

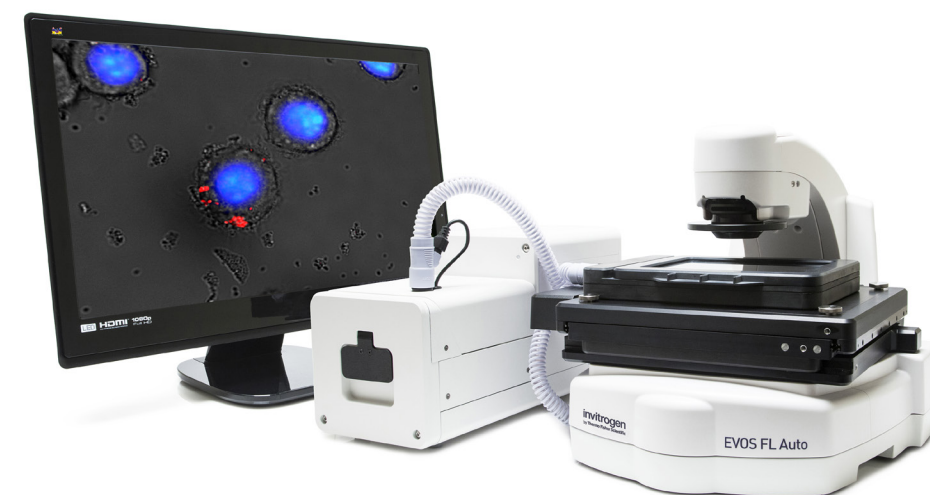
Phagocytosis visualized on the EVOS FL Auto Imaging System with Onstage Incubator

Introduction

Cellular internalization mechanisms are important to many areas and applications in cell biology, including phagocytosis of bacteria during the inflammatory response, uptake and clearance of apoptotic cells, receptor-mediated endocytosis, and screening of potentially biotherapeutic antibodies. The visualization of internalization both spatially and temporally can lend insight into basic cell biology and the development of compounds that may modulate these processes.

Historically, the ability to study these internalization pathways has been limited by both probes with significant constraints as well as unnecessarily complex imaging platforms that complicate live-cell imaging under physiologically-relevant conditions.

While traditional pH-sensitive probes are prone to photobleaching and require tedious washing or quenching steps, the photostable rhodamine-based pHrodo™ Red and Green dyes undergo a dramatic increase in fluorescence in response to an environmental shift



from high to low pH. Furthermore, both pHrodo dyes have a pKa of approximately 6.8, ideal for visualizing the pH change across the plasma membrane as cargo is internalized from the extracellular environment.

The EVOS™ FL Auto Imaging System is a fully automated, fluorescence and transmitted-light inverted imaging system that enables straightforward yet precise climate control and a variety of image acquisition and processing features that simplify live cell imaging over time. Here we demonstrate

simplified live-cell time-lapse imagery from the EVOS FL Auto Imaging System equipped with the Onstage Incubator to visualize the internalization of pHrodo Red-labeled *E. coli* by murine macrophages.

Materials

- EVOS FL Auto Imaging System (AMAFD1000)
- EVOS™ Onstage Incubator (AMC1000)
- Live Cell Imaging Solution (A14291DJ)
- NucBlue™ Live ReadyProbes™ Reagent (R37605)
- Gibco™ Dulbecco's Modified Eagle Medium (11995065)
- Gibco™ FBS (16000036)
- pHrodo™ Red *E. coli* BioParticles™ Phagocytosis Kit (P35361)

Methods

Murine macrophage (MMM) cells were plated at a density of 250,000 cells per well in 35 mm glass-bottom dishes. After approximately 20 hours under normal culture conditions, MMM cells were labeled with NucBlue Live ReadyProbes Reagent at 2 drops/mL of media for 20 minutes at 37°C to visualize nuclei. The cell culture media was then replaced with Live Cell Imaging Solution followed by the addition of pHrodo Red *E. coli* BioParticles at 50 µg/mL. Imaging was performed at 37°C with 5% CO₂ immediately after addition of labeled bacteria. Imaging was performed in the transmitted, DAPI, and Texas Red™ channels every 30 seconds over a period of 50 minutes with a 100x oil objective (1.4 NA) using the time-lapse function of the EVOS FL Auto Imaging System with Onstage Incubator.

Results and Discussion

Initially, pHrodo Red *E. coli* BioParticles were observed under brightfield illumination in the extracellular media (Figure 1A, arrows). Figures 1B and 1C show progressive uptake of the bacteria for approximately 15 and 28 minutes, respectively. As expected, red fluorescence continued to increase in macrophages engulfing cargo as pHrodo Red *E. coli* BioParticles were internalized into the relatively acidic environment of the phagosome over the course of 36 minutes (Figure 1D).

We have demonstrated a simple and robust approach to capturing phagocytosis of bacteria in this study, however, a variety of additional applications using pHrodo dyes with the EVOS FL Auto Imaging System have been established. These include zymosan particle uptake, acidification of cytosol during apoptosis, EGF- and transferrin-receptor internalization, and antibody internalization. The no-wash, fluorogenic nature of pHrodo-based assays combined with the simplified live cell imaging capabilities of the EVOS FL Auto system represents a powerful and accessible approach in visualizing a variety of internalization processes in cell biology.

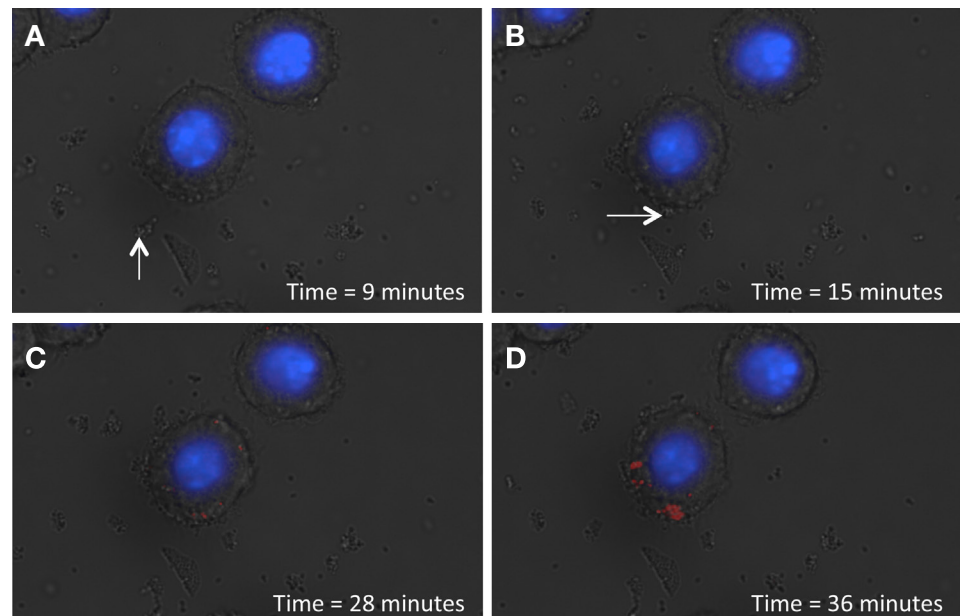


Figure 1. Time-lapse images showing uptake of pHrodo Red *E. coli* BioParticles by murine macrophage cells.

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