ECLIPSE Ti2

New inverted research microscope

Increasing data collection and fidelity by maximizing field of view

White, A. (2018) Nature Methods – Application Note.

With the release of the Nikon Ti2 inverted microscope, the world's first 25mm FOV became available. Now, Nikon has taken advantage of this improvement by building the largest FOV point scanner in the world, the A1 HD25®.

Recently there has been a general push to increase the throughput of microscopy to produce more data with less time in front of the system. For confocal fluorescence microscopy, much of this innovation has centered on making systems faster and more sensitive. However, because fluorescent samples have a limited photon budget, these approaches reach a practical limit as too much laser power is applied and/or not enough signal is generated.

Recognizing these limits, Nikon has worked to go beyond its market-leading speed, sensitivity, and image quality in targeting an additional approach to throughput: making a bigger picture. New, larger optical components to increase the standard confocal microscope system FOV to an incredible 25 mm FOV. The Nikon A1 HD25 sets a new standard in confocal microscopy.

Increased field of view substantially increases cell counts in a single image

(a) **18 mm field of view**
(20x objective)

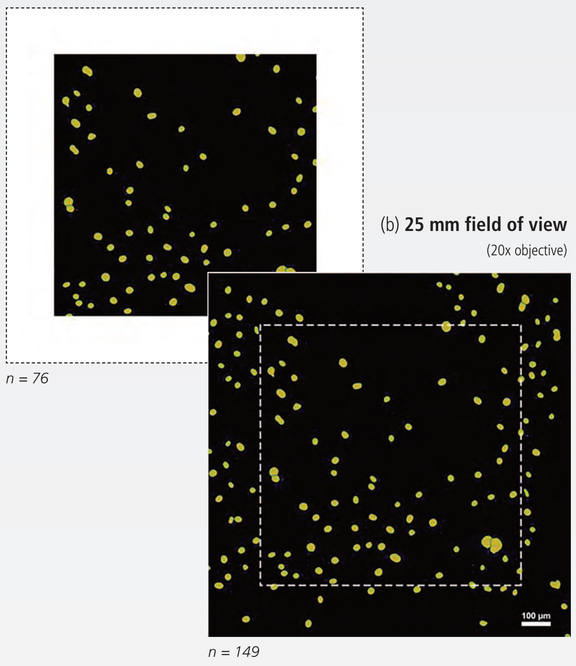

Fig. 1 Cell counts increase approximately twofold with use of a 25 mm FOV versus an 18 mm FOV. a,b, Images collected from a representative field of cells with an 18 mm FOV (a) and a 25 mm FOV (b). Cell counts are presented for each condition and show n = 76 at 18 mm and n = 149 at 25 mm.

Figure 1 shows that the improvement in FOV can lead to the collection of nearly twice the data in every single image with the potential to cut experiment times in half by requiring the collection of fewer images in order to achieve the same n. Higher counts provide better statistics over whole experiments. There may be a study in which a subtle change has occurred under a particular condition but the results of statistical analysis are inconclusive. In this situation, increasing the population size n might generate enough confidence for a conclusion to be drawn.

Figure 2 shows the cumulative effect of increased FOV on cell count during an experiment in which 47 images were collected at both 18 and 25 mm. The data shows that the cumulative cell count over these 47 images increases much more rapidly with the 25 mm FOV (Fig. 2a). In this case, average intensity measurements were collected from a subset of these images (Fig. 2b). Collecting more data simply by virtue of having a larger FOV means that in the same number of images, the much larger n produced leads to less statistical error (represented by error bars in Fig. 2b). In other words, capturing data with a large FOV drives a more rapid decrease in relative uncertainty as the total image count increases. Lower uncertainty means that better conclusions can be drawn when the experimental results are collated. A1 HD25 is about using the available tools and technology to produce better data, and more of it, resulting in more impactful research.

