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Rapid, high-throughput cell counting with the Vireo multi-camera array microscope

IN COLLABORATION WITH DR. PADMA RAJAGOPALAN AND LAB, VIRGINIA TECH

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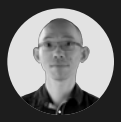

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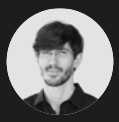

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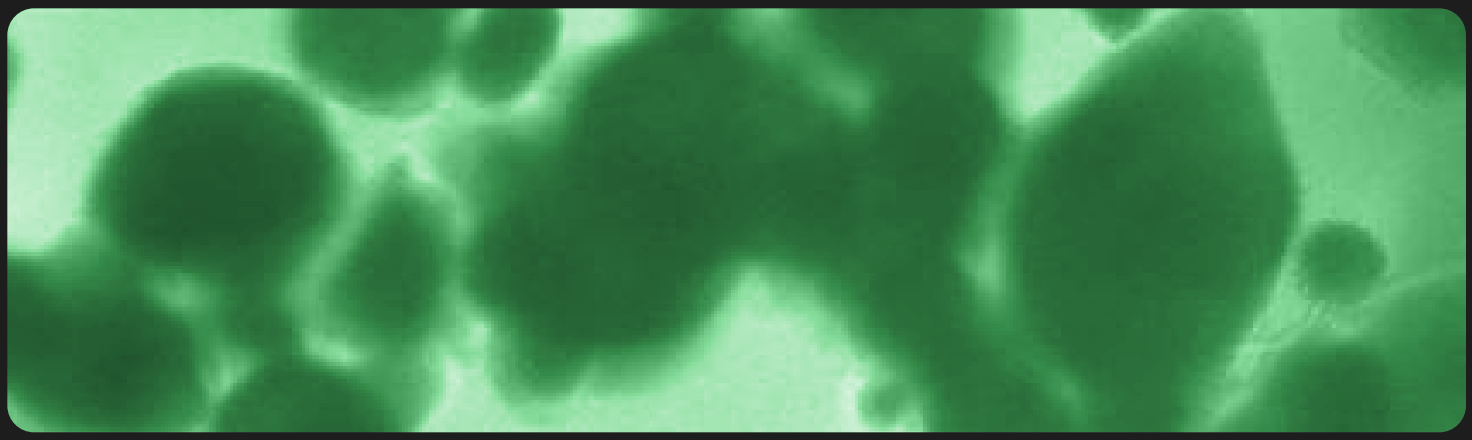


Abstract

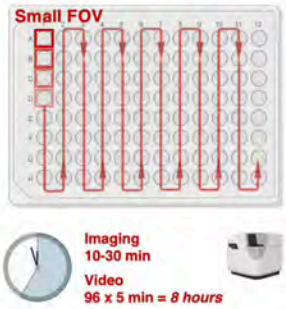
This white paper outlines a fast and accurate workflow for high-throughput cell counting using Ramona's new Vireo™ microscope. Leveraging Ramona's unique Multi-Camera Array Microscope (MCAM™) technology, the Vireo™ employs 24 compact microscopes and parallelized software to capture and process cell culture data at significantly faster speeds than existing technology. In this demonstration, Ramona's Vireo™ microscope 1) rapidly scans and fluorescently images an entire 96 well-plate contents in <30 seconds, and 2) applies automated software to locate and count all labeled cells (here, via Hoescht stain) in a matter of seconds. Users can visually inspect and adjust cell segmentation and counting parameters in real-time to ensure accurate and reproducible results. Users can then output cell counts and associated statistics for all 96 wells with the click of a button. Here, we demonstrate this workflow applied to both B16-F10 murine melanoma cells and hepatocyte-like cells, while detailing prescribed capture and processing methods for others to use to achieve matching results.

