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Computational optimisation of optical projection tomography for 3D image analysis

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Chapter 7

Conclusions & Discussion

Chapter summary

In this thesis, we have addressed seven research questions regarding four themes: 3D imaging, 3D reconstruction, 3D segmentation and applications in the biomedical domain. In this chapter we concisely answer and summarize these seven research questions from the research presented in the previous chapters. Subsequently, limitations that we have found are addressed and possible solutions with respect to data, algorithms and theory are discussed. Lastly, ideas on future research are presented.

7.1 Main contributions

The main contributions of the work presented in this thesis can be summarized by answering the seven research questions as follows:

RQ1: To what extent is it possible to increase the processing speed of OPT imaging and reconstruction in an integrated manner?

We have made an attempt to make the process of tomogram reconstruction convenient for users by offering an efficient and reliable way of 3D imaging for OPT. Our OPT system enables to acquire a tomogram and reconstruct a sample in the millimetre scale, e.g. zebrafish larvae, in a few minutes. By using the OPT reconstruction software as presented in Chapter 2 (cf. § 2.2.2), an OPT tomogram is uploaded to our computer cluster for reconstruction. The computations for reconstruction from tomogram to 3D image are parallelized over the cluster through a smart scheduling schema. With the current image size, the time for reconstruction is around one minute using 5 compute nodes of 8-core 2.66 GHz CPU+16G RAM and 8 nodes of 4-core 2.66 GHz CPU+16G RAM. The exact time used for a specific sample is determined by the CPU resources available in the cluster as well as the image intensity distribution of a sample. OPT users will receive the reconstructed data through a web interface (link) provided by the software after the completion of the computation. By the increased sample throughput, more samples can be processed and thereby the integrated system brings facilitates the statistical analysis of the biological samples.

RQ2: To what extent is it possible to reduce the artefacts of 3D image introduced during reconstruction process by misalignment of Centre of Rotation (CoR)?

In the exploration of OPT imaging and reconstruction, we found that the misalignment between the CoR and the image centre normally introduces ring artefacts around the object edges of the reconstructed slices. This is independent of the reconstruction algorithm used. This means that the prerequisite for a good quality reconstruction, i.e. the 3D image, is to correct for this misalignment. We investigated how to apply for this correction and we have presented a fast and accurate CoR correction algorithm, cf. § 2.3.1. The algorithm is implemented on the OPT tomograms and it corrects the sinograms in a straightforward way. Similar to the 3D reconstruction, the CoR correction is also implemented in a parallel manner. By using the proposed CoR correction before reconstruction, the ring artefacts are effectively eliminated from the reconstructed slices. Besides the qualitative comparison of the ring artefacts, quantitative improvement is analysed and evaluated cf. § 2.4.1 and § 2.4.2.

RQ3: Can the Point Spread Function (PSF) of the OPT imaging system be modelled and applied for deblurring of an OPT reconstruction?

This question is answered in Chapter 3. It is of interest to know the relationship between projection blur and imaging depth, therefore we propose a protocol to acquire a tomogram image set of a single fluorescent bead. Our protocol accommodates for a decrease in the probability of overlap between different point sources in a full 3D revolution. We model the PSF using a generalized 3D Gaussian model. We have simplified this model in a workable manner and relate the model to magnification. The model can be easily used for 3D image deconvolution and deblur from just the value of magnification. In Chapter 3 both qualitative and quantitative comparisons are given based on different magnifications and different specimens including zebrafish larvae, zebra finch embryo, chicken heart etc. Moreover, we found that the performance of the proposed deconvolution and deblur approach increases on samples imaged with larger magnifications. This is because smaller magnifications correspond to flatter 3D deblur models whilst larger magnifications relate to steeper, i.e. sharper, models. The results are shown in the modelling section of Chapter 3.

RQ4: Can the iterative reconstruction eliminate the streak artefacts produced in the fast reconstruction?

