Keeping pace with the pulse of life

ZEISS LSM Lightfield 4D

Instant Volumetric High-Speed Imaging of Living Organisms

zeiss.com/lightfield-4d

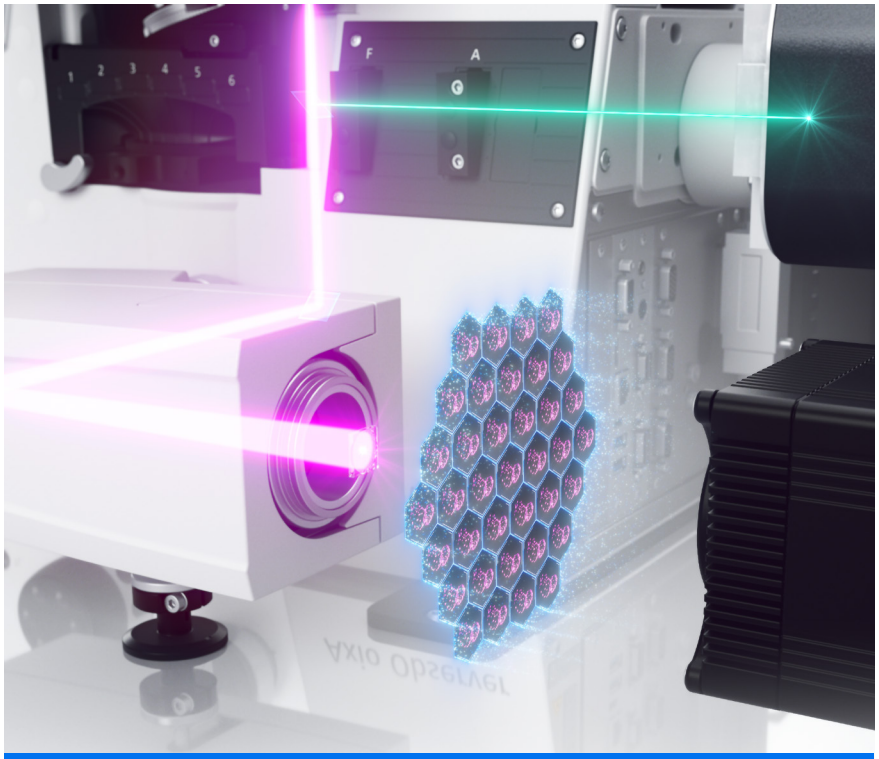
ZEISS

Seeing beyond

ZEISS LSM Lightfield 4D

Don’t miss the moment when life reveals its secrets.

Lightfield 4D is instant volumetric imaging at high speed. Acquire comprehensive 3D information with a single snap and say goodbye to any time delay within an imaged volume. For the first time, capture the fastest movements within whole organisms at up to 80 volumes per second – with all spatiotemporal information intact. Crawling larvae, beating hearts, flowing blood, and firing neurons can be studied in 3D at unprecedented speed to unravel the secrets of life.



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One snap. One volume.
Capture spatial signals and fast dynamics without compromise.

Minimum light exposure. Maximum information gain.
Observe entire organisms for as long as you want without altering the processes of life.

Fast acquisition. Increased throughput.
Examine multiple positions or numerous samples with instantaneous volumetric imaging.

One imaging platform. Endless possibilities.
Be innovative in your experiments and combine high-speed volume imaging with all the possibilities of an LSM.

The unique one-snap-one-volume acquisition minimizes light exposure and allows you to efficiently acquire thousands of volumes over extended periods of time without harming your sample. Reach new heights of productivity with the ability to capture multi-color images at multiple positions within or between whole organisms, organoids or spheroids, in a single acquisition run.

