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Fluorescence Image and Video Acquisition with the Vireo™ Microscope

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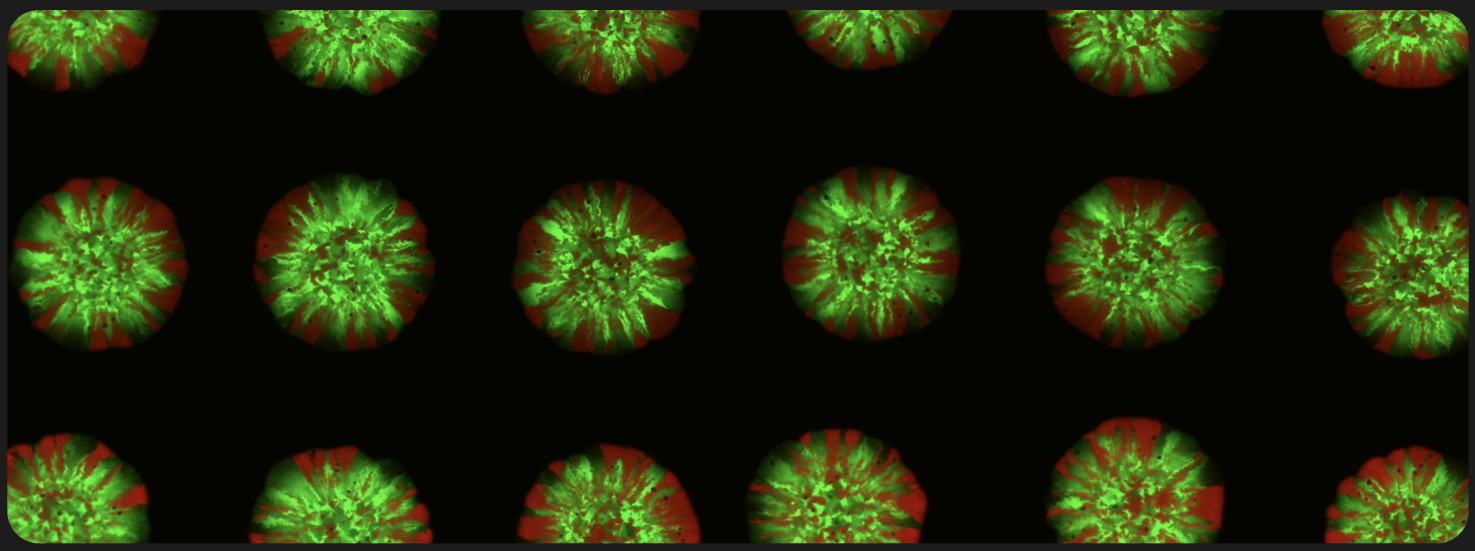


Ramona's new Vireo™ microscope accommodates rapid 4-channel fluorescence imaging of biological specimens like cell cultures, organoids, and small model organisms at high-throughput. Applications include fluorescence capture of Cell Painting assays, CellTiterGlo and Hoechts stains, video of GCaMP dynamics, and more (Figure 1). The following summarizes the Vireo's fluorescence imaging operation, configuration options, and performance. Additional details may be found at **https://www.ramonaoptics.com/products/vireo**.

