New Nikon-BioAxial Super-Resolution Platform Offering 90 nm Resolution with Conventional Fluorophores

Nikon

BioAxial

Nikon France and BioAxial are pleased to announce their partnership in bringing to market a new high-end live cell super-resolution microscopy system based on breakthrough conical diffraction technology. Our user-friendly platform seamlessly integrates the BioAxial CODIM100 beam shaping module into a high performance modular Nikon Eclipse Ti-E confocal fluorescence microscope and its native NIS-Elements imaging software. This turn-key platform combines top-of-the-range super-resolution performance with remarkable ease-of-use thanks to a streamlined workflow. Because of its unprecedented low photoxicity demonstrated jointly with Institut Pasteur (Paris, France) and its outstanding sensitivity, CODIM100 coupled to the Eclipse Ti-E confocal fluorescence microscope is the first super-resolution system specifically designed for live cell imaging applications.

Technology Overview

Conical Diffraction, Beam Shaping and the BioAxial Module

Conical diffraction occurs when light is transmitted along one of the two optical axis of a thin biaxial crystal. As shown in fig.1, a focused beam is diffracted to a slant cone within the crystal which is refracted into a perfectly circular cylinder upon exiting the back face of the crystal.

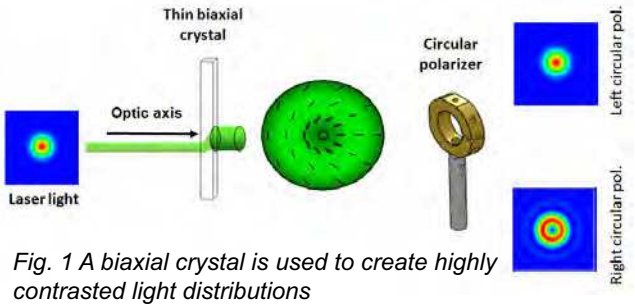

Fig. 1 A biaxial crystal is used to create highly contrasted light distributions

The conically diffracted light can be decomposed on two orthogonal polarization states. By introducing polarizing optics, the two beam components can readily be separated or mixed in different proportions to obtain a family of various and highly contrasted spatial distributions of the emerging beam.

When controlling the input and output polarization states, families of light distributions of the size of an Airy disc can be obtained (fig. 2).

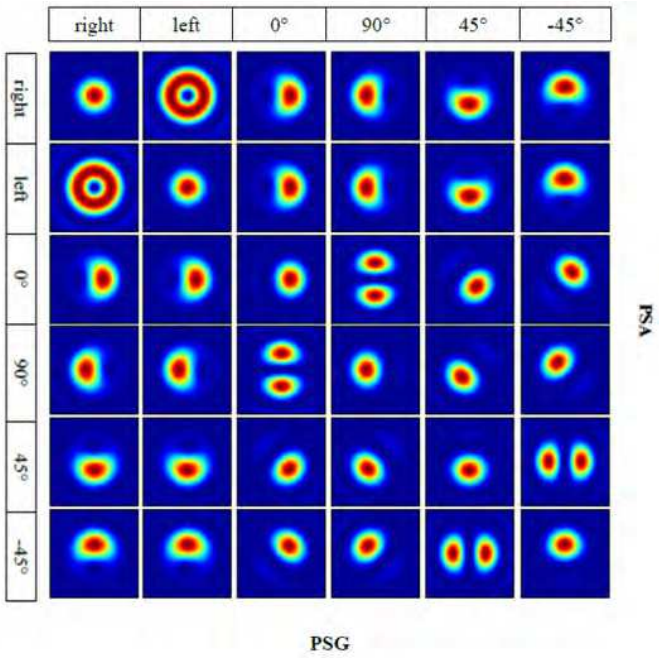

Fig. 2 Family of spatial distributions generated with a biaxial crystal and polarizing optics.

The "half-moons" distributions shown in fig. 3 are used as the excitation source for the fluorescence confocal microscope.

