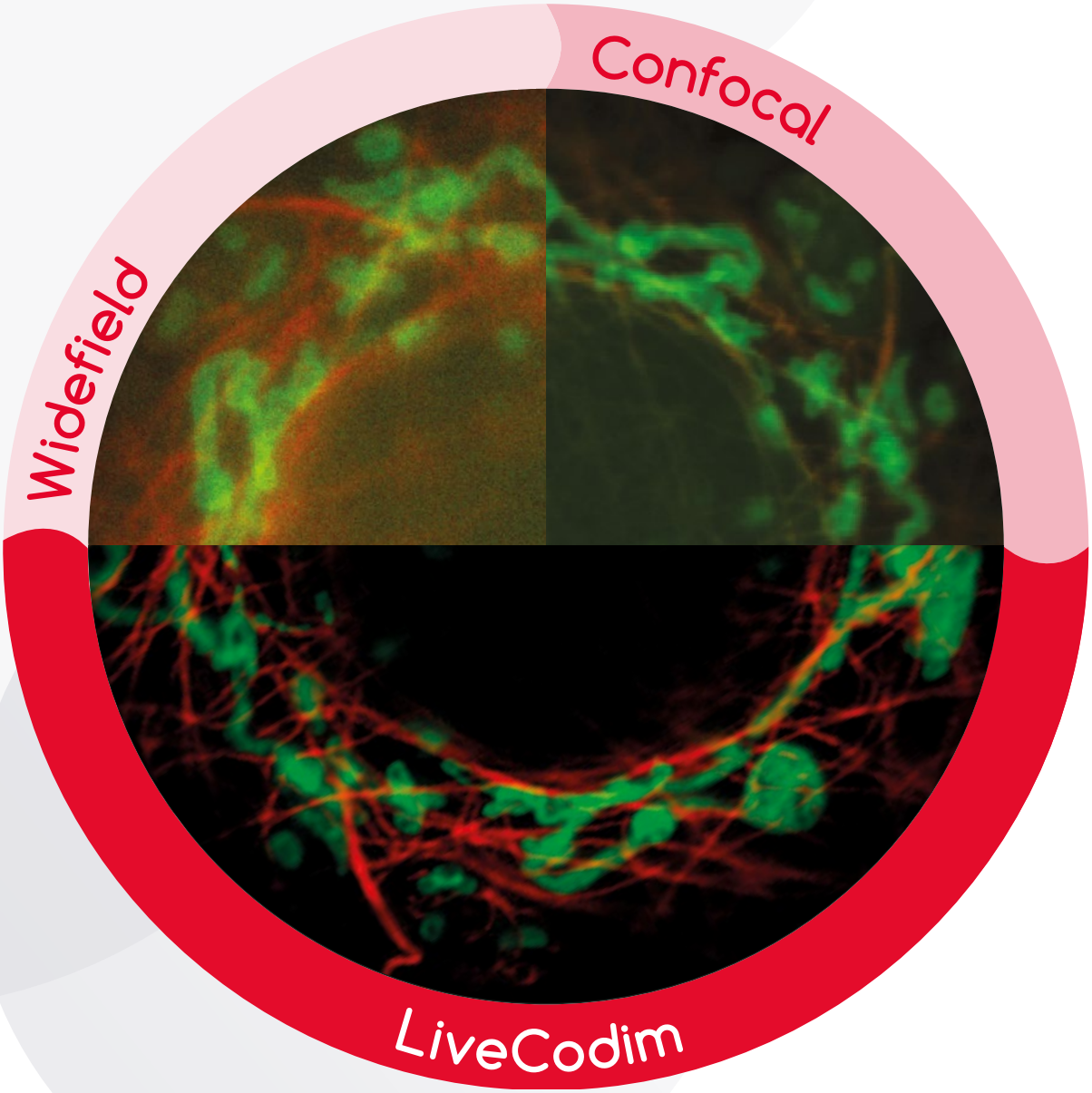
LiveCodim

Super-resolution imaging platform

A solution for widefield and confocal microscopes

telight

Revolution in super-resolution



LiveCodim is a universal, modular super-resolution imaging device. Via C-mount it will transform any widefield or confocal microscope into a **super-resolution imaging platform**. It is the solution for easy live imaging with high resolution and low phototoxicity.

