# Revealing life across scales – down to molecular details



# **ZEISS Elyra 7 with Lattice SIM**

Your Complete Super-Resolution System with Unprecedented Resolution



Seeing beyond

# Your Complete Super-Resolution System with Unprecedented Resolution

### The ZEISS Lattice SIM family

> In Brief

> The Advantages

> The Applications

> Technology and Details

> The System

> Service

Using microscopy to visualize biological structures provides insights into function. When imaging fixed structures, acquisition settings can be optimized for spatial resolution. However, when capturing dynamic events in living samples, higher acquisition speeds and low-light conditions must be balanced with resolution. The ZEISS Lattice SIM family balances sample size, imaging speed, and superresolution capabilities based on your application – from outstanding optical sectioning of tissues and developing organisms to high-speed imaging of living cells to resolution excellence at the molecular level.



### **ZEISS Elyra 7 with Lattice SIM**

ZEISS Elyra 7 includes a wealth of microscopy techniques to meet your experimental needs across scales, optimally matching resolution, speed, and sensitivity requirements to your demanding samples. Employ SIM Apotome for fast optical sectioning, Lattice SIM for super-resolution imaging, SIM<sup>2</sup> image reconstruction for resolution excellence down to 60 nm, as well as SMLM and TIRF for investigations at the molecular level. You can combine these techniques to multiply the insights from your specimen and to correlate the acquired data.

With ZEISS Elyra 7, not only do you gain unique SIM technology. You also maintain the use of standard dyes and fluorescent proteins, the ability to perform simultaneous two-color imaging with clean separation between channels, and the flexibility to choose from a variety of imaging modes to best suit the needs of your samples.

# Simpler. More Intelligent. More Integrated.

### > In Brief

### > The Advantages

- \_\_\_\_\_
- > The Applications
- > The System
- > Technology and Details
- i reennology and Detail
- Service

#### **Resolution excellence with Lattice SIM<sup>2</sup>**

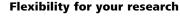
With SIM<sup>2</sup>, a novel image reconstruction algorithm raising the SIM technology to a new level, you make the best use of the available photon budget. You can now double the conventional SIM resolution and discriminate the finest subcellular structures, even those no more than 60 nm apart. Lattice SIM<sup>2</sup> comes with outstanding out-of-focus light suppression, giving you the sharpest sectioning in wide-field microscopy even for highly scattering samples. SIM<sup>2</sup> image reconstruction robustly reconstructs all structuredillumination-based acquisition data of your Elyra 7 – with minimal artefacts – for living and fixed samples.

#### Speed and efficiency for your experiments

While doubling the classic SIM resolution, the light-efficient Lattice SIM<sup>2</sup> gives you gentle imaging of living and fixed specimens at high speeds of up to 255 fps.

Combine Lattice SIM<sup>2</sup> and SIM<sup>2</sup> Apotome with Burst and Leap modes to make super-resolution acquisition faster than ever before. With SIM Apotome mode, even lossless acquisition can be achieved, meaning for every reconstructed image just one raw image is needed!

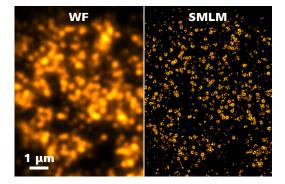
Or make use of Elyra 7 Duolink to image two differently stained structures simultaneously and use the multiple colors to boost resolution even further.



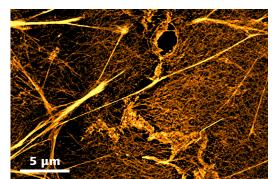
Elyra 7 handles virtually all types of samples, including photo-sensitive cell cultures, scattering *C. elegans* and plants or tissue sections of up to 100 µm thickness.

Elyra 7 includes several microscopy techniques: Lattice SIM<sup>2</sup>, SIM<sup>2</sup> Apotome, widefield, SMLM and TIRF. You can correlate images of the same sample acquired using any or all of all these techniques to multiply the insights from your specimen.

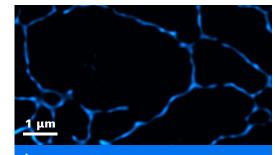
You can even combine Elyra 7 with a variety of other imaging systems such as LSM with Airyscan or scanning electron microscopy in a correlative workflow.



SMLM: Xenopus laevis A6 cells (epithelial kidney cells). Gp120, a nuclear pore complex protein arranged with eightfold symmetry was labeled with Alexa Fluor 647.



The Lattice SIM<sup>2</sup> image of Cos-7 cells labeled with phalloidin Alexa 488 shows the fine structure of the Actin network. Maximum intensity projection of Z stack is shown.



Click here to view this video

Lattice SIM<sup>2</sup>: Time lapse imaging of the endoplasmic reticulum (Calreticulin-tdTomato) in a Cos-7 cell reveals highly dynamic structural changes.

### > In Brief

#### > in brief

> The Advantages

### > The Applications

- , me applications
- > The System

#### > Technology and Details

- > Service

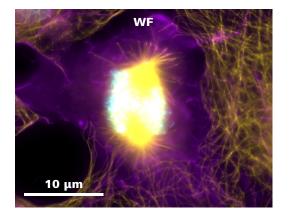
### Lattice SIM:

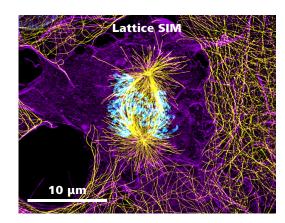
Your 3D super-resolution technique In classic SIM, the sample is illuminated with a grid structure that interferes with structures in the sample, creating Moiré fringes. These fringes contain high frequency information – that is, high resolution information – transformed down to low frequencies that can be resolved by the microscope. To achieve this effect in all directions, the sample is imaged at different rotational and translational positions (phases) of the grid pattern. The phase images are deconvolved into the resulting image, which will have twice the resolution in all three dimensions.

In Lattice SIM, the sample is illuminated with a lattice spot pattern instead of grid lines. Due to its intrinsic two-dimensionality, the lattice pattern requires only translational repositioning but no rotation. This leads to a dramatic increase in imaging speed. In addition, the lattice pattern provides higher contrast to allow a more robust image reconstruction. Since the sampling efficiency is doubled compared to classic SIM, half as much light exposure is needed making Lattice SIM a preferred live cell imaging technique. The lattice pattern gives better contrast: you maintain image quality at higher frame rates.

### Click here to view this video

Watch the movie for a quick comparison of classic SIM and Lattice SIM





Lattice SIM: Comparison of widefield and Lattice SIM images of a Cos-7 cell undergoing mitosis stained for actin (Phalloidin Alexa Fluor 568, magenta), microtubules (anti-beta-tubulin Alexa Fluor 488, yellow) and nucleus (Hoechst, blue). Images are maximum intensity projections of 30 planes of a total depth of 3.19 μm. Objective: Plan-Apochromat 63×/1.4 Oil

### Lattice SIM



### > In Brief

#### / III DHEI

#### > The Advantages

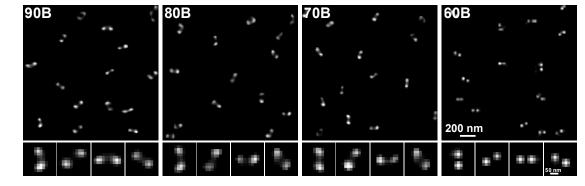
#### .....

- > The Applications
- > The System
- \_\_\_\_
- > Technology and Details
- > Service

### SIM<sup>2</sup> reconstruction: Double your SIM resolution

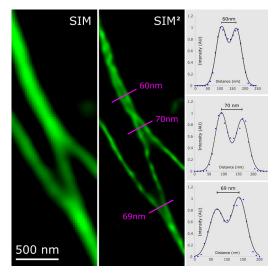
Dual-iterative SIM, or SIM<sup>2</sup>, is a groundbreaking image reconstruction algorithm that increases the resolution and sectioning quality of structured illumination microscopy data. SIM<sup>2</sup> is compatible with all SIM imaging modes and fully integrated in ZEISS ZEN software.

