

High Content Phenotypic Screening of Cell Paint Assays on Araceli Endeavor™



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Summary and Impact

The Compound Management and Screening Center (COMAS) has been implemented at the Max Planck Institute of Molecular Physiology in Dortmund as the central substance library of the Max Planck Society.

COMAS major research goal is to identify bioactive compounds as modulators of biological functions by using biochemical and cell-based high-throughput assays. Especially Phenotypic cellular assays hold great promise in identifying bioactive molecules from large compound collections. Furthermore, this approach should facilitate more translational investigation of primary- and patient-derived organoid tumors.

The speed at which you can deliver high content results is currently limited by the speed at which you can acquire plates.

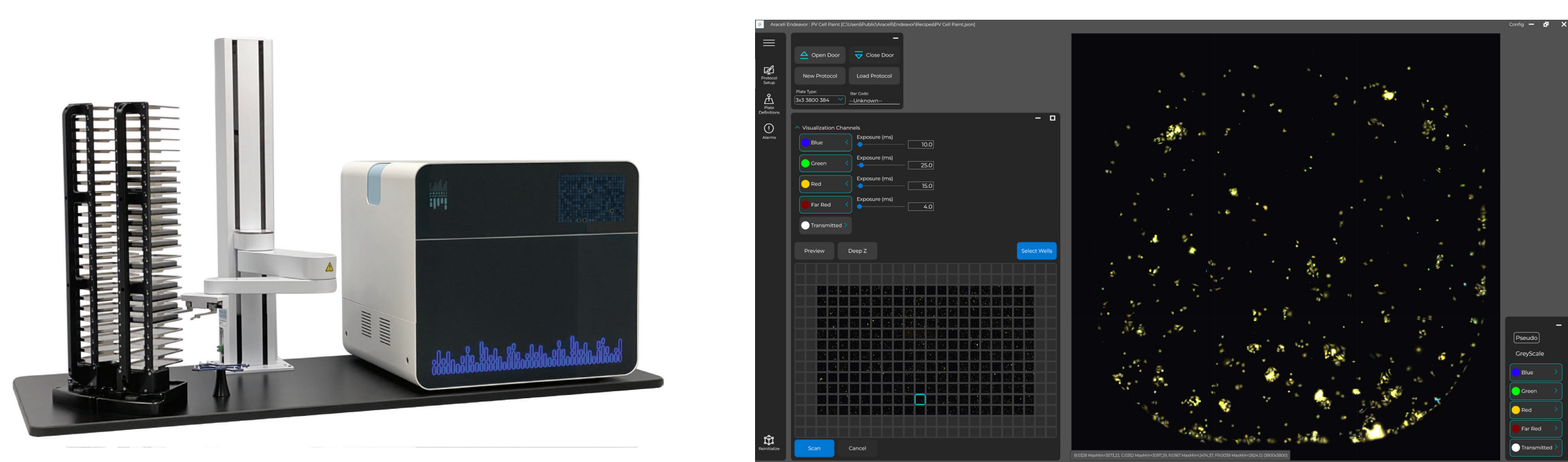
Using the new Araceli Endeavor™ we were able to complete the scan of forty-one 384well Cell Paint Assay plates (2.4x2.4mm) in four fluorescence channels at 0.27µm resolution in 7 hours.

In comparison to the currently implemented High Content Imager this results in an 94.9% reduction of the time used for image acquisition at similar resolution and well coverage. Endeavor's optimized autofocus is an important factor contributing to observed speed. During the batch run it showed 0.2% out of focus wells originating from dirt on the plate bottom or dust in the respective well.

Adding Transmitted light as fifth channel increased acquisition time only about 14% for the 2.4x2.4mm scan of the 384 well plate.

Comparison of nuclei counts with Araceli's analysis software Voyager and Cell profiler confirms accuracy of image acquisition. Prospectively, Araceli Bioscience targets to develop Voyager as a faster alternative to currently available analysis software, so the speed advantage of image acquisition will be fully realised in the end to end workflow.

