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High-throughput imaging and analysis of a scratch wound assay with a multi-camera array microscope

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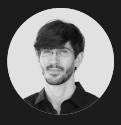

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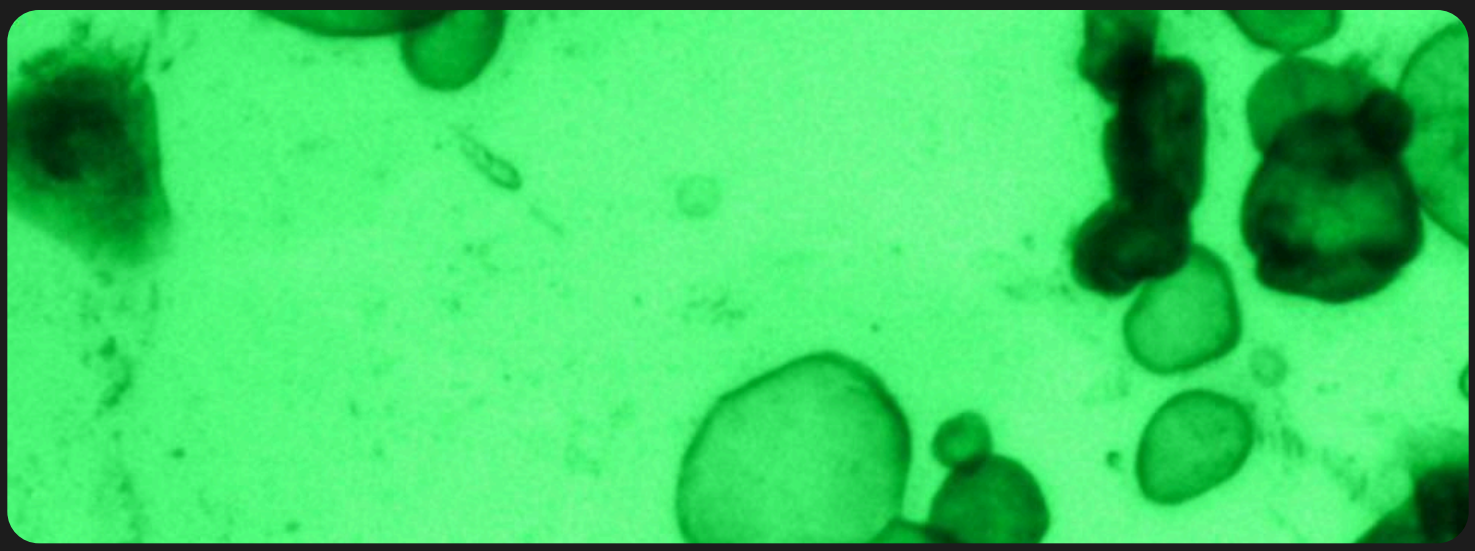

Abstract

Ramona's Vireo™ uses array microscope technology to image multi-well plates in standard formats with high speed and precision, and parallelized software to rapidly quantify biological measurements. The Vireo enables researchers to rapidly acquire images and video across multi-well plates with unprecedented speed and simplicity. Powered by Ramona's Multi-Camera Array Microscope (MCAM®) technology, the Vireo uses an array of 24 compact microscopes and parallelized software to reduce standard workflows that typically require 15 minutes or more to just a few seconds. In tandem with 24x synchronized array imaging, z-stacking and/or video acquisition, Vireo AI analysis software accurately quantifies incoming data to segment and assess scratch wound area over time. Each microscope within the array is equipped with its own compact objective lens and 13-megapixel (MP) digital image sensor, providing a combined 312-MP imaging system. Users can select between multiple bright-field and four-channel fluorescence imaging modalities with the click of a button.

In this white paper, we demonstrate that the Vireo accurately images and automatically measures 96 wound areas, as prepared in a 96-well plate wound assay format, with minimal user intervention. Full 96-well plate wound assays were scanned in under 15 seconds per time point, while integrated analysis automatically quantified wound area across all wells in near real time. Time-lapse imaging over 48 hours was used to monitor wound closure dynamics and characterize healing rates across three different cell types. These results demonstrate how the Vireo platform enables fast, scalable wound-healing assays with immediately actionable quantitative analysis, which can significantly accelerate experimental throughput and yield biological insight.

