Shape analysis for phenotype characterisation from high-throughput imaging

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Chapter 7

Conclusions and Discussion

In this thesis we have developed an architecture for multi-modal high-throughput axial-view imaging (MM-HTAI) including six new computational approaches for shape analysis in support of phenotype characterisation in life-sciences research. The shape analysis is conditioned by good 2D and 3D shape descriptions. The 3D shape description are further interpreted as 3D measurements represented as volume and surface area. Specifically, the 3D measurements are derived from our 3D modelling approaches and can serve as an assessment for size and shape in a biological model system, e.g., zebrafish. The proposed approaches are developed for high-throughput (HT) applications such as HT compound screening which requires massive and reproducible evaluations. In addition, we have developed a pipeline which incorporates well-designed image features and a graphical model to predict the kinship. This represents a particular example of taxonomy applied to a group of faces. We have extended our analysis with a CNN architecture for accurate taxonomy prediction in different datasets. These provide an insight into the behaviour of our system that can be transferred to shape analysis using popular model systems such as zebrafish. In this chapter, we summarise our answers to the six research questions (RQs) (Section 7.1). In the after we address the problem statement (PS) (Section 7.2). Next we discuss limitations of our current methods and subsequently propose possible solutions to address these new challenges (Section 7.3). Finally, we formulate recommendations for future research (Section 7.4).

7.1 Answers to the six research questions

In Chapter 2, we answered RQ 2: *To what extent is it possible to obtain an accurate 2D shape description for the zebrafish from the MM-HTAI architecture*? We stressed that an accurate 2D shape description for a zebrafish larva is of importance for both shape and phenotype analysis as well as for the subsequent 3D reconstruction method. A good 2D shape representation should clearly present the object as a whole. In this manner, we can accurately evaluate the shape variations of the object and identify anomalies. However, in the case of the zebrafish, the transparent part of an object especially challenges almost all the current segmentation methods. So, in this chapter, we focussed on the development of an efficient and robust hybrid method for zebrafish segmentation. With the developed method we are able to obtain a whole shape representation for the zebrafish from bright-field microscopy. Instead of investigating very complex systems, we

combined the merits of the unsupervised learning method i.e., mean shift and the edge based level set method. The mean shift algorithm is able to obtain an approximation for the whole shape of the zebrafish whereas the edge based level set method are able to retain the clear contour. The 2D shape approximation obtained by the mean shift algorithm also provides the level set method with a good initialization thereby accelerating the convergence for curve evolution. The implementation of this idea made our method suitable in bright field microscopy in HTI. Furthermore, we developed a process to split, align and stitch the two segmentation candidates. In addition, we also developed an efficient refinement on the hybrid result and obtained better 2D shape representations suitable for axial-view zebrafish imaging.

In Chapter 3, we answered RQ 1: *To what extent is it possible to develop an MM-HTAI architecture for the zebrafish larvae*? and RQ 3: *To what extent is it possible to obtain precise 3D shape description and derive accurate 3D measurements that are statistically relevant for the zebrafish from the MM-HTAI architecture*? Taking the 3D nature of the shape for an organism, the 3D shape analysis using 3D measurements of volume and surface area can obtain a more robust and stable assessment. This is only available with the help of a good 3D shape representation. The conventional 3D imaging modalities can obtain 3D images, while the low imaging efficiency of and complicated post processing should be addressed. So, we have implemented the MM-HTAI architecture based on the VAST BioImager and light microscopy to acquire axial-view images for the zebrafish. Next we have developed the shape-based 3D reconstruction method using a few amounts of axial-view images. This method is inspired by the multi-view stereo, and as such the 3D reconstruction was efficiently implemented. To guarantee a good shape-based 3D reconstruction, we have presented the voxel residual volume maximisation algorithm for camera calibration. From the 3D modelling, we have obtained the 3D measurements for the zebrafish larvae in three larval stages and reported the 3D measurements as statistical representations. The first merit of this work is providing an accurate shape reference to normalise the assessment in phenotype analysis. In addition, the statistical representations for the 3D measurements enables rapid shape screening for applications using zebrafish larvae. The other merit in this work is that we have obtained natural 3D visualisations for the zebrafish larvae which can be used as a shape basis for an integrated zebrafish atlas [175].