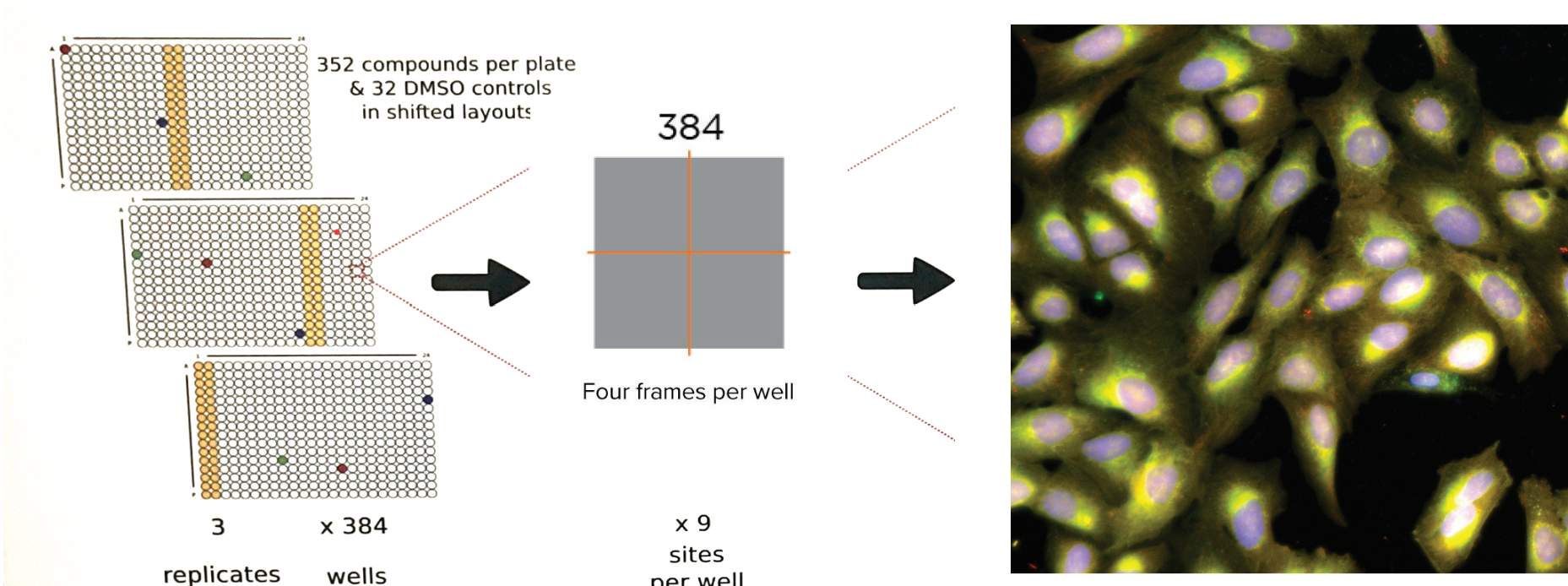
Endeavor System and Image Acquisition Software



Araceli Endeavor is a new high content imaging (HCI) platform designed for speed and ease of use. Capable of increasing throughput by more than 12 times industry standard, Endeavor delivers 0.27µm/pixel resolution and a 4400x4400px field of view. The image acquisition user interface is simple and intuitive to enable walk-up operation. Endeavor is also automation compatible, although the evaluation was performed manually.