Iterative reconstruction is implemented in a way that takes the observed projections, i.e. tomogram images, into account and uses it as a reference for updating the current reconstruction. If there are streak artefacts in the current reconstruction, through an iterative reconstruction workflow these will be propagated and reflected on simulated sinogram and be further compared to the observed projection. Aiming at minimizing the error between simulated and observed projection, the algorithm guarantees that the new reconstruction will converge in the correct direction. In Chapter 4, an example of streak artefacts in zebrafish is given and we present the effectiveness of iterative reconstruction on streak artefact elimination based on the results of multiple samples we have tested.

RQ5: How and to what extent the initialization and the number of iteration steps influence the results in iterative reconstruction?

We explore the effects of iteration steps and initial reconstruction having on the reconstructions. Thereby, we focus in particular on the zebrafish specimen. By using the reconstructions for segmentations, we measure the segmentation performance in zebrafish. By evaluating the segmentation performance, we quantify the influences of iteration steps and initial reconstruction and further optimise the parameters for the iterative reconstruction. To achieve this, a highly reliable segmentation algorithm is required, that takes the transparency of the samples into account. Based on the experimental results, we find that the combination of 10-iteration and FBP-initial

reconstruction has the best performance with the current preparation protocol and data. We further demonstrated that without a phantom for reference of quality, and empirical approach provides sufficient information on quality of the reconstruction.

RQ6: Is it possible to “learn” a 3D reference structure of zebrafish for 3D fluorescence quantification in zebrafish?

This research question deals with the application domain of OPT; here we have focussed on our typical model system, i.e. the zebrafish. In order to avoid influence of the variation in individual samples and imaging environment, i.e. exposure time and magnification, on tumour quantification for drug discovery, relative quantification is proposed and defined in Chapter 5. This quantification approach can be further generalized to other fluorescent signals in zebrafish. In terms of 3D relative quantification of fluorescence in zebrafish, we focus on the automated detection of reference structures. This detection is defined as “learn” as we aim to avoid the laborious manual labelling in a volume image. By using the current state-of-the-art volumetric segmentation approaches in biomedical imaging, we trained a robust segmentation approach to detect the two reference structures, i.e. *Body* and *Eye*. Based on the 38 training samples we have achieved promising results. For both reference structures we achieve an accuracy of over 90%. We think that the accuracy can be further improved by adding more data in the training procedure.

RQ7: How much 3D information can be achieved and identified from bright-field zebrafish OPT imagery and to what extent such identification can be automated?

From an unstained zebrafish 3D OPT bright-field image we are able to distinguish a number of well-defined regions in the volume. For a *5dpf* zebrafish we can observe four volume regions: i.e. *Eye*, *Head*, *Muscle* and *Belly*. Whilst in a *25dpf* zebrafish, a more advanced developmental stage, more comprehensive volume regions can be observed: i.e. *Eye*, *Head*, *Belly*, *Fin*, *Muscle*, *Blood vessel*, *Notochord & Spinal cord*, *Swim bladder* and *Intestine*, cf. § 6.3.1. In order to explore the automated detection of 3D structures, we trained 35 zebrafish samples aged from *5dpf* to *7dpf* and independently tested the automated detection method on 3 samples. The average accuracy for *Head*, *Muscle*, *Belly* and *Eye* is 54.96%, 70.39%, 39.16% and 0% respectively. Constrained by the transparency characteristics, data availability, benchmark labelling, computational resources and algorithm intelligence, automated 3D detection on zebrafish is still in its infancy and remains a challenge.

7.2 Achievements of research presented in this thesis.

All considered, we set out to study the use of OPT and pondered on how to maximize the information that can be obtained from an OPT image. In order to do so, we reviewed the quality of the images that are obtained from OPT.

The quality is influenced by the reconstruction algorithm, the artifacts that are introduced from the reconstruction and the artifacts that are introduced from the imaging. We have shown from our research questions that we have addressed, in addition, the speed of operation as we consider this an important asset of OPT imaging in a research workflow.

We have shown that the artifacts from the reconstruction, i.e. rings and streaks can be corrected in an efficient manner. The imperfections in the imaging causing deblur can be restored by a specifically designed process of deconvolution. It is clear from the algorithms that are designed and probed that OPT imaging is a typical form of computational imaging. It requires sufficient computational resources and smart algorithmic approaches in order to be efficient and valuable.