In Chapter 4, we answered RQ 4: *How can we efficiently deal with the translucency and transparency of specimen in light microscopy and still obtain a good 3D shape description from the MM-HTAI architecture*? We addressed the challenge that the shape-based 3D reconstruction method requires accurate 2D shapes for the zebrafish. However, in some cases, the boundaries for the specimen are not well-defined, which prevents the production of an accurate 2D shape representation. In Chapter 2, we have developed a new approach for accurate segmentation of zebrafish, though, this is not always feasible in all applications. In addition to our earlier work, we therefore have developed a two-phase method to address the problem. We first developed an improved volumetric representation as a confidence map which takes a confidence score for each voxel in 3D space. The confidence map is estimated from the textures of the original axial-view images. Next we have applied the region based level set method to explore the optimal 3D shape description over the confidence map. In comparison with the 3D measurements obtained from the shape-based 3D reconstruction which can be regarded as approximations of groundtruth, we have found that the proposed two-phase method can produce sufficiently accurate 3D measurements. We also have shown the feasibility of the method in high-resolution imaging settings.

In Chapter 5, we answered RQ 5: *How can we obtain a multi-modal 3D description and the corresponding measurements for the zebrafish from the MM-HTAI architecture*? We indicated that an accurate 3D shape description for organ development provides important measurements for toxicology. For example, quantitative endpoints like organ size or growth retardation are very much desired for a good assessment. As a result, we have developed a multi-modal 3D reconstruction for the zebrafish larvae combining whole-mount bright-field with organ scale modelling. With the help of our MM-HTAI architecture described in Chapter 3, we acquired the bright-field images representing the whole zebrafish and the fluorescent images representing the organs under study (e.g. liver). We have adapted our previous shape-based 3D reconstruction method to obtain the multi-modal 3D reconstruction and developed an alignment to fuse the 3D multi-models. We have reported the 3D measurements for the zebrafish and its liver and found a trend that a larger organism tend to have a larger liver.

In Chapter 6, we answered RQ 6: *To what extent is it possible that the classification models (or regression models) are able to validate the performance of the image features to characterise the phenotypes in support of shape analysis*? We have demonstrated that if we use higher magnifications and resolution in our

imaging e.g., tissue or cellular scale, the textures in images can convey informative features for shape analysis. Therefore, we made a pipeline which integrates various types of image features and classification models to validate feasibility of the image features in our application. In Subsection 6.1, we used local binary patterns (LBP) on human faces as present in images. From the extracted features, we trained a multi-class logistic regressor for kinship recognition according to the facial appearance similarity. A set of semantic kinship graphs were learned offline and applied at testing time to estimate the kinship in a group of people. In Subsection 6.2, we have proposed a CNN architecture which is suitable for taxonomy prediction for different datasets including butterflies, orchids and wood species. In order to accelerate the training process, we have presented a fine-tune strategy using the CNN models trained on a large scale of images. The results show the accuracy of the proposed methods compared to the baseline methods. This provides a good understanding for the performance of our methods in a large scale of texture based classification problems.

7.2 Answers to the general problem statement

From the answers to the six RQs, we will address the PS.

> **PS**: *To what extent can we develop a stable HTI architecture and produce a robust and accurate shape analysis for phenotype characterization from the HTI architecture?*

A feasible HTI architecture is necessary to ensure an efficient and sufficient sampling size for model system. However, in life-sciences, efficient acquisition of images is limited by the complicated manipulation of a small specimen like zebrafish larvae. The zebrafish are always positioned along their longitudinal axis; multiple axial-views are commonly used to depict the zebrafish larvae. The VAST BioImager has been developed for the purpose that one can easily manipulate a zebrafish larva in any arbitrary axial-view. This leads the HTI architecture to the HTAI architecture. In practice, the VAST BioImager can be mounted on various types of microscopes, such as bright-field, fluorescence and confocal, so we can use the VAST BioImager to manipulate the zebrafish and have the microscope produce the images. If we employ different types of microscopes, the HTAI architecture is translated into the MM-HTAI architecture. This architecture is able to obtain multi-modal images for a specimen presented to the observer in

an efficient manner. Of course, proper adaptions for the imaging software are required. *Therefore, we have handled the problem of the development of a stable HTI architecture by constraining the imaging in axial-view. In addition, the employment of multi-modal microscopy extends the architecture to the MM-HTAI architecture.*