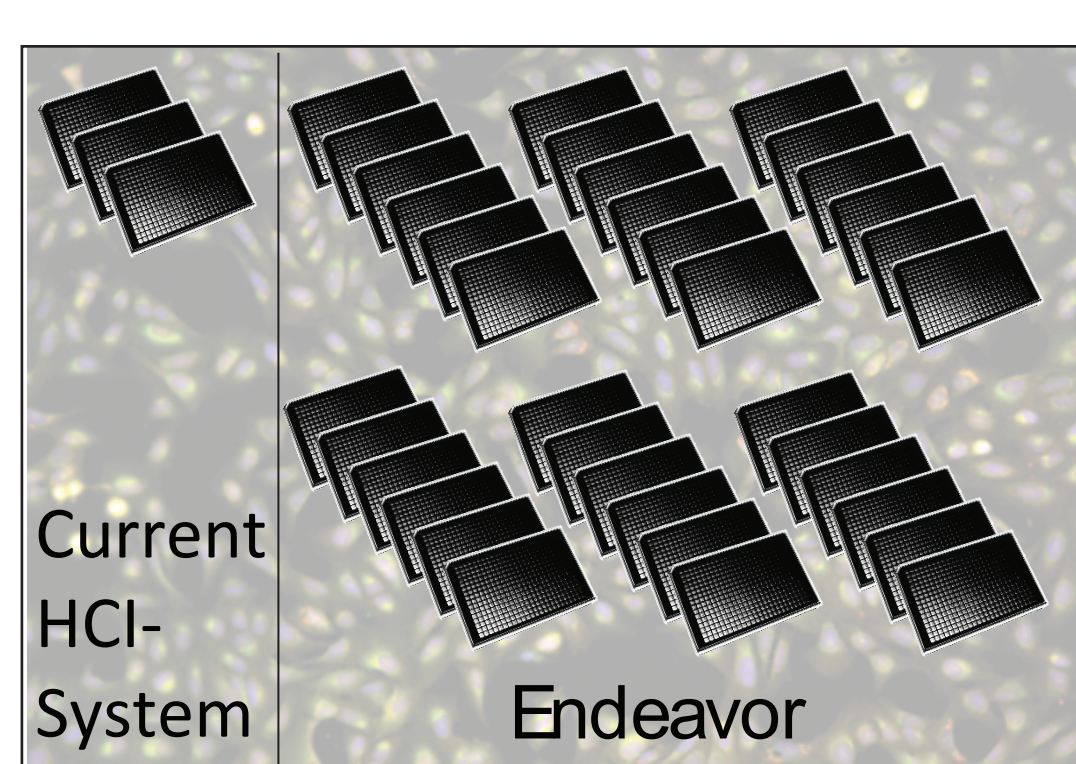
Cell Paint Protocol

U2OS cells were seeded at a density of 1600 cells per well. Compound treatment was performed with the Echo 520 acoustic dispenser (Labcyte). Cells were incubated with compounds for 20 h. First mitochondria were stained with Mito Tracker Deep Red. After fixation and permeabilization, the staining solution containing 5 microliter/ml phalloidin solution, 25 microg/ml ConcacavalinA, 5 microg/ml Hoechst 33342, 1.5 microg/ml WGA, 1.5 microM SYTO 14 and 1% BSA was added. Plates were washed three times with no final aspiration. Images were acquired on an Araceli Endeavor™ (Araceli Biosciences) in 4 channels (Blue: Ex 377-401/Em 416-446; Green: Ex 461-487/Em 501-517; Red: Ex 539-560/Em 577-607; FarRed: Ex 617-645/Em 662-700) with 4 sites per well at a resolution of 0.27µm.



