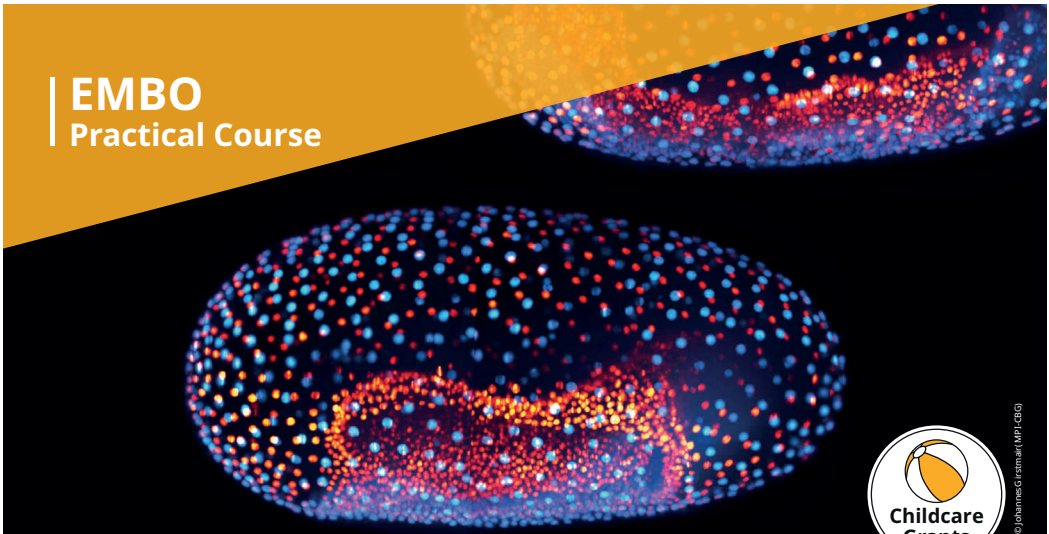
EMBO
Practical Course



Childcare Grants

Light sheet microscopy

15 – 26 August 2022 | Brno, Czech Republic

Abstract Book

meetings.embo.org/event/22-light-sheet-micro

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EMBO
Practical Course

Light sheet microscopy

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Abstract Book

EMBO

GENERAL INFORMATION

E 26	1 basement	1S23	Zeiss Lightsheet.Z1
D36	2nd	215	Luxendo MuVi SPIM
			Miltenyi Biotec UltraMicroscope Blaze
		216	Zeiss Lattice Lightsheet 7
			Viventis LS1
			3i Cleared Tissue LightShee
C02	1st	113	Leica Stellaris DLS
		114	Zeiss Lightsheet 7
E35	1 basement	1S118	ASI Lightsheet
			OpenSPIM
E35	1st	145	Lecture Room
	2nd	211	Students Office

WELCOME

Dear course participant,

Welcome to the EMBO Practical Course on Light Sheet Microscopy at the Central European Institute of Technology CEITEC in Brno. This course is the continuation of a successful series of EMBO courses held at MPI-CBG in Dresden. With the core group of organizers, Emmanuel Reynaud and Jan Peychl, we tried to foster the adoption of the light sheet technology in life sciences over the past decade. Light sheet microscopy is now an established technology that enables imaging of large biological specimens with minimal photo-damage and at unprecedented speeds. A brief look at scientific literature suggests that light sheet technology opened up new avenues to study cell biological, neurobiological and developmental processes across multiple scales.

The challenges associated with light-sheet imaging remain the same; need for new paradigms in sample preparation, throughput of long term time lapse imaging and the enormity of the data that the microscopes generate and that need to be analyzed computationally. At CEITEC, we will try to show you how to tackle these challenges. In comparison with previous courses, we will put more emphasis on imaging of plant samples and photomanipulation of the imaged specimens. The course will, as before, revolve around your samples, your scientific questions and your ideas.

We are looking forward to an exciting, intense and hopefully rewarding two weeks of light sheet microscopy in the heart of Moravia. Brno is the place where Mendel did his groundbreaking genetic experiments and nowadays it is a home of thriving academic/industrial ecosystem developing and producing state-of-the-art electron microscopes. Bringing light sheet imaging to this environment has a potential to combine the two technologies for the betterment of science and society.