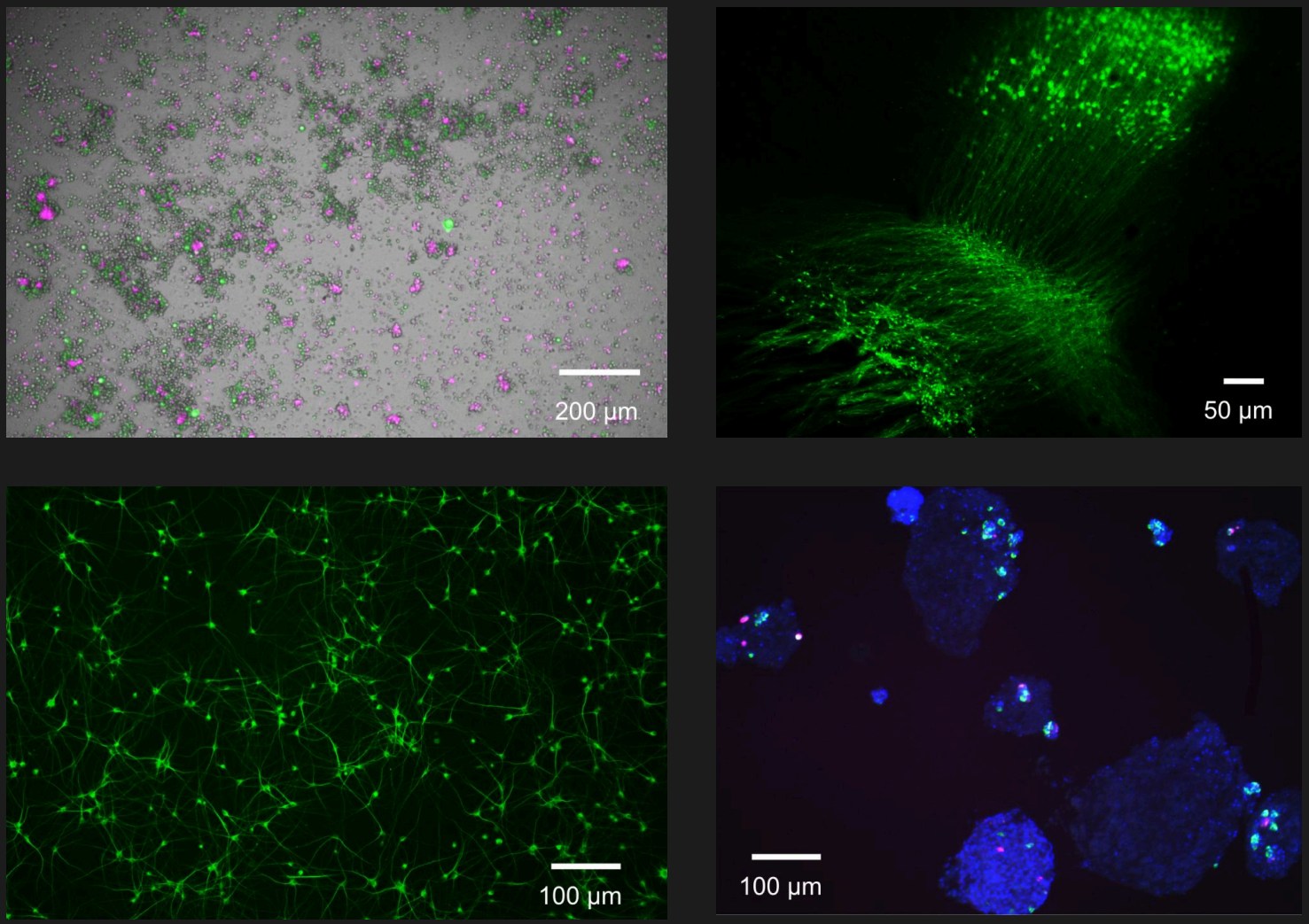


Figure 1. Several unique specimens captured via Vireo™ fluorescence imaging. (clockwise from top left) T-cell and tumor-cell co-culture in 96 well plate with blue and red fluorescence labels applied and overlaid atop co-acquired bright field image; thin tissue section of rodent brain with GFP-labeled neuronal projections; stained neuronal culture in 96 well plate, 4-channel labeled tumor organoids with 384 well plate.

Parallelized fluorescence imaging and video with 24 microscopes

To offer break-through imaging speeds, Ramona's Vireo™ uses 24 microscopes to synchronously capture images and video of biological dynamics within multi-well plates. For multi-channel fluorescence image capture, each of the Vireo's 24 unit microscopes is outfitted with its own set of 4 tightly packed excitation sources and 4 tightly packed excitation filters. Each unit microscope also has its own set of 4 tightly packed emission filters. The 24 microscopes

all capture one fluorescence channel at any given time. For multi-channel capture, small (<5 mm) mechanical translations are used to rapidly switch sources and filters across all 24 microscopes in parallel **(Figure 2)**. The result is an extremely fast fluorescence imaging process - allowing users to image all wells of a 96 well-plate in 4 fluorescent channels in < 2 minutes.