Introduction and Motivation

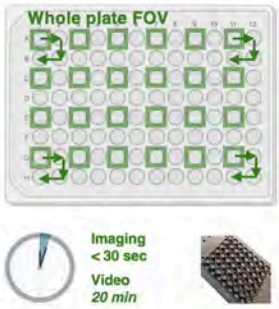
Rapidly and accurately counting cells at high throughput remains a challenging task for most laboratories today. First, current plate-scanning microscopes are slow at stepping through 96, 384 and 1536 well plates while capturing multi-modal data (e.g., both bright field and multi-fluorescence images). Second, data fidelity remains a big concern. Most microscopes rely on autofocus to bring each well into sharp contrast, which often leads to missed wells or significantly defocused features, making cell counting impossible. Third, current software methods for finding and counting cells across hundreds to thousands of images also remain slow and at times inaccurate across diverse cell culture conditions.

The Vireo: Image full well plates in <30 sec

Sequential image acquisition



Parallel Microscope Acquisition



4-Channel Fluorescence Specifications

CENTER EXCITATION	EMISSION PASSBAND	DETECTED FLUOROPHORE
380 nm (UV)	460/40 nm	DAPI
460 nm (Blue)	535/50 nm	GFP
560 nm (Lime)	610/80 nm	RFP, Texas Red, mCherry
633 nm (Red)	715/80 nm	Cy5

Figure 1: The Vireo™ is a compact, benchtop microscope with 24 parallelized imaging systems for ultra high-throughput workflows. As compared to standard microscopes that must step-and-scan through all 96 wells of a multi-well plate, the Vireo™ performs a rapid 2×2 scan to capture data from each well. 4-channel fluorescence imaging capabilities enable rapid, accurate cell counting across multi-well plates.

To address these limitations and facilitate high-throughput cell counting with a simple and quick process, Ramona has developed a new microscope **(the Vireo™, Figure 1)** that quickly images and computationally processes up to hundreds of wells in real-time. Users can now insert a multi-well plate containing cell cultures of interest, and within seconds obtain a detailed spreadsheet of per-well cell counts and associated statistics.

The Vireo™ contains a compact array of 24 microscopes, each outfitted with its own compact objective lens and 13 megapixel (MP) digital image sensor (312 MP total) to rapidly image full 96, 384 and 1536 well plates in a matter of seconds. The Vireo™ is based upon Multi-Camera Array Microscope (MCAM™) technology as detailed in several recent prior publications [1-4]. The Vireo™ is outfitted for several different modalities of image capture, which users can switch between with the click of a button. Options include several unique bright-field and 4-channel fluorescence imaging modalities **(see Figure 1)**. The Vireo™ also includes a stage-top incubator with temperature, humidity and environmental control for live-cell imaging studies. Additional specifications and details about this new instrument are available at: **https://www.ramonaoptics.com/products/vireo**.

Methods

Cell counting via nuclear Hoescht stain

To demonstrate the speed and accuracy of Ramona's new parallelized software for rapid cell counting, we first outline a standard process for a 96-well plate preparation using Hoescht nuclear staining, where fluorescence images are captured for subsequent count statistics. We note that similar strategies may be employed for alternative fluorescence channels (e.g., green channel with GFP stain; red channel with an RFP stain), for bright-field imaging of stained specimens, and for alternative capture areas (e.g., for the entire well area of a 96 well plate, as opposed to just the center area, or for a 384 well plate). Additional details regarding imaging and processing options are available at **docs.ramonaoptics.com**

(a) Full 96 well-plate scan, DAPI Channel (b) Zoom-in of 3 wells with masks (in red) (c) Cells with masks