Pavel Tomancak

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Useful Information

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Phone: 158

Phone: 150

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Organizational Information

Shall you have any questions regarding the course itself, you might need help with food & accommodation, logistics, or perhaps you seek advice on what to see in Brno - please, do not hesitate to contact our organizational team.
They are here to support you and make things smooth.

Please keep your badges with you throughout the course inside the Institute so that you can be identified when asked.

Wifi
ssid: MUNIguests
password: MUNIguests

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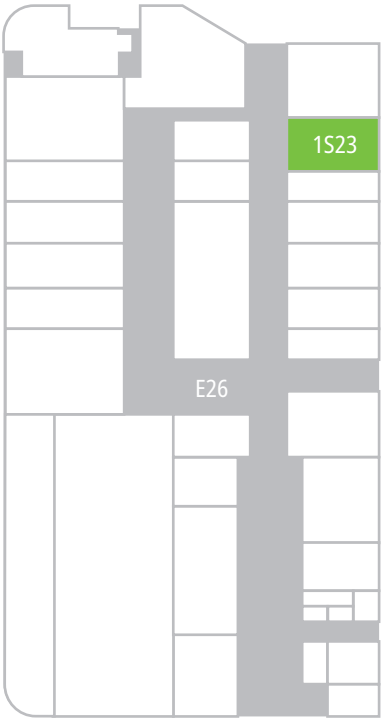
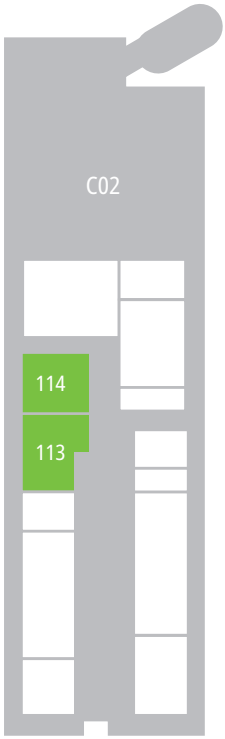
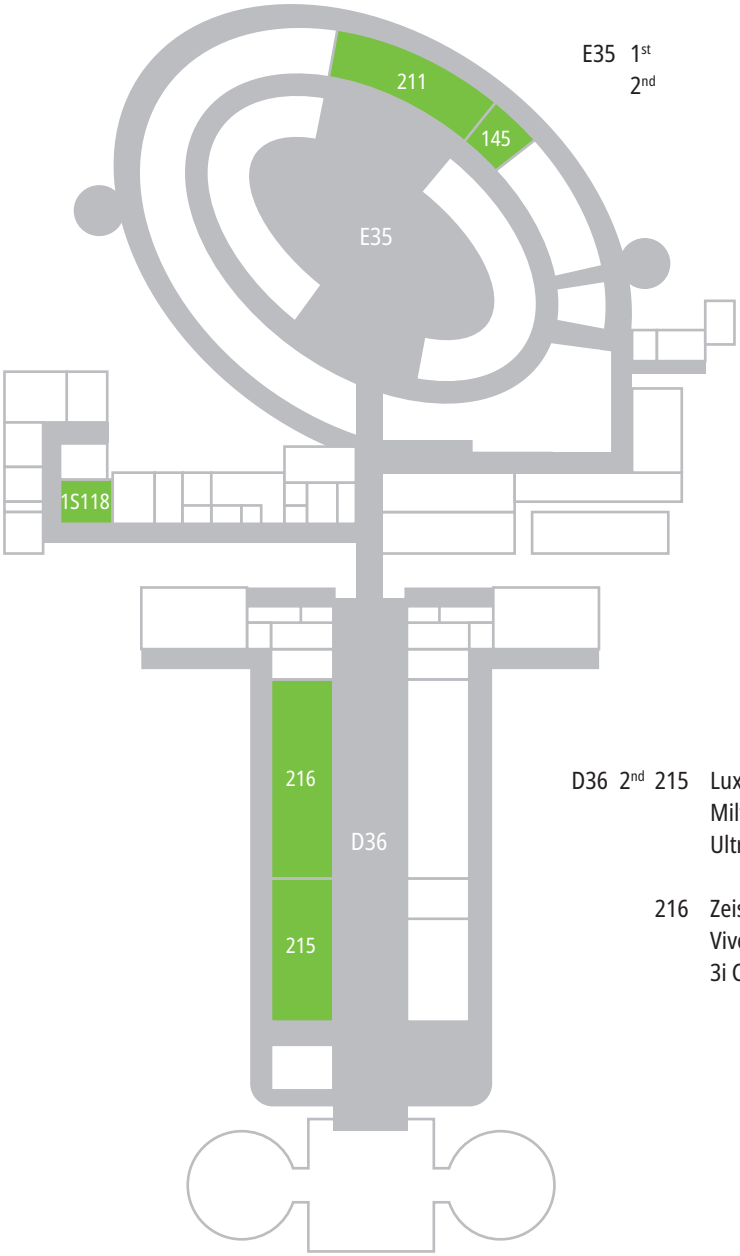
INSTRUMENT LOCATIONS

