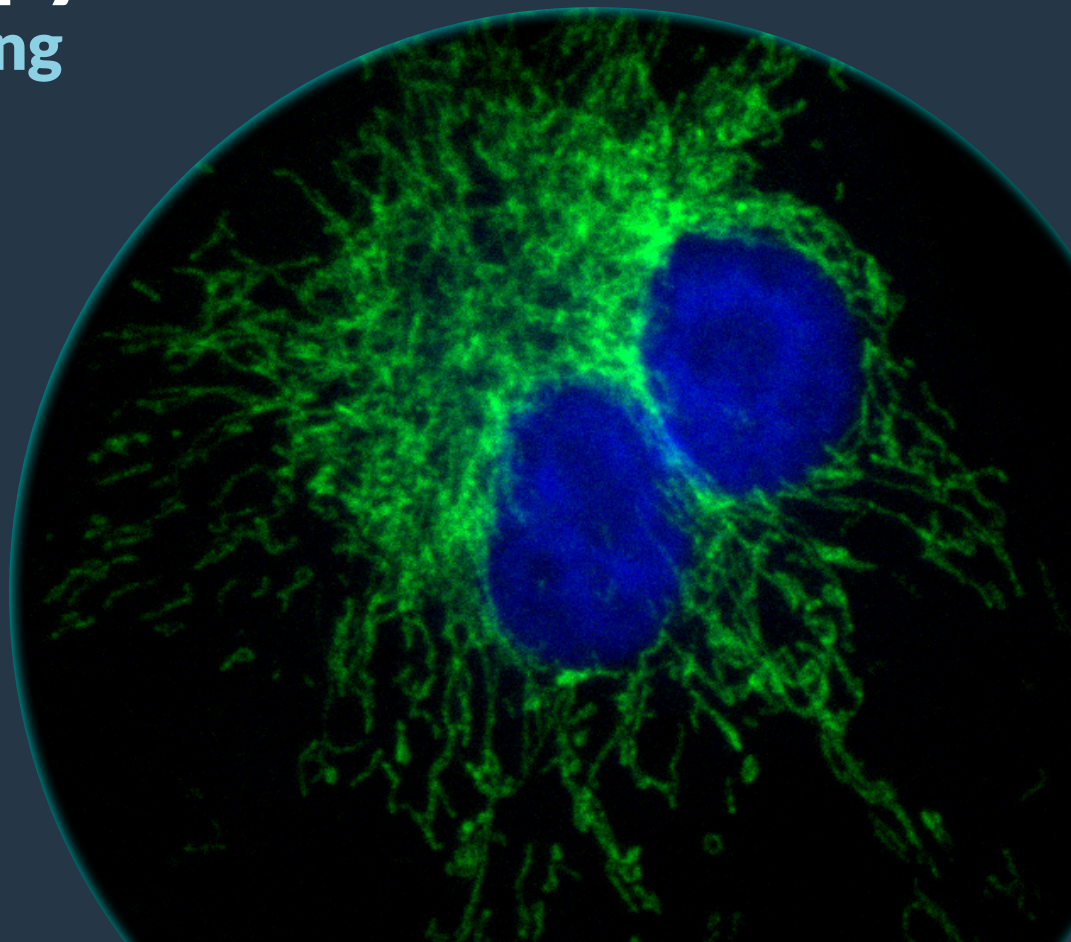
INSCOPER
More than Microscopy Software

Confocal.nl
the Dutch microscopy company

Confocal microscopy

with Inscoper Solution using Confocal.nl technology



TECHNICAL NOTE
#2021-11

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Confocal microscopy with Inscoper Solution using Confocal.nl technology

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Live-cell microscopy is nowadays widely used to image and better understand the fundamental nature of cellular function and organization. The development of diversified microscopy approaches, such as confocal microscopy, allows to image a large spectrum of cellular dynamic events with high spatial and temporal resolution. Here we present a new opportunity for biology researchers to perform confocal experiments on a widefield microscope, using the imaging devices from Confocal.nl coupled with the Inscoper control and image acquisition solution.