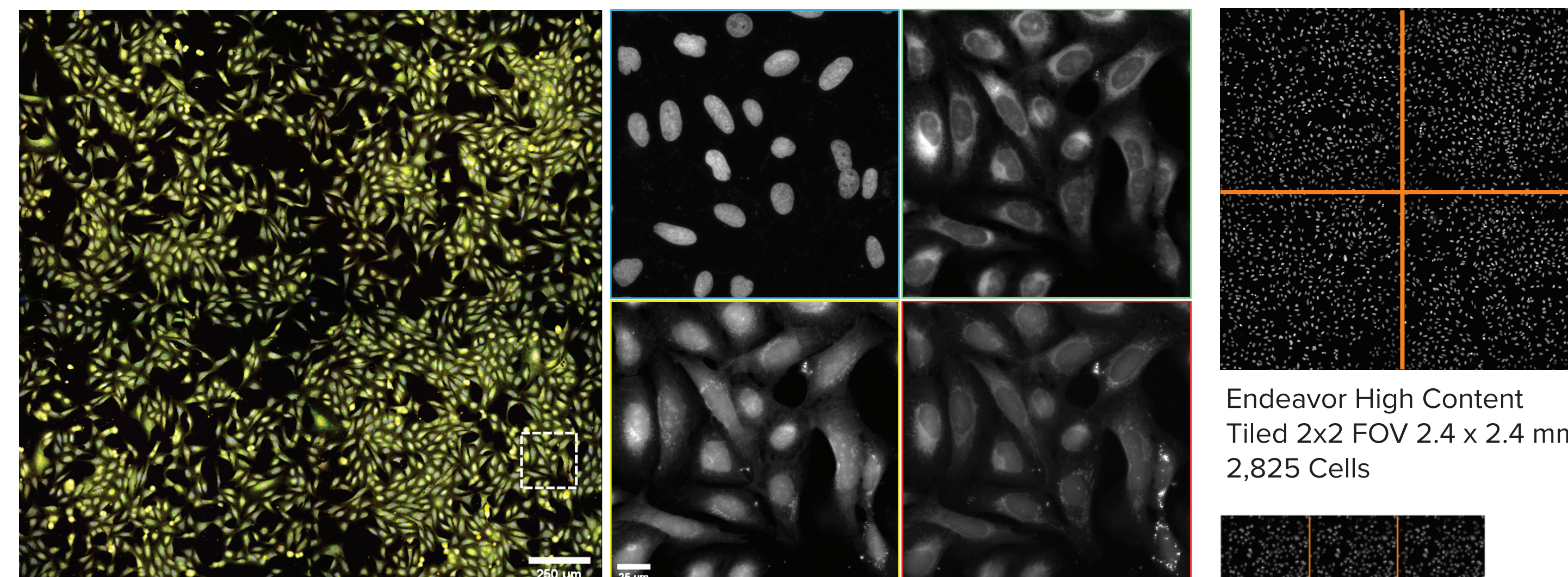
Araceli delivers at high speed resolution of 0.27µm. This allows visualization of subcellular structures like mitochondria, actin, and even down to nuclear structures.

Cell Based Screening Throughput per 8h Workday

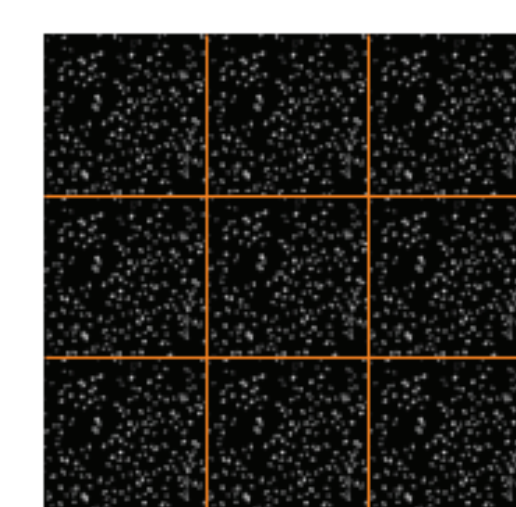


Using COMAS's current system, a batch of forty-one 384 well plates imaged in four fluorescent channels with 0.65 µm/pixel resolution requires 136 hours. Endeavor can image the same plates in only 7 hours while covering a comparable area per well. On a per day basis, this increases throughput 16x from 3 plates per 8 hour workday to 48 plates.