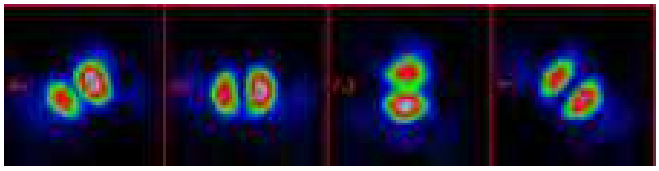

Fig. 3 Half-moons light distributions which are projected on the sample

Thanks to its powerful algorithm CODIM100 generalizes the concept of single emitter localization as shown in fig.4 (left and center) to multiple and denser emitters as shown in fig.4 (right).

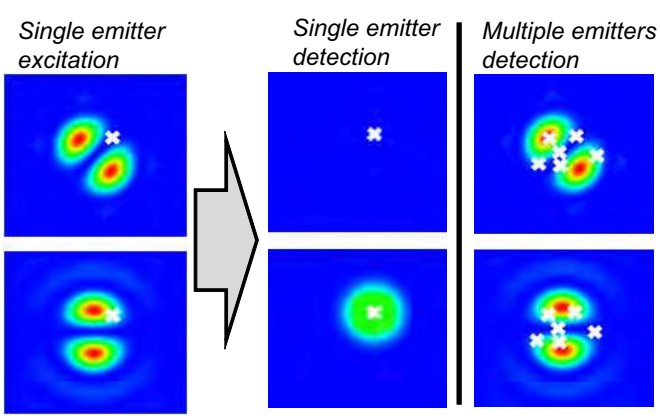

Fig. 4 Single emitter and generalization to multiple emitters

The sample is scanned as usual and up to 4 micro-images per scan point are collected. At the end of the scan, the series of micro-images are processed by the algorithm to reconstruct a super resolved image of the region of interest. Fig.5 shows the optical path in the different parts of the system.

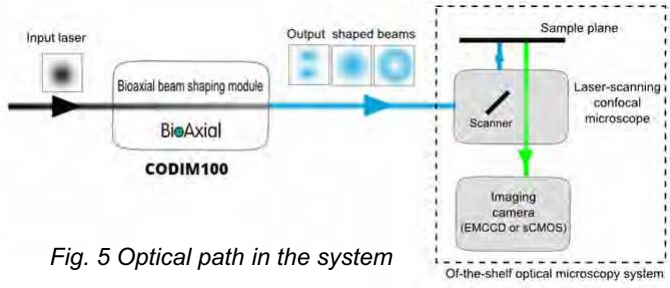

Fig. 5 Optical path in the system

Nikon's Perfect Focus System

When working at sub-diffraction resolution levels, any focus drift can ruin an image acquisition. To prevent this from happening the Nikon Ti-E has been equipped with the unique Perfect Focus System (PFS) which automatically corrects focus drift caused by thermal and mechanical changes as well as reagent addition in real time during prolonged periods of time-lapse imaging. Fig.6 shows the PFS working principle.

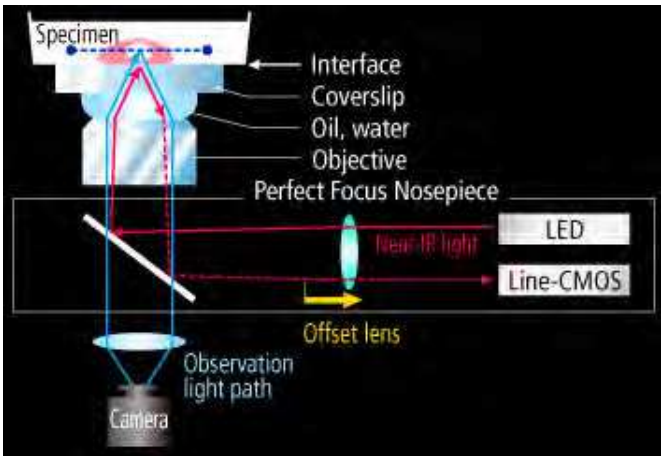

Fig. 6 Principle of the Nikon Perfect Focus System

Major Features and Benefits

Conical Diffraction Microscopy (CODIM) and the CODIM100 super-resolution module coupled to the Nikon Eclipse Ti-E confocal fluorescence microscope is a new technique which brings a series of features and benefits to biologists and microscopy experts, the most important ones being :