Unlike conventional reconstruction algorithms, SIM<sup>2</sup> is a two-step image reconstruction algorithm. First, order combination, denoising, and frequency suppression filtering are performed. All the effects resulting from these digital image manipulations are translated into a digital SIM point spread function (PSF). The subsequent iterative deconvolution uses this PSF. Similar to the advantages of using experimental PSF for deconvolution of hardware-based microscopy data, the SIM<sup>2</sup> algorithm is superior to conventional one-step image reconstruction methods in terms of resolution, sectioning, and robustness.



GATTA-STED Nanoruler 90B, 80B, 70B and 60B (GATTAquant, Germany) were imaged and processed with Lattice SIM<sup>2</sup> mode with a 63×/1.4 oil objective. Distances of 90 nm, 80 nm, 70 nm and 60 nm are resolved.





Images of Cos-7 cell stained with anti-alpha-Tubulin Alexa Fluor 488 were processed with the conventional SIM algorithms based on generalized Wiener filter and with the novel SIM<sup>2</sup> reconstruction. The images show an improvement of resolution for SIM<sup>2</sup> compared to SIM. The superior sectioning capability of SIM<sup>2</sup> is shown in the movie. Objective: Plan-Apochromat  $63 \times / 1.4$  Oil

#### > In Brief

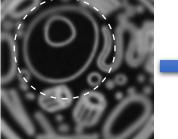
# > The Advantages

- > The Applications
- > The System
- ••••••
- > Technology and Details
- > Service

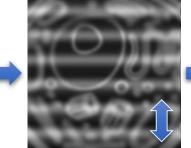
#### SIM Apotome: Flexible optical sectioning

Live cell imaging with a widefield system often suffers from out-of-focus blur or background signal. These effects can decrease contrast and resolution. The SIM Apotome acquisition mode uses structured illumination to give you fast optical sectioning of larger volumes with crisp contrast and high resolution in all dimensions. A grid pattern is used to illuminate and rapidly modulate the fluorescence signals in the focal plane. After acquiring three or five images with different grid positions (phases), these frames are combined into a resulting image which contains only information from the focal plane – your optical section.

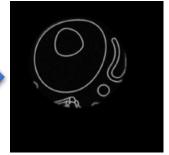
The SIM Apotome acquisition mode in combination with the SIM<sup>2</sup> reconstruction algorithm allows you to further tune the gentleness of fast live-cell imaging with high contrast and resolution. Or use your new optical sectioning speed to increase productivity when acquiring large sample areas or large volumes at different magnifications.



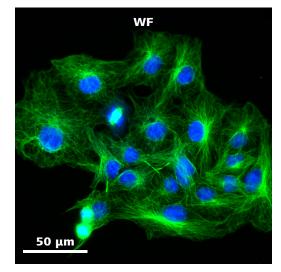
Widefield image with out-of-focus light. Signal from the focal plane is encircled by a white dashed line.

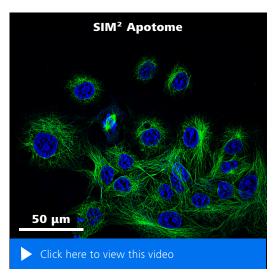


SIM Apotome acquisition at 3 or 5 different grid positions



Reconstructed optically sectioned image





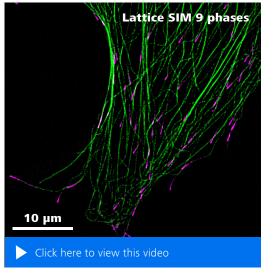
SIM<sup>2</sup> Apotome: Comparison of widefield and SIM<sup>2</sup> Apotome single plane images of Cos-7 cells stained for microtubules (anti-alpha-tubulin Alexa Fluor 488, green) and nuclei (Hoechst, blue). Objective: LD LCI Plan-Apochromat 25×/0.8 Imm Corr

### > In Brief

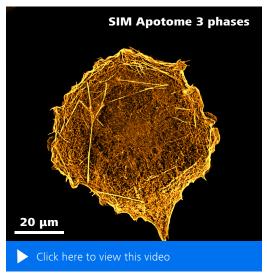
>	The Advantages
>	The Applications
>	The System
>	Technology and Details
>	Service

#### Balance your need for speed and resolution

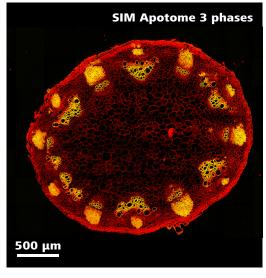
Higher imaging speeds and decreased light exposures are a constant demand in imaging experiments. At the same time, these acquisition settings affect the resolution of the resulting images, and these parameters must be balanced with the desired outcome. To increase speed and decrease light exposure with SIM techniques, the number of phase images acquired for the reconstruction of one final frame/volume are reduced. The robustness and flexibility of ZEISS Elyra 7 structured illumination patterns plus the image reconstruction software allow a significant reduction to the number of phase images required for Lattice SIM acquisition mode, and, importantly, this only causes a slight decrease in the resolution of the final images. Elyra 7 Lattice SIM acquisition can be operated at 9 phase images per frame instead of 13; SIM Apotome acquisition can be operated at 3 phase images per frame instead of 5; increasing the imaging speed by 44% or 66% respectively. The increased speed is particularly advantageous for gentle imaging of highly dynamic live cells where slower acquisition would result in motion blur and reduced resolution. In combination with Leap mode, the reduced phase acquisition of Lattice SIM decreases the number of phase images per final frame. This means that you only need three times as many phase images as the resulting number of reconstructed full frames, enabling gentle super-resolution imaging that is unprecedented.



Cos-7 cell expressing EMTB-3xGFP (green) and EB3-tdTomato (magenta) shows dynamic movement of microtubules. Imaged in Lattice SIM 9 phase mode. Objective: Plan-Apochromat 63×/1.4 Oil



Actin dynamics in a Cos-7 cell expressing LifeAct-tdTomato were imaged with the SIM Apotome 3D Leap mode over time. The image shows a maximum intensity projection of 30 planes over 3.4 µm depth. Objective: Plan-Apochromat 40×/1.4 Oil



SIM Apotome volume tile scan image of a Helianthus section. Sample: "Helianthus" from TS-Optics Set Dauerpräparate Botanik 25St. Objective: EC Plan-Neofluar 10×10.3

### > In Brief

#### .....

- > The Advantages
- > The Applications
- -----
- > The System
- > Technology and Details
- .....
- > Service

#### Boost the speed of SIM imaging even further

Elyra 7 already provides you with fast imaging speeds. But you can further increase the temporal resolution and productivity for 2D and 3D imaging by using the speed enhancement modes. The Burst mode and the Leap mode are compatible with Lattice SIM as well as SIM Apotome acquisition. Combined with SIM<sup>2</sup> image reconstruction, they enable you to capture highly dynamic processes at exceptional resolution in all three dimensions.

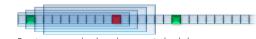
#### 2D Burst mode:

#### Get full temporal information

Burst mode processing uses the rolling window approach to let you observe processes in your living samples at up to 255 fps. Since Burst mode is a post-acquisition step, you have the flexibility to use it with previously acquired data sets. You decide how much temporal resolution is required for your data analysis.



Burst-mode processing



Events green and red can be separated only by Burst mode processing

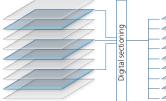


U2OS cell expressing an Rab5-mEmerald (green) and tdTomato tagged Golgi associated transport marker (orange). Simultaneous dual-color acquisition. Objective: Plan-Apochromat 63×/1.4 Oil

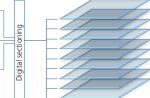
### 3D Leap mode:

#### Digital sectioning at a new level

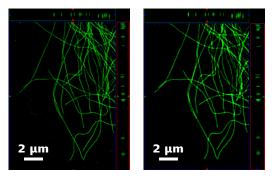
For demanding fast imaging in 3D, the Leap mode acquisition enables you to reduce your imaging time and lower the light exposure on your sample. This works by imaging only every third plane, for three-times higher volume imaging speed and three-times fewer light exposures. ZEN reconstructs the entire volume using a pixel reassignment approach.