Endeavor High Content at Speed and Resolution



Endeavor High Content Tiled 2x2 FOV 2.4 x 2.4 mm 2,825 Cells



Traditional high content Tiled 3x3 FOV 2.1 x 2.1 mm 1800 Cells

Contrary to traditional systems out in the market Endeavor does not sacrifice coverage or resolution in favour of speed. Images are acquired as 4K images on a sCMOS camera with a single field of view of 1.2 mm at a resolution equivalent to a 35 to 40x objective. The pixels on the camera sensor are 0.27 microns per pixel where traditional sCMOS have 0.64 microns per pixel. The large Field of View of the camera sensor requires half as many images to acquire the same number of cells as on the currently used High Content Imager.

Staying Focused at Lightning Speed

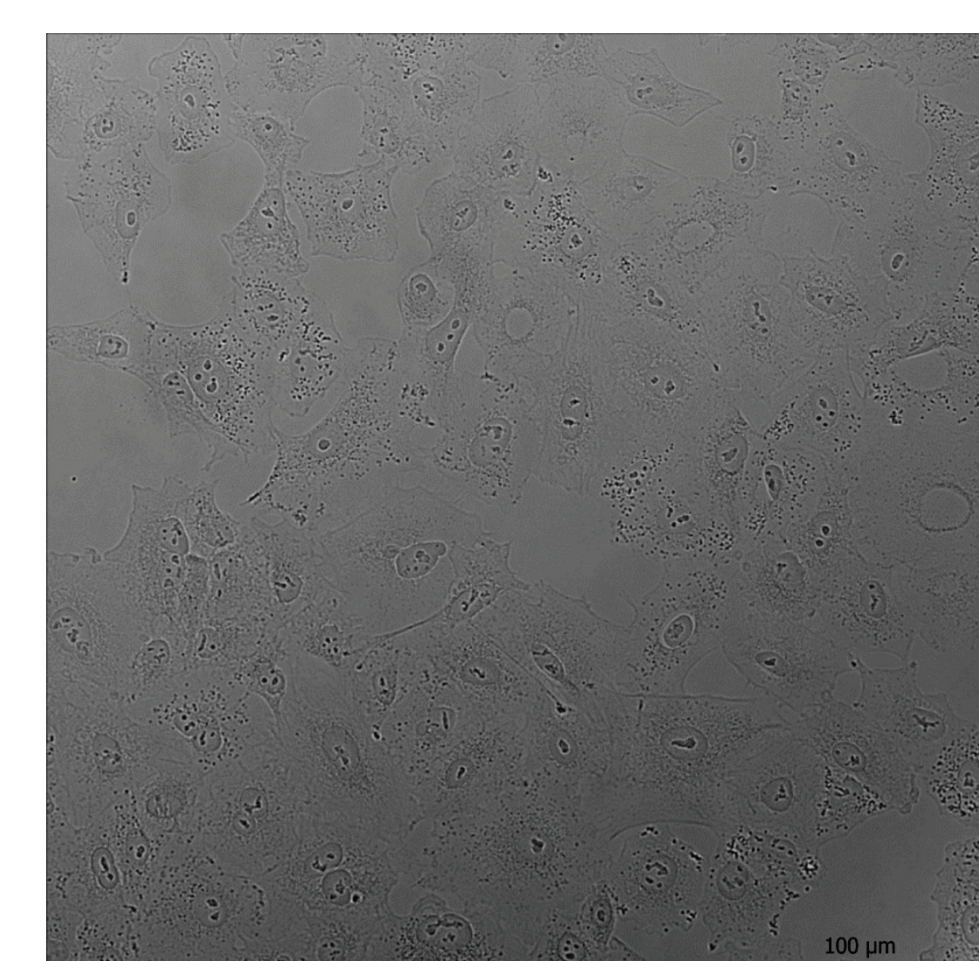
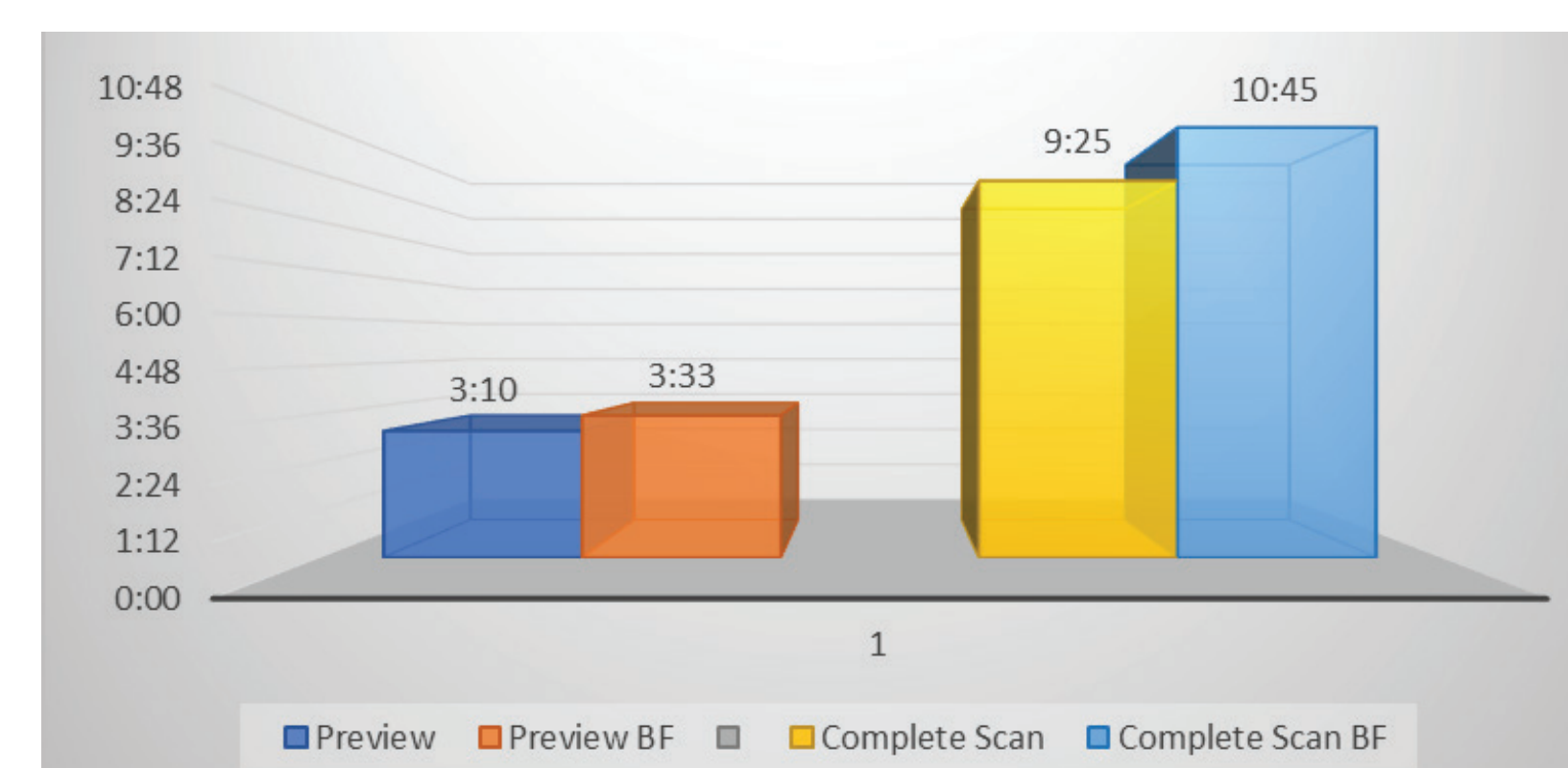
15,744 Wells Scanned
31 Wells Flagged*
0.2% Out of Focus Wells

The Optimized Autofocus Function contributes to the overall speed of Endeavor. This increased speed does not compromise accuracy. Only 0.2% of all wells ended up being out of focus.