E 26 1 basement 1S23 Zeiss Lightsheet.Z1



C02 1st 113 Leica Stellaris DLS
114 Zeiss Lightsheet 7





E35 1 basement 1S118 ASI Lightsheet OpenSPIM

E35 1st 145 Lecture Room

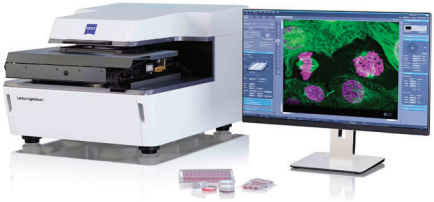
2nd 211 Students Office

D36 2nd 215 Luxendo MuVi SPIM
Miltenyi Biotec
UltraMicroscope Blaze

216 Zeiss Lattice Lightsheet 7
Viventis LS1
3i Cleared Tissue LightShee

INSTRUMENTS

Lattice Lightsheet 7



Illumination
Illumination: 13.3× / NA 0.4
Detection: 44.83× / NA 1.0
Beam shaping by cylinder lens and spatial light modulator (SLM)
Pre-defined Sinc3 beams with length [µm]×thickness [nm]:
- 15×550 (w/ side lobes) & 15×650 (w/o side lobes)
- 30×700 (w/ side lobes) & 30×1,000 (w/o side lobes)
- 100×1,400 (w/ side lobes) & 100×1,800 (w/o side lobes)

Excitation
LED (white & red) for transmitted light Laser (488 nm, 561 nm, 640 nm) for reflected light and epi-fluorescence

Detection
Hamamatsu ORCA-Fusion

Filters
Emission Filter Camera 1
- BP 570-620 + LP 655
- BP 495-550 + LP 655
- LBF 405/488/561/642
- ND filter
- Empty
- BP 495-570
- LP 570

Emission Filter Camera 2
- BP 570-610 IR+
- Empty
- BP 495-550 + BP 570-620
- BP 500-550 IR+

Secondary Beam Splitter
- Plate
- LP 565
- LP 640
- Empty

Sample types
- Sample carrier frame Dish 35: for 35 mm cell culture dishes
- Sample carrier frame Dish 35...40: for 35 – 40 mm cell culture dishes
- Sample carrier frame Slide: for slides 26 mm × 76 mm; also suitable for multi-well glass-bottom slides 26 mm × 76 mm
- Sample carrier frame Chamber slide: for LabTekR chambers 25 mm × 57 mm; also suitable for multi-well glass-bottom slides 25 mm×57 mm
- Sample carrier frame Multiwell: for multiwell microplates 85.48×127.76 mm

Incubation
yes

Special features

Location
D36/216

Lightsheet 7

Illumination
IDetection optics:
Water immersion:
- 5x / 0.16 foc (WD = 5.1 mm)
- 10x / 0.5 foc (WD = 3.7 mm)
- 20x / 1.0 foc (WD = 2.4 mm)

Clearing:
- Fluar 2.5x / 0.12 M27 (WD = 8.7 mm)
- 5x / 0.16 foc, n=1.33-1.58 (WD = 5.1 mm)
- Clr Plan-Neofluar 20x / 1.0 Corr nd=1.45 (WD = 5.6 mm)
- Clr Plan-Neofluar 20x / 1.0 Corr nd=1.53 (WD = 6.4 mm)

Illumination optics:
- 5x / 0.1 foc
- 10x / 0.2 foc

Excitation
405 nm, 488 nm, 561 nm, 638 nm

Detection
2x PCO edge 4.2 sCMOS camera,1920 × 1920; pixel size 6.5 µm × 6.5 µm

Filters
DAPI-GFP, GFP-Cy3, GFP-mCherry, GFP-Cy5, Cy3-Cy5

Sample types
- Water chamber (n=1.33)
- 20x Clearing chamber (n=1.35-1.58)
- Large sample chamber (n=1.33-1.58)
- Translucence chamber