Imaging only every third plane of the Nyquist sampled volume



Reconstructed planes



Cos-7 cells stained with anti-beta-tubulin Alexa Fluor 488. Images show XY, XZ and YZ view of the cropped volume image for Nyquist sampled (left) and digitally sectioned (right) images of the same sample area. Objective: Plan-Apochromat 63×/1.4 Oil

# **Expand Your Possibilities**

#### . . . .

### > In Brief

### > The Advantages

> The Applications

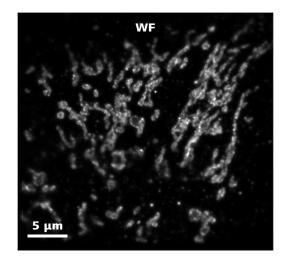
- ine , ppressions
- > The System
- > Technology and Details
- > Service

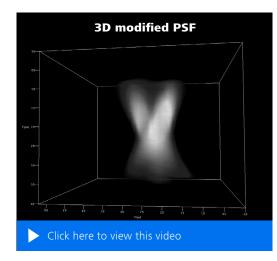
#### SMLM:

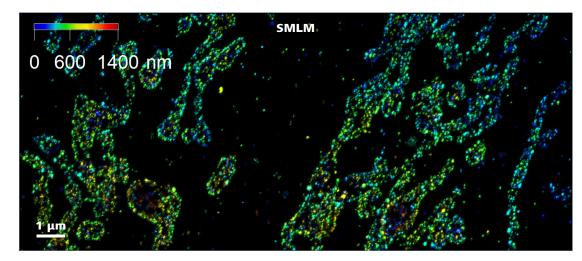
### 3D imaging at molecular resolution

In single-molecule localization microscopy (SMLM), fluorescent molecules are sparsely activated so that only one out of many will be in its on-state within a single point spread function (PSF). This lets you determine its center of mass with a localization precision that far exceeds the extension of the PSF. Once recorded, the molecule is turned to its off-state and the cycle of activation/deactivation is repeated until all molecules are captured. The localizations are plotted in a new image to create the super-resolution image. With Elyra 7 you can use SMLM techniques such as PALM, dSTORM and PAINT to achieve lateral localization precision of 10–20 nm. The ZEN software will seamlessly perform the image reconstruction of your data.

In addition, Elyra 7 provides you with 3D SMLM mode based on PRILM technology. The PSF is reshaped for encoding the Z position so while acquiring only one plane, you get volume information of  $1.4 \mu m$  depth at 20-40 nm axial resolution. Thus, you can acquire 3D data with consistent molecular precision.







3D PAINT image of mitochondrial membranes in BSC1 (kidney epithelial cells). The outer membrane protein Tomm20 was labeled using Ultivue – I2-650 imaging strand. Reshaped PSF encoding for Z information was used to create a 1.4 μm deep 3D PAINT image. Upper figures show the widefield microscopy image (left) of the area as well as the 3D image of the modified PSF (right) used for 3D SMLM. Objective: alpha Plan-Apochromat 63×/1.46 Oil

# **Expand Your Possibilities**

### > In Brief

### > The Advantages

- > The Applications
- > The System
- > Technology and Details
- .....
- > Service

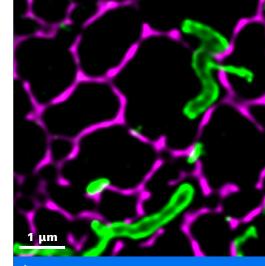
### Simultaneous two-color imaging

Investigation of living samples very often focuses on interactions of different proteins or organelles. Simultaneous imaging of the involved structures is key to proper understanding of these highly dynamic processes. Equip your ZEISS Elyra 7 with a Duolink adapter to operate two Hamamatsu ORCA-Fusion sCMOS cameras in parallel and use all the advantages that a widefield-based technology can offer:

- True simultaneous two-color imaging within your entire field of view – without any delays
- Acquisition of a super-resolved real-time snapshot of an entire living cell by picking a low exposure time
- Increased productivity of fixed cell experiments by doubling the information obtained at the same time
- Imaging of any possible color combination, with minimal signal crosstalk as enabled by the integrated multi-bandpass emission filters
- Acquisition of 4-color images without mechanical filter change – making your multi-color experiments even faster
- Multi-color SMLM experiments

### Hamamatsu ORCA-Fusion BT

This camera features a scientific CMOS (sCMOS) with a back-thinned sensor enabling peak quantum efficiency of ~95 %. With its ultra-low, uniform readout noise and CoaXPress interface for high acquisition speeds and exposure times down to 1 ms, it yields unparallel digital imaging results.



#### Click here to view this video

Cos-7 cell expressing the endoplasmic reticulum marker Calreticulin-tdTomato (magenta) and mitochondrial marker Tomm20-mEmerald (green) was simultaneously imaged for two colors. The movie shows high dynamic interactions of the ER and mitochondria. Objective: Plan-Apochromat 63×/1.4 Oil



*Elyra 7 Duolink sCMOS camera adapter for simultaneous twocolor acquisition with integrated multi-bandpass emission filter cubes for efficient image acquisition* 

# **Expand Your Possibilities**

#### > In Brief

- The Advantages
  The Applications
  The System
- > Technology and Details
- -----
- > Service

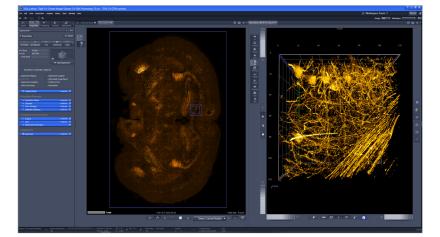
#### Translate your images into quantitative data

#### **ZEISS ZEN:** A journey through different scales

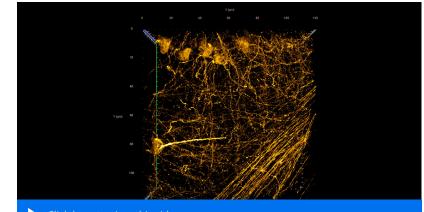
Biological samples often contain different types of information at different length scales. Collecting low to high resolution data in the same sample not only makes you more productive, but also allows you to interconnect your findings and create more accurate biological models based on your experimental findings. With AI Sample Finder, you automatically detect your whole sample even before starting your experiment – ensuring that you won't miss any relevant areas. The ZEN Connect toolkit enables you to combine different experiments recorded with various acquisition modes or systems – placing your experiments into the spatial context of the whole sample.

#### ZEISS arivis Pro: Advanced image processing and 3D reconstruction

Use the efficient ZEISS arivis Pro software for visualization and quantification of large 3D and 4D data sets. ZEISS arivis Pro not only renders volume images of almost unlimited size, but also provides advanced image processing tools such as volume fusion, channel shift, conventional and machine learning based segmentation, 3D tracking, and neuron tracing. Visualize your quantitative results within ZEISS arivis Pro or export all data for further analysis. The modular structure of ZEISS arivis Pro flexibly adjusts to your needs for advanced image processing and analysis.



Driven by ZEN (blue edition), ZEISS Elyra 7 is now fully integrated into the ZEN software ecosystem, providing workflow-oriented solutions for image acquisition, AI-based data processing, and advanced correlative analyses.



#### Click here to view this video

Murine brain expressing the neuronal marker Thy1-eGFP, imaged in SIM Apotome and Lattice SIM modes over a Z stack range of 170  $\mu$ m. The ZEN Connect project combines data sets recorded with 10× SIM Apotome, 25× SIM Apotome, 40× SIM Apotome and 63× Lattice SIM. The volume rendering shows a subset of the 63× Lattice SIM data set. Objective: Plan-Apochromat 63×/1.4 Oil. Sample courtesy of Herms Lab (MCN, University of Munich, Germany).

### > In Brief

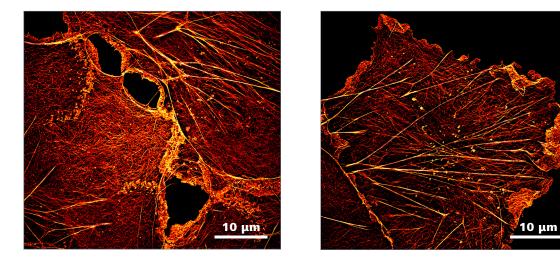
- > The Advantages
- -
- > The Applications
- > The System
- > Technology and Details
- , recimeregy and becan
- Service

#### Lattice SIM<sup>2</sup> – Simply Image More

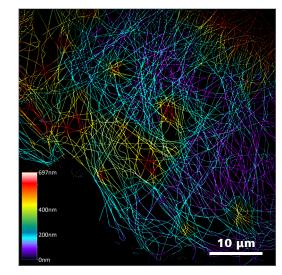
Study of the components of the cytoskeleton is a prominent research field in biology. Due to the fine structures of these components, for example the actin network or microtubular filaments, imaging far below 100 nm is often performed with super-resolution techniques.

