

Defocus Effects on OCTA Signals

A Control Parameter for the Assessment of Retinal Vessel Morphology

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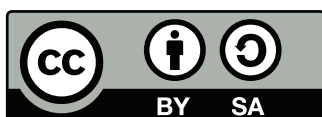
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Abstract

Angiographic imaging for the assessment of the human retinal vasculature is an essential tool in ophthalmology. Optical Coherence Tomography (OCT) Angiography (OCTA) is a contrasting strategy allowing to visualise the retinal vasculature non-invasively based on repeated OCT scans from the same location. Downstream vasculature quantification from OCTA is promising and was therefore extensively explored. Using this approach, diabetic macular ischemia, a poorly understood manifestation of diabetic retinopathy, is studied in this work. Differences in the vasculature density comparing patients and healthy controls, the size of non-perfused areas and relations among vasculature and local retinal sensitivity were assessed. The focus precision was found to be a significant predictor of vasculature quantification results.

Numerous image processing pipelines, metrics and application exploration studies were developed in the context of OCTA quantification. The use of diverse hardware platforms, diverse software implementations and diverse composition of patient groups result in a confusing overall impression, further complicated by the demonstrated limited comparability of quantification metrics among devices and device modes. The overarching rationale in OCTA quantification is revisited and complemented by the concept of the OCTA parameter space. Defocus is identified as one of the influencing factors that deserve additional control. Defocus effects are studied in a well controlled environment, a phantom. First, the influence of defocus on signal strength in OCT and OCTA is characterised. Microfluidic chip phantoms offering channels with well known dimensions were used. The signal strength consequences of defocus are qualitatively different looking at a laterally extended interface in comparison to a laterally limited particle. The effect of defocus on the velocity response in OCTA and the secondary role of the scan lens is studied.

The dependencies found in the phantom studies serve as a foundation to formulate theses for equivalent appearances in clinical imaging studies. Qualitative trends rooted in the presence of defocus are recognised in an image series taken under defocus conditions.

Proposals for a quality control ecosystem around OCTA quantification are discussed. The methodology of this work appears scalable. Allowing for a systematic investigation of the OCTA parameter space, informed decisions on parameter settings in the vasculature quantification pipeline will shed light on the root causes of inter device quantification differences as well as the OCTA signal generation process.

Zusammenfassung

Angiographische Bildgebung ist ein essentielles Werkzeug der Augenheilkunde für die Erfassung und Beurteilung der Netzhautgefäße. Die Optische Kohärenztomographie (OCT) Angiographie (OCTA) ist ein Kontrastverfahren, das aus wiederholten OCT-Aufnahmen des selben Ortes Gefäßdarstellungen nicht-invasiv ermittelt. Die nachgelagerte Gefäßquantifizierung ist vielversprechend und wurde vielfach erkundet. Diesem Ansatz folgend wurde in dieser Arbeit die diabetische makuläre Ischämie, eine kaum verstandene Manifestation der diabetischen Retinopathie, studiert. Unterschiede in der Gefäßdicke zwischen Patienten und Kontrollgruppe, der Größe nicht durchbluteter Areale und Zusammenhänge zwischen erhobener Gefäßgeometrie und örtlicher Lichtsensitivität wurden festgestellt. Die Präzision der Fokuseinstellung wurde als signifikanter Prädiktor der Ergebnisse der Gefäßquantifizierung ermittelt.

Vielfältige Bildverarbeitungslösungen, Metriken und Anwendungen wurden im Kontext der OCTA Quantifizierung entwickelt. Die Verwendung diverser Geräte, diverser Programmierungen und diverser Zusammensetzung von Patientengruppen resultiert in einem unübersichtlichen Gesamtbild, das durch den Nachweis eingeschränkter Vergleichbarkeit zwischen Geräten und Gerätemodi weiter verschärft wird. Die übergreifende Argumentationslinie der OCTA Quantifizierung wurde erneut überdacht und um das Konzept des OCTA Parameterraum ergänzt. Defokus wurde als unvollständig kontrollierter Einflussfaktor identifiziert. Defokuseffekte wurden in kontrollierter Umgebung, einem Phantom, studiert. Zunächst wurde die Signalstärke in OCT und OCTA in Defokusbedingungen analysiert. Mikrofluidische Chips mit Kanälen exakt spezifizierter Abmessungen wurden verwendet. Defokuseffekte unterscheiden sich maßgeblich, je nachdem ob ein lateral ausgeprägtes oder abgegrenztes Testobjekt verwendet wird. Der Effekt auf die Geschwindigkeitsantwort der OCTA und die Rolle der verwendeten Scan Linse wurden studiert.

