
Faculty: Central European Institute of Technology
         Brno University of Technology
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Student: Ing. Lenka Štrbková

Doctoral study program: Advanced Materials and Nanosciences
Field of study: Advanced Nanotechnologies and Microtechnologies

Supervisor: Prof. RNDr. Radim Chmelík, Ph.D.

Reviewer: Dr. Andreas Hoppe

PhD thesis title: Biophysical interpretation of quantitative phase image

Topicality of doctoral thesis:

The thesis is addressing an important problem in the field of quantitative microscopy and large scale image acquisition: The extraction and analysis of cell features from time-lapse images acquired through light-microscopy is challenging and prone to segmentation errors. As proposed in this thesis, the development and application of novel and robust quantitative cell feature and their analysis is a very important step towards a more objective and automated approach for large scale data analysis in quantitative microscopy. The application of coherence-controlled holographic microscopy (CCHM) appears to be well suited to assessing living cells without interference from any labelling techniques. Moreover, the distribution of dry mass which can be established from quantitative phase imaging allows the development of more objective cell features incorporating the temporal domain further reduce the variability and increase the stability and accuracy of the analysis as has been demonstrated with this work. In addition, the temporal domain allows for the study of cell behaviour which is important in many areas in cell biology research. The approach of using the newly developed cell features in a supervised machine learning approach enables to identify most suitable static and temporal features for a given classification as was successfully demonstrated in this thesis.
Meeting the goals set:

The thesis meets all its goals satisfactorily.

Problem solving and dissertation results:

The analysis is based on a supervised machine learning approach using state-of-the-art classification techniques. This allows for the identification and evaluation of the proposed new features from the quantitative phase imaging. The approach is then compared against using traditional morphometric features and a clear improvement in the classification rate is demonstrated.

The second part of the thesis is concerned with the development and evaluation of new cell features representing cell behaviour from time-lapse quantitative phase imaging. The approach taken is about constructing a suitable feature vector which represents time-lapse features and morphological cell features as well as motion features. A value-based approach representing features from just the image time series is provided as a meaningful comparison. Only the most significant features were kept from evaluating the error function from an SVM classifier which provided a good set of features. The value and feature based approach were applied to a time-lapse sequence of Epithelial cells undergoing epithelial - mesenchymal transition. In addition the feature-based and value-based classification was also compared against the static classification approach and showed that including time-lapse features clearly improved the accuracy and precision of the classification.

Importance for practice or development of the discipline:

The thesis makes a valuable contribution to the field of quantitative microscopy using a combination of cell imaging, feature extraction and machine learning. The work makes use of quantitative phase imaging which is shown to be well suited for label free imaging in quantitative microscopy. The proposed quantitative static and temporal features and subsequent classification allow for a wide variety of applications. For example, the temporal domain allows for the study of cell behaviour to classify malignant and benign cells. The proposed methods appears to be adaptive to different cell types. The choice of a supervised machine learning approach allows for specialist training opportunity in multi-disciplinary teams to improve the classification results. The proposed approach could also be useful in the study of developmental biology, cancer biology and drug testing.
Formal adjustment of the thesis and language level:

This thesis is a well-written, well-structured and well-illustrated with a clear expression and good command of the English language. I do not suggest any major formal adjustments.

Questions and comments:

The following areas could be explored during the viva:

- Cell sample size as a function of robustness.

- The incorporation of temporal information vs. static features.

- The selection of new cell features and their robustness: It would be interesting to see at which point the classification indicates a transition from the epithelial to mesenchymal state.

- Future areas of discussion: Automatic identification of suitable cell features in relation to different cell types.

- How could this work be taken forward to perhaps an unsupervised machine learning approach.

Conclusion:

This well-written thesis presents the results of original research and makes a valuable contribution to knowledge in the field of quantitative microscopy. The proposed methods have a wide range of applications and have shown to be an improvement on current techniques. The research appears to have been carried out in a professional manner as is reflected in quality of the dissertation and the presentation of results. I am very happy to recommend this PhD candidate for examination.

In my opinion, the reviewed thesis fulfills all requirements posed on theses aimed for obtaining PhD degree. This thesis is ready to be defended orally, in front of respective committee.

In London...date...20/01/2018.......

[Signature]
Dr. Andreas Hoppe