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Migration and interaction tracking for quantitative analysis of phagocyte–pathogen confrontation assays

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ABSTRACT

Invasive fungal infections are emerging as a significant health risk for humans. The innate immune system is the first line of defense against invading micro-organisms and involves the recruitment of phagocytes, which engulf and kill pathogens, to the site of infection. To gain a quantitative understanding of the interplay between phagocytes and fungal pathogens, live-cell imaging is a modern approach to monitor the dynamic process of phagocytosis in time and space. However, this requires the processing of large amounts of video data that is tedious to be performed manually.

Here, we present a novel framework, called AMIT (algorithm for migration and interaction tracking), that enables automated high-throughput analysis of multi-channel time-lapse microscopy videos of phagocyte–pathogen confrontation assays. The framework is based on our previously developed segmentation and tracking framework for non-rigid cells in brightfield microscopy (Brandes et al., 2015). We here present an advancement of this framework to segment and track different cell types in different video channels as well as to track the interactions between different cell types. For the confrontation assays of polymorphonuclear neutrophils (PMNs) and *Candida glabrata* considered in this work, the main focus lies on the correct detection of phagocytic events. To achieve this, we introduced different PMN states and a state-transition model that represents the basic principles of phagocyte–pathogen interactions. The framework is validated by a direct comparison of the automatically detected phagocytic activity of PMNs to a manual analysis and by a qualitative comparison with previously published analyses (Duggan et al., 2105; Essig et al., 2015). We demonstrate the potential of our algorithm by comprehensive quantitative and multivariate analyses of confrontation assays involving human PMNs and the fungus *C. glabrata*.

1. Introduction

The innate immune system is the first line of defense against invading micro-organisms, such as bacteria, fungi, parasites and viruses. It rapidly recognizes infectious agents and induces an inflammatory response that, among other things, leads to the recruitment of white blood cells, which are able to engulf and kill pathogens (Murphy et al., 2008). These are phagocytes, like macrophages and neutrophils, that recognize pathogens by specifically binding to cell-surface receptors (Flannagan et al., 2012) and take them up into their phagosome for killing by acidification and lysosomal proteins. However, pathogens have evolved strategies to evade the innate immune response and are able to establish infectious diseases that may even become systemic by dissemination via the bloodstream (Duggan et al., 2015b). On the other hand, many invaders – like human-pathogenic fungi from the *Candida* species – are able to persist as commensals in the human host and develop a pathogenic behavior only under specific conditions, such as immunosuppression of the host.

Microscopic analysis of confrontation assays can be used to quantify the interaction between immune cells and pathogens. In contrast to techniques based on flow cytometry, images provide a richer amount of spatial information at the single cell level. During recent years, computer algorithms have been developed that automatically perform image analysis and by that open up the possibility for high-throughput screening while retaining

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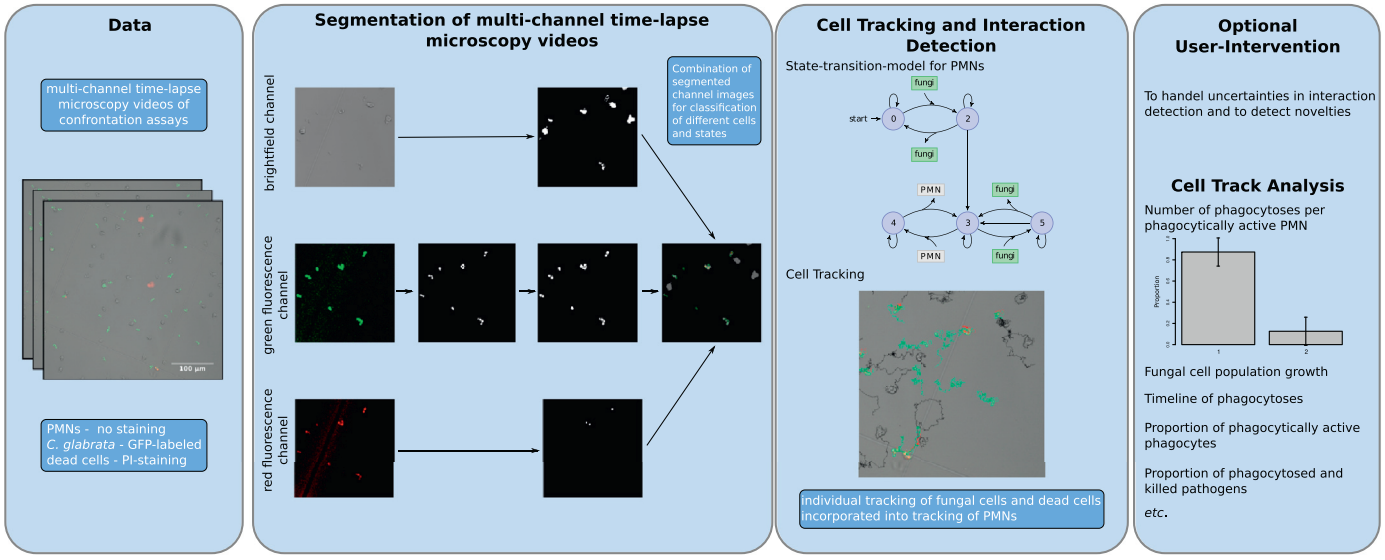

