NL5: outstanding deep 3D imaging and long term live cell imaging performance

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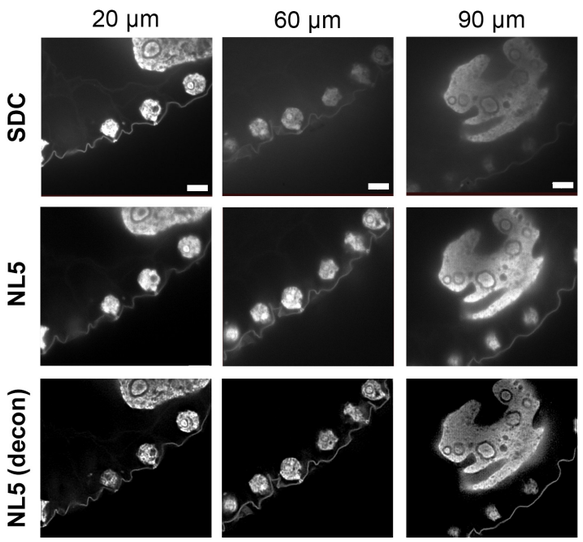
Introduction

The observation in vivo of biological processes is a key requirement in life sciences. 3D live-cell imaging is particularly informative but challenging at the same time.

Spinning Disk Confocal (SDC) microscopy has become very popular for cell biology researchers since it brings good resolution and a high acquisition speed, which allows the study of dynamic biological processes. However, it is limited in the imaging of thick specimens due to pinhole cross-talk. This phenomenon occurs when out-of-focus light goes through the surrounding pinholes limiting the confocality. In addition, dim signals are poorly detected by SDC since only 10% of the light passes through the disk [1]. This means that usually, the laser power needs to be increased to get a better signal to noise ratio (SNR), which is detrimental for long term live cell imaging.

At Confocal.nl we have developed an alternative solution with outstanding performance during deep 3D live cell imaging experiments. **The NL5 confocal system** presents a high acquisition speed (25 fps), with high resolution and contrast and it is live-cell friendly. Altogether this makes NL5 a good alternative to **overcome SDC limitations** and become the best option to study dynamic processes. Here we compare the SDC with the NL5 during deep 3D imaging and show the performance of NL5 during long-term live cell imaging experiments.



	SDC	NL5
Laser power (mW)	1.5	0.3
Light dose (µW/µm²)	9.5×10^{-3}	0.8×10^{-3}

Table 1. Laser power and light dose measured at sample plane with SDC and NL5.

Materials & Methods

For the acquisition of the Volvox sample a Nikon TiE microscope was used with CFI Plan Apo Lambda 100X 1.45NA oil objective. SDC imaging was performed with a well-known Spinning disc unit. For both datasets a Hamamatsu sCMOS camera was used. Autofluorescence was excited using a 561 nm laser. The z-interval was set to 200 nm.

To measure the laser power a Thorlabs S170C sensor was used at the sample plane.

The zebrafish dataset was captured using an Olympus IX83 microscope with 10x 0.45NA X-Line air objective. GFP was excited using a 488 nm laser. 540 micrometer Z-stacks with 5 micrometer intervals were captured every 10 minutes during a 24-hour time lapse.

"NL5 captured better SNR and less blur images than SDC when imaging thick specimens"

Results

NL5 showed better performance than SDC during deep 3D imaging experiments

A Volvox algae was used to acquire confocal sections of the 3D fixed specimen taking advantage of its autofluorescence. NL5 and SDC systems were both coupled to the same microscope and camera. SDC showed confocal resolution and good SNR in the sections close to the coverslip (1 to 20 um)(Fig. 1). However, the following sections presented more blur, poor SNR and less resolution. This effect was even more evident in the deepest sections of the stack, at 90 um (Fig. 1). On the other hand, NL5 presented similar resolution and SNR to SDC in the sections closer to the coverslip but it was superior in the deeper sections (from 20 to 90), showing higher SNR and less blur (Fig. 1).

