

High-Content Screening of Live-Cell Imaging and Analysis of *Mycobacterium marinum*-infected *Dictyostelium discoideum*

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Introduction

High content screening (HCS) has been useful for defining the functions of genes, proteins, and other biomolecules and also determine molecular mechanisms of life processes in living organisms. In TB research, HCS was used to identify potential early-stage anti-tubercular compounds based on *Mycobacterium tuberculosis* (Mtb)-infected macrophages¹.

However, extremely slow growth rate and strict safety conditions during Mtb handling pose a time consuming and expensive challenge while identifying suitable agents against the pathogen. These can be overcome by the *Dictyostelium discoideum*/ *Mycobacterium marinum* infection model. The amoeba *Dictyostelium discoideum* has long been used as a model organism for host-pathogen interaction studies due to its high conservation nature in innate immunity related genes and pathways. *M. marinum* is a close relative to Mtb, causing similar granuloma formations in fish and frogs although it is unable to cause any systemic infection in humans. Both organisms are fast-growing, inexpensive and easy to handle in the laboratory.

We intend a high content screening assay of live-cell imaging that allows to draw conclusions on proliferation rates and cell viability during infections under modifiable conditions.

Aim

To develop a high content screening assay for live-cell imaging analysis of the *Dictyostelium discoideum*/*Mycobacterium marinum* infection model to allow the simultaneous quantification of cell proliferation and viability in multiple samples.

Approach

Automated live-cell imaging and image analysis of amoebae infected with mCherry-expressing mycobacteria.

Purpose

To allow large-scale anti-mycobacterial compound screening in an inexpensive, easy to handle and genetically manipulatable host.

Methods

- ❖ *D. discoideum* AX2 cells, uninfected or infected with mCherry-expressing *M. marinum* at various multiplicities of infection (MOI), were monitored for 48 hours using the IncuCyte® live-cell analysis system.
- ❖ Phase contrast and fluorescence microscopy images of the infection were created in 1-hour intervals and the percentage of image area occupied by objects of interest (bacteria and amoebae) were determined for all time points.

Results

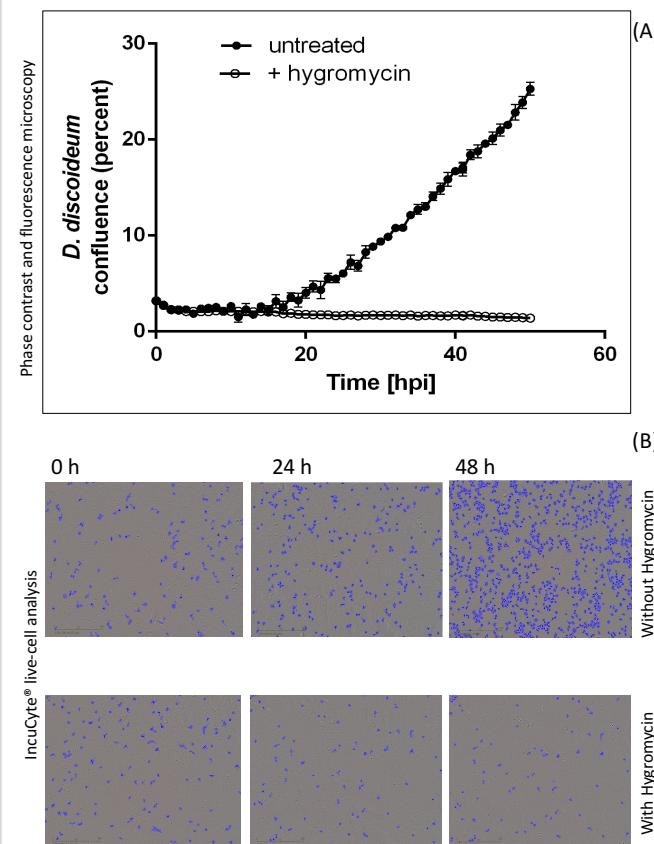


Figure 1 (A,B): Amoebic growth of untreated cells compared to cells treated with hygromycin as a proliferation inhibitor. Images show objects resembling viable amoeba detected by the cell quantification mask.

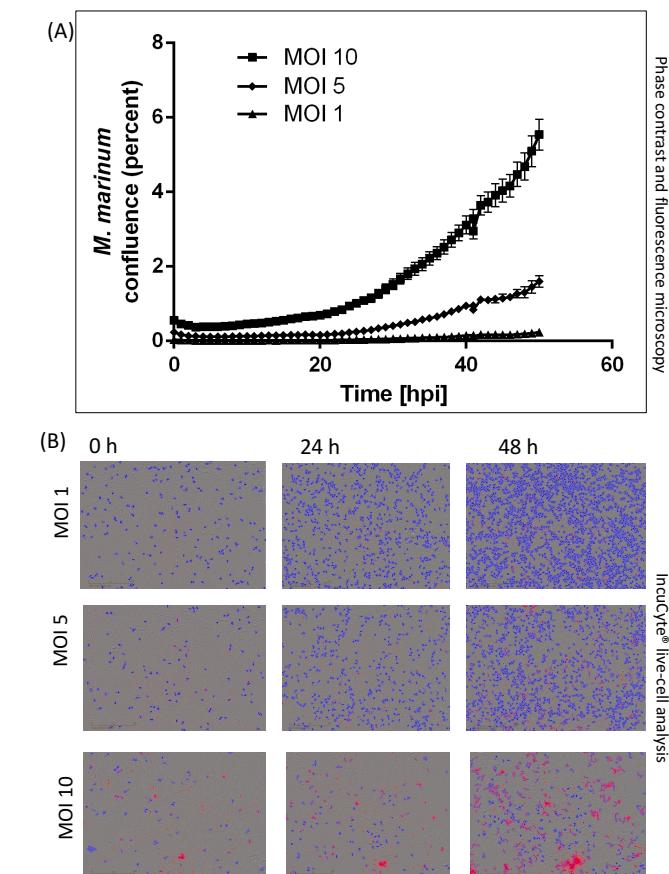


Figure 2 (A,B): Growth quantification of mCherry-expressing *M. marinum* infecting *D. discoideum* at different MOI. The confluence of red fluorescence was quantified by the IncuCyte® analysis software.

Summary & Conclusion

Using *D. discoideum* and mCherry-expressing *M. marinum*, we are developing a high-content viability assay that will allow us to monitor the events during amoeba-mycobacteria interaction under modifiable conditions.

- The careful determination of the MOI and host cell numbers is essential as high MOI levels may kill the host cells very fast to generate any reliable results.
- Our analysis suggests that too low MOI could also lead to high infection variability that can cause inconsistent experimental conditions generating incomparable data.

Conclusion

- The different perspectives provided by this approach to collecting data highlights the unique insight that each may add and the importance of considering the interaction of host-pathogen model.