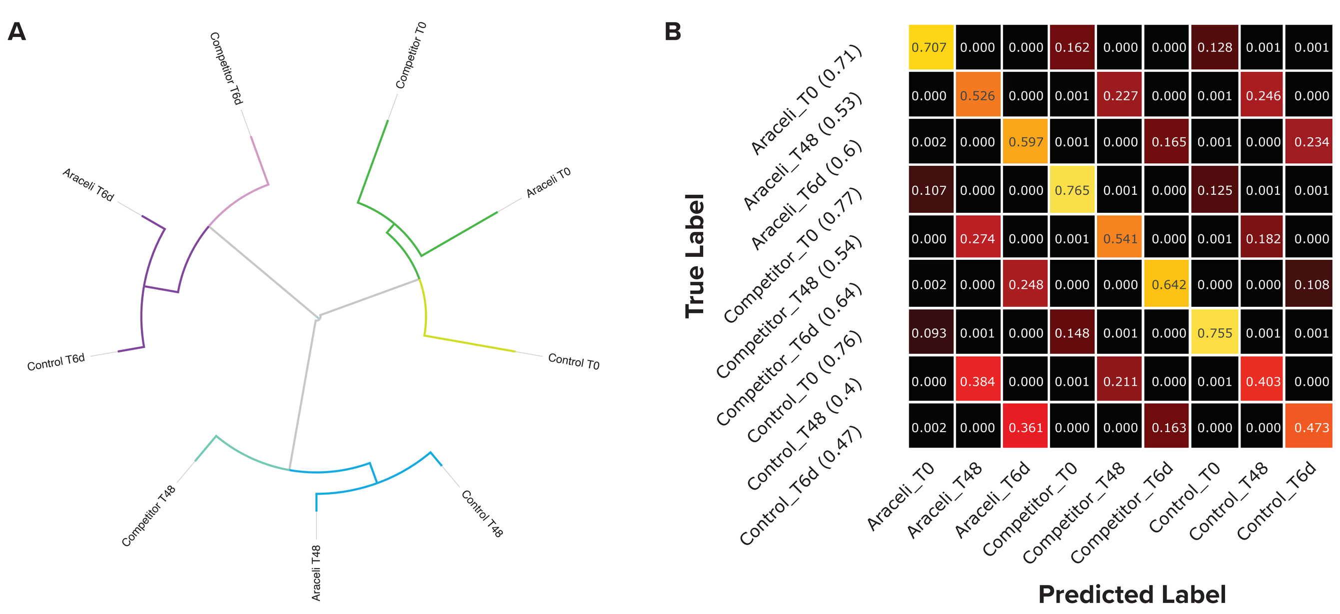


Fig.3: AI-based phenotypic analysis using ViQi AutoHCS reveals that fast time-point imaging is less perturbing. Samples imaged hourly on the Endeavor Pro ("Araceli") cluster closely with controls at 2 and 6 days after plating (**A**), demonstrating less morphological disruption compared to those left at room temperature for 4 hours ("competitor"). At 2 and 6 days, the confusion matrix indicates these fast imaged cells are less distinguishable from controls (**B**).

Use case: Imaging pooled screens at 100% well coverage, up to 5 million cells in <20 minutes in a 1-well plate

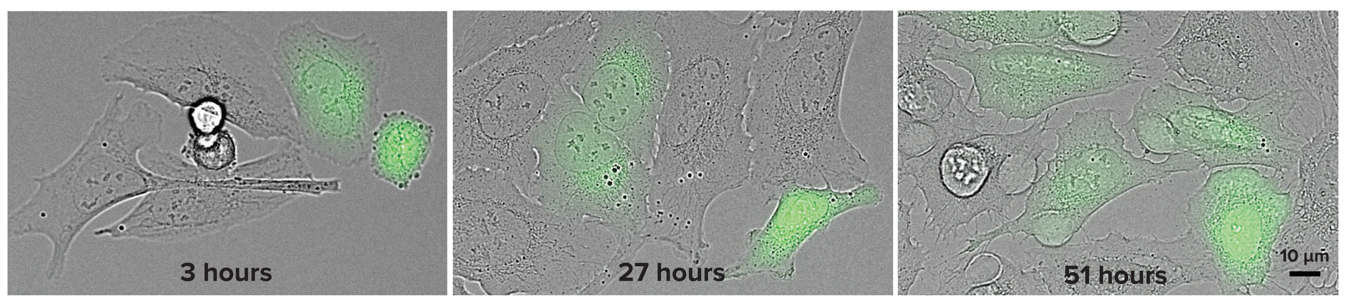


Fig.4: ROI of pooled cells, same location shown with brightfield and green fluorescence over 2 days.