Figure 1. SDC and NL5 images of a Volvox.
First and second panels show a comparison of the same Volvox confocal plane acquired with SDC and NL5. The third panel shows NL5 images after deconvolution.
Grey color: Volvox autofluorescence. Scale bar: 5 µm

Deconvolution was applied to further improve resolution using Microvolution and the PSF developed for NL5 (Fig. 1). Due to the high contrast images deconvolution was optimal, reaching 170 nm lateral resolution.

NL5 requires less laser power than SDC for a superior SNR images

High laser intensity is harmful for live cells, it generates phototoxicity and alters the behaviour of the live specimen, leading to less biologically relevant results. In addition, if a high laser intensity is needed in order to achieve an adequate SNR, the fluorophores can be quickly bleached. SDC is known to use less laser intensity than standard point-scanning confocals but long-term live cell imaging experiments can still be compromised when using SDC. Here, we measured the laser power and light dose used during the acquisition of the 3D Volvox image (Table 1). The Volvox sample imaged with NL5 had 16 times less light dose when compared to SDC. This difference in laser intensity at the sample plane will improve the results of live cell imaging experiments.

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NL5 requires less laser power than SDC for a superior SNR images

In order to demonstrate the capabilities of NL5 for long-term live cell imaging, a zebrafish embryo was imaged for 24 hours. Endothelial cells were labelled with endogenous GFP which allowed visualization of vessel formation in the embryo. All along the specimen vessel sprouting occurred as expected (Fig. 2). Focusing on the brain, we were able to observe the formation of functional vessels and how individual cells divided, moved and interacted with each other along full time lapse. In conclusion, no signs of phototoxicity were detected and the SNR was good along the full experiment, not showing any bleaching.

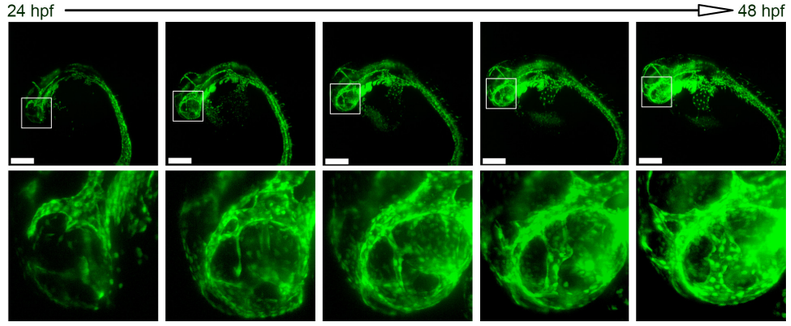

Figure 3. 24h hours live-cell imaging of a zebrafish embryo using NL5.
From left to right, 5 representative images of the vasculature formation in the embryo along the 24h (~6 hours between each image). White squares indicate the zoom in area shown in the panel below.
Green color: Endogenous GFP labelling endothelial cells. Scale bar: 200 µm

Conclusions

These experiments revealed a superior performance of NL5 when compared to SDC during deep 3D imaging. NL5 captured better SNR images with less blur than SDC, when going deeper than 20um in the sample. The high contrast images captured with NL5 were optimal for deconvolution, increasing the lateral resolution of images to 170 nm.

In addition, NL5 used less laser excitation power than SDC to obtain a better SNR of the same sample. This represents a great advantage for live-cell imaging experiments, as it was also proved by the zebrafish experiment. A zebrafish embryo (24 hpf) was kept alive under the microscope for 24 hours, while taking a total of 18000 images. The embryo development was not altered and no signs of phototoxicity or bleaching were observed. This demonstrates that NL5 is a good system for long-term imaging experiments with live samples.

References
[1] http://zeiss-campus.magnet.fsu.edu/articles/spinningdisk/introduction.html