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LiveCodim Components

Standard widefield microscope:

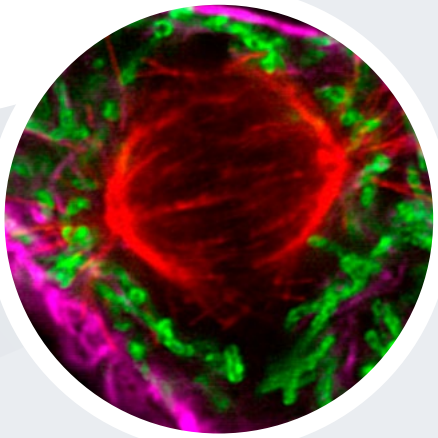
Compatible with anycommercially available or custom-designed microscope. Widefield illumination provided upon request.

LiveCodim complete scanning and super-resolution image acquisition unit

Optics for raster scanning, beam-shaping and image acquisition with Teledyne Photometrics camera.

C-mount

Camera mount attachment to microscope via either left or right side ports. May also be custom fitted for other ports. i.e. F-mount.



Controller

High-speed field-programmable gate arrays and high-voltage power supply.

Multichannel laser

Standard 405/488/561/640, 100 mW lines. Wavelengths can be adapted as required between 400 and 1000 nm.

From confocal to super-resolution

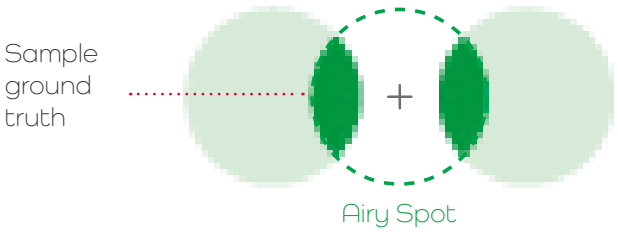
The advent of confocal microscopy offered a substantial step forward in biology by allowing for **improvements in spatial resolution and brighter, deeper imaging within tissues** through the use of laser-scanning technology.

However, light microscopy is limited by the diffraction of light through media. This limit is proportional to the wavelength of excitation light. For visible-range colors, only objects 200-400nm in size or distance apart can be resolved.

Super-resolution microscopy techniques have been developed to improve resolution but suffer from multiple drawbacks: they require complex optical set-ups with powerful lasers that induce photo-damage and require long acquisition times. This can make them difficult to use with living samples, limiting their ability to observe the dynamics of living cells and subcellular structures.

Confocal microscopes scan the sample with a diffraction-limited Airy spot as pictured above. The size of the Airy spot is proportional to the wavelength of light used for excitation and limited by the numerical aperture of the objective.

The illustration below shows the result of attempting to scan two points that are located closer together than the size of the Airy spot. Instead of the resulting image showing two true individual points, we see two spots blurred together.

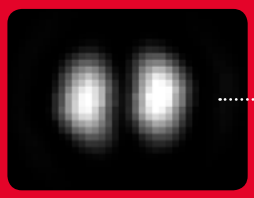


If two fluorescent objects are closer than the confocal spot size, they cannot be distinguished in the resulting confocal image.

Conical diffraction microscopy

Telight's LiveCodim offers a solution to both resolution and phototoxicity by using a **biaxial crystal to efficiently employ low-levels of light** and produce localized structured illumination patterns.

