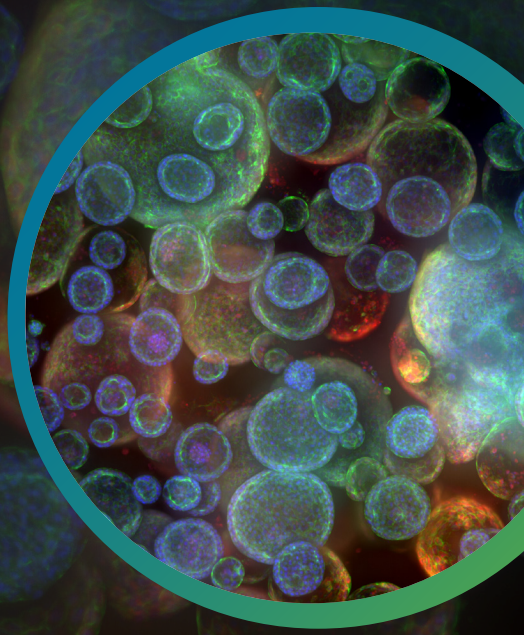
APPLICATION NOTE

Improve sensitivity, speed, and assay quality for complex biological assays using a high-content imaging laser-based system



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Introduction

With the increasing use of highly-complex cell-based 2D and 3D assays in biologic research, there is a pressing demand for improvements to the capabilities of automated high-content imaging. Here, we demonstrate improvement in assay sensitivity, quality, and speed of acquisition with the ImageXpress® Confocal HT.ai High-Content Imaging System. Using a high-power laser light source, the system significantly increases light throughput to the sample, which results in brighter images, increased sensitivity, and increased assay throughput. The impact is especially important for the assays where sensitivity and imaging time are the limiting factors. To demonstrate the practical impact of laser light sources, we present results from several complex biological assay systems: a GPCR activation assay, cancer spheroids, and lung organoids.

Benefits

- Increase image intensity and assay sensitivity with laser light source
- Reduce exposure time by 3-4X, and correspondingly reduce imaging time by 1.5-3X
- Ensure higher quality images for you assays

Methods

The Instrument

The ImageXpress Confocal HT.ai system is equipped with a multi-line laser light source with matched filters, spanning 405 nm to 730 nm excitation. This light source provides drastically increased illumination power to the sample in comparison to the previous-generation LED light source.

In the examples presented below, comparisons were done between the ImageXpress Confocal HT.ai system and the previous generation ImageXpress Micro Confocal system.

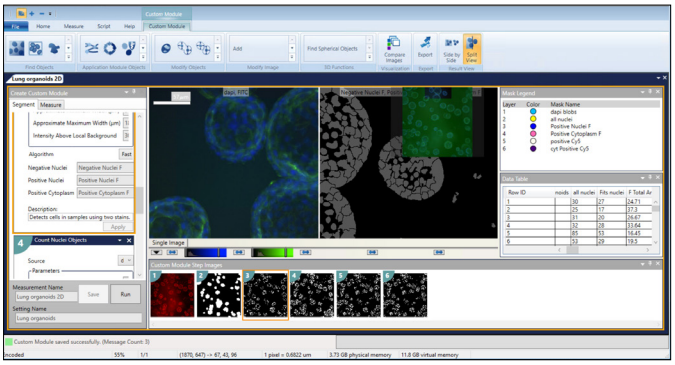
Cell assays

The Transfluor® Assay was performed as previously described using cell line expressing GFP-tagged beta-arrestin that associates with the receptor of interest upon activation[1]. Cells were stimulated with isoproterenol causing dose-dependent appearance of aggregated internalized receptors (pits), that were visualized with a 20X Plan Apo objective (60' spinning disc) in the FITC channel.