CONFOCAL MICROSCOPY FOR LIVE-CELL IMAGING

Live-cell imaging is used by scientists in a large spectrum of research fields, such as developmental biology, neurobiology and cell biology (Jensen, 2013). It is commonly used to improve knowledge of biological function through the study of both cellular and intracellular dynamics. However, some limitations have to be controlled to guarantee the robustness of these experiments. **Photobleaching** and **phototoxicity** are significant problems, altering cell integrity and fluorescent signal detection. To prevent their appearance, it is important to limit as much as possible prolonged exposure time and high-intensity excitation light. **Confocal microscopy** can be used using a fast scanner to perform **live-cell imaging** (Axelrod *et al.*, 1976; Bayguinov *et al.*, 2018). It provides researchers the ability to realize **three-dimensional imaging** of living cells and to **improve the spatial resolution** of their images, increasing the signal-to-noise ratio. Due to the high irradiance of laser beams in confocal microscopy, **a perfect synchronization and control of all devices of the microscope is required** to guarantee the minimal exposure time of the biological samples.

INSCOPER SOLUTION TO OPTIMIZE THE OPERATING OF THE MICROSCOPE

Inscoper is a **full image acquisition solution** for advanced video microscopes with fluorescence used in life science. Incorporating a specially-designed device, the Inscoper Solution provides a new user experience with **improved technical performance,** system integration and easy-to-use ***(Figure 1).***

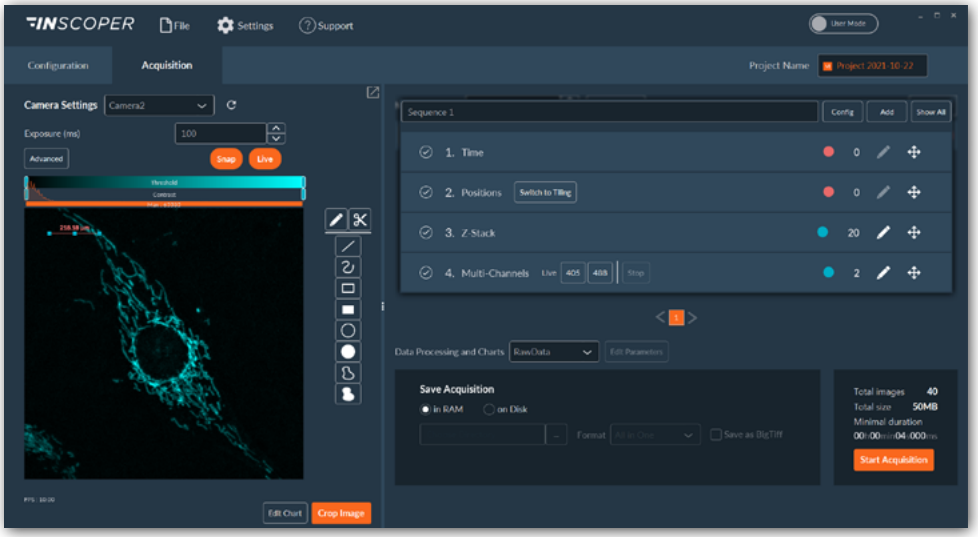

Figure 1: View of the Inscoper software
Multidimensional acquisition sequences are planned using the user-friendly Inscoper software interface. Live preview of the sample could be observed at the left part of the screen. Here, both "Multichannels" and "Z-stack" dimensions are selected.

Many users believe that image acquisition speed is only determined by the camera frame rate and the slow response time of microscopy devices. In fact, latency generated by imaging software is the major factor that reduces the response time of the devices, thus slowing the acquisition. The core of **Inscoper technology eliminates any software latency**, consequently tripling the frame rate compared to conventional approaches for a classical multi-dimensional acquisition (Time, XYZ, Channels).