In the chapters on optimisation of the images we have used several different samples typical for the range of magnitude common to OPT. In the chapters on application of OPT we use zebrafish and worked on typical manners to support the analysis of OPT images from zebrafish. In that we have invoked machine learning approaches that are state-of-the-art. The rationale behind the use of the machine learning in segmentation is to be able to automate these processes. That is, now that we can obtain good quality images from the OPT, we must develop the processing of these images. We have shown that is approach can be successful.

7.3 Limitations and possible solutions

Further to the presentation of the results we here consider some of the limitations as well as manners how to overcome these limitations. To that end we take for perspectives in the next paragraphs, i.e. data, hardware, algorithms and theory.

7.3.1 Data perspective

(1) The amount of biomedical image datasets, especially 3D images are, in general, relatively insufficient for existing computational architectures. Large image resources such as ImageNet ^[146] do have a better performance for this matter. In this data-limited context, typical for scientific research, we accelerate and facilitate the process of producing 3D OPT microscopy images. By employing the use of cluster and parallel computing with a fast reconstruction algorithm, i.e. FBP, we created a fast reconstruction system. It enables users to obtain 3D images fast with a reasonable quality for most cases,

yet it fails to avoid streak artefacts at locations where small and concentrated signals appear. To completely eliminate the possible streak artefacts, iterative reconstruction is applied on top of the fast reconstruction. Currently, the iterative reconstruction is, however, explored on GPU without parallel optimisation. This means that in terms of speed of 3D image data acquisition, the iterative reconstruction work is far from optimal. The combined optimisation of implementation on GPU and parallel computing for iterative reconstruction will be considered in the further research now that we know how to combine fast and precise reconstruction methods.

(2) In Chapter 3, the deblur experiments are implemented and reported on 25 3D images. The size of the data we have is far from sufficient with respect to requirements for statistical analysis and big data. The effectiveness of the proposed methods needed to be further verified as more data become available. In Chapter 4, parameters of iterative reconstruction are optimized based on experiments of zebrafish with two different clearing protocols. By using each protocol we image three zebrafish, which are utilized for reconstruction with different parameters. Even though the test performance of single zebrafish reaches up to more than 98%, with the training ratio of 20% and test ratio of 80%, it might be more convincing if more samples can be used. In Chapter 5 we train the two reference models based on 35 zebrafishes and achieve promising results on 3 test data, but there is room for improvement to achieve a higher accuracy. We will therefore consider keeping adding training samples as they become available and then regularly retrain the model, so as for the automated annotation in Chapter 6.

7.3.2 Hardware perspective

Imaging resources: The 3D imaging process is accomplished in a full revolution and samples are manually mounted in the FoV. Because of the manual operation, a perfect mount of the sample to meet the requirements for reconstruction cannot be guaranteed. To address this problem, three imaging parameters are introduced. The camera rotation and prism tilt in the microscopy synergistically determine the direction of CoR in the image space. Ideally, the CoR is supposed to be parallel with one of the image axes for reconstruction, but, in practice of imaging this is difficult to accomplish. The operator can, however, decrease the differences between them by adjusting two screws, a laborious operation. Another parameter is the prism rotation which determines the distance between CoR and image centre in the parallel axis. It is also difficult and time consuming to adjust this distance to an ideal value of 0. We solve this problem by presenting the CoR correction in Chapter 2.

From our perspective, there are two possible solutions for these problems. One is keeping the manual placement and fixing the cameral rotation and prism tilt screw after a satisfactory CoR direction is reached. The other solution would be replacing the manual mounting of the sample with a fixed automated mounting of the sample. In such way the three imaging parameters and sample mounting are fixed and optimized. This is

promising in terms of both imaging quality and efficiency. It requires, however, accurate mechanical motorized parts, for example how to guarantee the accurate and same position when placing each sample. This is absolutely challenging now but might be feasible in the near future.

7.3.3 Algorithmic perspective

(1) CoR correction: The CoR correction algorithm is dependent on the signal intensity of sample from the imaging system. This means that if signals are weak the tomogram cannot provide the algorithm with sufficiently strong signals to calculate the CoR. This is the main drawback of our CoR correction approach and currently we cannot establish a generic solution to avoid this drawback. However, the combination of bright-field and fluorescence channels can give sufficient information.

