A1R HD25: the latest in resonant scanning technology allows new live-cell imaging approaches

Traditionally, point-scanning confocal microscopes have used galvanometric (galvo) mirrors to generate high-contrast images of a variety of samples. While highly effective for routine imaging, this approach is not well suited for live-cell imaging. This Application Note focuses on the new Nikon A1R HD25 resonant scanner and its impact on live-cell applications.

Because of their inherent flexibility, point-scanning confocal systems have become workhorse instruments in labs worldwide. This flexibility comes from the use of galvo mirrors for laser excitation. Galvo mirrors can be driven variably on the basis of applied waveform, which gives researchers the ability to change optical zoom (and other parameters) on the fly, and means that image resolution can be optimized for just about any objective or application. This is not possible on a spinning disc or other wide-field system that uses cameras with a fixed pixel size. Typically, point-scanners are slower than camera-based systems. However, resonant scanning breaks the mold, maintaining the flexibility of a point-scanner while increasing overall frame rates.

Resonant scanning mirrors are driven by a different waveform than galvo mirrors, which require a linear sawtooth wave. A resonant scanner uses a high-frequency (~7.8 kHz for the A1R HD25) sinusoidal wave, which reduces stress on the motors and allows them to indefinitely function at a very high speed. For A1R HD25, this translates into speeds up to 30 frames per second (FPS) across the entire industry-leading 25-mm field of view (FOV). This Application Note focuses on the unique technological achievements of the Nikon A1R HD25 resonant scanner and how they enable live imaging.

Reduction of photobleaching and phototoxicity with resonant scanning

As stated, the key goal in the development of resonant scanning confocal systems was to improve scanning speed. A side benefit is a well-described reduction in the potential for damage from laser irradiation. Keeping this in mind, consider a sample with dynamics that can be adequately addressed by the collection of a volume of data every 30 minutes. In this situation, one can choose to use either a galvo or a resonant scanner for imaging. Figure 1 shows exactly this comparison: a 100-image *z* stack was collected every 30 minutes for 15 hours with the Nikon resonant (Fig. 1a–c) and galvo (Fig. 1d–f) scanners. These data show that resonant scanning resulted in images of the same quality, with less photobleaching and better sample viability over the 15-hour experiment.

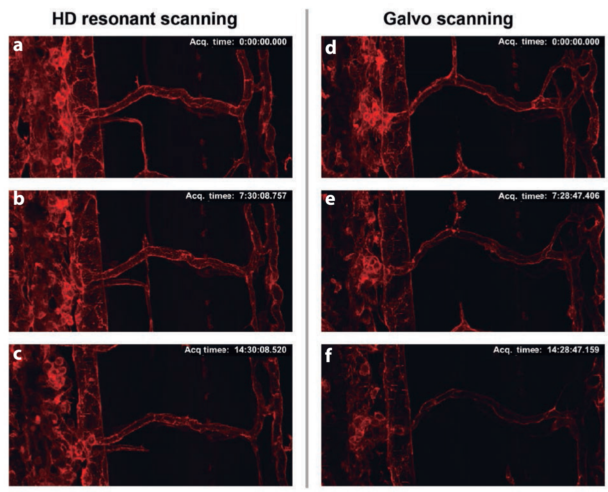

Figure 1 | Comparison of photobleaching with galvo and resonant scanners. **a**–**f**, 3D time-lapse images of trunk vasculature in a zebrafish larva expressing LIFEACT–mCherry in endothelial cells were acquired every 30 minutes over 15 hours with a galvo (**d**–**f**) and a resonant (**a**–**c**) scanner. Note that photobleaching was dramatically suppressed with the resonant scanner. Acq., acquisition. Images courtesy of S. Yuge and S. Fukuhara, Department of Molecular Pathophysiology, Institute of Advanced Medical Sciences, Nippon Medical School.

Traditionally, there has been a trade-off to choosing the resonant scanner in this kind of experiment: a reduction in signal-to-noise ratio (S/N) and image quality. Figures 1 and 2 demonstrate that with Nikon's HD resonant scanner, it is possible to take advantage of the benefits of resonant scanning without sacrificing image quality. The development of the A1R HD required a complete overhaul of the resonant scanner electronics. Part of what defines the resolution of a resonant scanner is how quickly the electronics can sample the image and create pixels. Higher sampling rates mean more pixels can be created in the same

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amount of time, and therefore the electronics can generate better resolution. Nikon increased sampling rates in the HD scanner, thus affording it the capacity to capture much sharper images at a maximum resolution of 1,024 × 1,024. The second major focus was improving the quality of the electronics to reduce their noise contribution in the final image. The reason for this is twofold: noise reduction not only improves the overall quality of the images but, with shorter pixel dwell times and potentially less signal, also is an effective strategy for maximizing S/N.