Die in den Phantomstudien erfassten Zusammenhänge erlauben die Formulierung von Thesen zu äquivalenten Vorgängen in der klinischen Bildgebung. Qualitative, in Defokus verankerte Trends wurden in einer unter Defokus aufgenommenen Bildserie wiedererkannt.

Vorschläge zur Ausbildung einer Qualitätssicherung im Umfeld der Quantifizierung der OCTA wurden diskutiert. Die Methodik der vorliegenden Arbeit erscheint skalierbar zu sein. Auf Grundlage eines umfassenden Wissens zum OCTA Parameterraum werden informierte Entscheidungen bei der Parametrisierung der Gefäßquantifizierung Licht auf die Ursachen der fehlenden Vergleichbarkeit zwischen Geräten sowie der Signalentstehung werfen.

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Abbreviations

DCP	deep capillary plexus 11, 23
DME	diabetic macular edema 13
DMI	diabetic macular ischemia 2, 5, 9, 13, 87, 97
DR	diabetic retinopathy 2, 13
DVC	deep vascular complex 13, 81 f.
ETDRS	early treatment diabetic retinopathy study 12
FAZ	foveal avascular zone 12
ICP	intermediate capillary plexus 11, 23
OCT	optical coherence tomography 1, 3, 15, 17 ff., 21, 56, 69 f., 73, 78, 91, 97
OCTA	optical coherence tomography angiography 1–5, 12 f., 15, 21 ff., 49, 51–57, 59 f., 69 ff., 78, 82, 87–90, 97
RPCP	radial peripapillary capillary plexus 11, 23
SVC	superficial vascular complex 13, 81 f.
svOCTA	speckle variance optical coherence tomography angiography . 21 f., 73 f.
SVP	superficial vascular plexus 11, 23

Introduction

The scientific progress on imaging techniques and their extensive application enabled and drove developments in the field of ophthalmology over decades [1]. The capabilities, limitations and the role of optical coherence tomography angiography (OCTA), a recently introduced angiographic imaging modality based on repeated optical coherence tomography (OCT) scans taken at one location as introduced in figure 1.1, are neither fully understood nor fully developed yet [2]. However, the method is likely to enable and contribute another pathway to discoveries and insights and to serve as a fundamental tool in the clinical practice [3]. Novel developments like the introduction of OCTA naturally come with a large number of explorative studies, leading to the formation of a nonuniform collection of explorative insights. The quantification of the retinal vascular geometry from OCTA was explored in numerous accentuations, sharing the promise of objective data analysis. The commercial availability of devices and analysis methods made the technology available to the clinical research community [2]. The following scientific vision motivates the presented work:

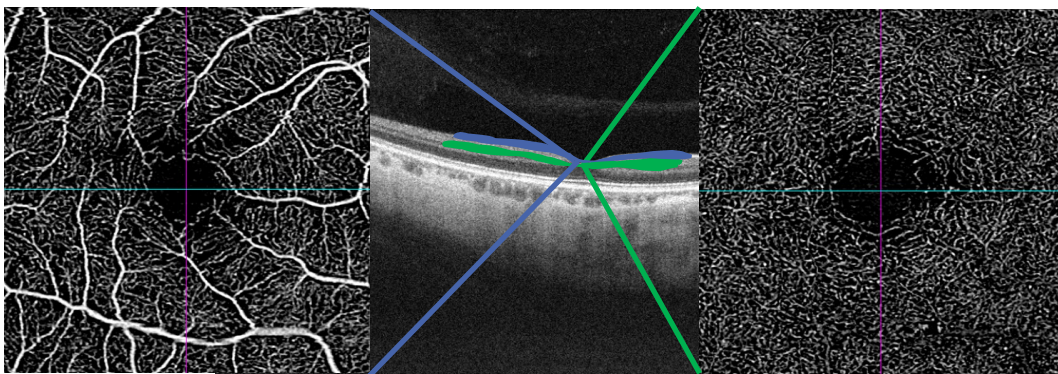


Figure 1.1: Visualisation of an exemplary optical coherence tomography angiography (OCTA) result. Repeated imaging of the retinal cross section, middle image, is performed. Multiple parallel scans are collected. Depth resolved visualisation of the signal fluctuation are presented as en face projections of the superficial layers, left, corresponding to the blue segmentation region in depth and the deep vascular complex, right, corresponding to the green segmentation area.