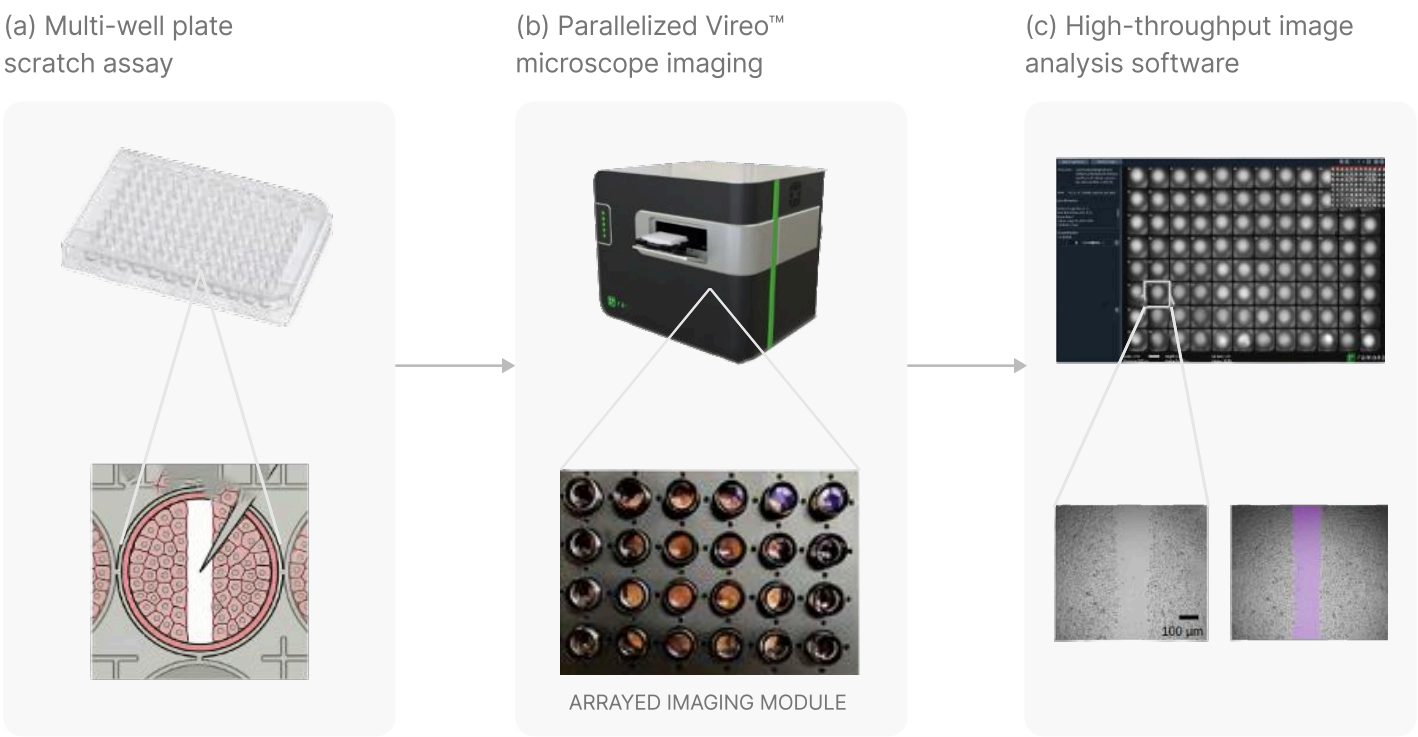

Figure 1: (a) Scratch wound (i.e., wound healing) assay with confluent cell layers prepared in a multi-well plate. (b) Vireo product images and (c) analyzes all wells within 15 seconds, offering a high-throughput imaging and analysis solution.