- Pooled live cellular imaging over multiple rounds
- To replicate cell pooling, 10,000 green-labeled U2OS cells added to 1 million cells in a one-well plate then imaged 3 hours, 1 and 2 days after plating
- Pooled screens necessitate well coverage, as each cell can be a distinct genotype
- **100% coverage (73cm2) and full resolution in 16-18 minutes/plate** for 2 channels
- Green cells analyzed with Araceli Clairvoyance™, representing 0.9% of total cell population after 3 days.
- Morphology data indicate an elongated phenotype at 51h

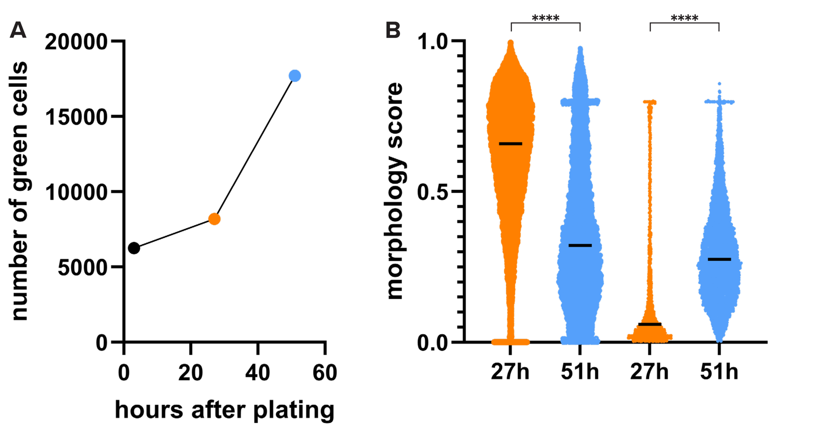
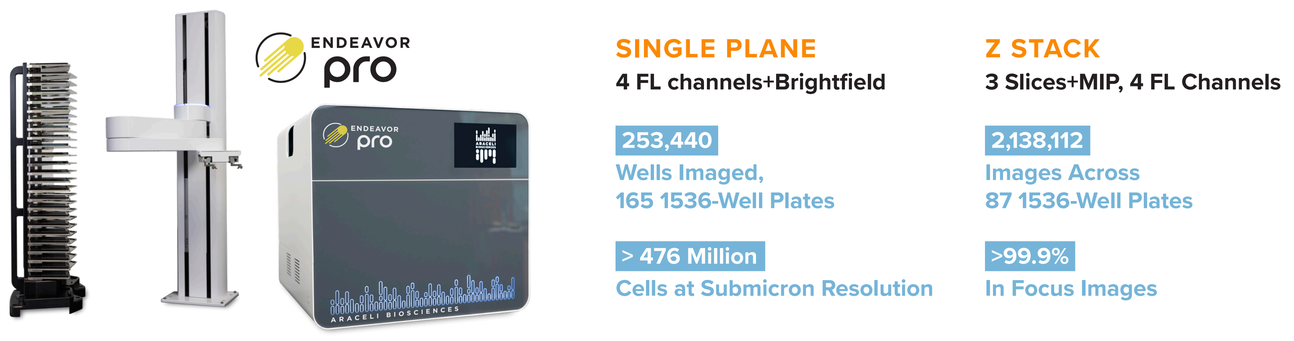


Fig.5: Single cell measurements of a minority population in a pooled sample over time. Green dyed cells (Fig.4) were detected via template matching and segmented with adaptive intensity measurements, filtering out objects <27µm2 and excluding the area 750µm from the sides due to edge effects. Cell counts over time (**A**) with a subset of morphology measurements plotted per cell (**B**). ****P<0.0001 (unpaired t-test)

How much can you image in a day?

24 HOURS OF AUTOMATED IMAGING



- Automation allows imaging to be scheduled: loading & unloading automatically from incubator. Endeavor Pro is automation-friendly with SiLA2 compatibility.
- 1536 well plates kept in a hotel stack, barcodes scanned and loaded/unloaded with robotic arm for 24hr.
- 75 terabytes generated in a single day: images saved directly onto network attached storage device.
- For z-dimensional data, 3 z slices imaged 1µm apart, saving both slices and the automatically generated maximum intensity projection with 4 fluorescent channels at 0.54µm/pixel (2x2 binning); single plane is at 0.27µm/pixel.

Methods overview

All data collected in vitro from human cell lines (A549 and U2OS) kept in standard conditions. Fixation for 15 minutes with 15% formalin. Live fluorescent labeling with Biotium ViaFluor488. Imaging done with 150-180µm bottom optical grade plates on the Araceli Endeavor® Pro. Analysis done with Araceli Clairvoyance™, except Fig. 3, which uses ViQi AutoHCS.

Conclusion: What can you do with live cell at scale?

Unlock new insights: Live cell imaging kinetically captures transient and dynamic processes, like protein translocation, cell killing, and drug responses, without the distortion or artifacts of fixation.

Solve scalability challenges: The Araceli Endeavor® enables high-throughput high-resolution imaging without compromising resolution or coverage. Thus high-throughput live-cell assays become a reality.

Minimize perturbance and maximize kinetic data: Short imaging times not only means more plates, timepoints, and more cells per day, but also less perturbance. Minimizing time out of the incubator, cells are less stressed and may deliver more accurate results, time-points are essentially the same between first and last well.

Enable the next generation of assay: This combination of speed with coverage enables pooled screening at scale. Simultaneous transmitted light & fluorescence imaging at submicron resolution paves the way for next-gen drug discovery with enhanced training data for machine-learning-driven phenotyping. Brightfield imaging offers non-perturbing, unbiased data lending itself to kinetic, AI-driven screens.

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