Using a laser light source for high-content automated imaging to improve sensitivity, speed, and precision for complex biological assays

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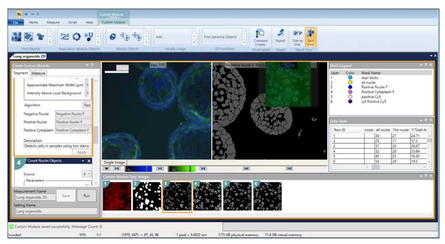
Summary

With the increasing use of highly-complex cell-based and 3D assays in biologic research, there is a pressing demand for further improvements in automated high-content imaging. In this study, we demonstrate improvement in assay sensitivity, precision, and speed of acquisition with a new configuration of the ImageXpress® Confocal HT.ai High-Content Imaging System, that utilizes laser light source. Using laser light source, the system drastically increases the power of illumination that results in brighter images, increased sensitivity, and increased speed and throughput speed of assays. The impact is especially important for the assays where sensitivity and timing are limiting factors. To demonstrate the practical impact of laser light sources, we present results from several complex biological assays.

Methods

The Instrument

ImageXpress Confocal HT.ai High-Content Imaging System with water immersion option



MetaXpress® High-Content Image Acquisition and Analysis Software

The new modification of the ImageXpress Confocal HT.ai system equipped with multiline laser light source with matched filters spanning 405 nm to 730 nm excitation wavelength that provides drastically increased illumination power to the sample.

Channel	LED/Laser (nm)	LED light source Power (mW)	Laser light source Power (mW)
Violet	377/405	200	400
Blue	NA/445		1000
Cyan	475/470	200	1000
Teal	NA/520		500
Green	543/555	300	1000
Yellow	560/555	200	1000
Red	631/640	155	900
NIR	NA/730		700

Cell assays

Transfluor® Assay was performed as previously described using cell line expressing GFP-tagged beta-arrestin that associates with the receptor of interest upon activation. Cells were stimulated with isoproterenol causing dose-dependent appearance of aggregated internalized receptors (pits), that were visualized by 20X confocal objective in FITC channel.

3D spheroids were formed with HTC116 colon cancer cell line using U-shape low attachment (Corning) plates (Sirenko, 2015). Spheroids were treated 48 hours with anti-cancer drugs, fixed and stained with DRAQ5, HCS CellMask Orange or AF555 Phalloidin, and Whole Cell Green or AF488 Phalloidin (Thermo Fisher Scientific). Spheroids were imaged using a Z-series of 20X confocal images 5 µm apart. Maximum projection images were used for cell analysis with MetaXpress software.

3D organoids were formed from primary human lung epithelial cells (ScienCells, Co) in Matrigel (Corning) domes prepared using Stem Cell Technologies kit and protocols. Mature lung organoids (after six weeks of development) were fixed and stained with Hoechst, MitoTracker Orange and AF488 Phalloidin. Organoids were imaged at 10X magnification with a Z-stack covering a range of 150–250 µm deep, and images analyzed using 3D image analysis tools of MetaXpress software.

Results

GPCR activation assay, Transfluor

G-protein coupled receptors are the largest class of pharmaceutical targets that play a major role in biological screening assays. The Transfluor Assay quantitates internalization of GFP-tagged beta-arrestin that associates with the receptor of interest upon activation, resulting in appearance of small fluorescent pits, that are counted and characterized by high-content imaging. Pits are relatively dim and require ~1 sec exposure. Using the system with lasers reduced exposure time by 3–4 times, and correspondingly reduced imaging time by 33% (1.5X gain in assay speed). In addition, assay Z'-values were also increased by 20%.