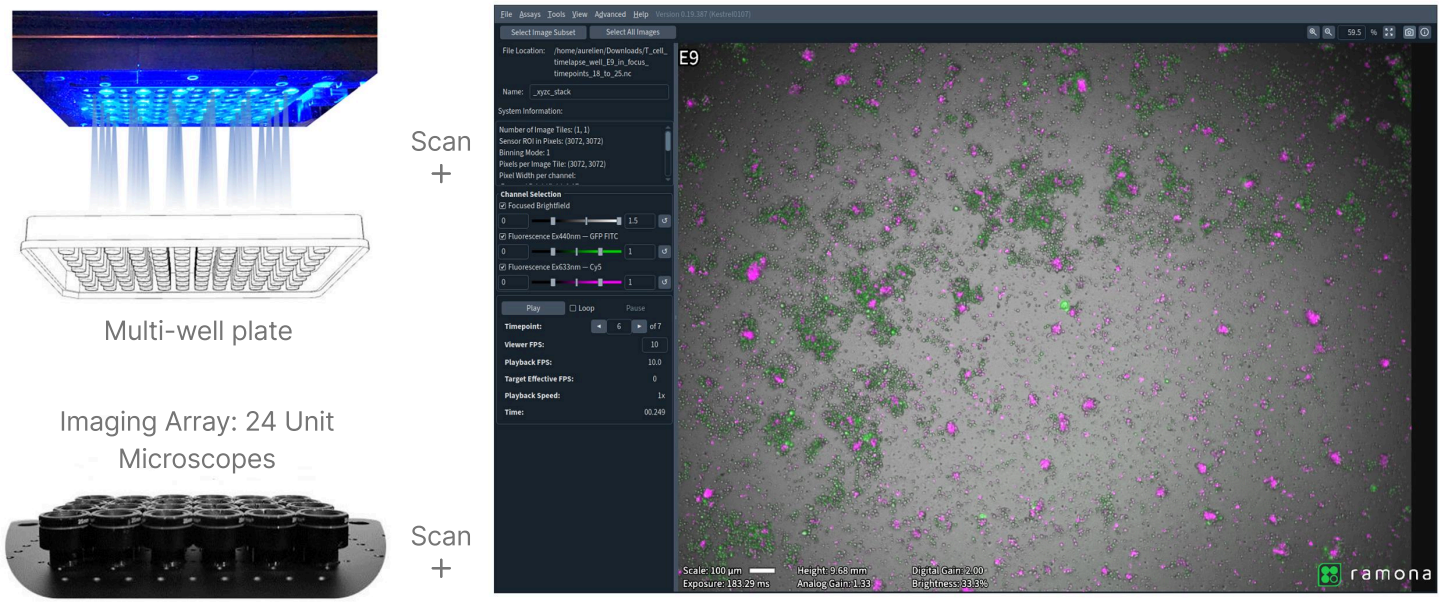


Figure 2: Schematic of Vireo™ Excitation and Imaging. Excitation Array (top) with 96 individual LEDs and excitation filters is scanned in small steps to provide 4-channel excitation across multi-well plates. Imaging array with 24 unit microscopes jointly scanned to capture entire plate areas (e.g., in 2×2 steps to cover a 96 well-plate). Ramona's software allows joint visualization and analysis of multi-channel data.

Configuration Options

Three primary configurations of 4-channel fluorescence filtering are currently available with the Vireo™, covering the standard blue, green, red and far-red spectrum. Additional and/or custom filtering schemes are available upon request. Please contact **info@ramonaoptics.com** to learn more and inquire about filter customization options.

