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

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Abstract

Invasive fungal infections are emerging as a signi cant health risk for humans. The innate immune system is the fi rst line of defense against invading microorganisms 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 bright field

microscopy (Brandes et al., 2015). We here present an advancement of this framework to segment and track di erent cell types in di erent video channels as well as to track the interactions between di erent cell types. For the confrontation assays of polymorphonuclear neutrophils (PMNs) and *C. glabrata* considered in this work, the main focus lies on the correct detection of phagocytic events. To achieve this, we introduced di erent 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., 2015a; 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 *Candida glabrata*.

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