High content imaging is now high throughput: End-to-end high content imaging and analysis of autophagy on a subcellular level in minutes

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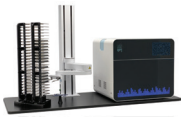
Measuring autophagic flux at high throughput speeds

- Cells eliminate intracellular waste and defective components through autophagy ("self-eating")
- A key therapeutic target for aging-related dysfunction, autophagy is implicated in neurodegeneration and cancer
- LC3b aggregation measures autophagic flux, resulting from increases in autophagy (stress) or lysosomal blockage
- High content imaging (HCI) allows for cell-level resolution, visualizing then counting aggregates within the cell
- Resolving aggregated LC3b is essential, cell lines can express endogenous LC3b in non-aggregated form
- Screening for autophagic flux requires speed for a many compound library, low variability and accurate detection

- This autophagy assay with the Araceli Platform shows:
  - Submicron resolution to accurately visualize aggregates
  - Elimination of variability with whole well imaging
  - <10-minute scan times for 96, 384 and 1536 plates
  - Comparable analysis to CellProfiler in <1/10 the time

Grow cells: 48 hrs
Treat cells: 12 hrs

Autophagy staining
Fix and stain: 4 hrs



Set up: <5 minutes
Imaging: 7-10 min/plate

ARACELI Voyager
CellProfiler™ cell image analysis software

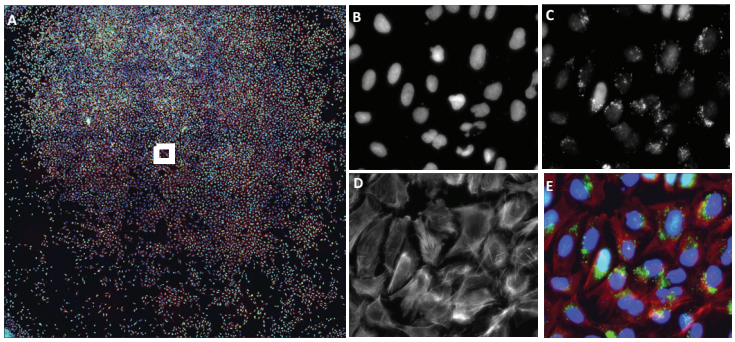
Compare analysis pipelines: 30min-3 days

Methods

Cell culture and autophagic flux assay
Human bone carcinoma (U2OS) cells were plated at 12500 cells/well in 96 well Grenier CellView plates and cultured for 48 hours in standard conditions (complete media, 5% CO, 37°C). Cells were treated with compound or DMSO in a 1:2 dilution series, 3 replicates/plate, for 12-16 hours. Cells were then fixed in 10% formalin for 15 minutes, permeabilized with 0.1% Triton x-100, then stained and counterstained for 60min/each in 1% BSA, with 1:1000 Rb anti-LC3b (L10382) and 1:1000 Gt anti-Rb Alexa 488 with 1:750 Hoechst and 1:1000 iFluor Phallodin-647. Cells were washed 4x between steps and all solutions are in PBS pH 7.4.

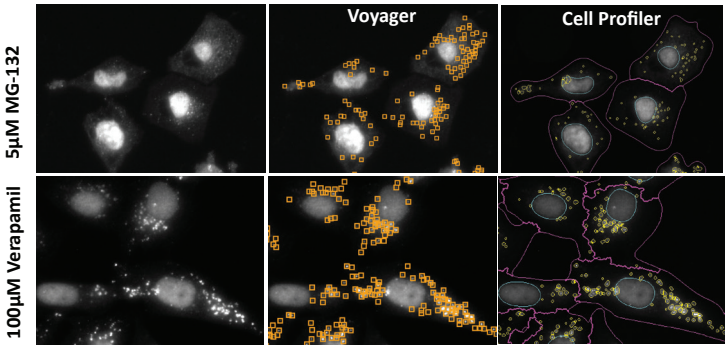
Treatments: **DMSO**: vehicle control; **[hydroxy]chloroquine**: autophagosome degradation inhibitor; **verapamil**: Ca^(2+) inhibitor; **MG-132**: inhibit proteasome