Fig. 1. Segmentation and tracking framework AMIT. This framework enables the analysis of multi-channel time-lapse microscopy videos and was applied to confrontation assays of PMNs and the fungus *C. glabrata*. The scheme shows the individual steps (segmentation and tracking), the optional user-intervention (UI), and the analysis of cell tracks.

the full information content of the images. This was applied to the study of confrontation assays with macrophages and fungal spores of *Aspgergillus fumigatus* (Mech et al., 2011; Kraibooj et al., 2015; Mattern et al., 2015) or fungal spores of *Lichtheimia corymbifera* (Kraibooj et al., 2014). Furthermore, the morphological switch of *Candida albicans* yeast cells to their hyphal form and the dynamics of invasion was quantitatively studied in confrontation assays with epithelial cells by automated image processing of microscopy image stacks (Mech et al., 2014).

Recent advances in imaging technologies have increased the potential of live-cell imaging in researching biological systems (Antony et al., 2013). Live-cell imaging is a powerful tool to study the interplay between immune cells and pathogens. It provides spatio-temporal information about dynamic processes and this may provide new insights into cell migration, phagocytosis and the virulence of pathogens. The quantitative high-throughput analysis of such video data represents an essential step in image-based systems biology of infection (Figge and Murphy, 2015; Medyukhina et al., 2015), which is, however, still hindered by the enormously time-consuming manual analysis and by the general lack of automated quantification approaches for phagocytosis assays.

To date, a large variety of tracking methods are available to analyze brightfield and fluorescence microscopy data (for reviews see: Kalaidzidis, 2007; Chenouard et al., 2009; 2014; and Meijering et al., 2006; 2009; 2012). The tracking methods can be roughly divided into three groups (for details see Brandes et al., 2015: deterministic frame-to-frame association, time-dependent model evolution, and probabilistic approaches. However, all these methods have in common that they were mainly applied to tracking one single cell type in the experiment.

Here, we present a novel framework, referred to as AMIT (algorithm for migration and interaction tracking), which is capable of tracking two different cell types and their interactions in phagocyte–pathogen confrontation assays. As a basis, we used our previously developed automated segmentation and tracking framework for non-rigid cells (phagocytes) in brightfield microscopy videos, which was rigorously evaluated (Brandes et al., 2015). We advanced this approach to segment and track phagocytes and pathogens in multi-channel live-cell microscopy videos as well as to detect interactions and higher-order events between these cell types. In particular, the main focus of this framework is the detection and quantification of phagocytic events to obtain quantitative biological information from live-cell imaging data.

The potential of our framework is demonstrated by its application to confrontation assays of immune cells (polymorphonuclear neutrophils (PMNs)) and fungal cells *C. glabrata* (see Fig. 1). To the best of our knowledge, there is currently no tracking framework available that tracks phagocytic events, implying that we cannot compare our framework to other state-of-the art methods. Therefore, we quantitatively compared the automatically obtained results to manually counted phagocytic events of the same videos and qualitatively compared our result with previously published analyses of confrontation assays between PMNs and *C. glabrata* (Duggan et al., 2015a; Essig et al., 2015). Further, due to the automated tracking of both cell types, the present analysis of confrontation assays is more comprehensive.

2. Material and methods

Within this section, we introduce the main principles of the AMIT framework. The microscopy time-lapse videos of confrontation assays consist of three different channels: the brightfield channel showing all cells in gray level and two fluorescence channels with GFP-labeled *C. glabrata* cells (green) and PI-stained dead cells (red) (see Fig. 1). The preparation of cells and acquisition of live-cell imaging videos of confrontation assays between PMNs and *C. glabrata* are described in Appendix A. An example video is available in the Supplementary (`video1.avi`) and raw data are available from the authors upon request.

First, we individually segment different cells – PMNs, fungi, and dead cells – from brightfield, green, and red fluorescence images, respectively (see Fig. 1). Brightfield images are segmented using the segmentation approach developed in Brandes et al. (2015). The segmentation approach for fungal cell clusters and dead cells as well as the splitting of fungal cell clusters in fluorescence images is described in Appendix B. Secondly, we combine the information from the segmented images to distinguish between different PMN states (Section 2.1). Then, fungal cell clusters and dead cells are tracked from segmented fluorescence images (Section 2.2). Finally, AMIT tracks PMNs and their interactions based on a state-transition-model and using cross-linking of tracks of fungi and of dead cells (Section 2.3). The framework is equipped with an op-