Image-based systems biology: A quantitative approach to elucidate the kinetics of fungal morphologies and virulence

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Abstract: *Aspergillus fumigatus* and *Candida albicans* are the major human-pathogenic fungi. There is a variety of experimental set-ups available to investigate the virulence and morphologies of both fungi. Imaging of these experiments using fluorescence microscopy yields vast amounts of image data which could not be analysed manually. Therefore, we applied the approach of 'image-based systems biology'. It comprises the automated image analysis with subsequent statistical feature analysis, followed by mathematical modelling. Application of 'image-based systems biology' to *A. fumigatus* phagocytosis assays and *C. albicans* epithelial invasion assays reveals important factors of the virulence of wild-type *A. fumigatus* and enables the quantitative description of the morphological transition of *C. albicans*, during invasion of the epithelium.

1 Introduction

Elucidation of communication and interplay between the fungal pathogens and the human host is of great interest. The spatio-temporal resolution of host-pathogen interactions provides vast amounts of biological data [Rit10]. For that, imaging technologies, such as fluorescence microscopy, are often utilised in microbiology [RC11, AKM$^+$11]. Thus, observation of large amounts of cell assays is possible leading to additional insights into biological processes which are, so far, not achievable with 'omics' data analysis alone. However, the very time-consuming and highly error-prone manual analysis of the large amounts of data represents the bottleneck of the analysis [JMK$^+$07, NHL$^+$06]. Therefore, an automated approach is at need since systematic studies of comprehensive mutant screenings cannot be performed otherwise. Automatic analysis of spatio-temporal data sets provides morphological features, as well as spatial and temporal dynamics of observed systems [RGH$^+$10]. These observations

represent a useful source for verifying or driving new hypotheses and, thus, can be incorporated into system models [CGT+08]. Integration of image analysis is the key of the 'image-based systems biology' approach. The gained quantitative and morphological features of the system under consideration, as well as interactions in the communication between host and pathogen during fungal infections are further statistically analysed to determine important characteristics of the system. Subsequently, the quantitative features are used to build and test mathematical models of certain processes. In this paper the application of the quantitative approach 'image-based systems biology' is highlighted on two different fungal experiments: (i) *Aspergillus fumigatus* phagocytosis assays [MTG+11] and (ii) *Candida albicans* epithelial invasion assays [MWL+].

2 Fungal virulence and morphology

First, the host-pathogen interactions between *A. fumigatus* and macrophages shortly after infection were investigated using phagocytosis assays of different *A. fumigatus* strains. In the early stages of infection *A. fumigatus* resides in its conidial form and is phagocytosed by macrophages. An automated image analysis algorithm was developed to successfully recognise conidia and macrophages [MTG+11]. The subsequent feature analysis revealed a decreased adhesion ratio for the pksP mutant compared to the wild type. Furthermore, the phagocytosis ratio increased as well as the formation of conidial clusters. We assume that due to the lack of the outer cell wall layer (rodlet/ melanin layer) α and β1-3glucans are exposed which enhance the recognition by macrophages and the increase in the aggregation behaviour. Finally, we rigorously validated the segmentation and classification algorithm, involving a quantitative comparison with a manual analysis by experts. This showed high precision and sensitivity scores and facilitated the adaptation to further experiments. Next, we extended and applied the algorithm to epithelial invasion assays of *C. albicans* [MWL+]. These assays were carried out hourly for the first six hours of infection. During that time *C. albicans* is initially in its yeast form and starts adhering to the epithelial surface. This is followed by hyphae formation which triggers a tighter adhesion, thus facilitating active penetration of the host surface or inducing endocytosis by the epithelium. To account for the different cell morphologies of *C. albicans* the automated image analysis algorithm was extended to recognise spherical and cylindrical cells. Following the 'image-based systems biology'

approach we statistically analysed the acquired features. The interpretation of these data was supported by two mathematical models, the kinetic growth model and the kinetic transition model, that were developed in terms of systems of ordinary differential equations. The kinetic growth model describes the increase in hyphal length and revealed that hyphae undergo mass invasion of epithelial cells immediately following primary hypha formation. Based on the kinetic transition model, the route of invasion was quantified in the state space of non-invasive and invasive fungal cells depending on their number of hyphae. This analysis revealed that the fungal decision to form primary hypha represents an ultimate commitment to invasive growth and suggests that *in vivo* the yeast to hypha transition must be under extremely tight negative regulation by yet unknown mechanisms that avoid the transition from commensal to invasive/pathogenic growth.

3 Conclusion

In this review we highlighted the findings of two investigations performed recently on infection processes of human-pathogenic fungi [MTG$^+$11, MWL$^+$]. In this context, we applied the 'image-based systems biology' approach for the first time. It comprises (i) analysis of large sets of microscopy image data in an automated fashion, (ii) statistical quantification of characteristic features on the basis of the high-throughput and high-content screening of image data, and (iii) integration of acquired spatio-temporal information into mathematical models. Our results are promising with regard to complementing traditional systems biology approaches based on gene-expression data and pave the way for new insights into fungal infection processes.

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