*15 of the 31 wells resulted from one plate with visible dirt on the bottom. After cleaning, the rescan worked without any wells flagged.

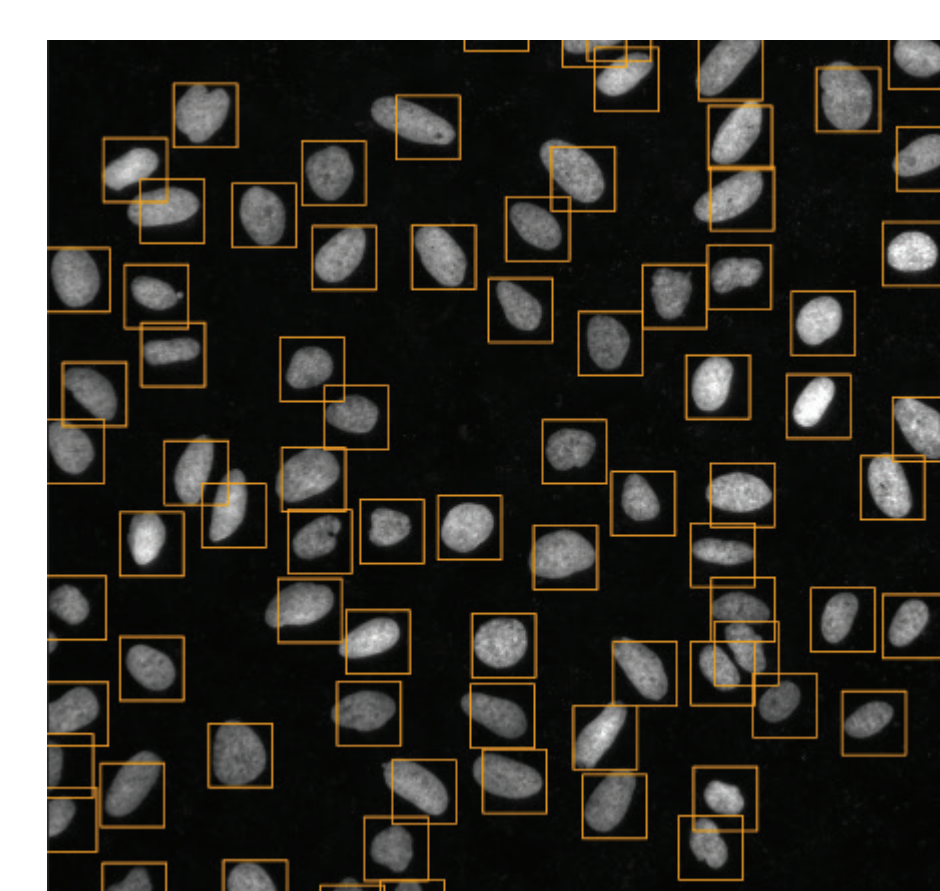
Transmitted Light as 5th Channel

Runtime comparison per plate average

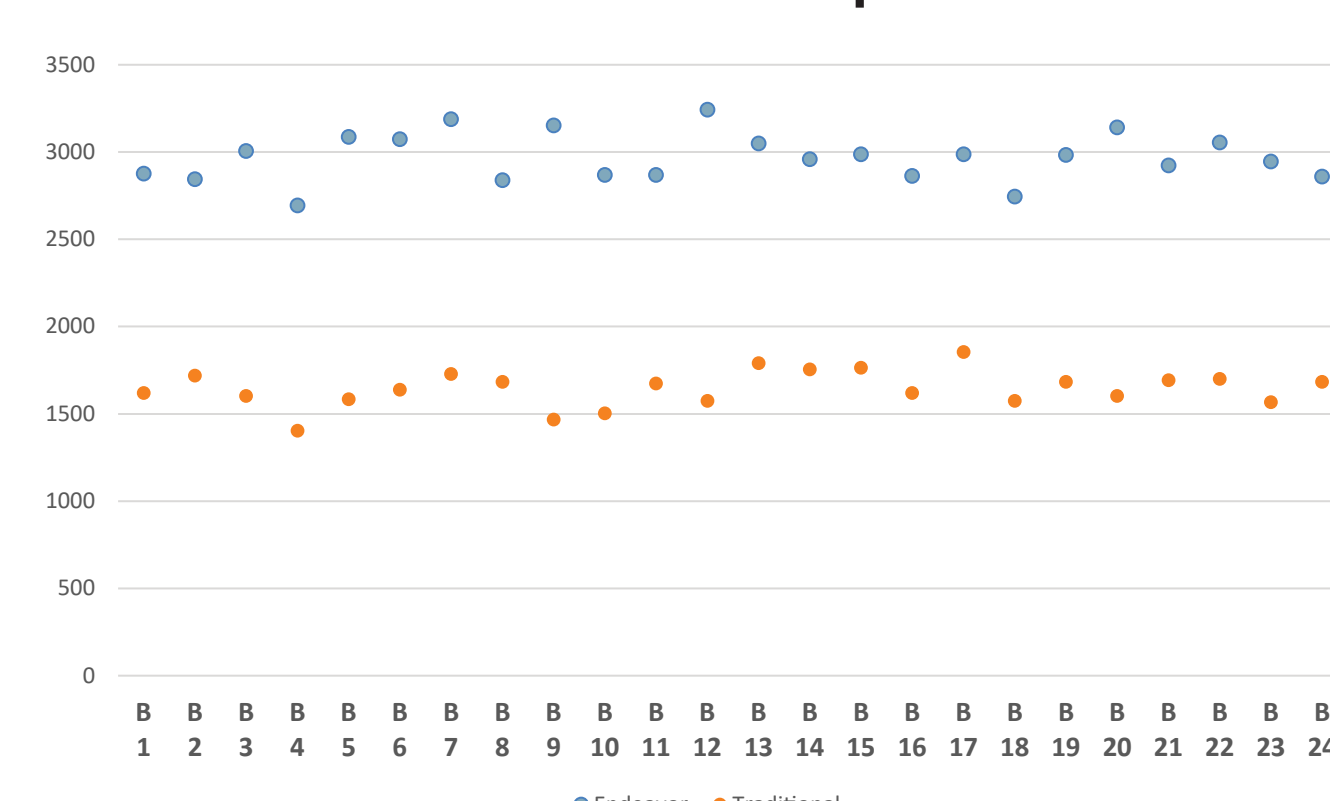


Araceli Endeavor can also acquire transmitted light images allowing for addition feature extraction or label free analysis. Adding transmitted light increased scan time only 14% for a 384 well plate (2x2 FoVs). If additional speed is needed, Endeavor can acquire images in Preview mode which acquires a single 1.2mm x 1.2mm field of view per well at the same 0.27µm/pixel resolution. A four channel preview scan of a 384 well plate took an average of 3:10 which increased to 3:33 with the inclusion of transmitted light. Transmitted light image provided courtesy of Araceli Biosciences.

Nuclei Count Comparison



Number of Cells per Well



Araceli Voyager analysis software was used to quantify the number of cells (nuclei) per well captured in the 2x2 Endeavor image. This was compared to a cell count performed on the traditional high count images (3x3) using the standard analysis software. Because of the larger imaging area of Endeavor, the total number of cells available for analysis in an Araceli scan is significantly higher. Example comparison of one row of cell counts from the same plate imaged on both systems.

Conclusion

In conclusion, Araceli Endeavor provides researchers with an exceptional combination of speed and ease of use, making it a valuable asset for high-throughput cellular analysis. Its 12-fold increase in speed compared to industry standards and 0.27µm/pixel resolution images offer unprecedented insight into the subcellular structures and functions of living cells. We believe that this new technology will significantly advance biomedical research and drug discovery by enabling scientists to generate higher quality data faster and more efficiently than ever before.