- Down to 90 nm resolution at 488 nm
- No need for special fluorophores
  - No change to sample prep facilitating scientists' adoption of the technology
- Low phototoxicity
  - Energy load < 1 µJ/µm² for long time lapse imaging of live samples

The new platform is very easy to use and can readily perform multicolor imaging. The system is inherently linear which allows quantitative image analysis. Its outstanding sensitivity makes it ideally suited for dim samples and genetically encoded fluorescent proteins.

Applications

Live Cell Imaging and Time-Lapse

To demonstrate the low phototoxicity and the very high resolution of the CODIM100, tubulin-GFP transfected glioma U373 cells (sample courtesy of **Dr. Cécile Leduc, Institut Pasteur**) were imaged over a period of 90 minutes. A time-lapse was assembled out of images taken every 6 minutes.

Fig.7 shows the region of interest in a conventional confocal image of the sample. Fig.8 shows a comparison of the region of interest images obtained in confocal microscopy vs CODIM. Fig.9 shows the time-lapse.

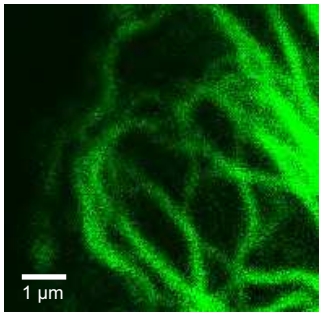

Fig. 7 Conventional confocal image

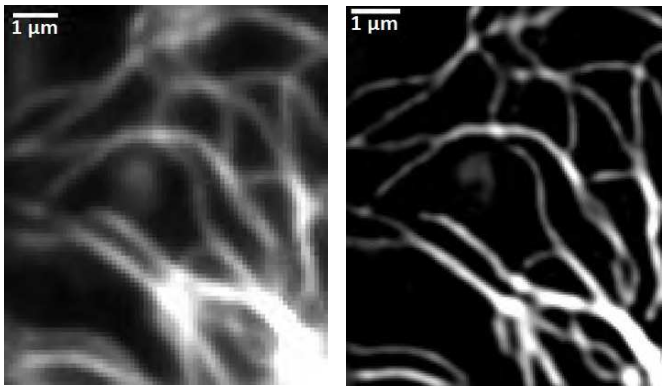

Fig. 8 ROI magnification (left) vs CODIM (right)