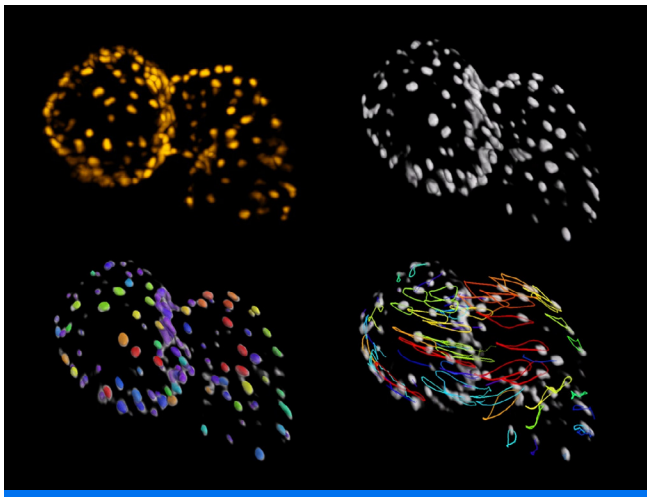
As an integrated part of ZEISS LSM systems, Lightfield 4D lets you effectively combine its fast volumetric imaging with any other LSM acquisition methodology: involving photomanipulation, super-resolution, spectral, and even molecular dynamic data can be added to each live imaging session.

One snap. One volume.

High-speed physiological and neuronal processes captured in 3D

Life moves. Many neuronal and physiological processes occur at very high speeds, making it difficult to accurately capture their spatiotemporal dynamics. Although established technologies have become faster, the required acquisition time still increases with sample volume, so fast processes like neuronal activity or heartbeats require a trade-off between volumetric information and image frame rate. With Lightfield 4D, you no longer have to compromise, as you can capture 80 volumes per second without time delay in 3D. This makes it possible to follow neuronal activity in zebrafish brains, track tissue movement in developing *Drosophila* embryos, and keep track of moving structures in crawling *C. elegans* larvae. The unique one-snap-one-volume imaging ensures that crucial events are not missed or distorted. Highly time-resolved particle tracking in complete volumes is finally possible. Start your experiments immediately – on your confocal and without the need to adjust sample preparation.

Investigating the morphology and cardiac wall movement of the developing zebrafish heart



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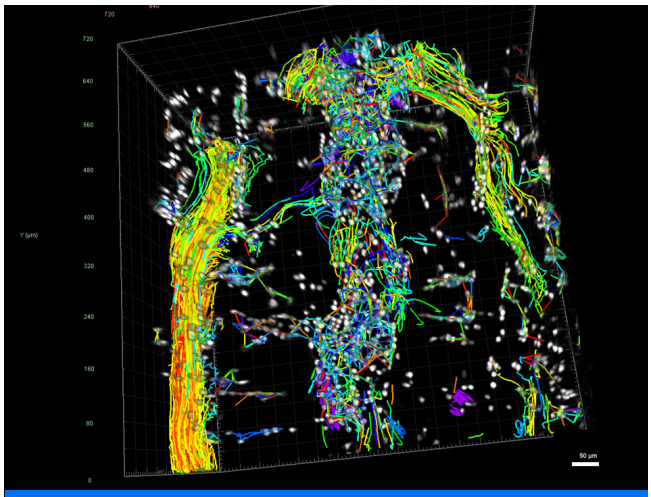
mCherry expression in cardiomyocytes acquired with ZEISS LSM 990 Lightfield 4D and Plan-Apochromat 20×/0.8 Air. Volume size: 723 × 723 × 430 µm³, exposure time 12 ms for 1.2 seconds in total. Sample courtesy of Stone Elworthy and Emily Noël, School of Biosciences, University of Sheffield, UK. Data acquired at Wolfson Light Microscopy Facility in the School of Biosciences at the University of Sheffield.

Analyzing embryonic heart morphology and movement in 3D is challenging as the heart is continually beating. The data was recorded from a 3 days post fertilization zebrafish larvae embedded in agarose. ZEISS Lightfield 4D allowed to image the heartbeat with 80 volumes per second. The movie shows 3 full heartbeats in 1.2 seconds, during which cardiomyocytes are temporally and spatially resolved. This allows for cell segmentation and tracking using ZEISS arivis Pro. It is clearly visible that the cardiomyocytes follow exactly the same trajectory in every heartbeat.