Incubation
yes

Special features

Location
C02/114

Lightsheet.Z1

Illumination
Dual-sided light sheet illumination with pivoting option, Illumination objectives: 5x/0.1 10/0.2

Excitation
405nm, 488nm, 561nm, 638nm

Detection
Single/dual-sided detection, 5x/0.16, 10x/0.5, 20x/1.0, 40x/1.0, camera 2x PCO Edge

Filters
LP 490, BP 420-470, BP 505-545, LP 510, BP 575-615, LP 560, LP 585, LP 660

Sample types
Sample chamber for live samples, agarose embedding holders. Sample chamber and optics designed for aqueous media (RI 1.33-). Short term (fast aquisition) and long term live imaging, 3D reconstruction

Incubation
yes

Special features

Location
E26/1S23

Stellaris DLS

Illumination
HC PL FLUOTAR 5x/0,15 IMM DLS
HC FLUOTAR L 25x/0,95 W DLS
HC FLUOTAR L 16x/0,60 IMM CORR DLS

Excitation
405, white pulsed laser 440 – 790nm

Detection
Hamamatsu ORCA

Filters

Sample types

Incubation
OKOLab incubation onstage

Special features

Location
C02/113

UltraMicroscope Blaze

Illumination
Uni- and bi-directional, Objective lenses 1.1×, 4×, 12×

Excitation
Laser beam combiner: max. 5 laser lines (405, 488, 561, 639, 785 nm), 50 mW–100 mW per diode

Supercontinuum white light laser 460–800 nm, 1–3 mW/nm

Detection
4.2 Megapixel sCMOS camera
5.5 Megapixel sCMOS camera

Filters
Seven filters ⌀ 43 mm

Sample types
Cuvette for UltraMicroscope Blaze (standard sample chamber)

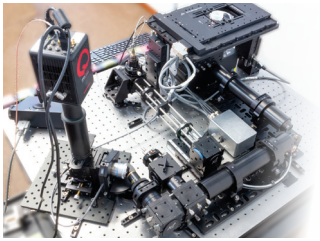
XXL chamber for UltraMicroscope Blaze

Incubation
no

Special features

Location
D36/215

Lightsheet



Illumination
static sheet through detection objective

Excitation
488/561/640nm

Detection
40x/1.1 WI objective with correction collar in inverted geometry, single sCMOS camera with total magnification 67x

Filters
matching excitation

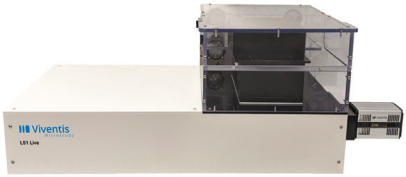
Sample types
aqueous live or fixed

Incubation
temperature and gas

Special features
fast volumetric imaging, compatible with multi-well plates

Location
E35/1S118

Viventis Microscopy **LS1 Live**



Illumination
Scanned gaussian light sheet of variable thickness between 2.2 to 4.4 um FWHM. Illumination with two 10X 0.2 NA

Excitation
488nm 60mW, 561 50mW, 638 100mW

Detection
25X 1.1 NA - field of view up to 900um.

Filters
Single band pass GFP // Double band pass GFP/mCherry(RFP) // Triple band pass GFP/mCherry(RFP)/FarRed

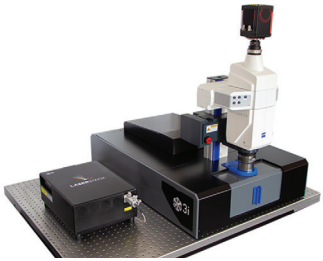
Sample types
Live sample or fix/expanded cleared sample in water-based solution (refractive index around 1.33).
Particularly suitable for organoids/spheroids/gastruloids and embryos imaging (i.e. mouse, c.elegands. zebrafish). Size limited by FOV of the microscope but tiling is possible during acquisition.

Incubation
Temperature controlled with heating above ambient temperature and CO_2 and O_2 controlled.

Special features
Sample is mounted in a dedicated FEP bottom dished where sample can be placed from top without need of embedding. Medium exchange is possible during acquisition.