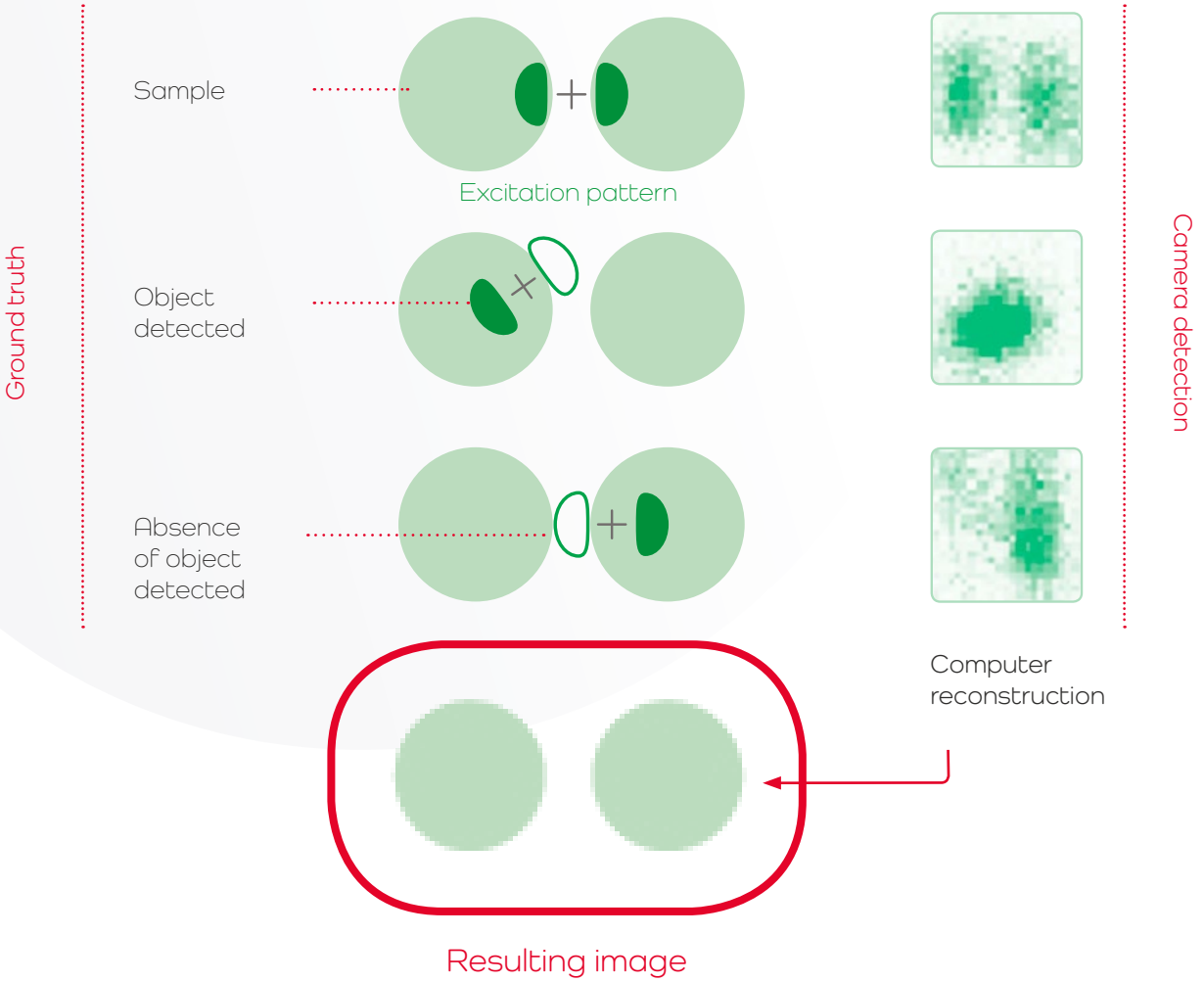
Our technology is based on **conical diffraction** produced within the crystal, allowing us to create an illumination pattern that has a sharply oriented exctinction, or a region of no light.