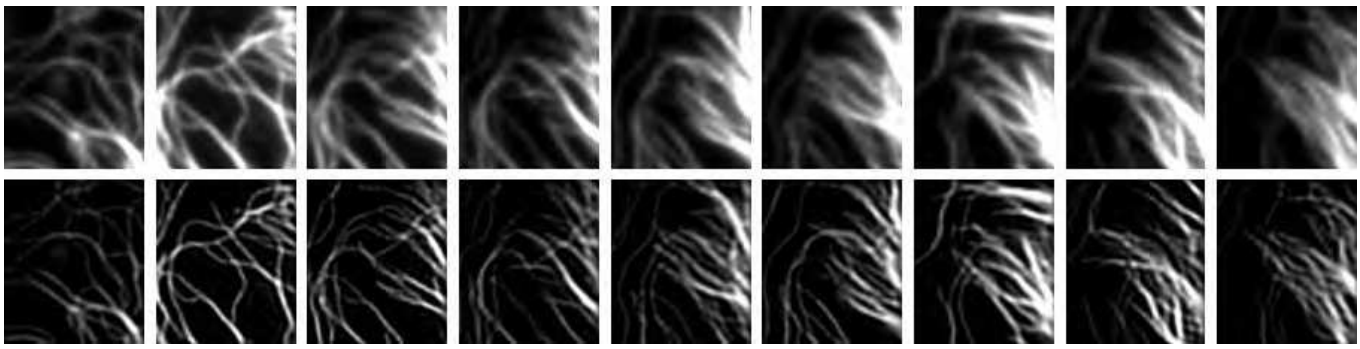

Fig. 9 Time-lapse of tubulin-GFP transfected glioma U373 cells. 90 minutes imaging. Top row is conventional confocal microscopy. Lower row is CODIM.

Correlative Electron-Light Microscopy using CODIM100

HeLa cells were transfected with a GFP-C1 probe. Importantly sample preparation was optimized for electron microscopy (EM) and not for light microscopy (sample courtesy of **Dr. Collinson and Dr. Peddie, Cancer Research UK, London Research Institute**). The expression of GFP-C1 in selected cells was very low to ensure physiological relevance of data. The dimest parts of the sample were imaged using conical diffraction microscopy (CODIM) followed by EM. CODIM imaging was performed with a 0.95NA 40x air objective since immersion objectives are not compatible with EM. Hence high resolution using air objectives was critical in this application.

Fig.10 shows a comparison of the region of interest images obtained in confocal microscopy vs CODIM.

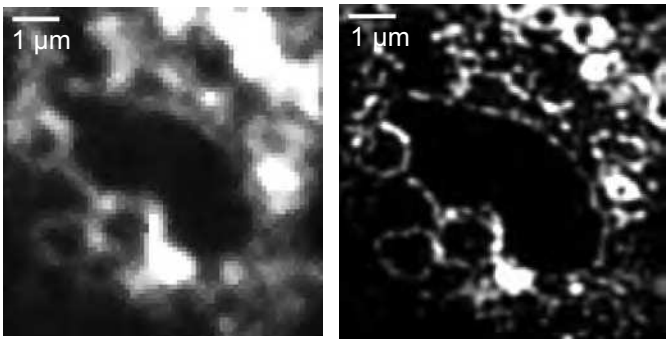

Fig. 10 ROI magnification (left) vs CODIM (right)

As shown in fig.11, the overlay of separate CODIM and EM images from the same ultrathin section improves CLEM protein localization precision.

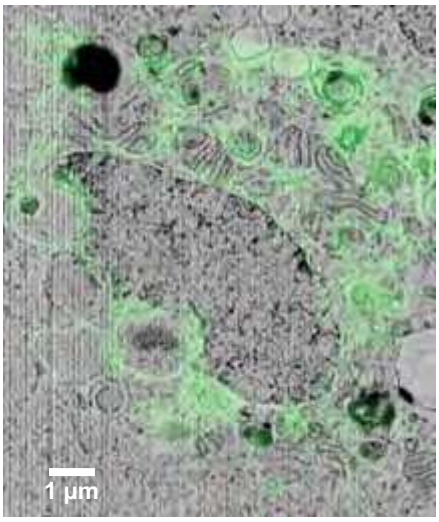

Fig.11 Overlay EM / CODIM

Faint Actin Structures imaged with CODIM100

Actin structures are challenging to image in general and even more in super-resolution because of low signal levels observed. Thanks to its broad dynamic range, CODIM100 was instrumental in visualizing the actin structure of this sample (courtesy of **Dr. Curet and Dr. Mamhudi, Sanofi**) which is home of many key protein interactions.

Fig.12 shows a comparison of the region of interest images obtained in confocal microscopy vs CODIM.