Integrated cell counts: 18 mm vs 25 mm FOV sampling

Average intensity after 3 images

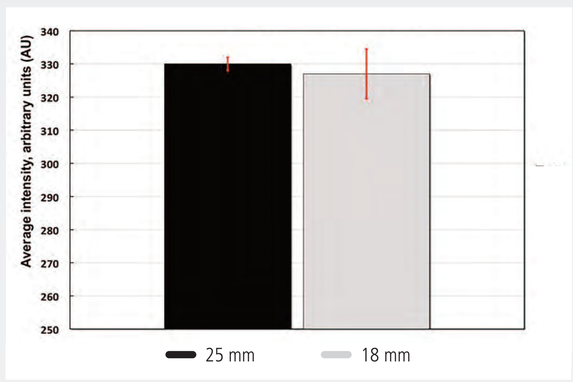

Fig. 2a Increasing FOV provides better statistics by increasing overall cell counts in a given experiment. This figure shows cumulative cell counts from 47 images collected with both a 25 mm and an 18 mm FOV. Summed data for each condition. **Fig. 2b**, A representative average intensity measurement from a subset of three images, demonstrating the smaller error generated at the higher n value. AU, arbitrary units

Faster and better image stitching

Large image stitching is another common application for confocal microscopes. With A1 HD25, not only does the large FOV allow for more rapid generation of stitched images, but the complete optical redesign means that these images are created with fewer artifacts (example shown in Fig. 3).

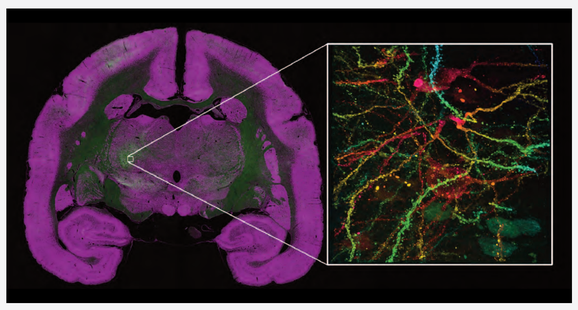

Fig. 3 Stitched overview image of marmoset brain captured with CFI Plan Apochromat Lambda 10X objective and detailed image of dendritic spines captured with CFI SR HP Plan Apochromat Lambda S 100XC Sil objective.

Summary

Many applications can benefit from Nikon's 25 mm FOV. The same improvements described above can be applied from wide field to confocal high content screening. This will result in the highest throughput system of its kind by maximizing data collection in every image. In the case of large model organisms such as zebrafish embryos, one is able to image the whole organism in a single FOV, and at a higher magnification than was previously achievable. Getting the whole picture allows the user to capture details at higher magnification and higher resolution than previously possible with a given size FOV.

With the world's first 25 mm FOV, the Nikon A1 HD25 is the latest in point scanning confocal technology. The data and examples described above have shown how this simple yet important optical change can allow researchers to optimize time spent in front of the microscope. The A1 HD25 means more and better data in every image, every day.

Hardware triggering: maximizing speed and efficiency for live cell imaging

Davis, M. A. (2017) Nature Methods – Application Note.

Live cell imaging experiments now require higher speeds and more data throughput than ever before. Nikon has robust tools that enable hardware triggering. This minimizes delays, synchronizes devices, and reduces the exposure of specimens to light.

A microscope imaging system is a combination of several hardware devices working together. These devices may include XY motorized stages, Z focus drives, piezos for fast XY or Z drive, filter wheels, light sources (both epifluorescence and transmitted), and detectors. Software can control the movement of these devices. However, the asynchronous nature of many devices with different native speeds means software must mediate device movement and image capture through a series of commands and callbacks. Latencies occur as a result of the interaction - the effects on the total acquisition time for large data experiments can be extremely negative. The timing clock on the host computer ultimately dictates performance. Thus, other processes being managed on the same computer can easily affect high precision timing.

Bypassing software latencies through direct hardware triggering in NIS-Elements

Most of today's high-performance detectors and peripherals have input and output ports (I/O) for direct voltage-mediated control of device mechanics. However, commercial software packages generally use serial communication, which relies solely on the PC clock and software control.