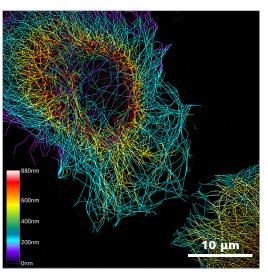
Lattice SIM<sup>2</sup> now allows you to gain much more structural information from your samples compared to conventional SIM techniques. It not only operates with a resolution of down to 60 nm but also provides markedly improved sectioning quality in your images. This novel, robust image reconstruction method efficiently separates signal and background – all without the need for tailor-made staining protocols or expert knowledge of complex microscopy techniques.

You can take advantage of the easy-to-use Lattice SIM<sup>2</sup> technology to unveil complex structural information and simply see more.



The Lattice SIM<sup>2</sup> images of Cos-7 cells labeled with phalloidin Alexa 488 were acquired with an alpha Plan-Apochromat 100×/1.57 oil objective. Maximum intensity projection of Z stack is shown.





The Lattice SIM<sup>2</sup> images of Cos-7 cells labeled via immunofluorescence with anti-alpha-Tubulin Alexa 488 are shown as color-coded projection. Data were acquired with an alpha Plan-Apochromat  $100 \times / 1.57$  oil (left) and Plan-Apochromat  $63 \times / 1.4$  oil (right) objectives. The images demonstrate the excellent sectioning capabilities of SIM<sup>2</sup> image reconstruction algorithm.

#### . . . .

> In Brief

### > The Advantages

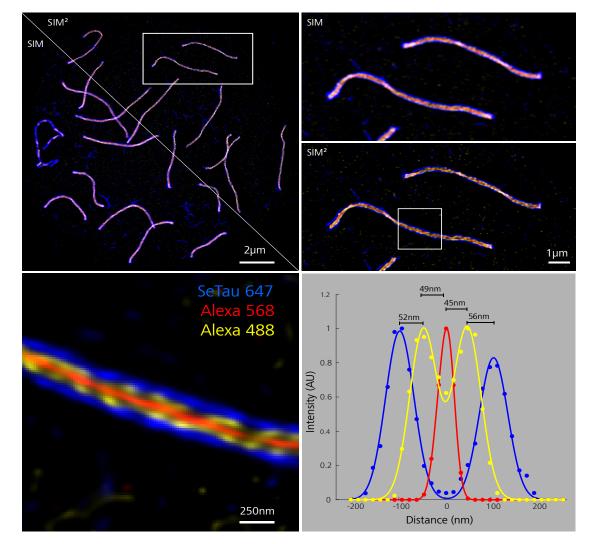
, me navantages

- > The Applications
- > The System
- > Technology and Details
- .....
- > Service

# Straightforward multi-color super-resolution imaging

Studying multiprotein complexes requires super-resolution imaging with multiple colors, which is not often attainable with conventional techniques. Lattice SIM<sup>2</sup> enables you to perform multi-color imaging at resolution down to 60 nm for conventionally stained samples.

The synaptonemal complex is a well-known structure in the nucleus of meiotic cells and consists of two lateral elements, which are connected to a central element by transverse filaments. Due to its small size, three-color imaging of the synaptonemal complex has previously been possible only using complex methods with elaborate sample preparation, such as super-resolution imaging of three-fold expanded samples using the expansion microscopy technique. Lattice SIM<sup>2</sup> resolves the two strands of SYCP3 (lateral elements) as well as SYCP1-C (C-terminus of transverse filaments) without special sample treatment or staining for distances well below 100 nm. More importantly, the three-color image provides structural information for the distances between the proteins SYCP3 and SYCP1. Even within the SYCP1 protein, the differently labeled N- and C-Terminus can be clearly separated with less than 50 nm resolution between the two labels.



Architecture of threefold labeled synaptonemal complexes from mouse testis visualized via immunolabeling of SYCP3 with SeTau647, SYCP1-C with Alexa 488 and SYCP1-N with Alexa 568 and Lattice SIM<sup>2</sup> mode. Sample courtesy: Marie-Christin Spindler, AG Prof Ricardo Benavente, Biocenter of the University of Würzburg. Objective: Plan-Apochromat 63×/1.4 Oil

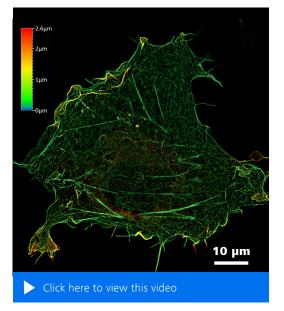
### > In Brief

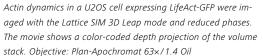
- .....
- > The Advantages
- > The Applications
- \_\_\_\_\_
- > The System
- > Technology and Details
- > Service

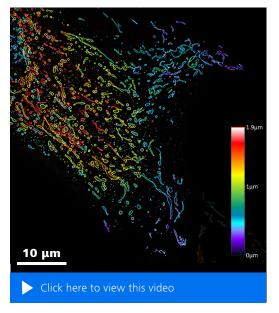
#### **Observe life's finest details**

ZEISS Elyra 7 was designed for the understanding of biological processes that require the observation of living samples in low light and high spatiotemporal resolution. Observe cellular, sub-cellular, and sub-organelle structures in living specimens in 2D and 3D over time. Whether you are interested in cytoskeletal dynamics, mitochondrial fusion and fission, or budding of the endoplasmic reticulum, it gives you both live cell compatibility and super-resolution.

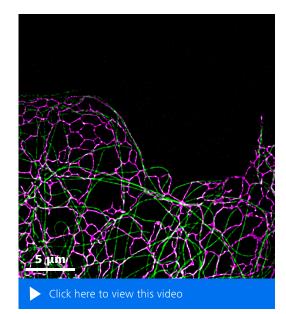
To image the fine structures of the cytoskeleton components, e.g., actin and ER networks or microtubular filaments, super-resolution imaging below 100 nm is required. Mitochondria are powerhouses of our cells, generating energy in the form of ATP to sustain the cell. These highly dynamic organelles constantly undergo fusion and fission events to ensure proper distribution of ATP across the cell. They are known to interact with the cytoskeleton and other subcellular compartments, e.g., microtubules, which they ride on to get to their destinations, or the ER, which wraps around mitochondria to initially constrict their diameter before fission events.







U2OS cell expressing Tomm20-mEmerald. Image shows a color-coded projection of the Lattice SIM<sup>2</sup> volume data set. Objective: Plan-Apochromat 63×/1.4 Oil



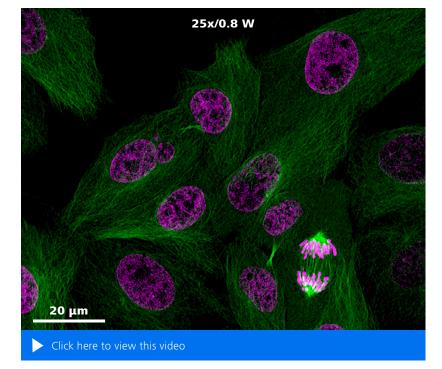
Simultaneous imaging of the endoplasmic reticulum (Calreticulin-tdTomato, magenta) and microtubules (EMTB-3xGFP, green) in a Cos-7 cell reveals highly dynamic interaction of these organelles. Objective: Plan-Apochromat 63×/1.4 Oil