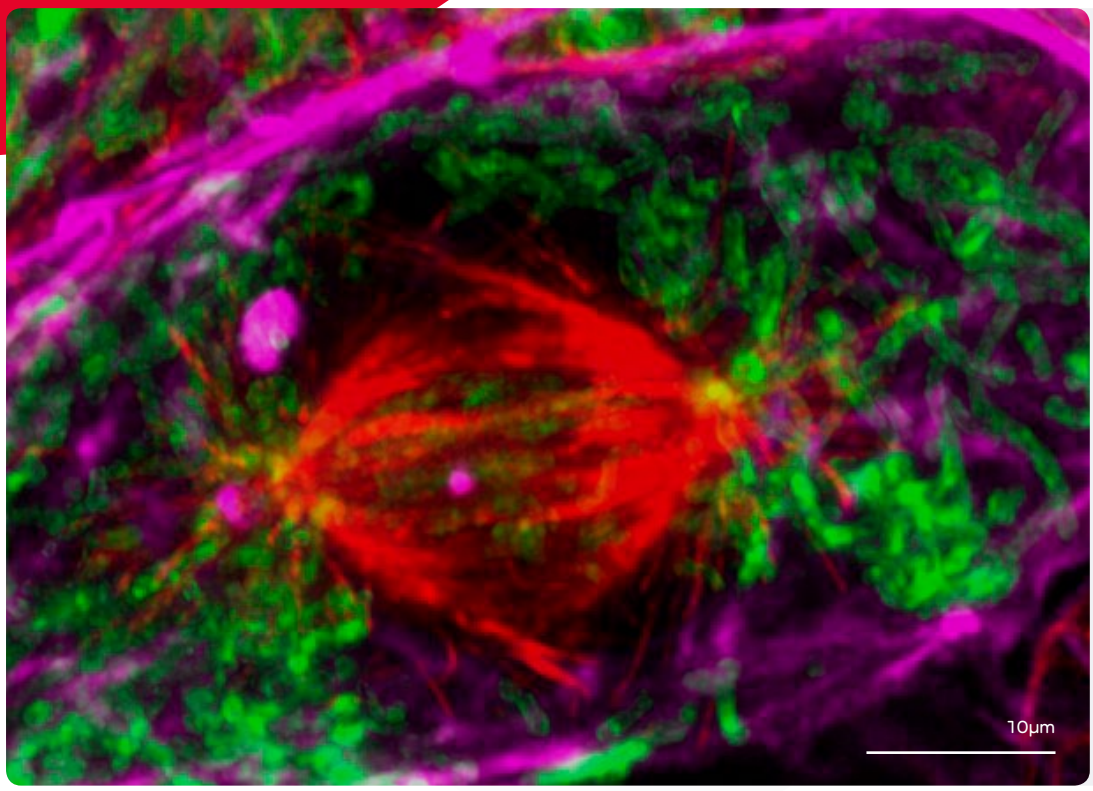
"Half-moon" distribution utilized by Telight LiveCodim

These structures take the shape of two "half-moon" distributions that are rotated in three orientations. The resulting fluorescence signal is collected on our camera and used to reconstruct the immediate surroundings of the point. Since the **region of darkness in the half-moon is sharper** than a confocal diffraction-limited excitation spot, we can use this information to reconstruct a super-resolved image.

Between efficient spatial sampling and low laser power, we protect the sample from photodamage without sacrificing speed: **10x10 µm images can be acquired in a second or less.**

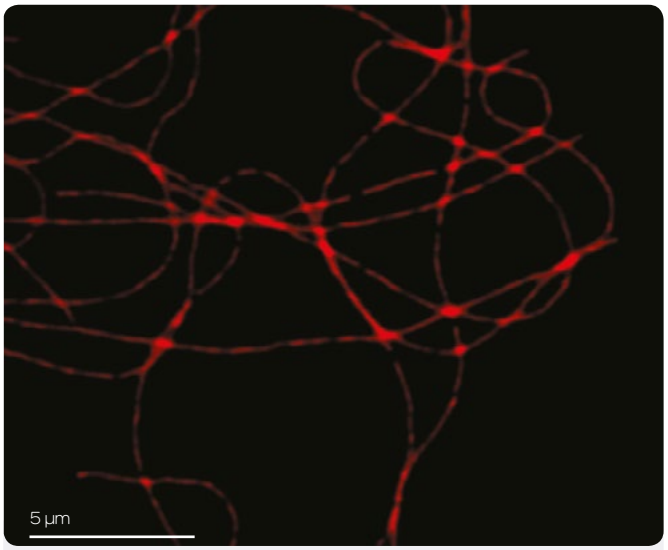


Resolution & 3D imaging



Mitotic spindle in a human fibroblast cell (huFIB). This image was built from 10 depth slices of the cell, generating a 3D representation of cell division.