CONFOCAL.NL PRODUCTS FOR SUPER-RESOLUTION AND FAST SCANNING

Inscoper recently integrated in its solution the two **confocal** systems from Confocal.nl (Amsterdam, Netherland): the NL5 and the RCM2 ***(Figure 2).***
Confocal.nl developed confocal microscopy approaches adapted for live cell imaging using **low laser intensity**. It prevents sample phototoxicity and photobleaching of fluorochromes. These innovative systems allow researchers to easily monitor long-term biological phenomena with **fast acquisition speed, high spatial resolution** without inducing sample alteration.

Figure 2: NL5 and RCM2 products from Confocal.nl controlled with Inscoper Solution
Confocal microscopy with Inscoper Solution using (A) NL5 and (B) RCM2. Both systems are controlled by the Inscoper Solution

NL5 is a camera-based **fast line-scanning confocal system** adapted for 3D live cell imaging with high sensitivity (up to 95% quantum efficiency) and resolution (170 nm after deconvolution, ***Table I***). NL5 can convert an epifluorescence microscope into a fast-scanning confocal. It contains a slit pinhole that increases acquisition speed by scanning lines instead of individual points (25fps). NL5 can decrease the exposure time during acquisition and prevent both phototoxicity and bleaching. Thus, it appeared to be a cost-effective solution that could be coupled to any research microscopy via a c-mount interface.

RCM2 is a camera-based **bidirectional re-scan confocal microscope** (RCM). It is a standard confocal microscope extended with an optical re-scanning unit that projects the image directly on a camera (De Luca *et al.*, 2013). After deconvolution, the resolution is further improved to 120 nm ***(Table I).***

Here, a Nikon Ti2 Eclipse microscope (Nikon, Tokyo, Japan) with a Plan Apo λ 60x 1.4 NA oil immersion objective (MRD01605, Nikon) was used. For these experiments, the camera was a digital CMOS Orca Flash 4.0 (C11440; Hamamatsu Photonics, Hamamatsu, Japan) and the light sources were an Oxxius laser (L4Cc; Oxxius, Lannion, France) for confocal imaging and a light engine from Lumencor (Spectra, Lumencor, Beaverton, USA) for bypass imaging.

	NL5	RCM2
Detector	Camera	Camera
Resolution with deconvolution	170nm	120nm
Sensitivity (% Quantum Efficiency)	Up to 95% QE	Up to 95% QE
Speed	25fps @full frame	2fps @512x512px
View of Field	330×330µm (40x)	220×220µm (40x)
Optimized for…	40-100x	40x (with high NA), 60x, 100x
ByPass mode	Yes (motorized)	Yes

Table I: *Comparison between NL5 and RCM2 system from Confocal.nl (data from www.confocal.nl)*

BIOLOGICAL APPLICATIONS

First, **NL5** was used to image nuclei from HeLa cells labelled with Hoechst. Images from bypass and confocal mode were taken and compared using the same microscope and camera ***(Figure 3)***. Confocal images presented a better x-y axis resolution compared to the bypass mode. For instance, compacted DNA (brighter spots observed in nuclei) exhibits more details with confocal mode of the NL5 with an increase **of the signal-to-noise ratio** (SNR). It is important to note that this result could again be improved using a deconvolution protocol (resolution after deconvolution according confocal.nl is 170 nm).