### > In Brief

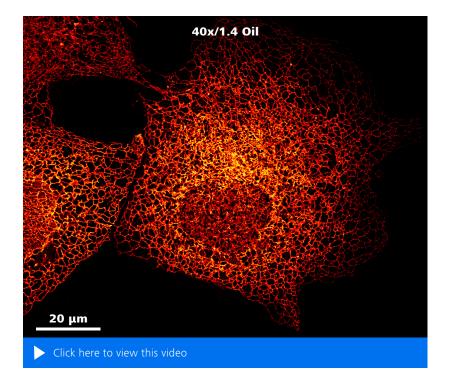
- > The Advantages
- -----
- > The Applications
- > The System
- \_\_\_\_
- > Technology and Details
- > Service

#### Excellent sectioning at incredible speed

SIM<sup>2</sup> Apotome is your flexible live cell imaging method for experiments that do not require the highest spatial resolution but rely instead on excellent sectioning quality. SIM<sup>2</sup> Apotome is superior to conventional confocal microscopy in terms of lateral and axial resolution as well as volume acquisition speed while it is also very gentle to your sample. Here, LLC PK1 cells expressing H2B-mCherry and  $\alpha$ -Tubulin mEmerald-GFP were continuously observed with a 25×/0.8 water immersion objective over 35 min, while undergoing mitosis. SIM<sup>2</sup> Apotome is compatible with objectives of different magnifications (10x, 20x, 25x and 40x). The high NA (1.4) 40x magnification images almost reach the resolution and sectioning capabilities of a conventional SIM microscope as demonstrated for Cos-7 cell expressing Calreticulin-tdTomato, while multiplying acquisition speed.



SIM<sup>2</sup> Apotome time lapse data of LLC PK1 cells expressing H2B-mCherry (magenta) and a-Tubulin mEmerald-GFP (green). Data shown as maximum intensity projection of 12 planes over 3.7 μm depth. Objective: LD LCI Plan-Apochromat 25×/0.8 Imm Corr



SIM<sup>2</sup> Apotome time lapse data of Cos-7 cells expressing the endoplasmic reticulum marker Calreticulin-tdTomato. Data shown as maximum intensity projection of 12 planes over 1.4 μm depth. Objective: Plan-Apochromat 40×/1.4 Oil

### > In Brief

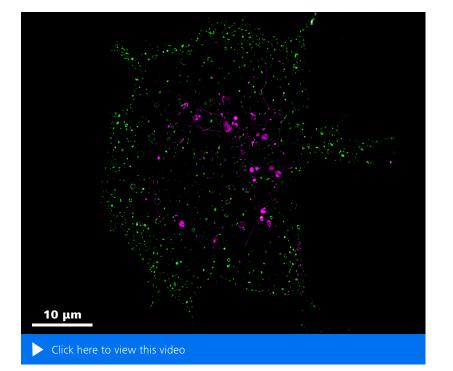
- III blief
- > The Advantages
- -----
- > The Applications
- > The System
- > Technology and Details
- .....
- Service

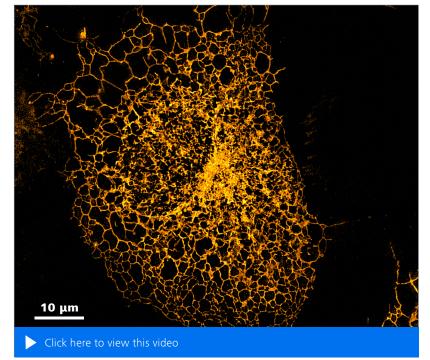
#### Super-resolution imaging at up to 255 fps

The diffusive and especially the ballistic movement of small vesicles in cells can be captured only when super-resolution and high-dynamic imaging are possible at the same time. With the Burst processing of 2D time lapse data, Elyra 7 is able to generate super-resolution images at 255 Hz in a large field of view and even acquire two colors simultaneously in both Lattice SIM and SIM Apotome acquisition modes.

#### Digital sectioning for 3D imaging three times faster

Elyra 7 Leap mode accelerates the volume imaging speed three times and at the same time decreases the light dosage on your sample. While still capturing all the finest details, the entire volume (18 planes) of the U2OS cell expressing Calreticulin-tdTomato was imaged at 38 volumes/min speed in Lattice SIM acquisition mode. For SIM Apotome acquisition mode, you can expect up to three times higher volume imaging speed.





U2OS cell expressing Rab5-mEmerald (green) and tdTomato tagged Golgi associated transport marker (magenta). Simultaneous dual-color acquisition with an exposure time of 1.5ms/phase for a FOV of  $1024 \times 1024$  pixel (64 µm × 64 µm). Objective: Plan-Apochromat 63×/1.4 Oil U2OS cell expressing calreticulin-tdTomato to visualize the endoplasmic reticulum. The time series shows a maximum intensity projection of the volume data set. Objective: Plan-Apochromat 63×/1.4 Oil

### > In Brief

#### .....

> The Advantages

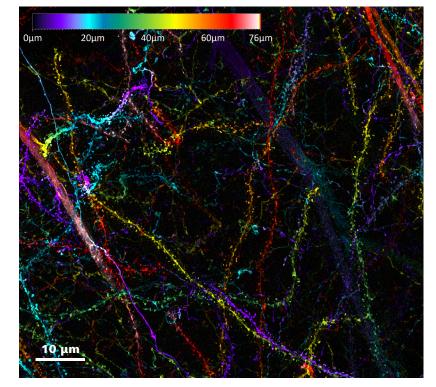
```
> The Applications
```

#### > The System

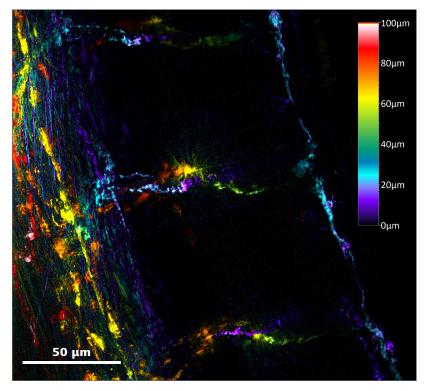
- > Technology and Details
- Service

#### Resolve the details hiding in the depth

Despite being a structured illumination-based microscope, Elyra 7 Lattice SIM<sup>2</sup> as well as SIM<sup>2</sup> Apotome also provide you with super-resolution and highquality sectioning in thick or scattering samples. The combination of robust illumination patterns and excellent image reconstruction technology enabled us to image throughout an entire murine brain section of ~80  $\mu$ m thickness expressing the neuronal marker Thy1-eGFP.



Murine brain expressing the neuronal marker Thy1-eGFP was imaged in Lattice SIM mode over a Z stack range of 75 μm. The image shows the color-coded projection of the volume data. Objective: Plan-Apochromat 63×/1.4 Oil. Sample courtesy of Herms Lab (MCN, University of Munich, Germany)



Zebrafish embryo expressing a vascular marker fli1-EGFP was imaged in SIM Apotome mode over a Z stack range of 100  $\mu$ m. The SIM<sup>2</sup> processed image shows the color-coded projection of the volume data. Objective: LD LCI Plan-Apochromat 25×/0.8 Imm Corr. Sample courtesy of Haass Lab (MCN, University of Munich, Germany)

> In Brief	
> The Advantages	

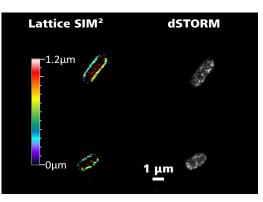
- \_\_\_\_\_
- > The Applications
- > The System
- > Technology and Details
- \_\_\_\_\_
- > Service

#### Correlative microscopy within the same system

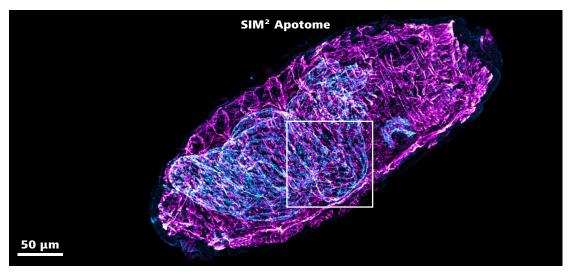
Correlative microscopy, where the same area of the sample is imaged using different techniques, has become an important tool for biological research. Imaging with Elyra 7 can be combined seamlessly with LSM 980 Airyscan or ZEISS electron microscopy solutions. Nevertheless, Elyra 7 itself contains three different imaging modalities – SIM Apotome, Lattice SIM and SMLM – providing the possibility to combine them when needed for sample scales varying by orders of magnitude.