Investigating the flow of insect blood cells (hemocytes) in the *Drosophila* hemolymph



Click here to view this video

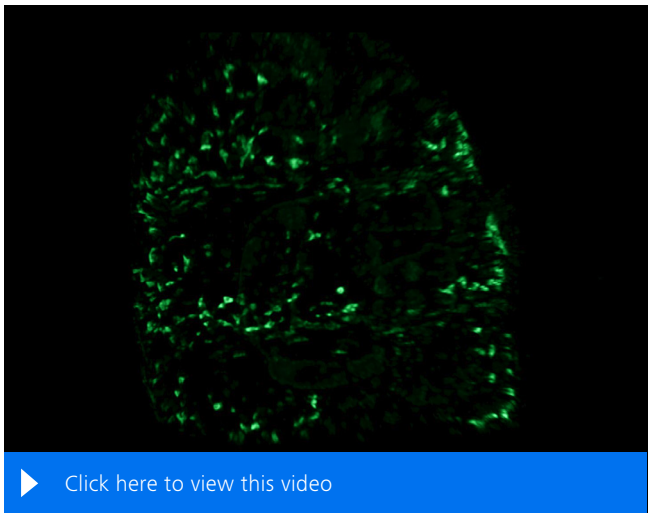
GFP-expressing hemocytes acquired with ZEISS LSM 990 Lightfield 4D and Plan-Apochromat 20×/0.8 Air. Volume size: 723 × 723 × 430 µm³, exposure time 12 ms for 2.5 seconds in total. Sample courtesy of Iwan Robert Evans, University of Sheffield, UK. Data acquired at Wolfson Light Microscopy Facility in the School of Biosciences at the University of Sheffield.

Investigating flow of hemocytes, the insect blood cells, through the hemolymph *in vivo* was almost impossible for researchers due to the fast three-dimensional movement. ZEISS Lightfield 4D offers the unique opportunity to image a large volume fast enough to follow this process under physiological *in vivo* conditions. Thanks to the unparalleled imaging speed of 80 volumes per second, cells are reliably resolved both spatially and temporally. The acquired data allow subsequent segmentation and automated tracking using ZEISS arivis Pro.

Minimum light exposure. Maximum information gain.
Gentle observation of entire organisms over extended periods of time

Collecting 3D information of living samples has always been a challenge, especially for large sample volumes. Optical sectioning requires sequential acquisition of single images to create a Z-stack. Each slice requires light exposure, which is not fully limited to the plane of illumination and can easily add up to harmful amounts across the volume. Lightfield 4D works differently: A complete Z-stack is acquired with a single illumination event, reducing light exposure and phototoxic effects to a minimum. Living samples can be imaged over long periods of time at high temporal density. This combination of outstanding 3D imaging speed and extreme gentleness allows you to follow the sample in multicolor over time without influencing the recorded living activity. You can observe developmental processes, cell migration, vesicle movement or other changes in tissues and organisms that take hours or even days to complete, and still achieve the temporal resolution needed to understand the dynamics.

Observing the formation of fat body tissue in a developing pupa of *Drosophila melanogaster*