Introduction

The scratch wound assay, also known as the wound healing assay, is a simple and widely used in vitro method for studying cell migration and collective cell behavior. In this assay, a pipette tip is used to create a “scratch” in a confluent monolayer of cells, generating a cell-free region that mimics a wound. Over time, surrounding cells migrate into this area, and wound closure over time is monitored by microscopy. By quantifying the rate and dynamics of this process, researchers can evaluate mechanisms that regulate cell motility and tissue repair. Thus, scratch wound assays are widely used in studies of cancer metastasis, tissue regeneration, and drug screening, offering a cost-effective and reproducible approach for assessing factors that influence cellular migration and repair processes.

For scratch wound assays, bright-field imaging is preferred to avoid concerns about phototoxicity or photobleaching of fluorophores during long-term live-cell experiments. The Vireo also integrates a stage-top incubator with precise temperature, humidity, and CO_2/O_2 control, enabling extended time-lapse imaging for live-cell observation, which we use here for scratch wound assays. Additional details are available in Ref. [1–4], with further specifications at **https://www.ramonaoptics.com/products/vireo.**

In the following sections, we describe how the Vireo can be used to measure wound closure in scratch wound assays through a step-by-step workflow. This includes configuring the microscope hardware and software to perform 48-hour time-lapse imaging within the integrated stage-top incubator, followed by automated analysis of scratch wound areas after image acquisition. Notably, this analysis can also be performed in real time during acquisition, enabling immediate monitoring of wound healing dynamics across the plate. Additional documentation and resources are available at **docs.ramonaoptics.com**.

Methods and Results

Cell cultures and image acquisition

A549 lung epithelial cells, B16-F10 melanoma cells, and human foreskin fibroblast (HFF) were plated into 96-well plates and grown to confluence. Scratch wounds were created manually in wells using a 200uL pipette tip followed by 2x washes with DMEM to remove cell debris. B16-F10 cells were incubated only in the high growth complete media (DMEM/10%FBS/2mM L-glut); A549 and HFF cells were incubated in two additional media formulations to encourage different growth conditions: reduced growth low-serum media (DMEM/2%FBS/2mM L-glut), or low growth serum-free media (DMEM+2mM L-glut). Plates were then loaded into the Vireo imaging system under incubation, and time-lapse acquisition was initiated in the MCAM Acquisition software after defining imaging parameters (brightness and exposure time). For this application, we captured time-lapse images in the brightfield channel at 4x magnification, and the Laplacian projection method was used to save only the best-in-focus plane. Images were acquired every 15 or 30 minutes for 24 or 48 hours, respectively, at 37°C and 5% CO_2 **(Figure 2)**.