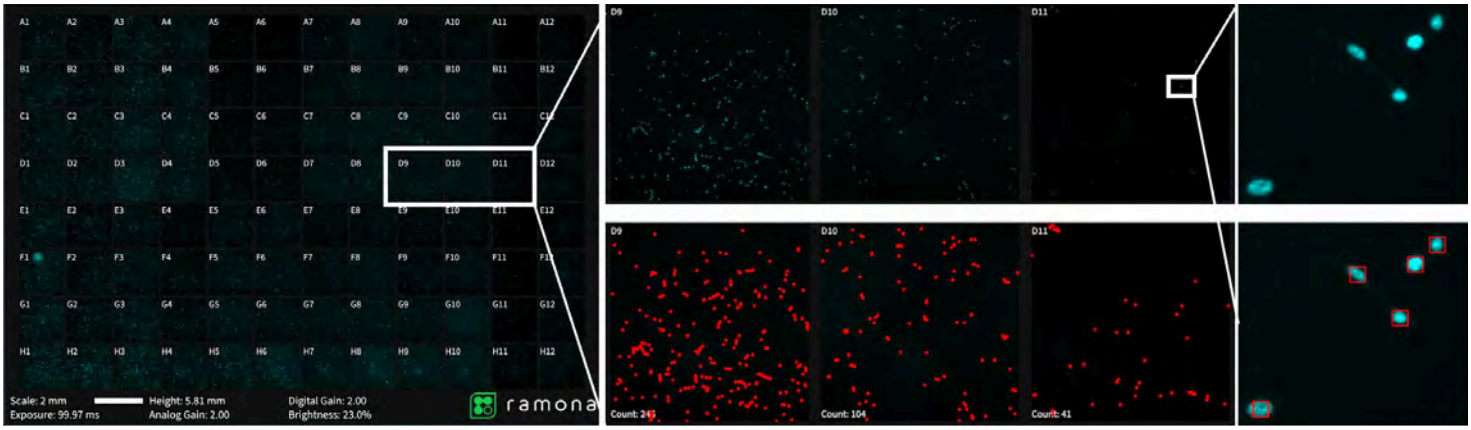


Figure 2: Rapid 96-well plate scan for cell counting via Hoescht nuclear stain. (a) Full well plate visualized with Ramona's software. (b-c) Zoom in showing nuclei (blue) and associated masks (red).

Step-by-Step Guide

1. **Prepare 96 well plate with cells of interest with nuclei labeled via Hoescht staining**

- Example used here: B16-F10 cells were grown in DMEM supplemented with 10% FBS + 1% P/S until confluent in a 70-mm dish. The cells were then trypsinized, plated onto a 96-well plate in various seeding densities and incubated at 37°C overnight. Cells were incubated with Hoescht-33342 diluted 1:1000 in growth media for 15-minutes followed by 1x washing with growth media.

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2. **Insert the 96 well plate into the Ramona Vireo™ microscope and configuring the system to perform a well plate scan with the following properties:**

- Scan with Vireo™ 10X lenses (4X lenses may also be suitable). See example 96-well plate scan in **Figure 2(a)**.
- Capture rapid z-stack to maximize image quality. Suggested # of z-stack slices is 5 with a suggested step size of 10 µm.
- Select XYZC stack, Select DAPI fluorescence channel, Select Laplacian projection

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3. **After scanning completes in 20 seconds (20X+ faster than standard single-lens microscopes), use Ramona's built-in software to perform nuclei counting with the following steps (either manually selected or scripted within an automated workflow):**

- Open acquired dataset in the MCAM Viewer software
- Select projection strategy for captured z-stack (Suggested: Laplacian)
- Select min/max contrast thresholds
- Select Assays > Cell Counting. A panel will open on the right
- Select min/max nuclei radii (you can measure cell radius by using the circle tool under "Tools")

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4. **Run "Cell Counting Assay" (built-in model). See detected results in Figure 2(b-c).**

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5. **Export counts to spreadsheet for downstream statistical assessment. The Cell Counting Assay returns both the number of detected cells per well (here, via cell nuclei) as well as additional parameters such as average brightness and area (see details below). Two files are generated, "analysis_metadata.nc" file containing the analysis results and a csv file summarizing the cell count on a per well basis**

Additional Options for Cell Counting

Here are some additional options available for cell counting that deviate from the above workflow:

- Alternative fluorescence channels besides DAPI may be used (GFP, RFP, etc).
- Alternative well-plate formats may be used (e.g., 384 and 1536 well plates)
- Scan Area may be controlled to image larger or smaller areas of each well

Please find more information about how the Vireo™ can meet your experimental needs at **ramonaoptics.com**