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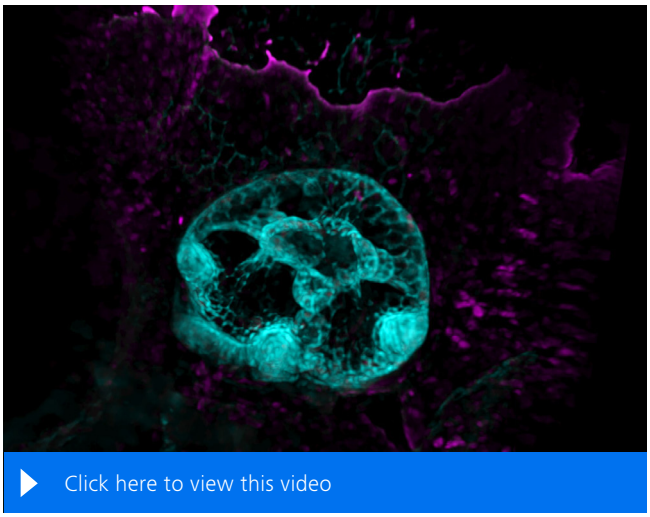
52h old Drosophila melanogaster pupa expressing cD8::eGFP in the progenitor cells of the adult fat body using the driver OK6-Gal4;Elav-Gal80, 15-hour overnight imaging including 12 positions and 10 animals, 500 ms exposure times per volume with 2-minute intervals. Courtesy of Ignacio Manuel Fernández Guerrero, Cellular Analysis Facility, MVLS-Shared Research Facilities, University of Glasgow. Data acquired at the Cellular Analysis Facility, University of Glasgow

Visualizing development of tissues and organs in intact animals enables better understanding of factors involved in their regulation and dysfunction. One example is the developing fat body forming during the pupal stage of *Drosophila*. ZEISS Lightfield 4D allows to keep pace with the cell movement, to provide robust data for 4D tracking. Acquisition speeds are fast enough to image multiple animals at each time point, facilitating higher volumes of data acquisition, allowing an increase in throughput while preserving image data quality. Finally, illumination is gentle enough to image overnight without sacrificing organism viability or fluorophore strength.

Long-term imaging of sensitive processes: Zebrafish ear undergoing developmental morphogenesis



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Zebrafish embryo, timelapse movie of developing otic vesicle, 2–3 days post fertilization, otic epithelial labelled with GFP-CAAX, nuclei with RFP. Every 2 minutes, volumes of 4 different zebrafish embryo ears were imaged over the course of 16 hours. Courtesy of Tanya Whitfield, Sarah Baxendale, School of Biosciences, University of Sheffield, UK. Data acquired at the Wolfson Light Microscopy Facility, University of Sheffield.

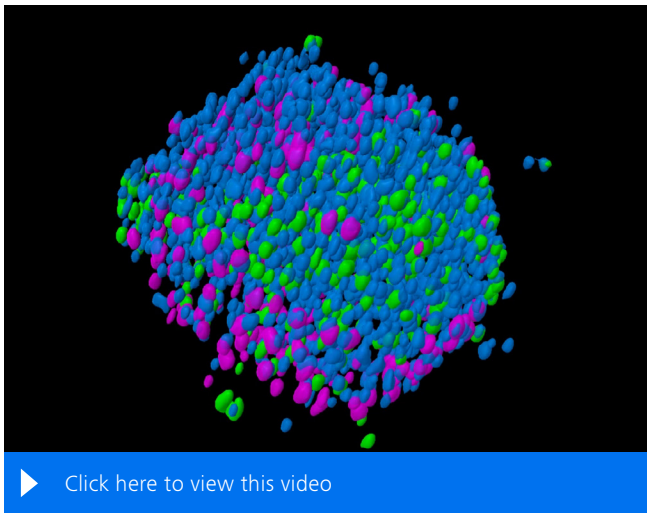
Morphogenesis of developing organs requires a complex coordination of varying regulators and genomic elements. Understanding the impact of these components is best enabled by screening animals with different genetic perturbations and observing dynamic organ patterning in real time. Lightfield 4D enables acquisition of light-sensitive processes with sufficient resolution to track epithelial cell morphological patterning. Its one-snap-one-volume imaging not only ensures that no developmental processes are missed or lost amidst the z-stacks but even allows multiple animals to be imaged in batch mode to capture all events and increase experimental throughput.

Fast acquisition. Increased throughput.