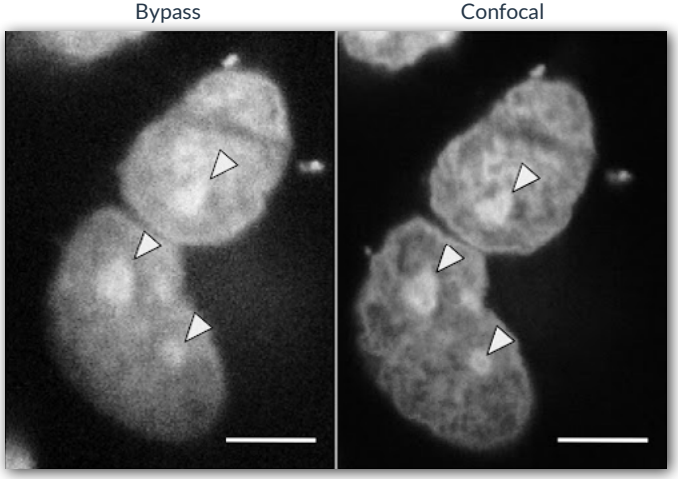

Figure 3: Two-dimensional confocal imaging using NL5 (Confocal.nl) controlled by Inscoper Solution
Fixed HeLa cells labelled with Hoechst (nuclei, gray). White arrows indicate condensed chromatin. Scale bar: 10µm.

NL5 was then used to image mitochondrial networks in fixed HeLa cells that were transfected with a GFP-fused mitochondrial protein and labelled with Hoechst ***(Figure 4)***.

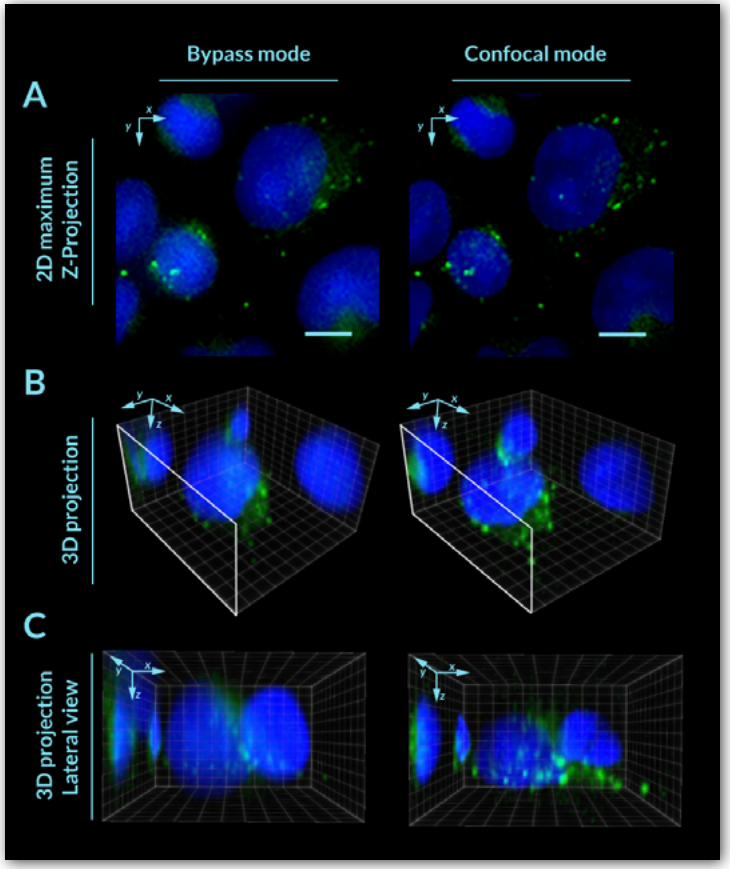

Figure 4: Three-dimensional confocal imaging using NL5 (Confocal.nl) controlled by Inscoper Solution
Imaging of fixed HeLa cells transfected with mitochondria-GFP (mitochondria, green) and labelled with Hoechst (nuclei, blue). Z-stack has been performed under the control of Inscoper Solution. Resulting images are presented using 2D maximum Z-projection (A), 3D projection with top (B) and lateral (C) views using the CleanVolume plugin of ImageJ software. White arrows represent x,y,z axis orientation. Scale bar (2D image): 10µm. Dimensions (3D projections) : 31x33x20µm.

Using the Inscoper user interface, a multidimensional imaging sequence was peformed, composed by a "Multi-channels" acquisition associated with a Z-stack (step: 0.5µm). Once again, better lateral resolution and SNR was observed ***(Figure 4A)***. Three-dimensional (3D) projection could be performed with all Z-stack images using the open-source ImageJ software (https://imageJ.nih.gov/ij/) or others. Here, 3D visualization was realized with the ClearVolume plug-in (https://clearvolume.github.io/) of ImageJ ***(Figure 4B)***.