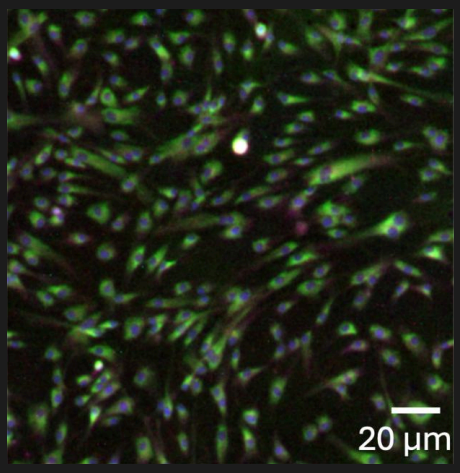
ARRANGEMENT A: LIME	ARRANGEMENT B: AMBER	ARRANGEMENT C: LIME-AMBER
Excitation: 395/25 nm **Emission:** 460/30 nm **Target:** DAPI	**Excitation:** 395/25 nm **Emission:** 460/30 nm **Target:** DAPI	**Excitation:** 395/25 nm **Emission:** 460/30 nm **Target:** DAPI
Excitation: 470/40 nm **Emission:** 535/50 nm **Target:** Alexa488, GFP, GCaMP	**Excitation:** 470/40 nm **Emission:** 535/50 nm **Target:** Alexa488, GFP, GCaMP	**Excitation:** 470/40 nm **Emission:** 535/50 nm **Target:** Alexa488, GFP, GCaMP
Excitation: 560/40 nm **Emission:** 630/62 nm **Target:** Alexa Fluor 555, tdTomato, RFP, DsRed	**Excitation:** 560/40 nm **Emission:** 630/62 nm **Target:** Alexa Fluor 555, tdTomato, RFP, DsRed	**Excitation:** 560/40 nm **Emission:** 630/62 nm **Target:** Alexa Fluor 555, tdTomato, RFP, DsRed
Excitation: 620/60 nm **Emission:** 715/80 nm **Target:** Cy5, Alexa647	**Excitation:** 620/60 nm **Emission:** 715/80 nm **Target:** Cy5, Alexa647	**Excitation:** 620/60 nm **Emission:** 715/80 nm **Target:** Cy5, Alexa647

Table 1: Three currently available 4-channel fluorescence imaging arrangements for Vireo™ microscope.

Performance Upgrades and Verification

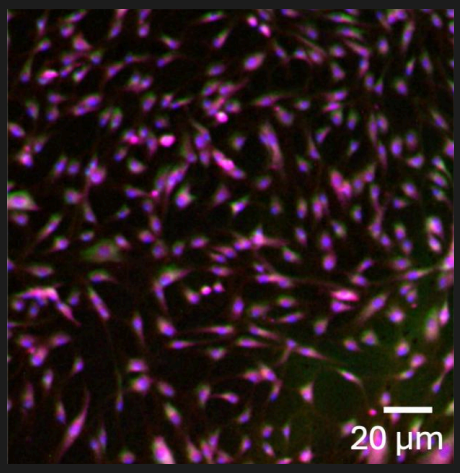
Ramona has upgraded its fluorescence unit in 2026 to further enhance its high-quality excitation. These upgrades allow faster capture of high signal-to-noise (SNR) ratio images and video - now offering matching SNR imagery with 50-70% shorter exposure times than our previous Vireo design **(see Figure 3)**. In addition, Ramona continues to create and support new imaging optics that offer enhanced performance. In 2026, a new 10X lens is available with enhanced resolution and light throughput **(Figure 3 right)**. The Vireo can be easily upgraded and/or enhanced with new lens options - please contact info@ramonaoptics.com to learn more about the best Vireo configuration to speed up your microscopic imaging with.

Vireo 4X (2025)



Avg. Exposure: 475 ms

Vireo 4X (2026)



Avg. Exposure: **260 ms**

Vireo 10X (2026)