Accelerated collection of information on large samples with multiple labels

Typically, acquisition time of large volumes is the critical factor that limits the throughput of imaging. Acquiring a large volume with a single image snap speeds up your experiments by multitudes. The unmatched speed with which Lightfield 4D captures multi-color volumes can be used to increase the productivity of experiments in a variety of ways: Image and analyze more samples than ever before in every session, immediately improving experimental statistics. Compare multiple different sample cohorts of wild type and genetically modified phenotypes, or samples with different drug treatments. Instead of hours, only minutes are spent collecting the data you need, leaving you more time for advanced analysis and investigation of your datasets.

Efficient volume imaging of cleared spheroids with subsequent cell counting

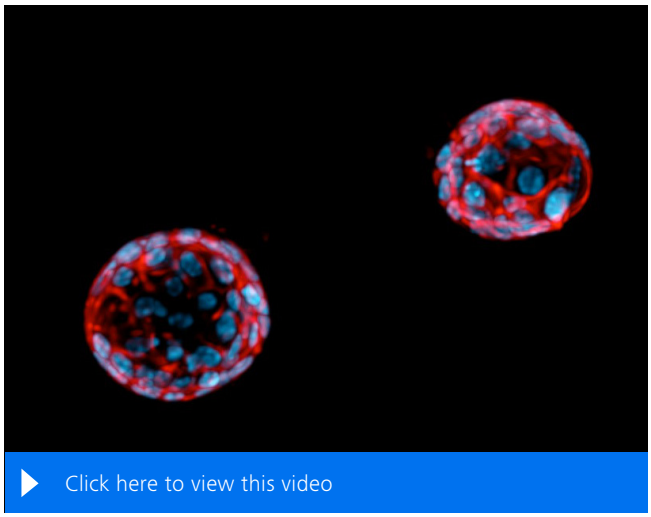


Click here to view this video

Cleared spheroid of a co-culture of HCT-116-GFP (colon cancer)/NIH-3T3-RFP (fibroblasts) cells stained with Hoechst for nuclei. Imaged in an InSphero Akura plate. Dataset was segmented using arivis Pro. Sample courtesy of InSphero AG. Schlieren, Switzerland

Organoids and spheroids can deliver more meaningful data than those derived from classical 2D cell culture models. However, traditional acquisition methods like confocal point scanning or the use of spinning disk systems take considerable time for the acquisition of z-stacks. The speed of Lightfield 4D imaging enables advanced screening applications where higher throughput is required and facilitates faster screening of many spheroids under similar and different conditions, like in compound screens and drug treatments.

Imaging cancer organoids with high speed to enable assessment of perturbations



Click here to view this video

Colorectal cancer organoids, actin cytoskeleton labelled with phalloidin (magenta), nuclei labelled with DAPI (blue). Captured with a 40× objective using 100 ms exposure time for each fluorophore. Courtesy of Nikki R. Paul, Cancer Research UK Scotland Institute, Glasgow. Data acquired at the Cellular Analysis Facility, University of Glasgow.

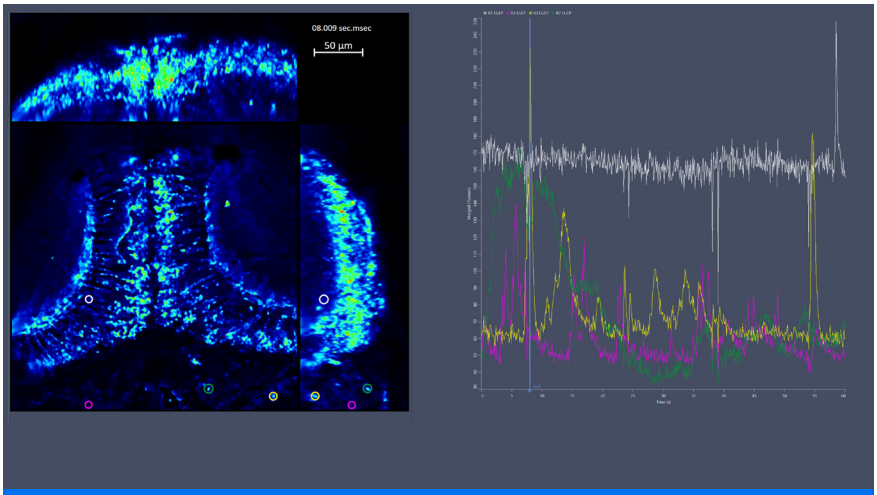
Organoids are popular biological models for analyzing properties within cancer systems, such as responses to drug treatments, extracellular environments, and immune cell interactions. Image acquisition of such large 3D structures and screening through large sample sets is particularly time consuming. Lightfield 4D enables 3D image acquisition of organoids at a rate of several per second, dramatically increasing the throughput for screening through large numbers compared to traditional microscopy methods.