Comparative analyses
Cytoplasmic spot detection and counting done in parallel between CellProfiler and Araceli Voyager™. For both, nuclei and cell segmented (see figure for Voyager details and example), with nuclei subtracted from cell to define cytoplasm, and spots counted within cytoplasm. For CellProfiler, images were preprocessed to eliminate illumination profile and background intensity offset and spots enhances using difference of gaussians; Voyager automatically flatfield/vignette corrects image from Endeavor. CellProfiler optimization of parameters done on test set of 3 images with full pipeline generating new image files, which were then visually assessed and rerun with new parameters. Voyager's assay development mode used to visualize and tweak each parameter stepwise with near instant analysis feedback. Z' calculated from effect size peak, compared to DMSO control.

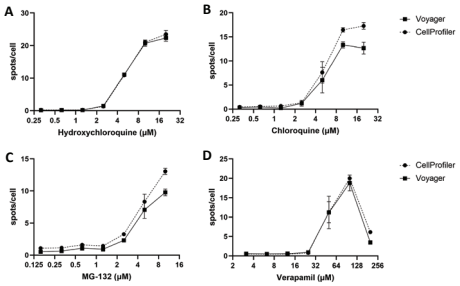


Full well, full plate high resolution scans in <10 minutes
96 well plates imaged at 0.27µm/pixel, 3 channels, with (A) full wells (~85% of well area) collected. Exposures set up to maximize dynamic range at drug concentration peak for each plate, 10µM chloroquine shown with Hoechst (nuclei, B), LC3B (autophagosomes, C), and phalloidin (actin, D); merged image (E).

Autophagic flux consistently detected with comparable levels in Voyager and CellProfiler



Z' Score	Chloroquine	Hydrochloroquine	MG-132	Verapamil
Voyager	0.8314	0.8503	0.8342	0.6325
CellProfiler	0.9037	0.8355	0.8799	0.9489

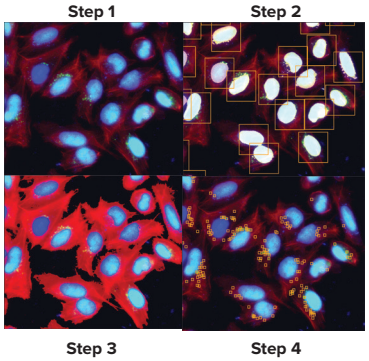


Z' (table) and dilution series of a) hydroxychloroquine, b) chloroquine, c) MG132 and d) verapamil, comparing spots/cell measurements in Araceli Voyager (solid line/box) and CellProfiler (dotted line/circle), with respective Z' calculated in table. Note: verapamil is cytotoxic at high dose.

Voyager analysis workflow saves days of assay set up, hours of compute time per plate

Analysis	Set up time	Analysis time	CPU/RAM (gb)
CellProfiler	2 days	4-5 hrs / plate	128 / 256
Voyager	< 15 min	10-20 min / plate	32 / 64

Voyager workflow
Crop of 5µM chloroquine treatment in Voyager assay development mode:
Step 1: Plates loaded into Voyager with automatic flat field/vignette correction;
Step 2: Identify and segment nuclei, using machine vision on Hoechst stain;
Step 3: Segment cells using actin stain, subtracting nuclei to create cytoplasmic mask;
Step 4: Identify spots with template match, optionally segment spots if area and intensity desired.



HCS and analysis at a submicron subcellular resolution at high throughput speeds

- Autophagic flux reliably detected with several known modulators in dilution
- Good Z' indicates robust effect size, minimal variability
- Plate to results delivered in under 30 minutes
- Araceli Voyager yield equivalent analysis to CellProfiler with:
  - 10% compute time at 25% compute power
  - Easy, straightforward and fast set up
  - Near-instant feedback to refine each step, and assay overall
- 0.27µm pixel size allows for spot detection at submicron scale
- Assay broadly generalizable to other quantitative spot-based HCI assays such as: subcellular protein localization, plaque formation, aggregation, FISH, phagocytosis

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