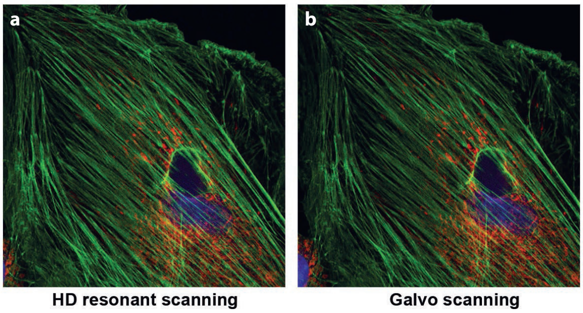

Figure 2 | Comparison of image quality. **a**,**b**, Examples of the image quality achievable with the A1R HD25 resonant scanner (**a**) and a galvo scanner (**b**). Image quality is maintained with the resonant scanner. Both panels show the same area of a fluorescently stained endothelial cell from bovine pulmonary artery.

Using the 25-mm A1R HD25 resonant scanner to capture high-resolution images of large live samples

An underappreciated aspect of several other approaches to high-speed and resonant scanning confocal systems is a substantial reduction in FOV. The reasons behind this stem from approaches to correcting for (or avoiding) the sinusoidal drive wave necessary for the resonant scanner to function. Nikon's approach has always been to maintain the same scan area for both resonant and galvo imaging. This is achieved through the use of a unique hardware-based pixel clock correction that physically tracks the movement of the mirrors. Instantaneous tracking of mirror position and velocity allows for the application of the corrections necessary for its industry-leading 25-mm FOV, 2 times the area of the previous A1R and more than 13 times that of some competitive technologies. Figure 3 shows an experiment in which the 25-mm FOV is a key advantage. In this dataset, 1,024 × 256 images were captured of an entire larval zebrafish at 30 FPS. Blood flow was then tracked with NIS-Elements software. Because high spatial and temporal resolution are both required for cell tracking in this context, it is only with the combination of the unmatched 25-mm FOV and resonant scan quality of the A1R HD25 that this experiment is possible.

Conclusion

With the A1R HD25, Nikon Instruments has focused on making meaningful enhancements to the flexibility of point scanners that can benefit all users. The HD resonant scanner allows researchers to choose resonant mode in experiments where previous versions of this technology might not have been compatible, as well as take advantage of the reduced phototoxicity. It is important to note that improved time resolution can also have a considerable effect on throughput for many imaging applications, especially when combined with the 25-mm FOV. The A1R HD25 offers users an instrument with unmatched flexibility that generates more high-quality data in less time than any other confocal system.

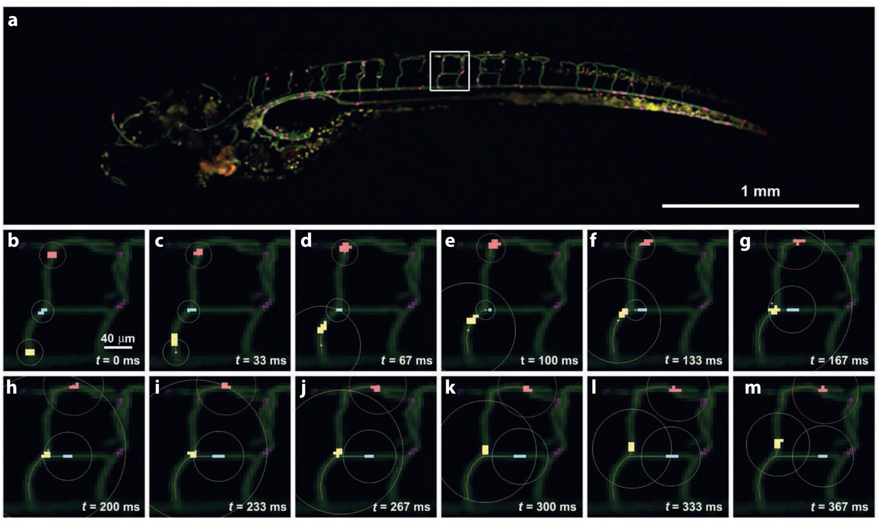

Figure 3 | Still images from 2D time-lapse imaging of blood flow in a whole zebrafish larva imaged with the A1R HD25. Images were acquired with a 4× objective and captured at 30 FPS with 2× averaging and a 1,024 × 256 scan size. **a**, A single frame from the time-lapse series showing a larva spanning the 25-mm FOV. **b**–**m**, Consecutive time points from a 12-frame section of the time-lapse series, acquired within the area outlined by the white box in **a**, showing tracking data for single blood cells. A galvo scanner would have had insufficient temporal resolution to accurately resolve these tracks in an otherwise similar setup. Tracking data were generated with NIS-Elements 2D tracking functionality. Sample courtesy of M. Marvin, Williams College.