Here, a detailed SIM<sup>2</sup> Apotome overview volume image of a drosophila larvae was combined with the superresolution Lattice SIM<sup>2</sup> volume image of a region of interest, putting the super-resolved area into full context.

Another advantageous combination is the pre-imaging of SMLM samples with Lattice  $SIM^2$  to easily identify interesting sample areas at resolutions of 60–100 nm, then perform the more time-consuming localization microscopy on suitable cells.



Lattice SIM<sup>2</sup> and dSTORM images of bacteria stained with a membrane marker coupled to Alexa Fluor 647. Sample courtesy of J. Nabarro, C. Baumann, G. Calder & P. O'Toole (Department of Biology & Bioscience Technology Facility, University of York, UK)



Lattike SIM<sup>2</sup> 10 μm

SIM<sup>2</sup> Apotome and Lattice SIM<sup>2</sup> images of D. melanogaster larva stained with HLH-54F-GFP (cyan) and Anti-Cut-Cy3 (magenta). Images show maximum intensity projections of 3D data. Sample courtesy of R. Palmer and G. Wolfstetter (University of Gothenburg)

### > In Brief

- III blief
- > The Advantages
- -----
- > The Applications
- > The System
- > Technology and Details
- .....
- > Service

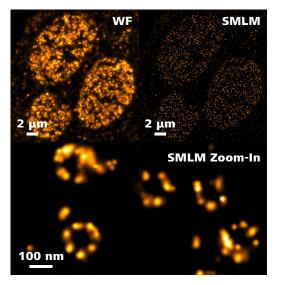
#### Single-molecule localization microscopy (SMLM)

SMLM encompasses techniques such as PALM, dSTORM, and PAINT. With high power lasers across the visible spectrum and dual camera detection, Elyra 7 allows researchers to gain access to a broad range of dyes, markers and fluorophores in almost any possible combination.

Elyra 7 enables precise quantification over a large field of view and an unprecedented Z-capture range. You now can acquire 3D data from a whole cell with molecular precision.

#### **Resolve molecular structures**

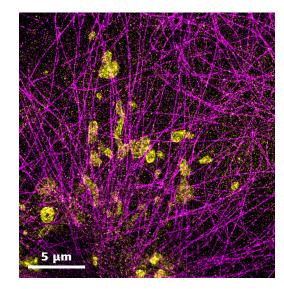
SMLM allows you to map precise locations of individual proteins.



SMLM: Eightfold symmetry of the nuclear pore complex in A6 cells. Gp210 was labeled with Alexa Fluor 647. Widefield image (top left), SMLM image (top right) and zoomed in region (bottom).

# Determine the relationships between molecules

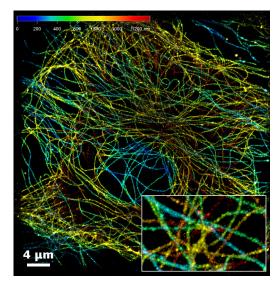
Detect two channels with molecular precision.



Dual-color 2D STORM of Cos-7 cells stained for microtubules (anti-tubulin-Alexa Fluor 647, magenta) and mitochondria (anti-TOMM20-CF568, yellow).

### Capture information in three dimensions

Untangle molecular relationships in Z with confidence.



SMLM: With Elyra 7 you can image a Z-depth of 1.4 μm in a single acquisition. 3D SMLM image of Alexa 647 α-tubulin color-coded for depth.

Sample courtesy of Michael W. Davidson, Florida State University, USA.

# The Lattice SIM product family

> In Brief

> The Advantages

> The Applications

> Technology and Details

> The System

> Service

#### Address all your super-resolution needs across scales

The ZEISS Lattice SIM product family gives you full access to super-resolution imaging for all research areas, from fast optical sectioning to the detection of highly dynamic processes and quantification at the molecular level.



**ZEISS Lattice SIM 3** Reveal cellular behavior and inter-cellular dynamics

Lattice SIM 3 is specifically designed to meet the imaging requirements of multicellular organisms and tissue sections. This system exploits the full potential of the SIM Apotome technology: fast optical sectioning at superior quality, large fields of view with access to smaller regions of interest, near-isotropic resolution, and the gentlest super-resolution imaging possible.



**ZEISS Lattice SIM 5** Reveal the vibrant sub-organelle network of life

ZEISS Lattice SIM 5 has been optimized for single cell imaging as well as capturing subcellular structures and their dynamics. Powered by the Lattice SIM technology and the SIM<sup>2</sup> image reconstruction algorithm, ZEISS Lattice SIM 5 provides you with outstanding super-resolution capabilities down to 60 nm in both living and fixed cells.



**ZEISS Elyra 7 with Lattice SIM** Reveal life across scales – down to molecular details

ZEISS Elyra 7 includes several microscopy techniques: Lattice SIM<sup>2</sup>, SIM<sup>2</sup> Apotome, SMLM and TIRF. You can combine these techniques to multiply the insights from your specimen and to correlate the acquired data. With its focus on single molecule localization microscopy, ZEISS Elyra 7 gives you resolution excellence down to the molecular level.

# **Your Flexible Choice of Components**

- > In Brief
- > The Advantages
- · me Advantages
- > The Applications
- > The System
- > Technology and Details
- > Service



#### 1 Microscope

- Axio Observer 7 (inverse stand)
- Stage top incubation
- Motorized XY stepper scanning stage
- Z-Piezo stage insert
- 2 camera ports or one camera port with Duolink

#### 2 Objectives

- C-Apochromat 63×/1.2 Water (DIC)\*
- Plan-Apochromat 63×/1.4 Oil (DIC)\*
- alpha Plan-Apochromat 100×/1.46 Oil (DIC)\*
- alpha Plan-Apochromat 100×/1.57 Oil HI Corr (DIC)\*

4

- alpha Plan-Apochromat 63×/1.46 Oil
- C-Apochromat 40×/1.2 W
- Plan-Apochromat 40×/1.4 Oil (DIC)\*
- LD LCI Plan-Apochromat 25×/0.8 Imm Corr
- Plan-Apochromat 20×/0.8 Air
- EC Plan-Neofluar 10×/0.3 Air

#### 3 ZEISS Elyra 7 Illumination and Detection

- Fiber coupled solid state or diode pumped solid state lasers
- Available lines:

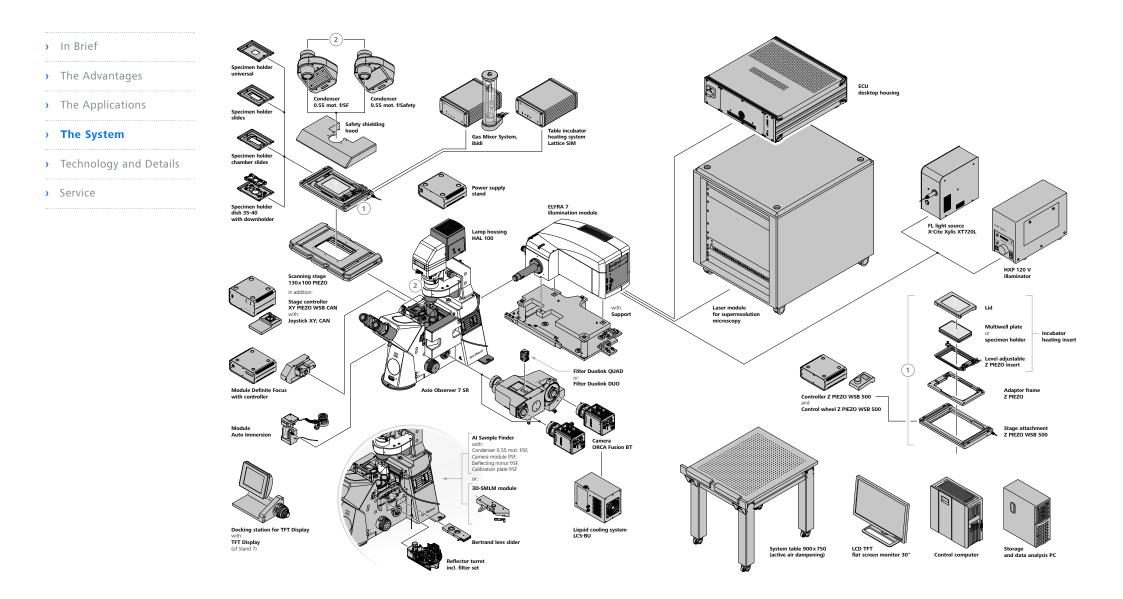
405 nm diode (50 mW), 488 nm OPSL (100 or 500 mW), 561 nm OPSL (100 or 500 mW), 642 nm diode (100 mW), 640 nm OPSL (500 mW)