Nikon's NIS-Elements software has been utilizing I/O connections for quite some time, and continues to expand capabilities. This software provides hardware triggering functionality by using these direct I/O voltage-mediated connections to quickly drive devices. Synchronization of device control with acquisition is paramount, so sensing of the detector's status (exposing or not exposing), as well as keeping count of frame number, is crucial. All of these signals are managed through digital acquisition interfaces (DAQs) for flexibility and convenience. NIS-Elements supports National Instruments NIDAQmx series devices (totaling more than 40 options) for this purpose, greatly reducing latency.

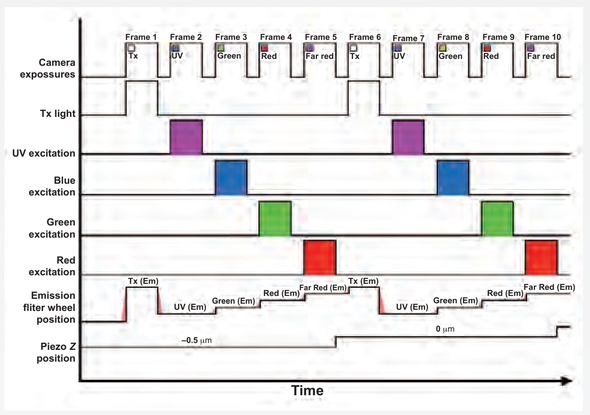

Fig. 4 Illustration of NIS-Elements GUI for NIDAQ control. This directly shows best experimental sequence

How NIS-Elements performs hardware triggering

With the detector exposure signal being observed by the NIDAQ and with peripheral hardware devices also connected, hardware triggering becomes nearly transparent. One defines multidimensional experiment parameters, and NIS-Elements sets up the sequence on the NIDAQ card. Once the detector starts, the NIDAQ moves devices between exposures and controls illumination during exposures. NIDAQ allows for precise illumination control, reducing the duration of illumination, keeping this time to an absolute minimum. With LED or laser sources, on/off times are in the range of tens of microseconds. Hardware triggering also executes multiple-device control efficiently by starting all of the devices simultaneously, improving the overall speed. NIS-Elements can even place device movements during the readout time of detectors running in overlapped ('live') acquisition modes, in which the detector is always running.

Ti2-E: a hardware-triggered microscope stand

The Ti2 offers the first stand in which all motorized components on the stand can take advantage of hardware triggering or latency reduction. Key capabilities of the Ti2-E include native direct connection capabilities without NIDAQ to detectors, allowing for several impressive features, including very fast autofocus routines. Inclusion of a NIDAQ initiates the microscope hardware by direct hardware triggering as well, therefore allowing customized triggered configurations to include both microscope hardware and ancillary devices from several vendors. For example, transmitted and epifluorescence light sources, dichroic mirrors, piezo Z drives, and emission filters may all be controlled together.

NIS-Elements is capable of being used in stimulation/activation experiments, high-content acquisition experiments, and custom experiment interfaces such as illumination sequencing. Users have complete flexibility over triggered device controls, experiment order, and patterns. Device configuration for hardware triggering is easy through NIS-Elements with the Ti2-E triggered microscope stand: predefined connectors and cables for XYZ devices, fluorescent LEDs, transmitted light LEDs, filter wheels, laser units and photostimulation devices are easily accessible.

Summary

Nikon's NIS-Elements hardware triggering is a robust and effective measure to minimize the exposure of samples to light, reduce or remove latencies between exposures, and greatly decrease the overall experiment duration.

Table 1 | Comparison of experiment durations with typical hardware and experiment parameters

	Experiment duration	
Experiment	Software triggering	Hardware triggering
3 , 6 Z positions	40 s	18 s
96 stage positions, 3 , 10 Z positions	12 min 15 s	6 min 21 s
Time lapse: 10 loops, 3 , 15 Z positions	1 min 38 s	49 s
Time lapse: 10 loops, 3 , 10 Z positions[a]	1 min 13 s	10.7 s

Hardware triggering significantly improves the overall experiment time. In the examples from table 1, the camera exposure time was longer than the camera readout time. The last row presents a comparison for which the camera exposure time was the same as its readout time, and the camera was able to run in overlapped (100% duty cycle) mode.