Location
D36/216

3i Cleared Tissue LightSheet



Illumination
dual-sided scanned infinity beam
two sets of excitation objectives
5x/0.14NA
10x/0.28NA

Excitation
488nm
561nm
640nm

Detection
1.0x/0.25NA
1.5x0.37NA
Hamamatsu Orca Flash 4.0 v3

Filters
Quad band-pass 446/523/600/677

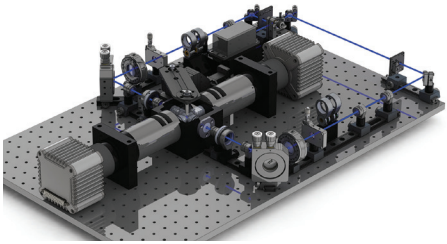
Sample types
cleared, any type of clearing media

Incubation
heating

Special features

Location
D36/216

OpenSPIM OpenSPIM



Illumination
Dual-sided illumination and two detection axes

40x water-dipping objective, N.A 0.80

Excitation
Two laser lines (488 and 561) coming from a multiple wavelength laser system (Stradus VersaLase)

Detection
Two Andor sCMOS Neo 5.5 cameras

Filters

Sample types

Incubation
Yes

Special features

Location
E35/1S118

LUXENDO
A BRUKER COMPANY

MuVi SPIM light-sheet

Illumination
Scanned Gaussian Beam light sheet microscope, Multi view SPIM - dual illumination / dual detection, Vertical sample mounting, Live and cleared sample imaging

Available objectives – exchange of objectives requires 15 minutes, therefore a pooling of applications is preferred minimizing the exchange of octagons/objectives
- 16X 0.8NA detection live samples
- 20X 1.0NA detection live samples
- 10X 0.5NA detection cleared samples
- 20X 1.0NA detection cleared samples

Excitation
405nm (>40mW), 488nm (>40mW), 561nm (>40mW), 642nm (>40mW)

Detection
3X sCMOS ORCA Flash4 3.0 Hamamatsu
Camera configuration:
- Left: Single camera detection
- Right: Dual camera detection – short & long wavelength
- 10X 1.0NA detection cleared samples

Filters
BP 418-462, LP 466, LP 498, BP497-554, LP 572, BP 580-627, LP 656, BP 655-704

Sample types
Live & fixed samples – up to 1.5 mm (l) × 1.5 mm (d) × 10 mm (h) – required imaging resolution dependent as objective combination may limit samples size

Cleared samples – up to 12 mm (l) × 15 mm (d) × 19 mm (h) – required imaging resolution dependent as objective combination may limit samples size

Incubation
Temperature control: 18 – 40 °C
CO2 control: 0-20%
O2 control: 0-20%
Humidity control

Special features
- Pivot scan for reduced shadowing effect
- Line mode acquisition
- Elongated light sheet for homogenous illumination
- Photo manipulation module for photo activation and ablation
- Dual color module

Location
D36/215

PROGRAMME

Lectures within the EMBO Course Light Sheet Microscopy

Building E35
Room 145
No registration necessary

Date/Time	Title	Speaker	Institution
15/08/2022 17:30–18:30	Introduction to Light Sheet Microscopy	**Ernst Stelzer**	Buchmann Institute for Molecular Life Sciences
16/08/2022 17:30–18:30	Light-sheet Imaging of Non-model Species	**Tassos Pavlopoulos**	IMBB-FORTH Greece
17/08/2022 9:00–10:00	Light Sheet Imaging of Mouse Embryos	**Kate McDole**	MRC LMB
18/08/2022 9:00–10:00	3D Image Analysis with Open Tools	**Robert Haase**	PoL Dresden
19/08/2022 9:00–10:00	Light Sheet Imaging of Insect Embryos	**Akanksha Jain**	ETH Zurich in Basel
22/08/2022 9:00–10:00	Light Sheet Imaging of Organoids	**Gopi Shah**	EMBL Barcelona
23/08/2022 9:00–10:00	Light Sheet Imaging of Bacterial Biofilms and Viruses	**Pavel Plevka**	CEITEC, Brno
24/08/2022 9:00–10:00	Light Sheet Imaging of Plants	**Jozef Samaj**	Palacky University Olomouc
25/08/2022 9:00–10:00	Light Sheet Imaging of Non-model Species	**Tassos Pavlopoulos**	IMBB-FORTH Greece

NOTES

CEITEC Atrium

SEE YOU LATER