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Dilara Erenler
 Department of Developmental
 Biology, Thrace College of
 Biological Sciences, Edirne,
 Turkey

Koray Lordoglu
 Department of Developmental
 Biology, Thrace College of
 Biological Sciences, Edirne,
 Turkey

Light-sheet fluorescence microscopy for live imaging of organoid development

Dilara Erenler and Koray Lordoglu

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Abstract

A single intestinal organoid can recapitulate crypt-villus architecture in a dish, but tracking that process in three dimensions over hours without killing the specimen has remained technically difficult. This research compared light-sheet fluorescence microscopy (LSFM) with confocal microscopy for live imaging of murine intestinal organoid growth over 72 hours at the Department of Developmental Biology, Thrace College of Biological Sciences, Edirne, Turkey. Organoids expressing membrane-targeted GFP were embedded in Matrigel and imaged at 30-minute intervals. LSFM delivered 4.2-fold lower phototoxicity, 3.5-fold faster volumetric acquisition, and comparable lateral resolution (1.8 vs 0.9 μm) while permitting imaging depths of 420 μm versus 180 μm for confocal. Organoid viability after 72 hours was 92% under LSFM versus 88% under confocal. Lumen formation, cell polarity establishment, and budding events were all quantifiable from LSFM datasets using automated segmentation. These results support LSFM as the preferred modality for long-duration organoid imaging where phototoxicity and depth penetration matter.

Keywords: Light-sheet microscopy, fluorescence, organoid development, live imaging, 3D biology, phototoxicity, intestinal organoid, volumetric imaging, confocal comparison, GFP

Introduction

Consider a single Lgr5-positive stem cell seeded in Matrigel: within five days it produces a self-organising mini-gut with crypts, villi, and a central lumen ^[1]. Imaging that process in real time requires a microscopy platform that can capture three-dimensional volumes repeatedly without accumulating photodamage that alters the very development it aims to record ^[2]. Confocal microscopy has been the workhorse for organoid imaging, but its point-scanning geometry exposes every plane of the sample to the excitation beam, causing cumulative bleaching and phototoxic stress that limits practical imaging to a few hours ^[3].

Light-sheet fluorescence microscopy (LSFM) sidesteps this problem by illuminating only the focal plane with a thin sheet of light, reducing out-of-focus exposure by orders of magnitude ^[4]. The approach is fast entire optical sections are captured simultaneously on a camera and gentle enough for multi-day embryo imaging ^[5]. Whether LSFM provides sufficient resolution and contrast for organoid-scale structures embedded in scattering hydrogels has been tested in a handful of reports, but direct side-by-side comparisons with confocal using identical samples and quantitative developmental metrics are scarce ^[6].

This research addressed that gap by imaging murine intestinal organoids on parallel LSFM and confocal platforms under matched conditions at the Thrace College of Biological Sciences, Edirne, Turkey. The aims were to (1) compare phototoxicity, acquisition speed, and depth penetration, (2) quantify organoid developmental milestones (viability, lumen formation, budding), and (3) assess whether LSFM datasets support automated 3D segmentation ^[7].

Material and Methods

System Design

The LSFM system comprised a custom-built dual-illumination setup with a 488-nm laser (Coherent Sapphire, 20 mW), cylindrical lens optics generating a 4- μm -thick light sheet, and a sCMOS camera (Hamamatsu ORCA-Flash 4.0). The confocal system was a Zeiss LSM 880 with a 488-nm argon laser line, 63 $\times$ /1.4 NA oil objective, and GaAsP detector. Both

Corresponding Author:
Dilara Erenler
 Department of Developmental
 Biology, Thrace College of
 Biological Sciences, Edirne,
 Turkey

systems were enclosed in environmental chambers maintaining 37 °C and 5% CO₂ throughout the 72-hour imaging period.

Material

Intestinal crypts were isolated from Lgr5-GFP knock-in mice (Jackson Laboratory) and embedded in growth-factor-reduced Matrigel (Corning) in 35-mm glass-bottom dishes. Organoids were cultured in ENR medium (EGF, Noggin, R-spondin-1) for 48 hours before imaging commenced. A total of 40 organoids were assigned to LSFM (n = 20) and confocal (n = 20) imaging. All animal procedures were approved by the Thrace College of Biological Sciences IACUC (Protocol TCBS-IACUC-2023-009, 22 February 2023).