(2) 3D PSF modelling: The 3D PSF in Chapter 3 is modelled based on a fluorescently labelled bead of fixed size, which is larger than but close to the resolution of the imaging system. In such case we approximate the model built from the sphere as the PSF model at the specific resolution, but a theoretical PSF is considered more powerful. In this work we assume that PSF model is linearly related to sphere size. The effect of the bead size on the modelling and deblur is not taken into account. But the theoretical relation between them needs to be further investigated. With respect to the magnification effect, the model is constructed based on 6 different magnifications. Subsequently, we estimated the PSF model on the 3D image according to magnification consistency, which is theoretically regarded as reasonable. However, if we critically think about this rationale, we need to prove the optimality of implementation based on magnification consistency. This means performance comparison of deblur with different magnifications implemented on the same 3D image needs to be considered, for experimental evidence of the abovementioned optimality.

7.3.4 Theoretical perspective

(1) 3D PSF modelling: In Chapter 3 we generalize the 3D Gaussian model with more parameters for the PSF modelling and deblur tasks. In practice, it works well for both tasks. The generalized 3D Gaussian model we used for deblur is supported by previous work and experimental observations, which is based on statistical assumption rather than imaging theory. This means that there is a lack of theoretical proof showing the expediency and effectiveness of PSF modelling from the aspect of imaging process. This question motivates us to think if there is another model working for OPT image deblur, benefiting from the inference of the imaging process. To answer this question, knowledge of optical physics and inferential mathematics are required. This might be an interesting and challenging research to continue with.

(2) 3D segmentation: The challenge of zebrafish segmentation in 3D image is a good estimation of the location of the surface based on the limited surface information such as pigments. The ground truth of zebrafish model for training are estimated based on human knowledge, i.e. manual labelling. There is no benchmark model for a zebrafish, so the manually labelled model is regarded as the closest to a benchmark. Under this assumption, we trained the zebrafish model based on the approximate benchmark and used it for evaluation. From practice in machine learning we know that when sufficient data is available the learnt model can achieve a very high accuracy but never reach to 100%, compared to manually labelled benchmark. One possible addition is to combine information from phenotypes of zebrafish such as smoothness, connectivity, etc., to evaluate the estimation of the rather transparent surface.

7.4 Outlook

Some 3D imaging techniques are non-invasive and at the same time provide sufficient interior information of the biological sample. This makes 2D imaging of physical sections less necessary. Stepping up one dimension, i.e. from 2D to 3D, with large image sizes introduces a big challenge for both hardware and software.

For image acquisition in OPT, the automated mounting of samples is a promising direction of research. Given automated mounting, a calibration becomes obsolete with the assumption that samples can be ideally placed for obtaining an approximately perfect reconstruction. Another point is that automated mounting will shorten the overall time for imaging each sample. Both aspects contribute a large decrease in imaging time and accelerate the imaging process. Taking advantage of this automation process, the system will be suitable for high throughput screening. But the challenge is that the progress of automation is determined by the collaborative development of mechanical engineering and computer technology.

The fast reconstruction algorithms in the OPT system have yielded reconstructions for hundreds of samples. From these samples we observed two kinds of artefacts in our work. But there might be more artefacts for OPT imaging. The scientific literature in this field is still limited. A systematic analysis of artefacts in OPT would be an interesting research topic. To this end an example can be taken from models that have been used for artefacts reduction in CT imaging.

The research presented in this thesis has addressed several research areas, from microscopy, algorithms, image processing and analysis, distributed and parallel computing to machine learning and AI. This shows that research can progress if state-of-the-art methodology is merged and applied. In the future progress for the field of OPT, this will also be the case. Merging the different aspects of OPT in a smart manner will allow application of OPT in biomedical research to flourish and grow.

In the workflow of imaging the OPT takes a niche for its ability to produce good images of samples in the range of magnitudes of millimetres. Efforts to further optimize

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this will be beneficial in understanding of biological phenomena as well as in imaging workflows in general.