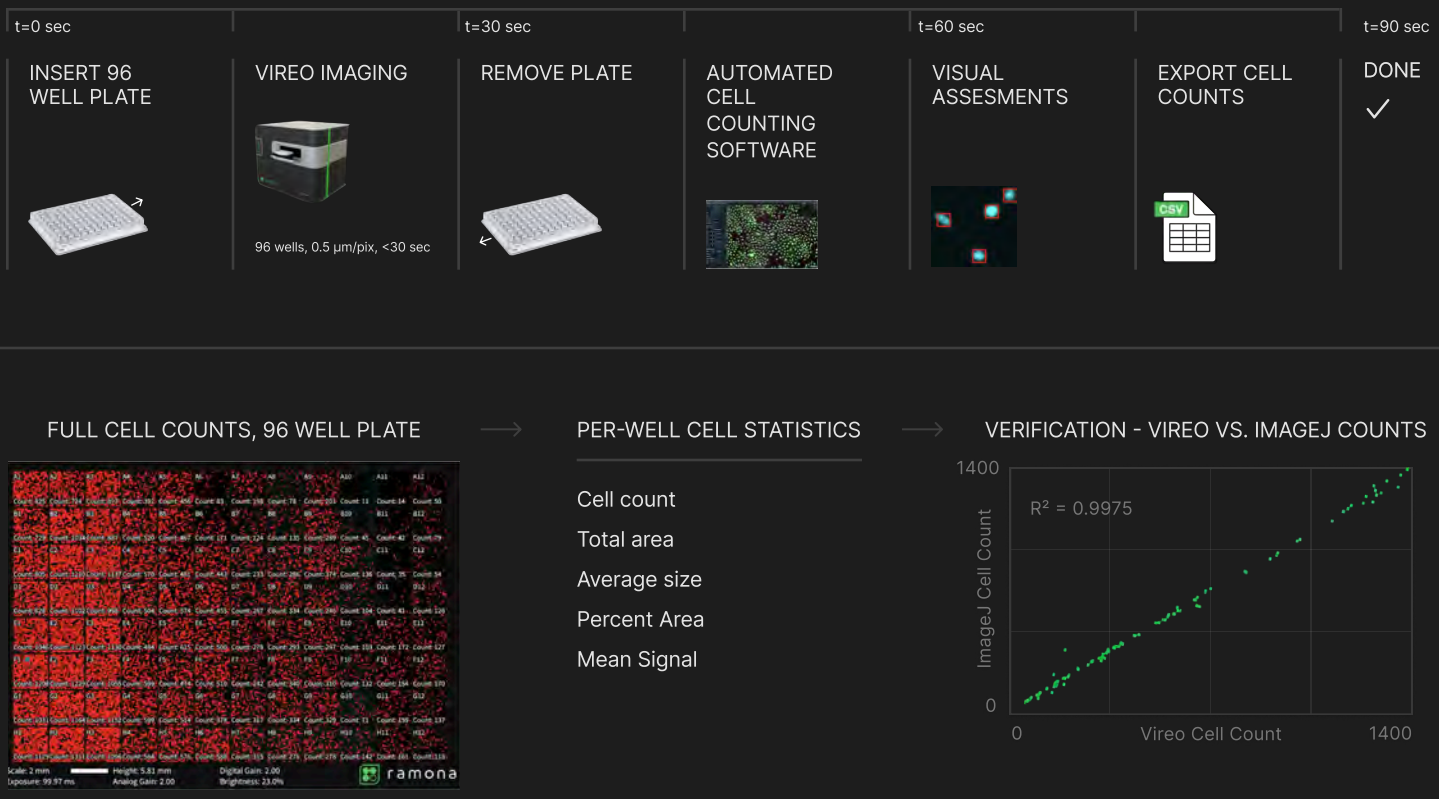

Figure 3: (top) Timeline of cell counting workflow with the Vireo™ microscope. (bottom) Full counts for 96 well plate displayed in Ramona's visualization software are output into a spreadsheet with associated measures and statistics. The 96-well plate's counts were compared to individually performing cell counting with well-known open-source software (ImageJ, each well = 1 green dot), highlighting extremely close agreement, but here in a fast and automated manner.

Cell Counting Accuracy Assessment

Ramona's custom-built machine learning model for cell detection and counting leverages the latest developments in foundational AI models to provide highly accurate counts. Example images captured by the Vireo™ of a 96 well-plate of B16-F10 cells are shown in **Figures 2-3**, with cell nuclei segmentation masks shown within the user interface for transparent quality assessment. Below, we detail quantitative validation of our imaging pipeline and cell counting software.

