

# Qualitative and quantitative analysis of phagocytosis

## Introduction

Cellular internalization mechanisms are important to many areas of cell biology, including phagocytosis of bacteria during the inflammatory response, uptake and clearance of apoptotic cells, receptor-mediated endocytosis, and uptake of antibody-drug conjugates. The ability to visualize internalization, both spatially and temporally, can lend insight into basic cell functions and the development of compounds that can modulate these processes.

Macrophages are one of the main cell types responsible for phagocytosis of bacteria during the inflammatory response. The rate at which the innate immune system can internalize and clear bacteria is dependent on several factors, including the presence of drugs or chemical compounds. One of these compounds, cytochalasin D, is a known cytoskeletal disruptor that inhibits the ability of macrophages to internalize cargo.

While traditional pH-sensitive probes are prone to photobleaching and require tedious washing or quenching steps, cells stained with the photostable rhodamine-based Invitrogen™ pHrodo™ Red and Green dyes can be imaged in the presence of the dyes with no need for wash steps. Furthermore, the pHrodo dyes undergo a dramatic increase in fluorescence in response to an environmental shift from high to low pH. Both pHrodo dyes have a  $pK_a$  of approximately 6.8, which is ideal for visualizing pH changes across the plasma membrane as cargo is internalized from the extracellular environment.



Here we demonstrate simplified time-lapse imaging of live cells using the Invitrogen™ EVOS™ FL Auto 2 Imaging System equipped with an Invitrogen™ EVOS™ Onstage Incubator to visualize the internalization of Invitrogen™ pHrodo™ Red *E. coli* BioParticles™ Conjugate by murine macrophages. In addition, we show quantitative analysis of the effects of cytochalasin D on macrophage phagocytosis using Invitrogen™ Celleste™ Image Analysis Software.

## Materials

- EVOS FL Auto 2 Imaging System (Cat. No. AMAFD2000)
- EVOS Onstage Incubator (Cat. No. AMC1000)
- Invitrogen™ Live Cell Imaging Solution (Cat. No. A14291DJ)
- Invitrogen™ NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605)
- Gibco™ Dulbecco's Modified Eagle Medium (DMEM), high glucose, pyruvate (Cat. No. 11995065)
- Gibco™ Fetal Bovine Serum (FBS), certified, US origin (Cat. No. 16000036)
- pHrodo Red *E. coli* BioParticles Conjugate for Phagocytosis (Cat. No. P35361)
- Celleste Image Analysis Software (Cat. No. AMEP4816)
- Invitrogen™ CellTracker™ Deep Red Dye (Cat. No. C34565)
- Invitrogen™ EVOS™ 20x Objective, fluorite, coverslip-corrected (Cat. No. AMEP4698)
- Invitrogen™ EVOS™ Light Cube, RFP or Texas Red™ (Cat. No. AMEP4652 or AMEP4655)
- Invitrogen™ EVOS™ Light Cube, DAPI (Cat. No. AMEP4650)
- Invitrogen™ EVOS™ Light Cube, Cy<sup>®</sup>5 dye (Cat. No. AMEP4656)
- Olympus™ 60x Oil Objective, apochromat, coverslip-corrected (Cat. No. AMEP4694)

## Methods

### Time-lapse imaging of live cells

Murine macrophage cells were plated at a density of 50,000 cells/well in 35 mm glass-bottom dishes. After approximately 20 hours under normal culture conditions, the cells were labeled with NucBlue Live ReadyProbes Reagent at 2 drops/mL of medium for 20 minutes at 37°C with 5% CO<sub>2</sub> to visualize nuclei. The cell culture medium was then replaced with Live Cell Imaging Solution, followed by the addition of pHrodo Red *E. coli* BioParticles Conjugate at 50 µg/mL. Immediately after addition of the labeled bacteria, imaging was performed at 37°C with 5% CO<sub>2</sub> in the transmitted light, DAPI, and Texas Red channels every minute over a period of 40 minutes using the time-lapse function of the EVOS FL Auto 2 Imaging System with Onstage Incubator and an Olympus 60x Oil Objective (1.4 NA). Quantitative analysis of the images was performed using Celleste Image Analysis Software. Note: For more accurate quantitation, a CellTracker dye can be used to stain the entire cell.