One imaging platform. Endless possibilities.

Innovative experimental design through high-speed volume imaging combined with all the possibilities of an LSM

Laser scanning microscopes (LSM) have proven to be the most versatile microscopy systems. They combine super-resolution and spectral imaging with high-quality optical sectioning of large samples, along with the capability to incorporate additional fluorescent information and molecular dynamics measurements. Take your experiments to the next level by pairing this remarkable flexibility with the gentle and instant volume imaging of Lightfield 4D: Monitor neuronal activity in 3D at high speed, and supplement this with super-resolution structural details captured with Airyscan. Track macrophage movement during a wound healing assay and add high-resolution details of the wound site to your investigation. Leverage the photomanipulation capabilities of your LSM for bleaching, photoactivation, photoconversion, or ablation experiments, followed by gentle volume imaging. Accomplish all of this on the same microscope as part of the same experiment without ever moving your sample.

The thinking zebrafish: Analyzing neuronal activity in developing organisms



The video shows calcium signaling, a reporter for neuronal activity, in the zebrafish brain. Changes in reporter intensity happen on a fast timescale. Thanks to the large volume and speed of Lightfield 4D, neurons more than 50 µm apart from each other can be recorded at the same time.

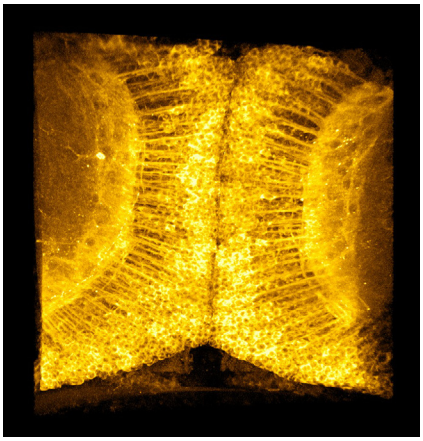
Data recorded from a zebrafish larvae 4 days post fertilization expressing the calcium reporter GCaMP6; imaged with ZEISS LSM 990 Lightfield 4D and LD C-Apochromat 40×/1.1 Water immersion; image volume: 361 × 361 × 109 µm³; 10 volumes per second for 1 minute (661 time points); exposure time 91 ms; intensity coding LUT (low intensity blue, high intensity red to white).

Additional high-resolution data was acquired using the Airyscan CO-8Y mode.

Sample courtesy of Anton Nikolaev, University of Sheffield, UK . Data acquired at Wolfson Light Microscopy Facility in the School of Biosciences at the University of Sheffield.

Imaging calcium signaling as proxy for neuron activity is a widely used technique in many model systems. These signals occur rapidly, in milliseconds, requiring high temporal resolution. Additionally, the brain is composed of densely packed neurons and glial cells, making it difficult to achieve high spatial resolution. Many imaging techniques struggle to achieve both high spatial and temporal resolution simultaneously. Often, calcium signaling is recorded in a single plane only or in a very small volume. Yet, to understand the functioning of neuronal circuitry, it is essential to track neuronal activity simultaneously in as many neurons as possible. ZEISS Lightfield 4D allows to record significantly larger volumes with more than sufficient speed to track neuronal activation. Simultaneous firing of neurons sitting 100 µm or more apart can be captured, giving completely new insights into neuronal circuitry.