3D spheroids were formed from an HTC116 colon cancer cell line (ATCC) using U-bottom low attachment (Corning) microplates as previously described[2] (Sirenko, 2015). Spheroids were treated for 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 in 3D (Z-series with 5 µm steps) using a 20X Plan Apo objective. Cell analysis was performed on maximum projection images in MetaXpress® High-Content Image Acquisition and Analysis Software.

3D organoids were formed from primary human lung epithelial cells (ScienCells) in Matrigel (Corning) domes, prepared using a Stem Cell Technologies kit and protocols[3]. 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 in 3D (Z-stack covering a range of 150-250 µm deep) and the images were analyzed using MetaXpress 3D image analysis tools.

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



MetaXpress High-Content Image Acquisition and Analysis Software

		LED light source	Laser light source
Channel	LED/Laser (nm)	Power (mW)	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 (optional)	560/555	200	1000
Red	631/640	155	900
NIR	NA/730		700

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 activated receptor of interest. This internalization results in the appearance of small fluorescent pits that are counted and characterized by high-content imaging. Pits are relatively dim and require ~1 sec exposure with an LED light source. The laser light source of the ImageXpress Confocal HT.ai system reduced exposure time by 3-4X, and correspondingly reduced imaging time by 33% (1.5X gain in assay speed). In addition, assay Z' values also increased by approximately 20%.