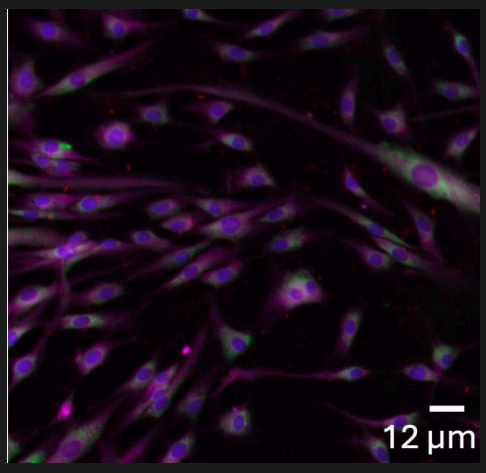


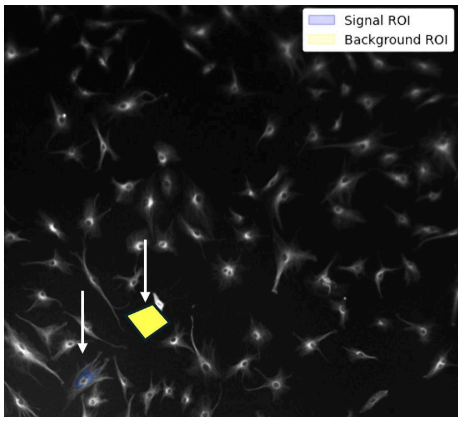
Figure 3: Multi-color fluorescence images of the same human osteosarcoma cells acquired under two fluorescence illumination configurations: (left) via a Vireo 4X from 2025 and (middle) an updated Vireo 4X from 2026, where the latter now offers a significantly shorter average exposure time and thus much faster performance. (right) Example image from Vireo with upgraded 10X lens exhibits higher resolution and SNR.

We have quantitatively benchmarked the performance of the Vireo's upgraded 4-channel fluorescence against an EVOS step-and-scan microscope (Thermo Fisher Scientific) outfitted with a 10X objective lens. EVOS filters were selected for matching target fluorophore detection (specifically, of DAPI (blue), Alexa Fluor 488 Phalloidin (green), and MitoTracker Red CMXRos (red)). Acquisition parameters on both systems were selected to obtain maximum fluorescence signal before detector saturation with <50% excitation brightness and standard digital gain settings with 2 unique cell culture specimens of interest. 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. EVOS exposures are approximately 3X lower due primarily to the use of more tightly focused epi-excitation. Image signal-to-noise (SNR) ratio was computed by selecting image areas with uniform fluorescence signal **(see two examples in Figure 4)** and computing the ratio of the pixel mean and standard deviation (SD) within that identified area (SNR=mean/SD).

SRN Performance, Vireo vs. EVOS

CHANNEL	VIREO SNR (RAMONA)	EVOS SNR (THERMO)
Blue SNR (DAPI)	8.49	6.78
Green SNR (Alexa 488)	8.71	6.57
Red SNR (Mito Red)	6.54	3.70
Far Red SNR	7.07	-

Example Vireo ROI (Blue)



Example Vireo ROI (Far-red)

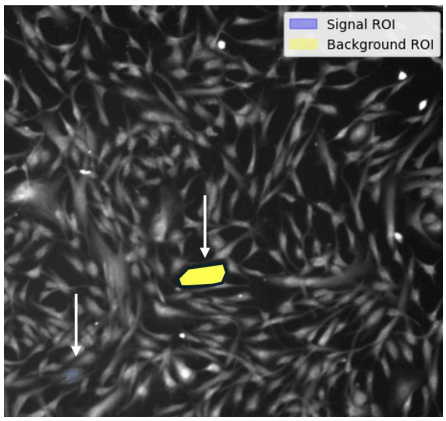


Figure 4: Assessment of Vireo fluorescence imaging performance. Table comparing average SNR on matching stained cellular specimens captured by the Vireo and EVOS highlights similar performance, but with the Vireo offering 24X parallel capture streams. Example Vireo images of two unique cellular specimens blue (middle) and far-red (right). Colored areas noted by white arrows used to compute SNR (blue) and background (yellow).

A summary of results is presented in Figure 4. **The average per-channel fluorescence image 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.** The corresponding average SNRs from the same specimens from the EVOS 10X microscope was 6.78, 6.57, and 3.70 for blue, green and red (far-red channel not available). These results highlight a slightly higher image SNR with the Vireo™, albeit with longer required exposure times. The overall takeaway from this comparative experiment is that the Vireo™ offers 4-channel fluorescence imaging performance that is on-par with currently widespread digital fluorescence microscopes, but here with the unique ability to acquire 24 image and video streams in parallel. Additional quantified performance specifications are available upon request by emailing **info@ramonaoptics.com**.