To have a closer look at the specific neuronal morphology, high-resolution images of the regions of interest can be acquired with the same ZEISS LSM, employing its confocal or Airyscan super-resolution capabilities.



Light-field microscopy by ZEISS

Your insights into the technology behind it

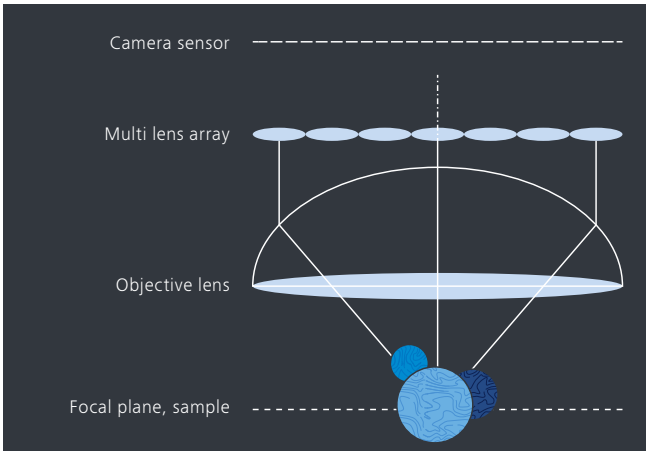
To truly capture the essence of biological processes, imaging must be done in 4D, as both volume and time are essential for investigating living systems. This concept is not new; many optical sectioning techniques have been developed over the past decades to attempt to meet this requirement. However, these methods typically rely on sequential image acquisition to create Z-stack images of volumes, which introduces time differences within the sample volume, severely limiting the imaging speed and the spatiotemporal accuracy of the acquired data.

Lightfield 4D offers a unique solution by imaging an entire volume at an exact point in time, without any time delay. Instead of capturing single 2D images at different time points, a micro lens array positioned in between objective and camera generates 37 individual images, collecting all of the 3D information at the same instant. Each of these different views provides both spatial and angular information which serves as the foundation for creating a Z-stack through deconvolution-based processing. In this way, Lightfield 4D can generate 80 volume Z-stacks per second.

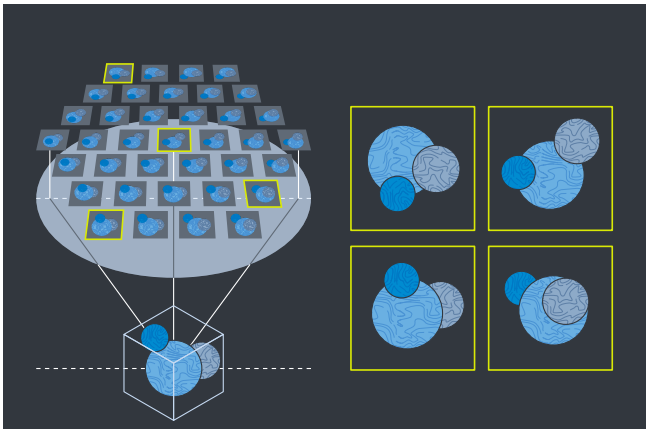
In addition to the uniquely high speed of volume acquisition, this method is notably gentle on living samples. By utilizing a single illumination event for each generated volume, it eliminates the need for repeated illumination to capture individual image pixels or 2D images in order to acquire a sample volume, keeping light exposure short and to a minimum. This combination makes Lightfield 4D the perfect method to capture fast processes, as well as image data from multiple living samples, over long periods of time.

The resulting volume size depends on the selected objective lens. Its magnification and numerical aperture (NA) determine the area depicted and the reconstructed Z-range. A variety of objective lenses are compatible with achieving the ideal measurements for the desired sample volume and resolution for Lightfield 4D.