Actually, a complete shape analysis requires delicate shape features, such as shape context [148], statistical shape models [60] and other features including convexity, compactness, curvature, moments etc. [176]. All the shape features should be investigated from the geometrical primitives of a shape. As a result, the prerequisite for shape analysis lies in available and accurate 2D/3D shape description. With the help of the MM-HTAI, we have sufficient data for unbiased shape analysis. However, existing methods fail to generalise the shape description for the zebrafish. So, we have developed the six new approaches towards a reliable shape analysis both in 2D and 3D: (1) the hybrid segmentation method for zebrafish segmentation, (2) the shape-based 3D reconstruction method, (3) the two-phase 3D reconstruction method in light microscopy, (4) the multi-modal 3D reconstruction, (5) the graphical model for kinship recognition, and (6) the adapted CNN architecture for image based taxonomy.

Now the question is: How can we validate the robustness and accuracy of the obtained shape descriptions? We have designed three strategies to answer this question. In Chapter 2, the first strategy is the employment of the groundtruth shape description as manually annotated contours in 2D. We have found that the shape description obtained by our method matches well with its groundtruth counterpart. In Chapter 3, the second strategy is to compute the scalar primitives for a shape including volume and surface area in 3D. We have introduced a known-size calibration particle and our method have yielded very accurate diameter, volume and surface area for these particles. This knowledge can be transferred to validate the 3D shape description of the zebrafish. In Chapter 6, the last strategy is developed to validate the performance of visual features through classification models. This resembles the behaviours of the visual features for the phenotype characterisation. *Therefore, we have handled the problem of robust and accurate shape analysis for phenotype characterisation by the production of robust and accurate shape descriptions and 3D measurements. The research community could use our results for further shape analysis as required in their fields.*

7.3 Limitations and possible solutions

We believe that the methods proposed in this thesis will be able to handle the six RQs and address PS. However, we have to concern the limitations of our approaches.

(1) From the hybrid segmentation method, we can obtain very accurate zebrafish segmentation results in bright-field imaging and we have measured such with segmentation accuracy and F1 score. We have to realise that in our application, there is always only one subject which is oriented in its longitudinal direction. It is also important for our method that the imaging condition should be well controlled such that the zebrafish in the images is depicted as a whole. This is an example which is difficult to be generalised by conventional methods. In this context, we can consider our method as a dedicated exploration for the conventional methods. However, we have not yet validated the performance of our approach under more challenged circumstance, such as the images (A) with serious lighting variation and (B) with multiple objects which are positioned in different orientations. To address these new challenges, we may incorporate orientation detection and multi-initialisation to our current method.

(2) We have indicated that the shape-based 3D reconstruction approach depends on good 2D shape representations. The visibility of a point on the zebrafish surface to an image plane e.g. profile-view is ambiguous. This will complicate the segmentation and subsequently result in an inaccurate 3D shape. We should note that in the case of zebrafish, for some axial-views, e.g., ventral and dorsal, the 2D shapes are more observable. In our shape-based 3D reconstruction method, we have to investigate a proper threshold for the visibility of each 3D point to estimate the optimal 3D surface. (A) The first limitation of the method lies in a trivial investigation for a proper threshold. Furthermore, the camera calibration model is somewhat sensitive to initialisation. From our imaging architecture, we can obtain good estimations for the intrinsic configurations including focal length, CCD sensor size and pixel size from the camera of the camera specification. We can also provide an approximate estimation for the extrinsic configurations i.e., the camera poses according to the pinhole camera projection model. Indeed, the operations have sufficiently improved our camera calibration which has been successfully applied to our setup. (B) We have admit that if a good initial estimation for the camera parameters is unavailable, especially for the camera pose, we cannot any more guarantee a good performance of the method. In our applications,

the shape of our organism system is longitudinal and holds a convex surface. Our method can obtain an accurate convex-hull for an organism. (C) However, we have to realise that the method cannot deal with estimations for concave parts on the object surface. Although most of the biological models own a convex surface, we should consider to address this challenge in our method.