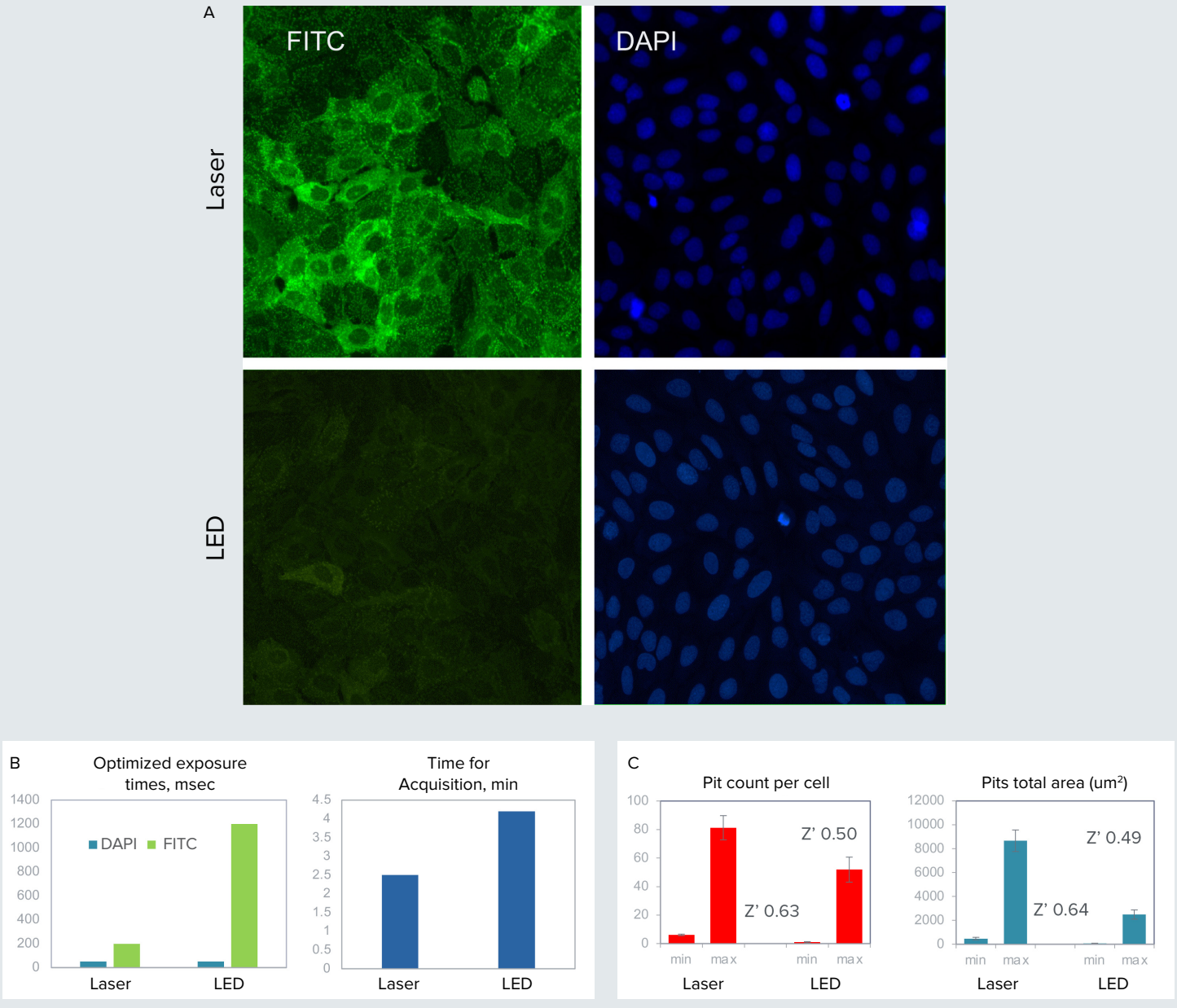


Figure 1. *A. Small dots (pits) in green formed upon receptor activation with isoproterenol. Nuclei were stained with Hoechst (blue). Images were acquired using the same exposure times for LED and laser light sources (optimized for lasers). B. Optimized exposure times and duration of imaging were substantially reduced using the laser light source. C. The assay quality (Z' value) was compared for images taken with laser and LED systems.*

3D cancer spheroids

In the second assay, we compared a standard imaging system ImageXpress Confocal 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-series images were acquired with a 20X Plan Apo objective to a spheroid depth of 150 µm (31 steps at 5 µm step size). Image analysis was performed on the maximum projection images to count nuclei and determine spheroid area. 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 for each light source, 3D spheroid acquisition was performed in approximately half the time using lasers vs LED, depending upon number of channels collected and fluorophores used.