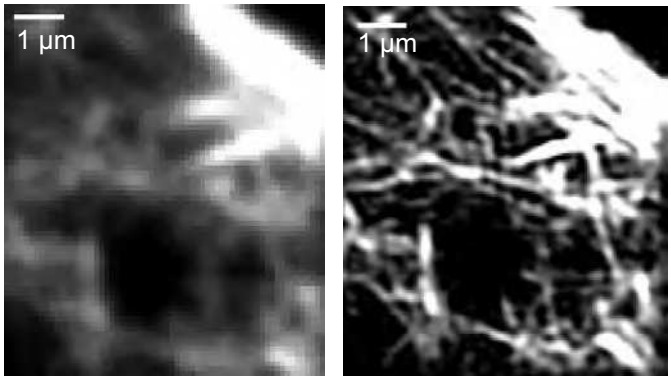

Fig. 12 ROI magnification (left) vs CODIM (right)

Tubulin-Alexa488 in U20S Cells

Fig.13 shows a conventional confocal image of tubulin labeled with Alexa-488 in U20S cells (sample courtesy of **Dr. Gabor Csucs, ETH Zürich**). The red rectangle shows the region of interest which has been magnified in the image shown in fig.14 (left). The image of the same ROI in fig.14 (right) obtained with CODIM100, strikingly demonstrates the gap in resolution by resolving the full network of tubulin which was invisible.

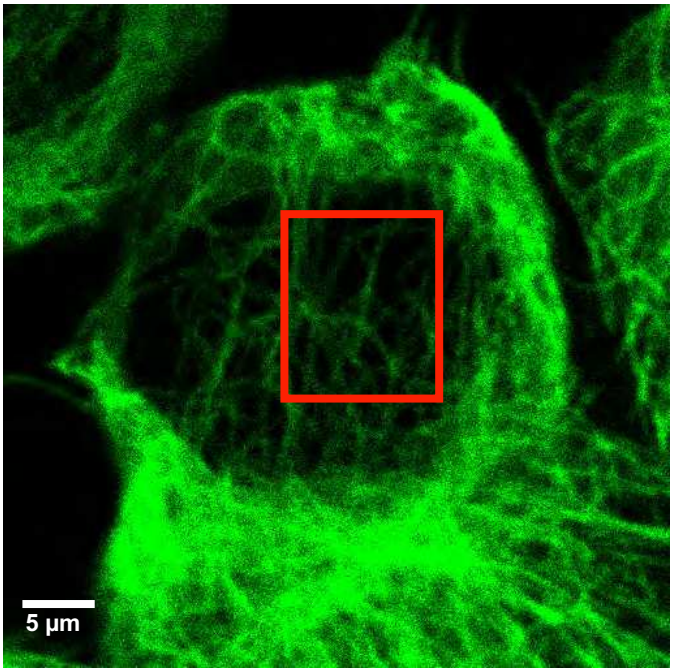

Fig. 13 Conventional confocal image

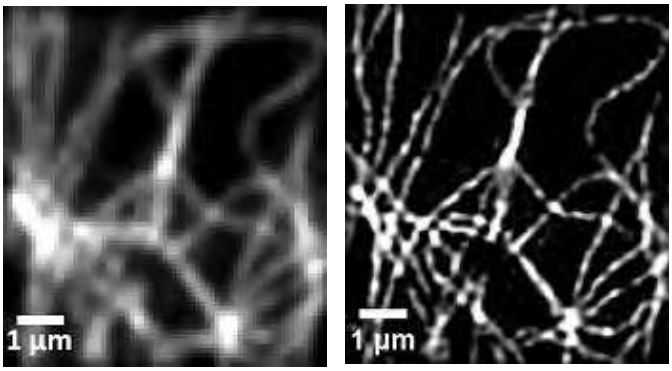

Fig. 14 ROI magnification (left) vs CODIM (right)

Protein-Protein Imaging using CODIM100

FluoCells (ref. F14781, Life Technologies) containing BPAE cells with red-fluorescent Texas Red-X phalloidin for labeling F-actin and green-fluorescent BODIPY FL for labeling microtubules were used in this experiment.