(3) In addition to our previous shape-based 3D reconstruction method, the improved two-phase method is less dependent on 2D shape representation. We should realise that this approach needs a 2D shape approximation for a sufficient texture sampling for the object and background in the original axial-view zebrafish images. This can, however, easily be addressed by the mean shift algorithm in our application. (A) We still need to test our method in diverse model systems which probably are difficult to handle with the mean shift algorithm. Another important issue is to investigate a proper c-level set for the optimal 3D surface estimation. We know that the sampling size of our dataset is sufficiently large i.e., 60 subjects using our imaging condition. We can use this dataset for a good estimation of the optimal c-level set. When a various lighting condition is employed, the colour distribution will be quite different. (B) It will be difficult to generalise the texture distribution using our current dataset. To solve this problem, we have to sample more subjects in more complex imaging condition to enlarge our dataset, so that we may obtain a more generalise estimation. In Chapter 4, we have evaluated the method on our dataset and a supplement dataset produced from the microscope. Both evaluations show accurate 3D measurements in comparison with the baseline method. (C) However, we have not validated the method for other model organisms, i.e., daphnia, etc..

(4) In principle, the confocal laser scanning microscope (CLSM) is widely used to acquire 3D images in plan-parallel slices. However, with standard equipment, this imaging method is very time-consuming and the image quality is subjected to the strength of the fluorescent markers. Our multi-modal 3D reconstruction method takes the shape-based 3D reconstruction as the basis and produces natural 3D modelling on the scale of organs using a regular fluorescence microscope. The developed method is efficient, which is, however, to a certain extent hampered by the quality of the fluorescent images. Some axial-view images for the organ, zebrafish liver in this work, fail to depict a whole shape. This is caused by by the self-occlusion (the thick yolk occludes the liver from the view of dorsal). As stated in previous section, we have to investigate a proper threshold for the estimation of the optimal 3D surface in our method.

(5) We have proved the feasibility of the features and graphical models in our current taxonomical datasets on detailed texture scale. (A) We, however, realise that for each dataset the amount of examples was still limited. On the one hand, we need more data to ensure a more general and robust fitting model; on the other hand, we probably need to consider a weakly supervised strategy to obtain a good model from a small number annotated instances. This is significant in life-science research in which collecting carefully labelled dataset is very expensive and sometimes cumbersome. (B) In addition, further application of the approaches will require an evaluation of the experimental settings of our method with respect to zebrafish imaging which is, at the moment, not available yet.

7.4 Future research

Based on the discussion of our current work, we provide six recommendations for future research.

We have separately developed several new approaches for corresponding tasks i.e., 2D shape representation acquisition, 3D shape reconstruction, multi-modal 3D reconstruction on multiple scales. Our first recommendation is to integrate all the individual modules into one framework (software) in support of shape and phenotype analysis. The framework should be able to communicate with the imaging architecture. In fact, the user only needs to prepare specimens and load the specimens in the imaging device. The whole system will capture axial-view images, pre-process the acquired images, obtain 2D shape for the subject, optimise the camera configuration and generate 3D shape representation for the whole-mount and organ scale of the zebrafish larvae. In the end, accurate 3D measurements of volume and surface area are done for each 3D modelling task.

In our current platform, the imaging process for the zebrafish larvae is accomplished in a sequential fashion. Once the imaging is done, the 3D reconstruction can be performed offline. Our second recommendation is to accelerate the whole pipeline by parallelisation. A straightforward manner is to accelerate the computation by distributing the computations for each subject to different CPU cores. Importantly, the most computationally expensive process is to keep track of the projection for each voxel in 3D space to each of the axial-view images. This is densely operated especially for the camera system calibration due to the massive evaluations of the objective function. Our third recommendation is to employ a

parallelization scheme by accelerating the computation under the condition that all the voxels in 3D space are independent. This can also be accomplished by the employment of GPU.

Our fourth recommendation is to apply our unified framework on diverse model organisms and subsequently evaluate its performance of a generalization. We have shown the successful application of our methods on the zebrafish larvae. We believe that similar results may be achieved on different model systems.

Currently, we have acquired the 3D models for whole-mount zebrafish larvae and some of its organs, i.e., the liver and cartilage. Our fifth recommendation is to apply our method for more organ systems 3D modelling like the zebrafish blood vessels and heart. We hope to finally integrate all the 3D models to comply with the zebrafish [19] and with other modalities like OPT [22]. Then, the zebrafish atlas can be used for accurate modelling and visualisation of gene expression and the development of various diseases.

In addition, our last recommendation refers to the imaging for the zebrafish under experimental conditions. With the control group we have collected, we will evaluate features and classification models on the zebrafish images enabling real texture based phenotype analysis.