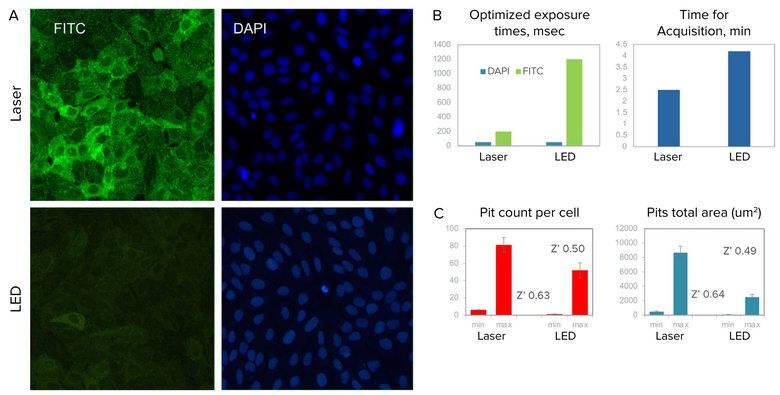


Figure 1. A. Small dots (pits) in green appeared upon receptor activation with isoproterenol. Nuclei stained with Hoechst (blue). Images were taken using same exposure times for LED or laser light sources (optimized for lasers). B. Optimized exposure times and duration of imaging were substantially reduced by using lasers. C. The accuracy of analysis was compared for images taken with laser or LED systems.

Imaging 3D cancer spheroids

In the second assay we compared a standard imaging system with LED excitation to one with laser excitation. HCT-116 spheroids were grown for four days in round bottom plates, with some wells receiving anti-cancer compound treatment with 5 µM of either Cytochalasin D or Nocodazole for the last two days. Spheroids were fixed and stained with either DRAQ5 (nuclei), Whole Cell Green, and Alexa Fluor 555 Phalloidin (actin); or DRAQ5, HCS CellMask Orange (whole cell), and Alexa Fluor 488 Phalloidin (actin).

Z-stacks of 31 confocal images were acquired 5 µm apart with a 20X PA objective to span 150 µm object depth. Image analysis was applied to count nuclei and determine area of spheroids. The images were taken using an exposure that yielded a 14 bit image whether acquired using LED or Laser excitation. By independently optimizing exposure times, 3D acquisition was done in up to half the time using lasers vs LED, depending upon number and channel of the wavelengths collected as well as intensity of the stains.

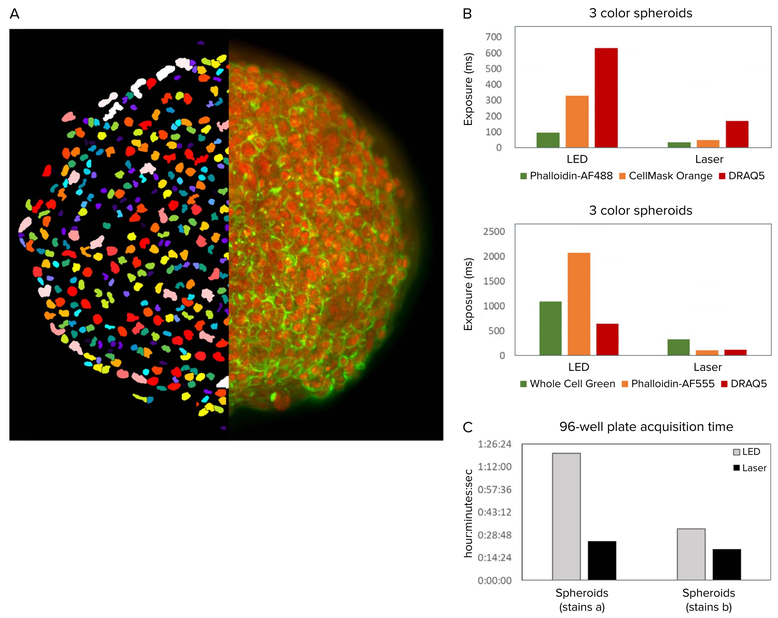


Figure 2. A. DRAQ5 and Phalloidin-AF488 overlay of an untreated spheroid plus nuclear segmentation and area masks of the 2D projection image. Low magnification QC of spheroid staining could be used to locate treated and control spheroids in the well for accurate centering before acquiring at a higher magnification. B. Exposure times optimized to acquire images of equivalent brightness. Different exposures were required for different staining protocols. C. Speed of acquiring a 96-well spheroid plate in the ImageXpress Confocal HT.ai system at 20X using LED vs laser light sources.