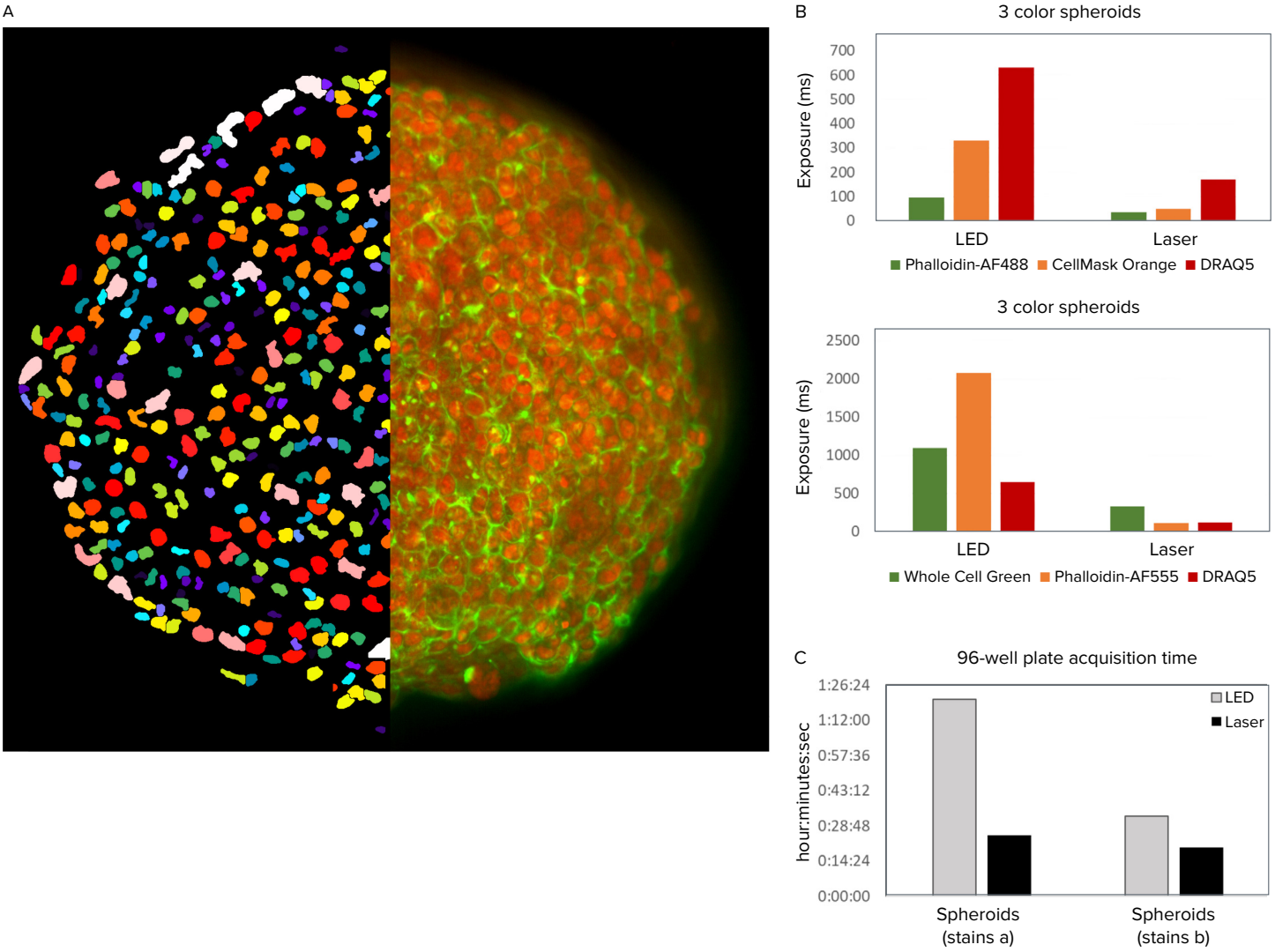
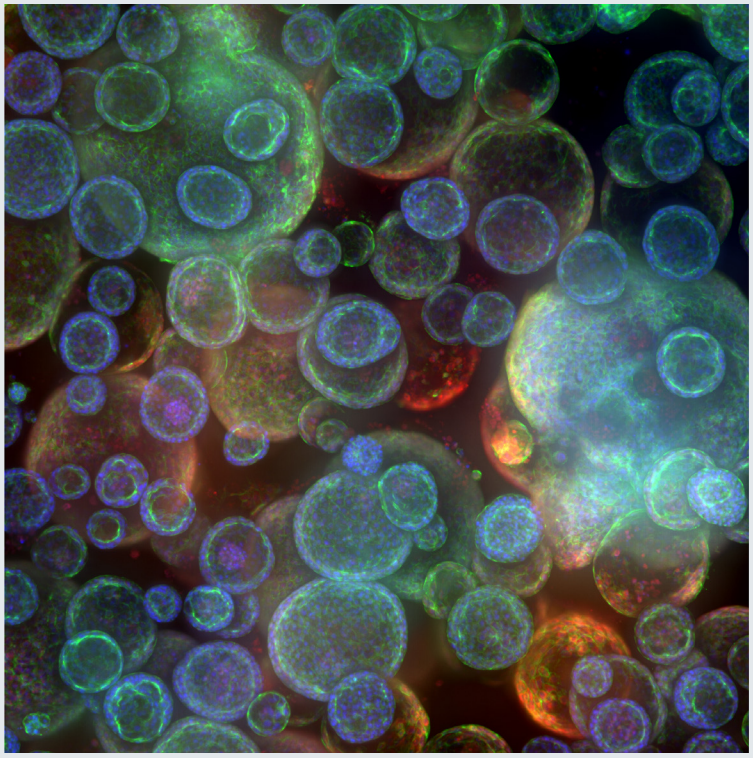
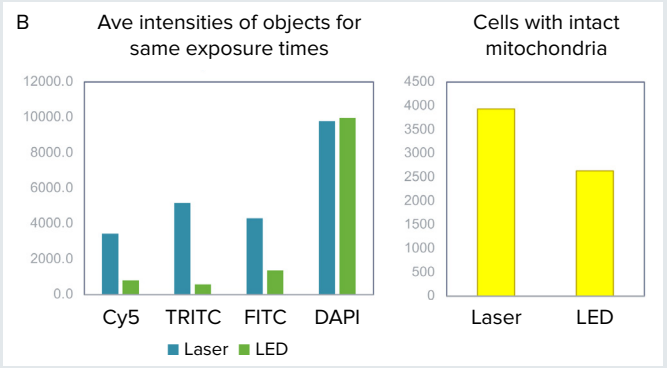