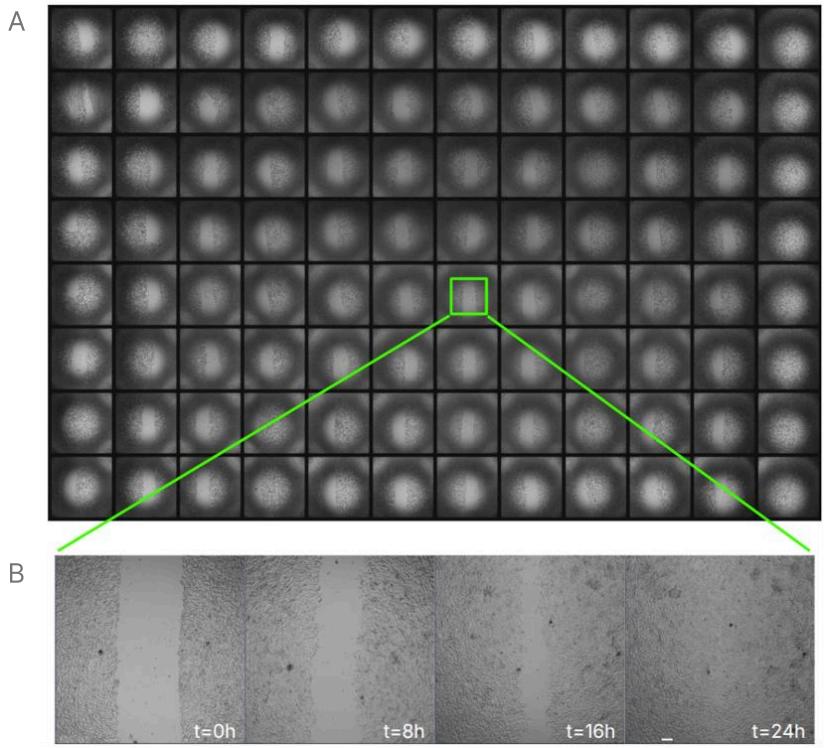

Figure 2: An overview of 96 wells with B16-F10 melanoma cells and scratch wound at t=0 (A), and zoomed-in images at t=0h, 8h, 16h, 24h (B). Scale bar: 100um.

Image Analysis

Once time-lapse acquisition was completed, time-lapse images were opened in the MCAM viewer software. A computer vision model trained on cell-free areas within the initial scratch wounds was then applied to these images to compute scratch-wound areas over time across all wells. The analysis outputs include a metadata file containing artificial masks indicating AI-identified wound areas **(Figure 3A, B)**, typically within less than 15 seconds for a 96-well plate at any single time-point, and a csv output that can be conveniently opened with any statistical software, such as GraphPad Prism or Microsoft Excel for downstream analysis. For example, **Fig 3C** showed largely consistent wound areas across all scratched wells, and 8 wells that were not scratched showed no areas detected.

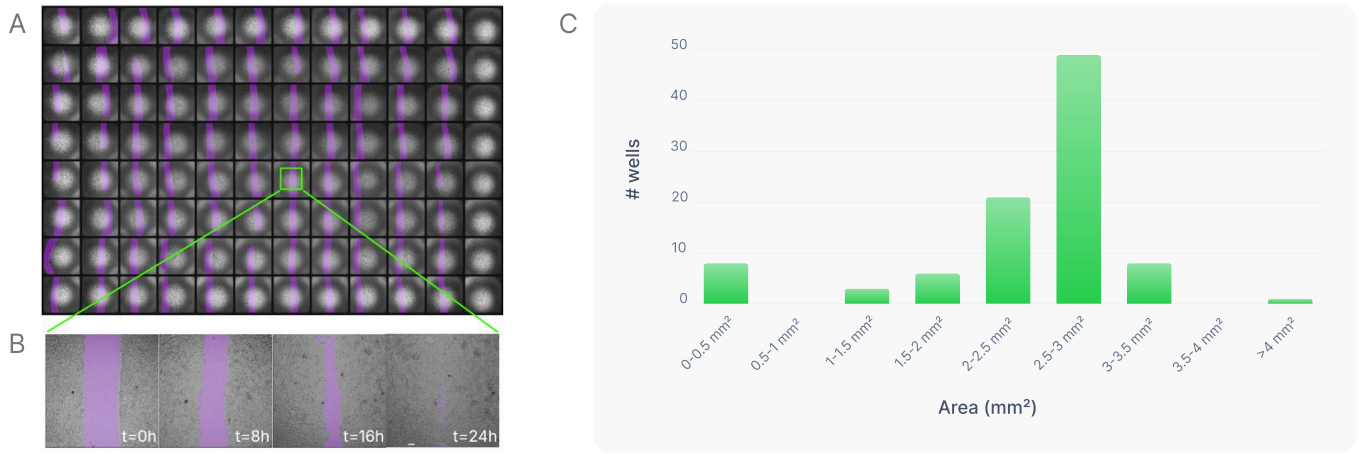

Figure 3: Ramona's custom vision model segments wound areas over time and generates masks for visualization/validation while outputting the percent wound area per total field area in a csv format. An overview of 96 wells with scratch wound area masks at time=0, with wells in the last column unscratched (A). Zoomed-in images of wound closure from t=0h, 8h, 16h, 24h, with scratch wound area masks in magenta (B). Distribution of wound areas at t=0 across 96 wells (C). Scale bar: 100um.