Results

Imaging 3D lung organoids

The organoid culture started from primary lung epithelial cells, and then were grown in Matrigel domes using reagents and protocol from Stem Cell Technologies (see Methods section). Briefly, cells were expanded in 2D, then mixed with GF reduced Matrigel and seeded into Matrigel domes in 24-well plates, or other plate formats.

Organoids are a very useful tool for disease modeling and assessment of compound effects. Automated imaging and analysis of organoids are important for the improvement of quantitative assessment of phenotypic changes in organoids, as well as the increase of throughput for experiments and tests. Confocal Imaging and 3D image analysis are especially useful for capturing complexity of 3D biological assays.

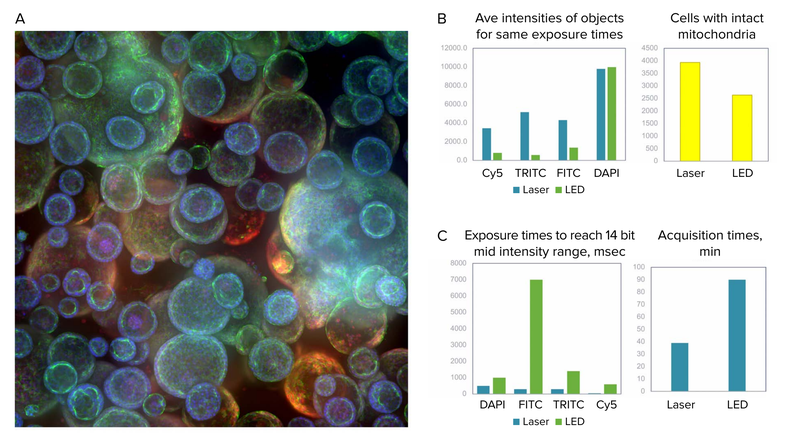


Figure 3. A. Confocal image of organoid culture (maximum projection), 10X. B. Images were taken using same exposure times for LED or laser light sources, then cell count and average intensities of objects (cells) shown for representative images. C. Exposure times were matched for 14 bit intensity range for both laser and LED. Optimized exposure times and duration of the experiment were substantially reduced by using lasers.

We have evaluated the impact of lasers on imaging of 3D organoid samples. Organoids comprised spherical objects with complex cavities and vesical structures that were imaged using 20–30 Z-planes through the Matrigel with 10X–40X magnification. Organoids were treated with compounds known to damage lung tissues, and were stained with markers visualizing mitochondria, cytoskeleton, and cell junctions. 3D analysis was done to count and characterize organoids and cells inside organoids. We were able to observe increased intensity, resolution, and sharpness of objects that resulted in more accurate analysis. Importantly, the speed of imaging was increased 2.3X (51–57% time reduction) due to 8X decreased total exposure time.

Implications of laser light source for assay speed and throughput

To test the impact of lasers on assay speed and throughput we have compared exposure times and acquisition speed for 10 independent assays performed by five scientists. The data below demonstrate decrease in exposure times and increased speed (time reduction) for 10 different assays.

Assays	Total exposure time per site, msec LED	Lasers	Decrease in exposure times (fold)	Increase in speed (fold)
1	1400	800	1.8	1.53
2	700	230	3.0	1.86
3	700	230	3.0	1.71
4	9500	1150	8.3	2.31
5	1250	250	5.0	1.54
6	5010	1000	5.0	2.03
7	1020	350	2.9	1.38
8	1340	370	3.6	1.54
9	3830	560	6.8	3.21
10	1200	255	4.7	1.55

Conclusions

- Using a laser light source significantly increased the power of illumination that resulted in increased brightness of images and increased assay sensitivity, which is especially important for dim samples.
- The imaging system with lasers substantially decreased exposure times, which resulted in increased speed and throughput. Confocal imaging with Z-stacks especially benefited from decreased exposure times.
- We characterized several biological assays and demonstrated improved brightness and image quality, which resulted in increased assay sensitivity and imaging speed.

MOLECULAR DEVICES