Microscope image performance comparison

We compared Vireo™ microscope fluorescence image quality, including resolution and SNR for a fixed exposure time, to alternative microscope images captured using an EVOS step-and-scan microscope (Thermo Fisher Scientific) outfitted with a 10X objective lens. We have verified that the approximate 1 µm optical two-point resolution of the Vireo™ (0.5 µm/pixel) is effectively equivalent to the same resolution of any ideal 0.3 NA 10X objective lens microscope

at 600 nm, which can also at best offer 1 μm resolution (0.5 μm/pixel). We co-captured 4-channel fluorescence images of 2 matching cell culture specimens with the Vireo™ and the EVOS 10X. For the Vireo, per-channel exposure times ranged from 200 ms - 450 ms. For the EVOS, per-channel exposure times ranged from 50-150 ms (approx. 3X lower, due primarily to more tightly focused excitation), The average per-channel fluorescence image signal-to-noise (SNR) ratio captured by the Vireo™ was 8.49, 8.71, 6.54, and 7.07 for blue, green, red and far-red channels respectively, while the SNR was 6.78, 6.57, and 3.70 for blue, green and red with the EVOS 10X microscope (far-red channel not available). This highlights a 1.25X, 1.32X and 1.76X higher image SNR with the Vireo, albeit with longer required exposure times.

Cell count software comparison

We next compared the accuracy and speed of our cell counting software to ImageJ - a well-known open-source software method for cell counting. Using Ramona's software, all 96 well counts from Fig. 3 were computed and returned as a spreadsheet in <30 sec. After exporting all captured image data into .tiff format, we then fine-tune and applied ImageJ's built-in software to sequentially perform cell counting per well. The results are plotted at the bottom of Figure 3, where we found an extremely close match between both counts (R = 0.9975). The 96 wells exhibited a distribution of cells from 8 to 1311, with a mean of 466 cells per well. To examine the average error of cell counts between Ramona's software and ImageJ's cell count software, we computed the normalized standard deviation between Ramona's cell count output and ImageJ's cell count output for each well, and averaged these standard deviations across all wells. The result was 10.88, suggesting that on average, Ramona and ImageJ's software differed in count by 11 cells out of 466. The normalized root mean squared error was thus 0.0223 (~2%). This error can most likely be assigned to both Ramona's software and ImageJ's software.

To further investigate the source of this error and verify Vireo™ cell count accuracy, we imaged a a separate but similarly prepared 96-well plate with slightly higher cell density. We compared Ramona's count output to a fully manual cell count (see Fig. 4(a)). For the single randomly selected well shown in Figure 4, Ramona's software output a count of 1850, while the manual count returned 1865, highlighting a fractional difference of 0.008 (5/1850 < 1%).

Additional Verification - hepatocyte cells

We have verified cell counting accuracy across a wide range of cell types, well plate formats, and dyes. An additional example of cell counting performed in collaboration with the Rajagopalan Lab at Virginia Tech is shown in Figure 4(b), where we imaged a 96 well plate of hepatocyte-like cells with multiple stains applied in 4 channels. Accurate cell counts were extracted once again via a DAPI nuclear stain.

(a) Manual Verification (ImageJ)

(b) Verification with Rajagopalan Lab (VA Tech)