1.1 Vision: Routine Imaging for Open, Accessible, Credible Science

The current routine visit to a clinic includes assessment of the patient's history, imaging and treatment planning, adjustment or verification. Previous imaging sessions are only accessible on request, if the patient sticks with the same clinic or brings previous findings. The use of patient data for research and quality control is complicated to navigate and therefore limited.

However, the electronic patient record was introduced in Germany already. Access to patient data for scientific studies is planned as an opt-out system, receiving support of patients despite not being ubiquitously known yet [4]. Additionally, registries like the IRIS registry [5], the UK Biobank [6] and oregis [7] demonstrate the value added by large scale, anonymised registries. The development of an infrastructure exploiting data which is routinely collected in the given use case as well as for scientific studies is progressing. OCTA with its noninvasive operating mode, excellent resolution and fast acquisition process [2] is an attractive modality for such registry studies, potentially even for routine data acquisitions secondary to clinic visits occurring anyways. Acquired data will be helpful in later stages of the patient's life. Donated data allows for scientific studies, allowing access to large cohorts and longitudinal data collected without substantial risks for patients and donated upon their declared wish.

The scientist's perspective on routine collection of OCTA data, accessible for scientific studies upon the patient's wish is promising. Large datasets enable studies with increased statistical power, allowing for extensive longitudinal studies, detailed cohort and sub-cohort analysis without the burden of time consuming and expensive study specific data collection. Building upon the same large datasets, enhanced comparability and transparency in method development is to be expected. The quantification of OCTA data is an interesting example. OCTA quantification extracts insightful key figures from OCTA data, e.g. the density of vascular networks. Results are especially insightful if they are directly related to the vasculature anatomy and especially valuable if it offers insight in disease presence or progression. With regard to both, the quantification of OCTA results enables the use of established statistical methods and straightforward longitudinal monitoring.

Diabetic macular ischemia (DMI) is a fuzzy term referring to a reduced macular vascular supply state in diabetic retinopathy patients as compared to healthy individuals. Research on DMI is still in its infancy despite the potential to incorporate it in the progression classification of diabetic retinopathy (DR), the leading cause of blindness in the working age population [8]. Since the vascular phenomenology may be captured, OCTA quantification studies looking at DMI patients contribute valuable knowledge to the scientific and clinical community.

However, significant differences in vasculature assessment and quantification among devices as well as among inbuilt device modes were demonstrated [9–12]. Some authors recommend using the same device, a pragmatic measure for the management of single patients and single centre studies. However, assuming the quantification of OCTA findings is directly equivalent to the quantification of the patient’s retinal vasculature geometry appears to be unjustified. On the contrary, the imaging strategy seems to affect the imaging results and downstream quantification.

Consequently, understanding the details of signal creation and the impact of measurement setup specifics or settings will be crucial to enable the introduced vision of scientific use of the electronic patient record. The need for sub-cohort definition following the OCTA device used is unattractive since the attractive large number of datapoints would vanish. The demonstrated lack of comparability appears as a technological hurdle to be overcome. Contributing knowledge on the underlying physical mechanisms is therefore a significant step towards the presented vision. Identifying the root causes of the demonstrated variability promises to deliver information needed for the informed design of imaging devices, quality control and assurance tools and impacts on downstream imaging and quantification. The demonstration of significant differences among quantification outcomes from various devices, but also different modes within the same device has been an important contribution. The lack of knowledge on root causes prevents standardisation and complicates the introduction of quality assurance tools. Accordingly, the identification and management of relevant control variables and disturbances in OCTA imaging is worthwhile despite being in its infancy.

After the vision for routine assessment of the retinal vasculature was set, the potential for vasculature quantification based on OCTA is explored. Interestingly, automated quantification of dye based angiographic assessments was proposed when OCT was just invented [13]. However, algorithms and pipelines aiming at vasculature quantification from OCTA images were developed aiming for an objective evaluation of the vasculature [14].