Figure 2. *A. 2D projection image of DRAQ5 and Phalloidin-AF488 overlay of an untreated spheroid (right half) and nuclear segmentation mask (left half). B. Exposure times optimized to acquire images of equivalent pixel intensities. Different exposures were required for different staining protocols. C. Speed of acquiring a 96-well spheroid plate in the ImageXpress Confocal HT.ai system at 20X using LED vs laser light sources.*

A





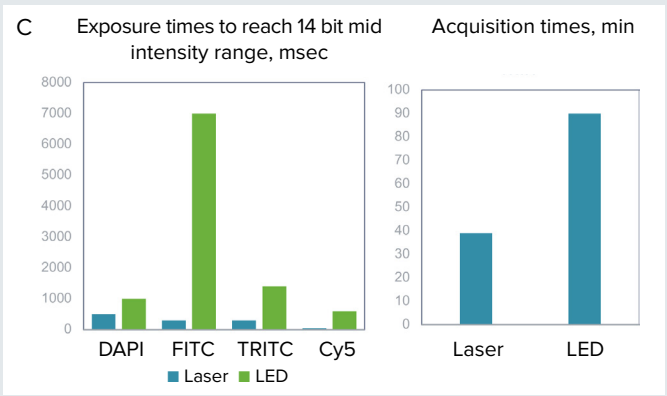


Figure 3. *A. Confocal image of organoid culture in Matrigel (maximum projection), 10X. B. Images were taken using same exposure times for LED and laser light sources. Cell count and average intensity of objects (cells with intact mitochondria) shown for representative images. C. Exposure times were matched (14 bit intensity range) for both laser and LED. Optimized exposure times and duration of imaging were substantially reduced by using lasers.*

3D lung organoids

The lung organoid cultures were started from primary lung epithelial cells, and then grown in Matrigel domes using reagents and protocol from Stem Cell Technologies. Briefly, cells were first expanded in 2D, then mixed with growth factor 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 methods for quantitative assessment of phenotypic changes in organoids, and for increasing the throughput of organoid experiments. Confocal Imaging and 3D image analysis are especially useful for capturing the complexity of 3D biological systems.

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 volumetric analysis was performed to count and characterize organoids and cells inside the organoids. We were able to observe increased pixel intensity, resolution, and sharpness of objects that resulted in higher quality analysis. Importantly, the speed of imaging was increased 2.3X (51-57% time reduction) due to an 8X decrease in total exposure time.

Implications of laser light source for assay speed and throughput

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

	Total exposure time per site, msec		Decrease in exposure times (fold)	Increase in speed (fold)
Assays	LED	Lasers		
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

Conclusion

The illumination power of the laser light source significantly increases image intensity and assay sensitivity, which is especially important for dim samples. The imaging system with lasers substantially decreased exposure times, which resulted in increased imaging speed and assay throughput. 3D imaging especially benefited from the laser light source. We characterized several biological assays and demonstrated higher image intensities and improved image quality, which resulted in increased assay sensitivity and imaging speed.

References

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