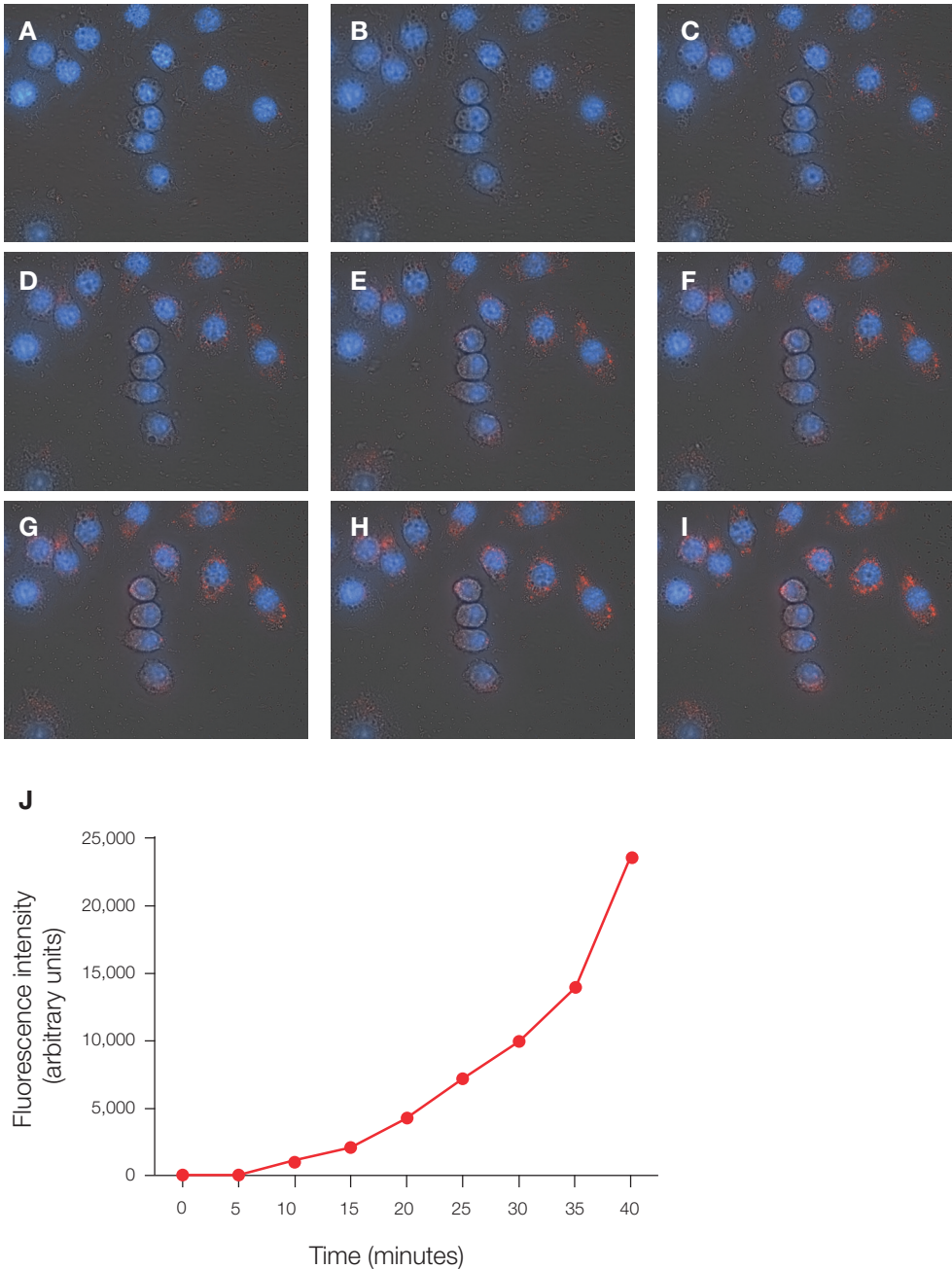
### Quantitative imaging and analysis of live cells

Murine macrophage cells were plated at a density of 15,000 cells/well in a 96-well plate. After approximately 20 hours under normal culture conditions, the cells were labeled with 1 µM CellTracker Deep Red Dye in serum-free medium for 30 minutes at 37°C to visualize whole cells. Cells were placed in complete medium and incubated at 37°C with 5% CO<sub>2</sub> for 3 hours. The cell culture medium was then replaced with Live Cell Imaging Solution followed by the addition of cytochalasin D and pHrodo Red *E. coli* BioParticles at 50 µg/mL. Cells were then incubated for 60 minutes at 37°C with 5% CO<sub>2</sub>. After incubation, automated imaging of samples was performed using the EVOS FL Auto 2 Imaging System with Onstage Incubator in the DAPI and RFP channels using an EVOS 20x Objective across 6 fields in each well. Quantitative analysis of the images was performed with Celleste Image Analysis Software.