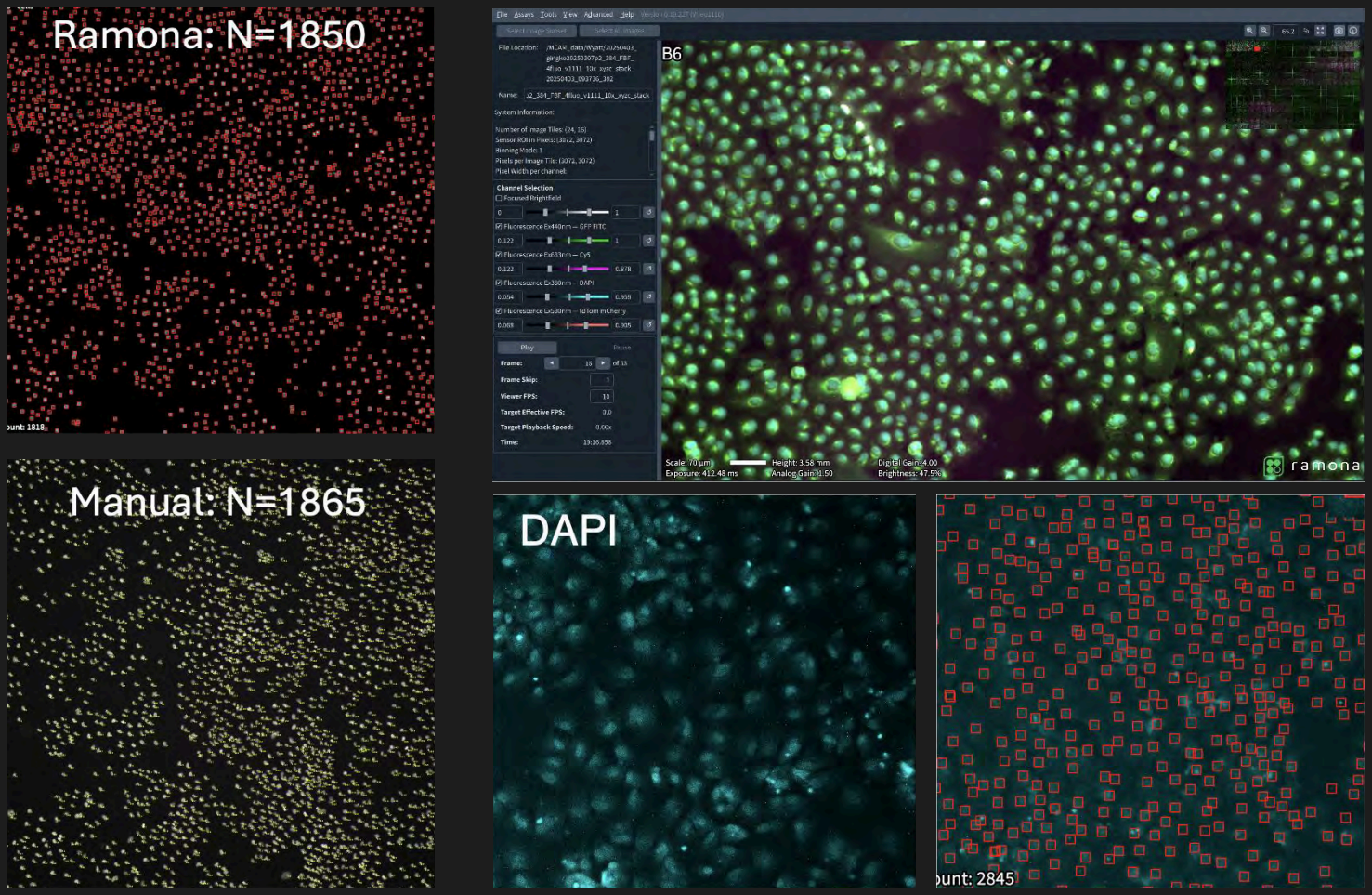

Figure 4: (a) Results of manual count verification. (b) High-throughput cell counting implemented on the Vireo™ with alternative specimens.

Conclusion

We have demonstrated here how Ramona's new Vireo™ microscope can count cells via fluorescence scanning with matching resolution and accuracy as compared to alternative microscopes, but now at significantly faster speeds (20X+ faster). This offers the new ability to perform full well-plate cell counting in seconds, rather than many minutes. Please contact **info@ramonaoptics.com** for more information.

References

[1] K. C. Zhou et al., “Parallelized computational 3D video microscopy of freely moving organisms at multiple gigapixels per second,” Nature Photonics 17, 442–450 (2023).

[2] M. Harfouche et al., “Imaging across multiple spatial scales with the multi-camera array microscope,” Optica 10(4), 471-480 (2023).

[3] K. Kim et al., “Rapid 3D imaging at cellular resolution for digital cytopathology with a multi-camera array scanner (MCAS),” NPJ Imaging 2, 39 (2024).

[4] K. Kim et al., “Parallelized brightfield and fluorescence imaging of organoids using a scalable multi-camera platform,” BioRxiv (2025). Available at: https://doi.org/10.1101/2025.10.07.681020