Lateral view of the 3D projection appeared diffrent between the two modes ***(Figure 4C)***. Indeed, z-axis resolution appeared better using confocal mode compared to bypass. For example, nuclei shape appeared more precise without the blurry diffusion of the signal observed with bypass. Similarly, mitochondrial spots appeared brighter with a higher SNR. Furthermore, NL5 can perform time-lapse acquisition or screen multiwell plates. It could also be coupled with other microscopy techniques that require fast imaging, like FRAP (Fluorescence Recovery After Photobleaching) for example.

In a second time, we observed BPAE cells labelled with MitoTracker and Hoechst (mitochondria and nuclei, respectively) using the **RCM2** system. Inscoper Solution fully controls all the devices from the RCM2, except the Confocal/Bypass mode switch that is not motorized. On this system, this switch needs to be manually done using a handle on the device before starting the acquisition. Acquisition sequence basically starts in bypass mode to select the area of interest in the sample. Then, the user can switch to the RCM mode to perform high-resolution images.

Controlled by the Inscoper Solution, RCM2 succeeded in performing multidimensional images of the mitochondrial network from BPAE cells ***(Figure 5)***.
Moreover, time-lapse could also be realized using RCM2 to explore with a 170 nm resolution (120 nm after deconvolution) dynamic biological processes like mitochondrial network (genesis, fusion/fission/ movements, ...) or cytoskeleton remodeling (microtubules or actin polymerisation/ depolymerisation).

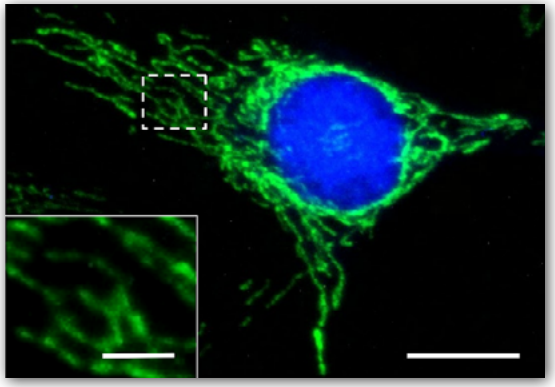

Figure 5: Mitochondrial network imaging using RCM2 (Confocal.nl) controlled by Inscoper Solution
Fixed BPAE cells labelled with MitoTracker (mitochondria, green) and DAPI (nuclei, blue). The area inside the square with dotted line is zoomed in the bottom left corner of the image. No deconvolution protocol has been performed here. Scale bar: 20µm (large image) and 5µm (zoomed image).

SUMMARY

The two systems from Confocal.nl (NL5 and RCM2) have been added to the list of equipment fully controlled by the Inscoper Solution. Both confocal microscopy devices appeared to be perfectly suited for live cell imaging thanks to respectively their low phototoxicity and high sensibility (high SNR). Thus, the Inscoper Solution and Confocal.nl products are compatible with multiple microscope stands. This makes it very universal and allows it to work with basically any fluorescent microscope.

BIBLIOGRAPHY

1. Jensen, E. C. Overview of Live-Cell Imaging: Requirements and Methods Used. *Anat. Rec.* **296**, 1–8 (2013).
2. Axelrod, D., Koppel, D. E., Schlessinger, J., Elson, E. & Webb, W. W. Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophys. J.* **16**, 1055–1069 (1976).
3. Bayguinov, P. O. et al. Modern Laser Scanning Confocal Microscopy. *Curr. Protoc. Cytom.* **85**, 1–17 (2018).
4. De Luca, G. M. R. et al. Re-scan confocal microscopy: scanning twice for better resolution. *Biomed. Opt. Express* **4**, 1–13 (2013).

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