The purpose was to evaluate the performance of CODIM100 in colocalizing proteins of interest better than with conventional confocal microscopy. In this application 488 and 561 nm excitation wavelengths were used.

Fig.15 shows a conventional confocal image of the sample while fig.16 shows a dramatic gain in resolution offered by CODIM100.

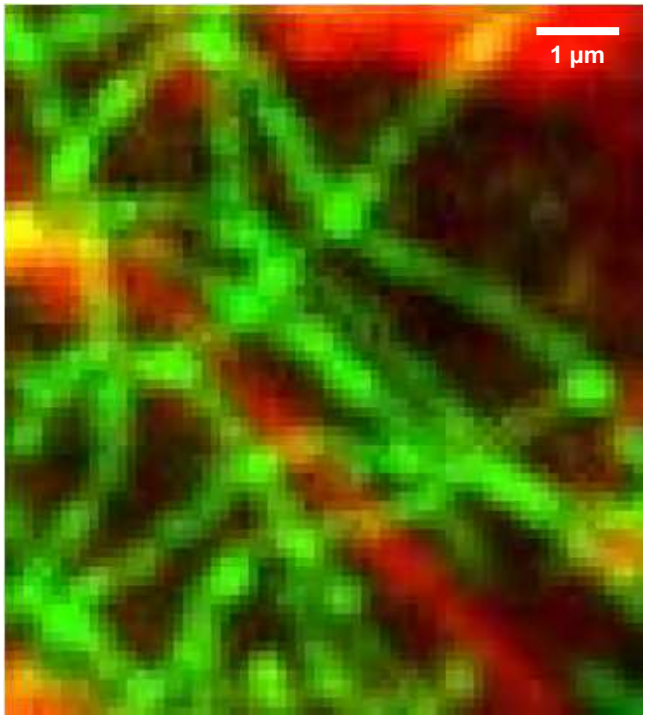

Fig. 15 ROI magnification of conventional confocal image

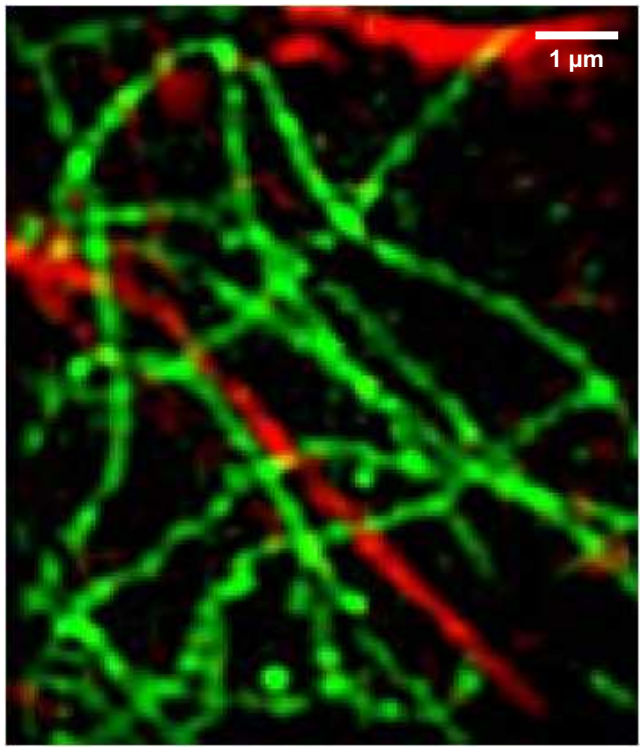

Fig.16 CODIM image of same ROI

For more information about the Nikon-BioAxial super-resolution platform, products and applications, do not hesitate to call your nearest BioAxial or Nikon France representative. You may also want to visit the BioAxial and Nikon websites at www.nikoninstruments.com and www.bioaxial.com

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