Image AnalyDifferential Effects of FBS on Wound Closure Rates of A549, B16-F10, and HFFsis

Using B16-F10 melanoma cells as an example, we reported a 90% wound closure after 24 hours in high-growth complete media **(Figure 4A)**. A549 lung epithelial cells and human foreskin fibroblasts achieved 60% and 73% wound closures, respectively, after 24 hours, and human foreskin fibroblasts reached complete wound closure at 48 hours **(Figure 4B, C)**. In addition, decreasing fetal bovine serum to 2% or omitting it in culture media significantly slowed the wound closure for A549 cells but not human foreskin fibroblasts, which showed a slight delay in wound closure **(Figure 4B, C)**.

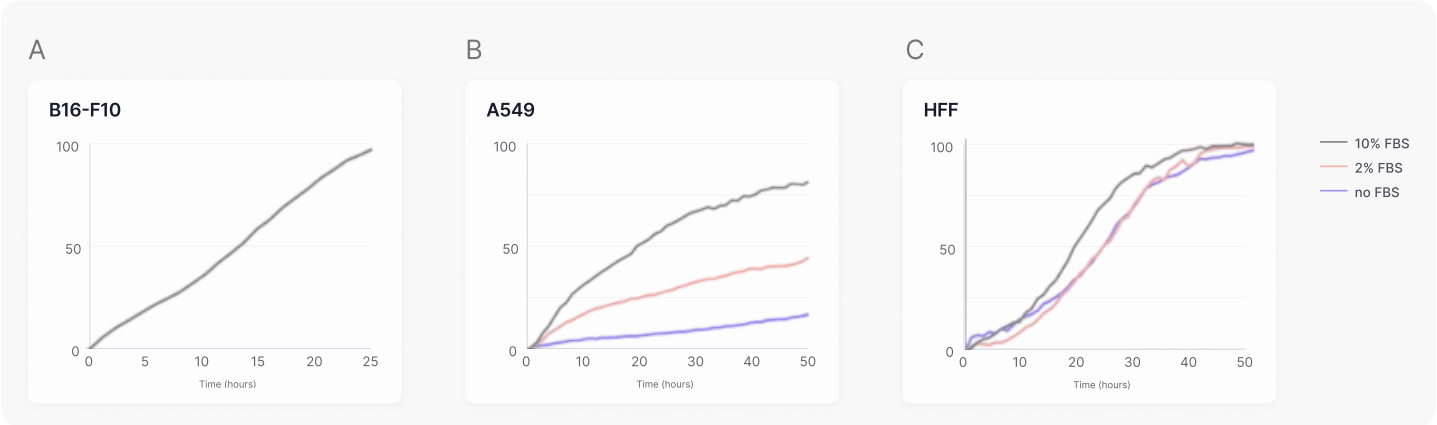

Figure 4: Wound area closure over time reveals a cell type-dependent response to reduced serum conditions. Changes in B16-F10 mouse skin melanoma cell wound areas were tracked through 24 hours (A), while changes of wound areas of A549 lung epithelial cells and human foreskin fibroblasts with different media formulations (black: DMEM/10%FBS/2mM L-glut; red: DMEM/2%FBS/2mM L-glut; blue: DMEM/2mM L-glut) were tracked through 48 hours (B, C). The rate of wound area closure was calculated by $\%\ \text{area closure} = \frac{A_{t=0} - A_t}{A_{t=0}} \times 100\%$

Conclusion

In this study, we demonstrated how the Vireo enables rapid, high-throughput measurement of wound-healing dynamics through automated imaging and analysis. A full 96-well plate was scanned in under 15 seconds, while integrated software simultaneously quantified scratch wound areas, allowing measurements to be performed live during image acquisition rather than through time-consuming post-processing of hundreds of time-lapse images. By combining fast plate-scale imaging with Ramona's custom AI-based vision model, researchers can continuously monitor wound closure over extended time-lapse experiments with minimal manual analysis.

Together, these results demonstrate how the Vireo platform combines rapid plate-scale acquisition, real-time analysis, and long-term live-cell imaging.

For more information about the Vireo and its capabilities, please visit: **https://www.ramonaoptics.com/products/vireo** to learn more.

References

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