- Lasers shared between Lattice SIM and SMLM
- Hamamatsu ORCA-Fusion BT sCMOS camera

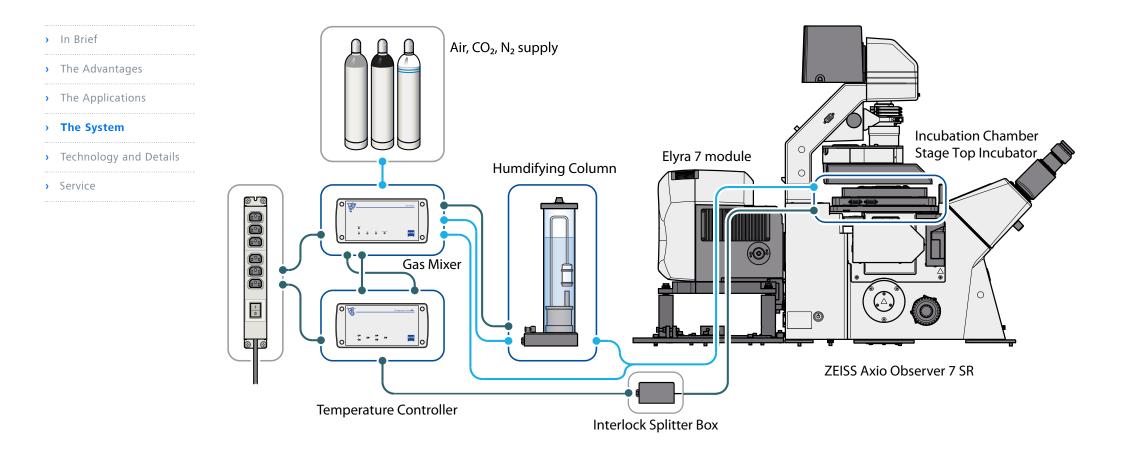
#### 4 Software

- ZEN (blue edition)
- SIM toolkit
- SMLM (PALM/dSTORM) module

# **System Overview**



# **Incubation Setup**



	Microscope		
In Brief	Stand	Axio Observer 7, motorized inverted microscope for superresolution microscopy	
The Advantages	Z-drive	DC servomotor, opto-electronically coded; smallest Z step 25 nm	
The Applications	XY Piezo scanning stage	motorized; range 130 mm × 100 mm; max sped 100 mm/s; resolution 0.2 µm; reproducibility: $\pm$ 1 µm; absolute accuracy $\pm$ 5 µm; suitable for mounting frames K 160 × 110 mm and Z-Piezo Stage insert	
The System	Z-Piezo stage insert	for XY scanning stage, max travel range 500 μm; smallest Z step size 5 nm, sample holders available for standard 3"×1" slides LabTek chambers, multiwell plates and 36 mm glass-bottom dishes;	
Technology and Details		level-adjustable and universal stage insert available for standard slides, glass-bottom dishes and LabTekTM chambers.	
Service	Optical Filters for Lattice SIM and SMLM		
	Filter sets reflector turret	Four exchangeable filter sets available for multi-channel Lattice SIM and SMLM; each filter set with four precisely mounted ACR-coded <sup>(1)</sup> filter modules for superresolution microscopy on a motorized six-position turret; two positions in each turret compatible with standard Push & Click filter modules, e.g. for visual sample observation.	
	Dual filter sets for Duolink optimized for dual color and double dual color applications	Filter sets are optimized for dual camera applications, maximum sensitivity, minimal cross-talk and reduced autofluorescence.	
	Filter slider	Manual filter slider with two positions (for emission filters or a Bertrand lens); fits into camera adapter of the microscope's side port; emission filters exchangeable for customizing detection conditions.	
	Lasers		
	Laser module for Elyra 7	Laser coupling with polarization-maintaining single mode fiber (no adjustment of laser coupling by users required).	
	Laser lines	405 nm (50 mW), 488 nm (100 mW or 500 mW), 561 nm (100 mW or 500 mW), 642 nm (100 mW) or 640 nm (500 mW); 405 laser can be attenuated by up to 100000 fold (used for activation and back-pumping); high power lasers (500 mW) can be 10 fold attenuated (488, 561, 642)	
	Cameras		
	sCMOS	Hamamatsu ORCA-Fusion BT; sensor pixel count: 2304×2304, effective: 1288×1288; pixel size 6.5 µm×6.5 µm; QE: up to 95 % @ 540 nm); water cooling (regulated sensor temperature −8 °C); dynamic range: 16 bit; binning: 1×1, 2×2, 4×4; Frame rates: 89 fps (fast) @ full frame in Laser WF modality	
		Liquid cooling system for sCMOS camera (Hamamatsu ORCA-Fusion BT)	

	Elyra 7 for SMLM	
<ul><li>In Brief</li><li>The Advantages</li></ul>	Illumination module	Type L and LS: Fully motorized Epifluorescence (EPI), high inclined and laminated optical sheet (HILO) and total internal reflection illumination (TIRF); simultaneous TIRF illumination with VIS and 405 nm laser lines;
> The Applications		individual triggering of lasers for synchronizing dye activation and illumination to camera read-out and transfer times; motorized TIRF angle adjustment; motorized TIRF field adjustment with three field size options
> The System		Type LS: Five different grating frequencies for Lattice SIM for optimal matching of illumination pattern to laser wavelength and objective lens;
> Technology and Details		Motorized exchange of gratings in multi-color Lattice SIM; one grating for SIM Apotome; fast piezo actuated phase stepping of gratings
> Service	3D-PALM module	Double phase ramp in pupil plane of back aperture of objective providing for phase ramp imaging localization microscopy (PRILM); z capture range typically 1.4 µm
	Camera	Up to two Hamamatsu ORCA-Fusion BT sCMOS cameras mounted on right side port
	Objective lenses (SMLM)	alpha Plan-Apochromat 100×/1.46 Oil DIC*, alpha Plan-Apochromat 100×/1.57 Oil-HI DIC* Corr (2D-PALM), alpha Plan-Apochromat 63×/1.46 Oil, Plan-Apochromat 63×/1.4 Oil DIC*, C-Apochromat 63×/1.2 W Corr DIC* (3D-PALM) ACR <sup>(1)</sup> coding (optional; Objectives with NA > = 1.46 suitable for TIRF and HILO illumination)
	Imaging modes	Widefield (WF) mode (sample illumination with thermal light source or LED), Laser WF mode (sample illumination with laser), SMLM mode for single-molecule localization microscopy
	Field of view (SMLM)	Maximal field of view 50.9×50.9 μm² (with alpha Plan-Apochromat 100×/1.46 Oil DIC*, 1.6× tube lens, full chip recording); 128.22×128.22 μm² (with Plan-Apochromat 63×/1.4 OIL DIC*, 1.0× tube lens, full chip recording); HP field 2× smaller, uHP field 2×√2 smaller than TIRF field
	Localization precision (SMLM)	Typically 10 nm – 20 nm lateral, 20 nm – 40 nm axial, given sufficient signal-to-noise & density
	Multi-color imaging (SMLM)	Detection of up to two different fluorescent labels (simultaneous with Duolink or quasi simultaneously by fast sequential laser switching)
	Acquisition speed (SMLM)	sCMOS (dSTORM) and widefield mode > 100 frames per second full chip; 200 frames per second (512×512 pixels)
	Data recording and analysis (SMLM)	Full software control of SMLM imaging; Definite Focus z-drift control
		Online SMLM processing for simultaneous data acquisition and analysis; manual editing of parameter settings for optimal results in SMLM with different fluorophores; feature-rich rendering of SMLM localization tables; export and import of localization tables for custom filtering; correction algorithms for lateral and axial drift; chromatic aberration correction (based on fiducial markers or prominent structures)
		Multi-emitter fitting algorithms allow to analyze overlapping signals with high precision. Up to 10 times higher labeling densities are possible speeding up acquisitions by the same factor.