- Microtubules of the spindle
- Mitochondria
- Cortical actin filaments



A cropped capture of a 20x20 µm image of microtubules in a huFIB cell. Microtubules were stained with AlexaFluor555 anti-tubulin antibodies. Here, with the high resolution capabilities of LiveCodim, we can visualize the discrete nature of antibody labeling and the microtubules appear non-continuous and compartmentalized.

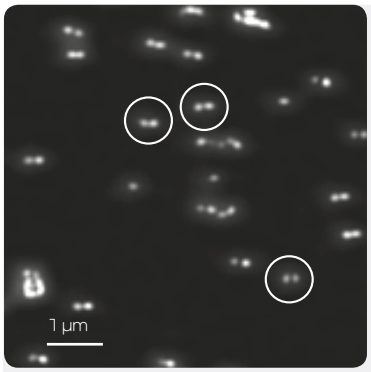
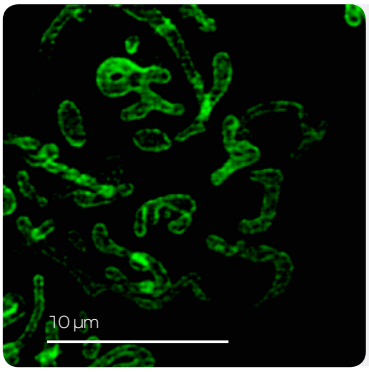
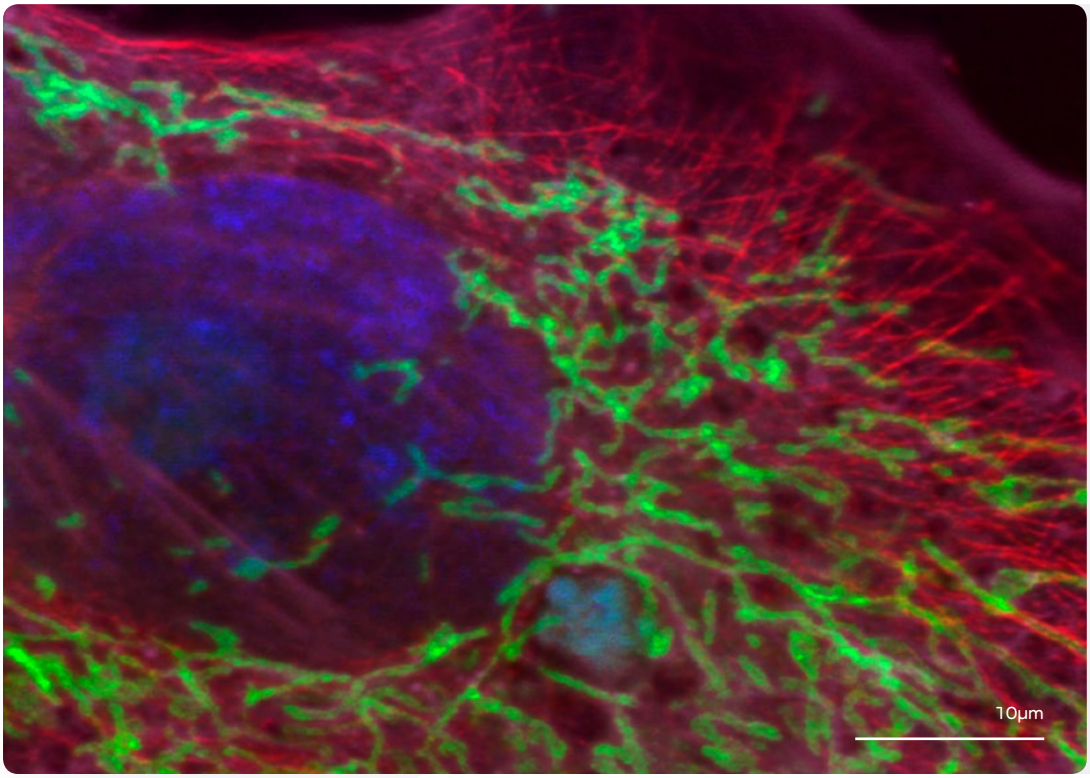


Image of nano-ruler bead pairs that are separated by 90 nm.
Close-ups show 3 different bead couples with clear separation of the beads.