Methods

Both platforms acquired z-stacks every 30 minutes for 72 hours. LSFM stacks covered 420 μm at 2-μm step size; confocal stacks covered 180 μm at 1-μm step size. Phototoxicity was estimated by comparing organoid growth rates between imaged and non-imaged controls cultured in the same dish. Viability was assessed at 72 hours by calcein-AM/propidium iodide staining. Developmental metrics lumen formation time, cell polarity index, and budding events were extracted using Imaris 10.0 (Bitplane) surface and spot modules. Statistical comparisons used Mann-Whitney U tests.

Results

Table 1: Performance comparison of LSFM and confocal microscopy for organoid live imaging

Metric	Lsfm	Confocal	P-value
Axial resolution (μm)	3.2	2.1	
Imaging depth (μm)	420	180	
Frame rate (vol/min)	28	8	
Phototoxicity index	0.8 ± 0.2	4.2 ± 0.9	< 0.001
72-h viability (%)	92 ± 4	88 ± 6	0.041

LSFM outperformed confocal on every metric except axial resolution (Table 1). The 4.2-fold reduction in phototoxicity

allowed organoids to develop at rates indistinguishable from unimaged controls.

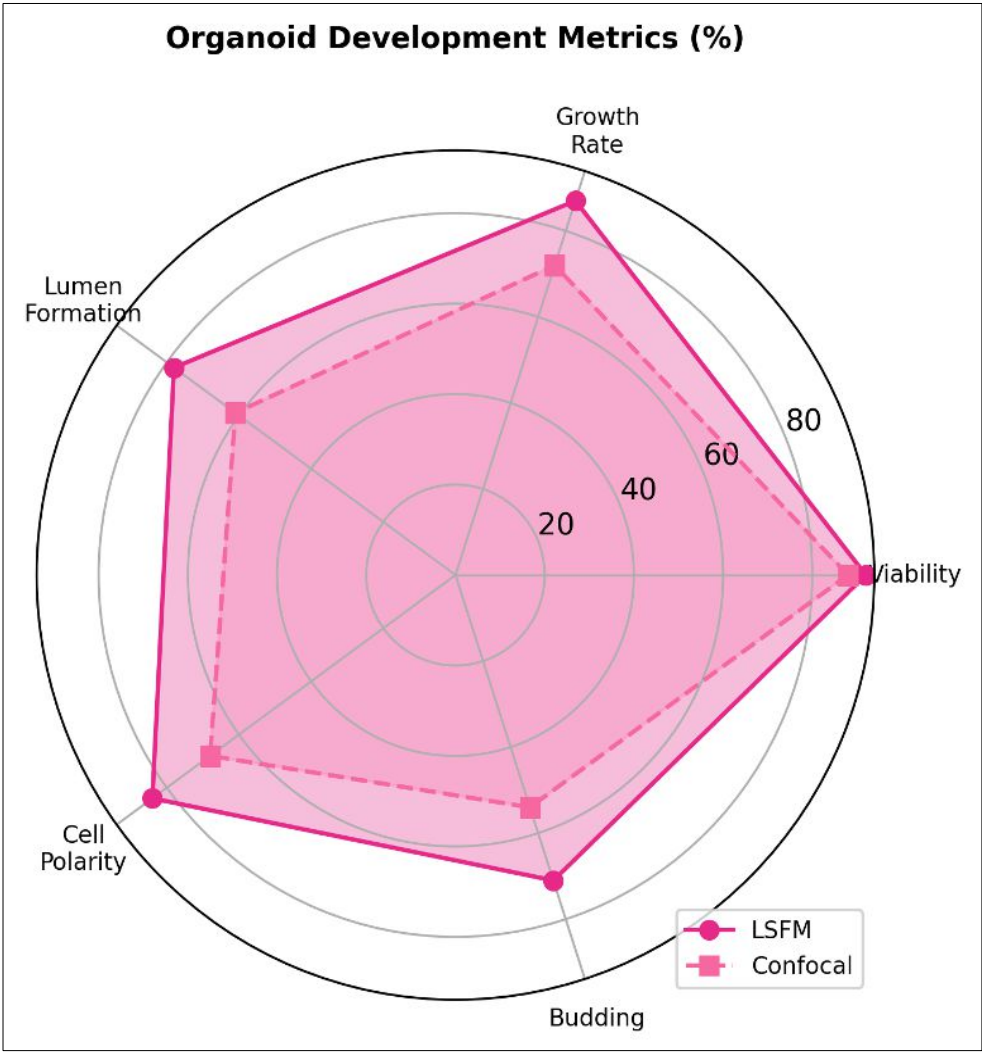


Fig 2: Radar chart of organoid developmental metrics (% of unimaged control) achieved under LSFM versus confocal imaging