## Results

Uptake of pHrodo Red *E. coli* BioParticles Conjugate by murine macrophage cells was visualized using the EVOS FL Auto 2 Imaging System with Onstage Incubator. As the labeled bacteria were phagocytosed into the cells and exposed to the acidic environment of phagosomes, red pHrodo fluorescence was observed as the dye became

activated (Figure 1A–I). The time-lapse images of *E. coli* internalization were quantified using Celleste Image Analysis Software. Over time, more pHrodo fluorescence signal was detected, indicating more *E. coli* were internalized by the murine macrophages (Figure 1J).

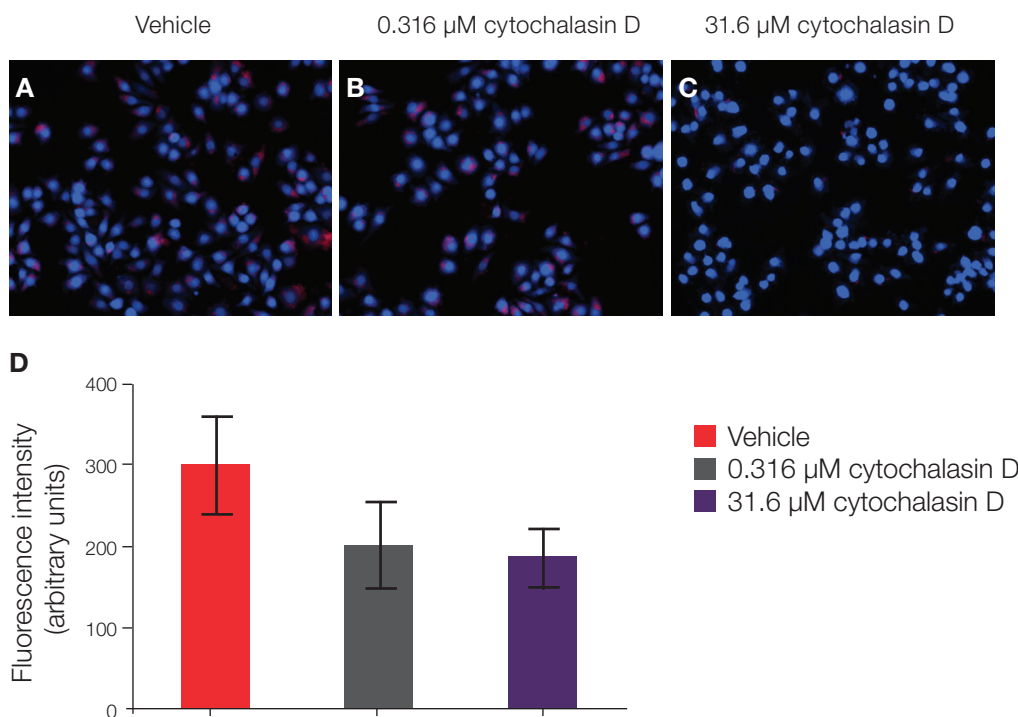


**Figure 1. Temporal visualization of *E. coli* phagocytosis by murine macrophages.** Time-lapse images show uptake of pHrodo Red *E. coli* BioParticles Conjugate by murine macrophage cells. Cells were labeled with CellTracker Deep Red Dye (pseudocolored blue). Images were taken at (A) 0 min, (B) 5 min, (C) 10 min, (D) 15 min, (E) 20 min, (F) 25 min, (G) 30 min, (H) 35 min, and (I) 40 min. (J) The images were analyzed using Celleste Image Analysis Software to quantitate the change in average fluorescence intensity over time. The pHrodo Red fluorescence increased over time, indicating that the murine macrophages progressively internalized the bacteria.

Changes in bacterial phagocytosis can also be visualized and quantitated using the same system of pHrodo dye-labeled *E. coli*, the EVOS FL Auto 2 imager, and Celleste Image Analysis Software. Using this system, we were able to visually and quantitatively validate that cytochalasin D, an inhibitor of actin polymerization, prevents macrophage uptake of bacteria in a dose-dependent manner (Figure 2).

## Conclusions

The ability of the EVOS FL Auto 2 Imaging System with Onstage Incubator to take time-lapse images of live cells in a controlled environment allows for easy visualization of phagocytosis. Inhibition of this actin-dependent process by cytochalasin D can readily be visualized with the EVOS FL Auto 2 Imaging System and quantitated using Celleste Image Analysis Software.



**Figure 2. Quantitative analysis of inhibition of phagocytosis in live cells.** Murine macrophages were stained with CellTracker Deep Red Dye (pseudocolored blue), treated with pHrodo Red *E. coli* Bioparticles Conjugate, and (A) DMSO (vehicle control) or (B, C) cytochalasin was added. Cells were then imaged on an EVOS FL Auto 2 Imaging System with Onstage Incubator. (D) Fluorescence intensity was quantified using Celleste Image Analysis Software.

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