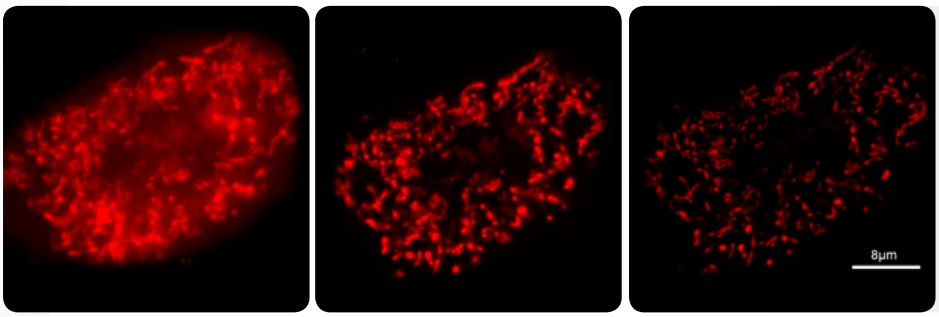
21x21µm field of view of TOM20 Alexa-Fluor488 - labeled mitochondria in a huFIB cell.



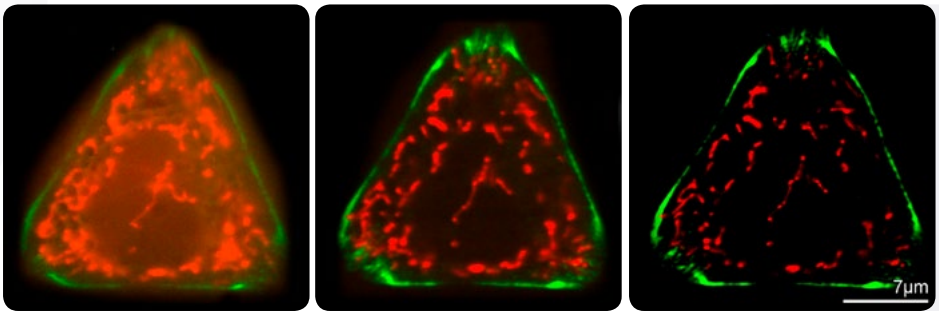
HuFIB cell, S-phase.
4-color acquisition:

- DAPI staining of nucleus
- Microtubules
- Mitochondria
- Actin

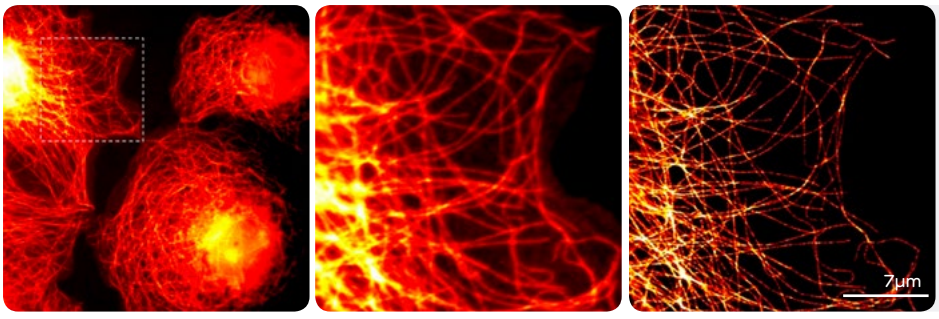
Widefield | Confocal | LiveCodim



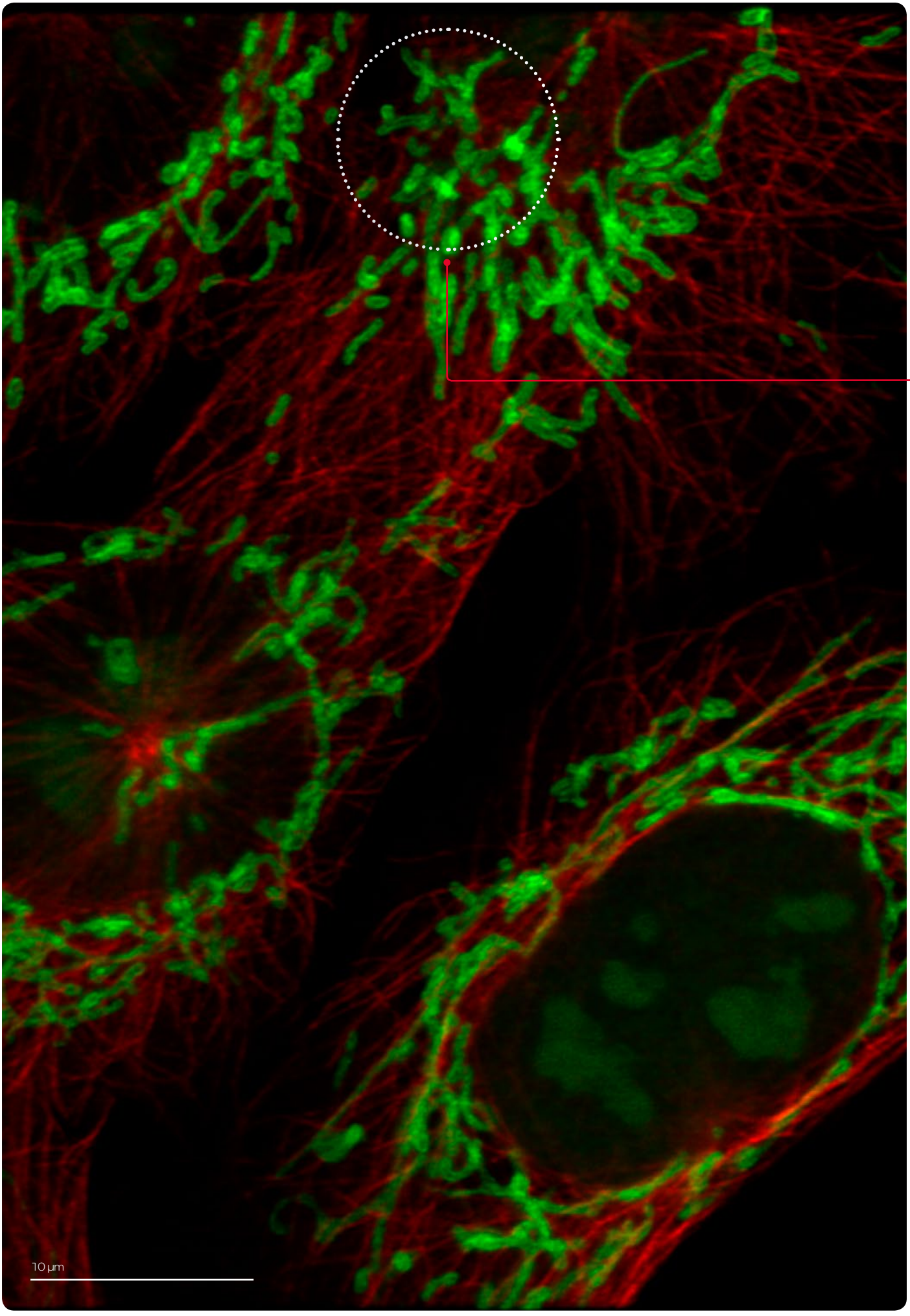
MDCK cell mitochondria (inner membrane) imaged with MitoTracker™ Orange



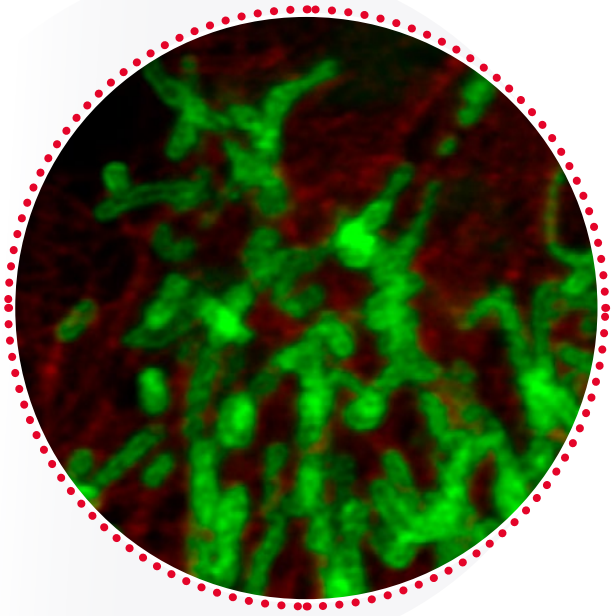
- MDCK cell focal adhesion
- Mitochondria