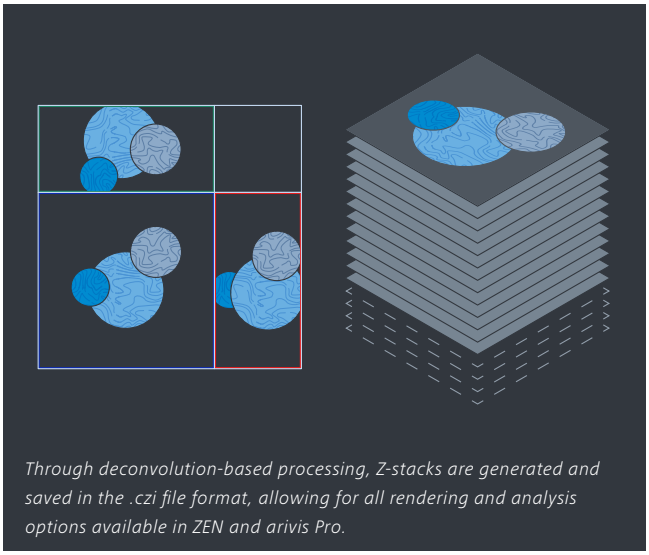
The generated Z-stacks are saved in the standard .czi file format used by ZEN, allowing for all the same rendering and analysis options as for any other Z-stack created in ZEN. For reproducible, reliable, and trusted research, all 37 individual images are saved as raw data for your instant and future access.



A micro lens array positioned in between objective and camera generates 37 individual images, collecting all of the 3D information at the same instant.



Each of 37 different views provides both spatial and angular information which contributes to the volumetric information of the sample. Lightfield 4D can generate 80 of such volumes per second.



Through deconvolution-based processing, Z-stacks are generated and saved in the .czi file format, allowing for all rendering and analysis options available in ZEN and arivis Pro.

Choose your platform

Combine light-field microscopy with LSM flexibility

ZEISS LSM 910

Understanding the fundamentals of life

Compact confocal microscope for innovative imaging and smart analysis

→ zeiss.com/lsm-910

ZEISS LSM 990

Freedom to explore

Top-class multimodal imaging combined in one confocal system

→ zeiss.com/lsm-990

Lightfield 4D (available with ZEISS LSM 910 and ZEISS LSM 990 on ZEISS Axio Observer)

Magnification	40×	25×	20×	10×	
RI Immersion	1.333	1.333	1	1	
Field of View	20.4 mm				
Object Field Size	361×361 µm²	585×585 µm²	720×720 µm²	1444×1444 µm²	Variance of up to 2% from system to system
Z-Stack Range	109 µm	278 µm	430 µm	1712 µm	calculated
Aquisiton Speed	up to 80 Volumes per Second				
Excitation Wavelength Range	405–740 nm				
X/Y Resolution *	2.2 µm	3.5 µm	4.4 µm	8.8 µm	measured, deconvolved
Z Resolution *	2.8 µm	8.4 µm	13.6 µm	57 µm	measured, deconvolved with optimal number of iterations
Voxel Size XYZ	0.7×0.7×0.9 µm³	1.12×1.12×2.7 µm³	1.4×1.4×4.4 µm³	2.8×2.8×18 µm³	
Stack Size XYZ *	512×512×121 Pixel³	512×512×103 Pixel³	512×512×99 Pixel³	512×512×95 Pixel³	

Recommended Objectives for Lightfield 4D

C-Apochromat 40×/1.2 W Corr M27
Plan-Apochromat 40×/1.3 Oil DIC M27
LD LCI Plan-Apochromat 40×/1.2 DIC M27
LD C-Apochromat 40×/1.1 W Corr
LD LCI Plan-Apochromat 25×/0.8 Imm Corr DIC M27
Plan-Apochromat 20×/0.8 M27
EC Plan-Neofluar 20×/0.50 M27
Plan-Apochromat 10×/0.45 M27
Plan-Apochromat 10×/0.3 M27
EC Plan-Neofluar 10×/0.3 M27

* Measured with beads in agarose (RI =1.378) with air or water immersion respectively and excitation/detection wavelength (label) 488 nm/525 nm (eGFP)

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