### > In Brief

•••••

- > The Advantages
- > The Applications
- -----
- > The System
- > Technology and Details
- > Service

Illumination module	Fully motorized Lattice SIM imaging; five different grating frequencies for Lattice SIM for optimal matching of illumination pattern to laser wavelength and objective lens; motorized exchange of gratings in multi-color Lattice SIM; one grating for SIM Apotome fast piezo actuated phase stepping of gratings.
Camera	Up to two Hamamatsu ORCA-Fusion BT sCMOS cameras mounted on right side port
Imaging Modes	Widefield modes for illumination with thermal light source or LED and lasers, Lattice SIM using two dimensional grid SIM mode (two- and three-dimensional Lattice SIM), SIM Apotome mode using one dimensional grid for z-sectioning
Objective lenses (Lattice SIM)	Plan-Apochromat 63×/1.40 Oil DIC*, C-Apochromat 63×/1.20 W Corr, alpha Plan-Apochromat 63×/1.46 Oil, ACR <sup>(1)</sup> coding (optional), alpha Plan-Apochromat 100×/1.57 Oil-HI DIC* Corr
Objective lenses (SIM Apotome mode)	Plan-Apochromat 40×/1.4 Oil; C-Apochromat 40×/1.2 W; EC Plan-Neofluar 10×/0.3 Air; Plan-Apochromat 20×/0.8 Air; LD LCI Plan-Apochromat 25×/0.8 Imm Corr DIC*
Resolution (Lattice SIM)	Lateral resolution (XY): down to 120 nm, axial resolution (Z): down to 300 nm (typical experimental FWHM values with objective lens Plan-Apochromat 63×/1.40 Oil DIC*, subresolution beads of 100 nm diameter and excitation at 488 nm; resolution is sample and SNR dependent)
Resolution (Lattice SIM <sup>2</sup> )	Lateral resolution (XY): down to 60 nm, axial resolution (Z): down to 200 nm (typical experimental FWHM values with objective lens Plan-Apochromat 63×/1.40 Oil DIC*, subresolution beads of 100 nm diameter and excitation at 488 nm; resolution is sample and SNR dependent)
Resolution (SIM <sup>2</sup> Apotome)	Lateral resolution (XY) down to 140 nm for 40× 1.4 oil objective; lateral resolution (XY) down to 265 nm for 25× 0.8 Imm Corr objective; (typical FWHM measurements on sub-resolution beads of 100 nm diameter and excitation at 488 nm; resolution is sample and SNR dependent)
Multi-color (Lattice SIM and SIM Apotome mode)	Detection of up to four different fluorescent labels (sequential detection) and simultaneous dual color detection with DuoLink
Max. Field of view (Lattice SIM)	80.14×80.14 μm <sup>2</sup> , full-frame recording (1280×1280 effective px) with Plan-Apochromat 63×/1.40 Oil DIC*
Max. Field of view (SIM Apotome mode)	126×126 μm², full frame recording (1280×1280 effective px) with Plan-Apochromat 40×/1.40 Oil 203×203 μm², full frame recording with LD LCI Plan-Apochromat 25×/0.8 Imm Corr DIC*; 252×252 μm², full frame recording with Plan-Apochromat 20×/0.8 Air; 505×505 μm², full frame recording with EC Plan-Neofluar 10×/0.3 Air
Acquisition speed (Lattice SIM)	19 SIM image frames per second at 512×512 px resolution and 1 ms exposure time (13 phase images per one SIM image) 28 SIM image frames per second at 512×512 px resolution and 1 ms exposure time (9 phase images per one SIM image)
Acquisition speed (SIM Apotome mode)	51 sectioned frames per second at 512×512 px resolution and 1 ms exposure time (camera limited) (5 phase images per one sectioned image); 85 sectioned frames per second at 512×512 px resolution and 1 ms exposure time (camera limited) (3 phase images per one sectioned image);
Leap mode and Burst mode	Leap and Burst modes are combinable with both the Lattice SIM and SIM Apotome. Leap mode increases the frame rate by a factor of 3 for 3D image acquisition. Max. 255 image frames per second at 512×512 px resolution and 1 ms exposure time are available for 2D data after Burst processing.
Data recording and analysis (Lattice SIM and SIM Apotome mode)	Full software control of Lattice SIM imaging; Multi-tracking (sequential multi-channel data acquisition with freely configurable change of gratings (Lattice SIM), or one common grating (SIM Apotome mode), filters and excitation lasers between tracks); Simultaneous dual color imaging with one grating; Lattice SIM and SIM Apotome mode imaging in user-defined sub-array regions (ROI imaging); Leap mode for 3 times faster imaging with excellent sectioning; Extension of imaged area possible with tile scanning and stitching. Burst mode processing for 2D time series data sets for Lattice SIM and Aptotome mode to increase effective frame rates by a factor of 15 and 5, respectively.

\* DIC indicates type of objective not imaging modality

<sup>(1)</sup> ACR (Automatic Component Recognition); Elyra 7 systems and ZEN imaging software automatically recognize ACR-coded components.

### > In Brief

>	The	Advantages

- > The Applications
- > The System
- -----
- > Technology and Details
- > Service

System information	All imaging modes combined in one system
Illumination module	Sample illumination in all widefield and superresolution modes by a single, highly integrated illumination module
	(with same set of lasers and a single Elyra laser module).
Camera	Up to two Hamamatsu ORCA-Fusion BT sCMOS cameras mounted on right side port
Software	
Standard	ZEN imaging software (64-bit); operating system: Microsoft Windows 10
	Full software control of image data recording in all imaging modes (including widefield, superresolution);
	Full software control of image data recording in all imaging modes (including widefield, superresolution);
	Full software control of image data recording in all imaging modes (including widefield, superresolution); Software-controlled switching between imaging modes.
Software packages	Full software control of image data recording in all imaging modes (including widefield, superresolution); Software-controlled switching between imaging modes. Full software control of data recording (multi-channel imaging, time series, z-stack)

Accessories	
Definite Focus	Holding focus to compensate axial drift, typical z-position accuracy: 30 nm; Specified limits of Definite Focus 3: 0.2 × DOF (Depth of field: DOF $\approx \lambda$ /NA <sup>2</sup>
Incubation	Stage-top incubation possible with safety lock
Duolink for attachment of two cameras of the same type	Allows attachment of two cameras of the same type to the microscope.
Storage PC with 55 TByte storage capacity	Direct streaming of data and parallel processing while streaming of data possible



Elyra 7 with Lattice SIM meets the requirements according to IEC 60825-1:2014 and is a laser class 3B device. Interlocks on customer interfaces prevent access to the laser radiation.

# **ZEISS Service – Your Partner at All Times**

Your microscope system from ZEISS is one of your most important tools. For over 175 years, the ZEISS brand and our experience have stood for reliable equipment with a long life in the field of microscopy. You can count on superior service and support - before and after installation. Our skilled ZEISS service team makes sure that your microscope is always ready for use.

### **Procurement**

- Lab Planning & Construction Site Management
- Site Inspection & Environmental Analysis
- GMP-Qualification IQ/OQ
- Installation & Handover
- IT Integration Support
- Startup Training

> In Brief

> The Advantages

> The Applications

> Technology and Details

> The System

> Service

# Operation

- Predictive Service Remote Monitoring
- Inspection & Preventive Maintenance
- Software Maintenance Agreements
  - Operation & Application Training
  - Expert Phone & Remote Support
    - Protect Service Agreements
      - Metrological Calibration
      - Instrument Relocation
        - Consumables
          - Repairs

# Retrofit

- Customized EngineeringUpgrades & Modernization
- Customized Workflows via ZEISS arivis Cloud

# **New Investment**

- Decommissioning
- Trade In



### Carl Zeiss Microscopy GmbH

07745 Jena, Germany microscopy@zeiss.com www.zeiss.com/lattice-sim