The dashed square in widefield (left) defines a 20x20µm region of interest (ROI) that is shown as scanned with the confocal (middle) and CODIM (right) features of LiveCodim.


10 µm

LiveCodim Features



Human fibroblast cells

- AlexaFluor488 anti-TOM20 labeling of the outer mitochondrial membrane.
- AlexaFluor561 anti-tubulin labeling of microtubules.

1

Super-resolved

90 nm lateral resolution acheived through our unique use of conical diffraction beam shaping and data processing algorithms.

2

Muti-color

LiveCodim's super-resolution capabilities are achromatic: any visible-range to near-infrared excitation wavelengths can be customized for multichannel imaging. All colors can be imaged simultaneously on living samples.

3

Depth scanning

LiveCodim can achieve resolution at depths up to 500 micrometers into biological samples with an axial resolution identical to confocal (500 nm). 3D images can be acquired with the z-stack feature.

4

Modular

LiveCodim is an add-on module that connects to your microscope via a camera C-mount side port. It is compatible with any commercially available scientific microscope as well as specialized devices fitted with incubators or custom-built set-ups.

5

Easy to use

The software user-interface is simple and straightforward: the user may view the sample with widefield fluorescence and run confocal and CODIM super-resolution acquisitions.
3D acquisitions with z-stacks as well as time-lapse imaging are readily available. All image results are saved in a TIFF format and can be visualized within the LiveCodim application or with open-source ImageJ.

Key elements

Specifications

Widefield light source	CoolLED - 16 wavelengths from 365 nm to 770 nm
Laser lines	405 / 488 / 561 / 640 nm (customizable on request)
Lateral resolution	90 nm at 488 nm wavelength
Axial resolution	500 nm at 488 nm wavelength
LiveCodim field of view	60 x 60 µm with 60x 1.4 NA objective
Acquisition speed	1s for 10x10 µm image at 488nm
Software	Automated and adaptive super-resolution image processing|: • Fast switching between image acquisition modes • Intuitive user-friendly interface
File format	OME-TIFF universal format
Fluorophore compatibility	No specific requirement, 400 – 1000 nm wavelength range
Dimensions	57x47x145 mm

Microscope requirements

Port	Right or left C-mount port Other ports can also be adapted for fitting
Filter turret	Automated
XYZ stage	Recommended automated
Objective	60/63x 1.4 NA (or higher) oil immersion for maximum resolution provided upon request
Optical table	Recommended

More details about LiveCodim specifications and relevantpublications can be found at our website **www.telight.eu**.

Application examples

Cell biology

LiveCodim is exceptionally suited for cellular and molecular biology studies, offering live 3D acquisition with low photodamage for the following types of studies:

- Cytoskeleton, cellular structure, colocalization
- Focal adhesion
- Cell cycle, membrane trafficking, signaling
- Visualizing protein and subcellular structure dynamics

Virology Immunology

High-resolution live-imaging for particle tracking and protein interactions are essential for characterizing:

- Immune cell differentiation and activity
- Virus entry and infection
- Drug uptake and delivery validation

Neurobiology

Multi-color imaging is highly useful for a range of colocalization research that includes investigating:

- Synaptic structure and composition
- Neuronal development
- Neuronal activity may be probed in tandem with voltage or calcium-reactive dyes

Plant biology

LiveCodim offeres high-resolution with depth penetration into tissues which is ideal for classifying:

- Plant cellular structure
- Plant embryogenesis and development

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BioAxial's LiveCodim has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 811988 – LiveCodim

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