Increasing data collection and fidelity by maximizing confocal field of view

For years, the field of view (FOV) of confocal systems has been limited by the FOV of the microscope they are attached to. With the release of the Nikon Ti2 inverted microscope, the world's first 25-mm FOV became available. Now, Nikon has taken advantage of this improvement by building the largest FOV point scanner in the world, the A1 HD25®. This Application Note focuses on the impact of this technology on simple, everyday experiments.

Microscope-based image creation is an important research tool that has continually evolved since the 1600s. Today's systems utilize a variety of technologies, such as high-end cameras, LEDs, lasers, and confocal point scanners, with the goal of acquiring better data. As part of this, recently there has been a push to increase the throughput of these systems so that more data can be produced 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. Utilizing the astounding optical quality of Nikon glass, the company has created new, larger optical components to increase the standard confocal microscope system FOV to an incredible 25 mm. This Application Note focuses on the effects of this increased FOV on everyday research and shows why the Nikon A1 HD25 is the new standard in confocal microscopy.

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

One of the most common types of microscope imaging experiment is a simple cell-count assay. Such assays are performed in a variety of ways, and are used to investigate questions pertaining to cell or tissue growth/death, the effects of drug treatments, and the consequences of environmental/applied stresses. When the desired model for such an experiment is best suited for analysis with a microscope, a researcher must create samples for all conditions and collect images from each. Image analysis is then required to count cells for each condition. In most cases, in this type of experiment a researcher has to sit in front of a microscope and capture enough images for each condition to produce a high enough cell count (*n*) to address a given question. So, how does FOV affect this common microscope system use case?

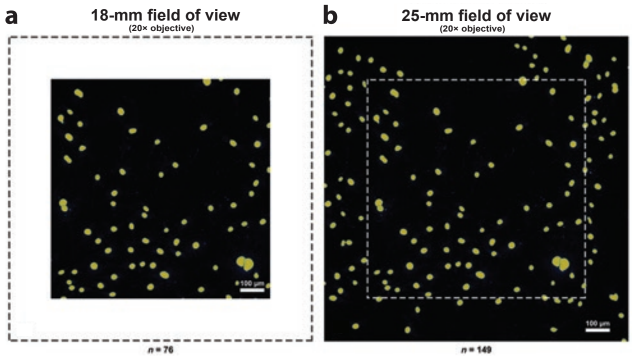

Fig. 1 | Cell counts increase approximately twofold with use of a 25-mm FOV versus an 18-mm FOV. a,b, Images collected from a representative field of cells with an 18-mm FOV **a** and a 25-mm FOV **b**. Cell counts are presented for each condition and show $n = 76$ at 18 mm and $n = 149$ at 25 mm.

Figure 1 shows a representative comparison of data produced when both a 25-mm FOV and an 18-mm FOV are used to count nuclei in a single field. With the 18-mm FOV, 76 cells were counted in the frame shown in Fig. 1a. Simply increasing the FOV to 25 mm increased this count to 149 cells when the same region was imaged (Fig. 1b). Immediately, one can see that this small improvement in FOV can lead to the collection of nearly twice the data in every single image. This has the potential to cut experiment times in half by requiring the collection of fewer images in order to achieve the same *n*. Say, for this example, that an *n* of 1,500 cells is desired for each condition. With the 25-mm FOV this can be achieved with ~10 images, whereas at 18 mm about 20 images are needed for every condition to achieve the same counts.

Adam White

Nikon Instruments Inc., Melville, NY, USA. e-mail: adam.white@nikon.com

Increased field of view provides higher cell counts and better statistics over whole experiments

Of course, saving time might not always be the goal when data are being generated for a confocal microscopy experiment. Consider, for example, 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. First, the obvious conclusion based on the data presented above is 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 as well (Fig. 2b). The less obvious, though equally important, conclusion is that 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.

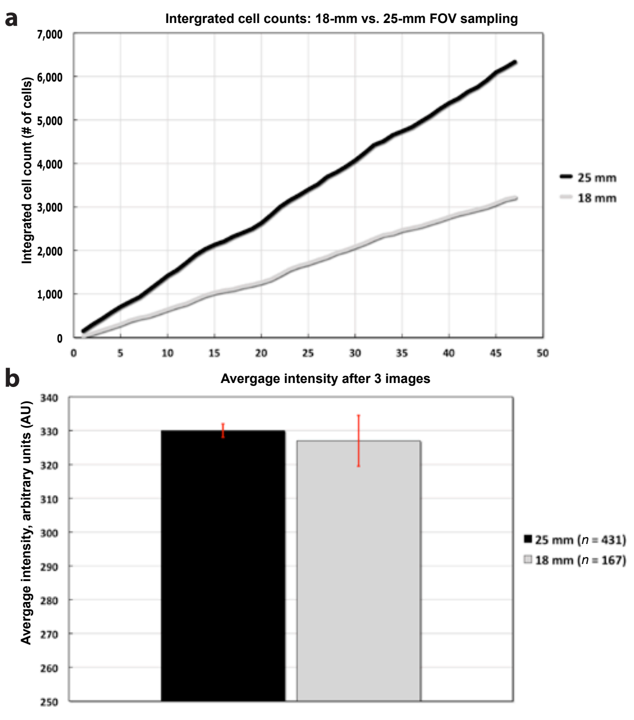

Fig. 2 | Increasing FOV provides better statistics by increasing overall cell counts in a given experiment. This figure shows cumulative cell counts from 47 images collected with both a 25-mm and an 18-mm FOV. **a**, Summed data for each condition. **b**, A representative average intensity measurement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

Conclusion

Importantly, although they are not covered in this note, the Nikon A1 HD25 can benefit many applications beyond cell counting. For example, the same improvements described above can be applied 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 may be 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 resolution than previously possible with a given-size FOV. 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 | Whole mouse embryo captured at 10× magnification and stitched using A1R HD25.

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.