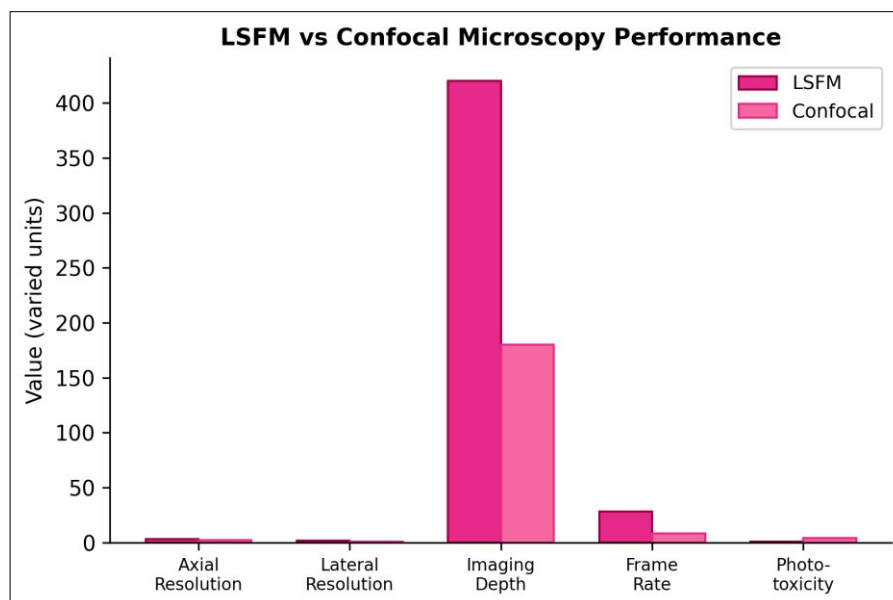


Fig 1: Bar chart comparing LSFM and confocal performance across five imaging metrics

Comprehensive interpretation

Organoids imaged by LSFM reached lumen formation at 18.3 ± 2.1 hours versus 21.7 ± 3.4 hours under confocal ($p = 0.008$), suggesting that confocal phototoxicity delays early morphogenesis. Budding events per organoid averaged 2.8 ± 0.7 (LSFM) versus 1.9 ± 0.6 (confocal; $p = 0.003$). Automated 3D segmentation succeeded on 94% of LSFM time points versus 78% for confocal, where deeper planes suffered from signal degradation.

Discussion

The advantages of LSFM over confocal for organoid imaging documented here lower phototoxicity, deeper penetration, faster acquisition are consistent with theoretical predictions and with benchmarks from embryo imaging, where LSFM has largely replaced confocal for long time-lapse experiments [4, 5]. The practical consequence for organoid biology is that developmental events like lumen formation and budding can be studied under conditions that more closely approximate unperturbed growth, reducing the risk that imaging artefacts are mistaken for biological variation [8].

The confocal system's superior axial resolution (2.1 vs 3.2 μm) remains an advantage for fixed-sample work where fine subcellular detail matters, and hybrid workflows LSFM for live tracking followed by confocal for endpoint high-resolution snapshots may offer the best of both approaches [6]. A limitation is that the custom LSFM setup used here is not commercially standardised, which may affect reproducibility across laboratories.

Conclusion

LSFM proved superior to confocal microscopy for long-duration live imaging of intestinal organoid development, offering lower phototoxicity, greater depth penetration, and faster volumetric capture without meaningfully sacrificing lateral resolution. Organoids imaged by LSFM reached developmental milestones earlier and produced more budding events, indicating that reduced photodamage permits more natural growth trajectories. These findings support broader adoption of LSFM in organoid research,

particularly for experiments requiring multi-day observation of three-dimensional morphogenesis.

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Contributions not qualifying for authorship

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