1.2 Scientific Questions

The following overarching, broad question is driving the presented work and a significant portion of the scientific discussion on OCTA quantification:

How to design retinal vasculature quantification methodology to support diagnostic and monitoring procedures?

Since Diabetic Macular Ischemia sparked the interest of the author as well as the clinical research community [8], the scope of the research question is focused on the assessment of this poorly understood condition. OCTA emerges as a novel angiographic imaging modality, offering unprecedented insights despite the frequent artefacts [15, 16]. Equipped with the ability to assess the retinal vessels in a depth-resolved manner, excellent resolution

and noninvasive nature, it is an ideal candidate for the assessment. Further on, numerous quantitative metrics derived from OCTA findings were described.

How to quantify the retinal vasculature to assess Diabetic Macular Ischemia?

Which OCTA metrics shed light on Diabetic Macular Ischemia?

Which insights on Diabetic Macular Ischemia arise?

Please note that the quantification of the retinal vasculature geometry exceeds the claim that OCTA results are quantified. Although some aspects of this work may be considered engineering rather than science, the need for scientific progress is uncovered right away. OCTA imaging is far from being fully understood. Although plausibility studies [17] and comparisons with histology [18] were performed, the details of signal creation remain poorly understood [2]. A large number of hardware platforms, device scan rates, employed scan patterns defining the various scan sizes and lateral sampling density options, angiography algorithms and quantification strategies were developed, leading to a rich, yet confusing overall picture [19].

How to develop OCTA towards an even better understood, more precise modality?

How to systematically investigate the OCTA behaviour following on the variation of technical details (parameters)?

1.3 Problem Statement

In order to advance the claim of OCTA quantification from image quantification to the quantification of retinal vessels, several problems preventing the immediate implementation need to be addressed.

1. The OCT hardware and software implementation in commercial devices are unstandardized. The effects of hardware and software choices on downstream vasculature quantification are unknown and their large number complicates the identification of associations.

Proposal: Identify a list of potential influences. Rank for practical significance in everyday clinical applications. Find a way to study the prioritised influences and characterise their role. Combine impacts to understand the full picture.

2. The interest in findings is strong, forcing the contribution of immature quantitative studies. The inherently interdisciplinary nature of the research further complicates the conduction of coherent, reproducible studies. However, reproducibility, verification and falsification are the cornerstones of scientific activity.

Proposal: Contribute to transparent research practices, spread expertise in accessible ways, make the realisation of valuable contributions as straightforward, simple and rewarding as possible. At the same time, oppose intransparent and obviously erroneous approaches.

3. The novelty of the approach comes with lacking nomenclature and vocabulary suitable to undoubtedly describe the unprecedented findings.

Proposal: Continuously develop a shared, transparent, intuitive vocabulary.

1.4 Structure of this Work

This dissertation is structured in the following way: Fundamentals and the State of the Art are presented in part I. Studies on DMI applying OCTA quantification are presented in part II. Among them, relationships among imaging parameters, focus precision in particular, and downstream quantification results were found. This finding sparked interest in the role of technical constraints coming with the device choice, potentially explaining parts of the variable quantification outcomes from various devices imaging the same patient. The concept of the OCTA parameter space is introduced and an experimental setup for its exploration is developed in part III. Exemplarily, the influence of defocus on the OCTA signal and images is characterised in part IV. Early explorations on quality assessment tools beyond the signal strength assessment are discussed in part V along with concluding remarks on the work.

PART I

FUNDAMENTALS AND STATE OF
THE ART

Fundamentals in Ophthalmology

Studying the human eye and its diseases is at the core of ophthalmology. On the one hand, studying ophthalmology is mandatory to maintain the vital function of the eye and to bring relief to those affected by occurring diseases. On the other hand, it has been a fascinating field of research on its own for centuries. Covering the knowledge on the eye is out of reach, the interested reader is referred to extensive introductions like [20]. Engineers and physicists may prefer the technical presentation in [21, 22].

The following section introduces the most important concepts needed for a first orientation on the anatomy and function of the eye. A more detailed introduction of the healthy retinal anatomy and vasculature will follow. Diabetic Retinopathy is introduced with a focus on changes in the macular vascular system, especially diabetic macular ischemia (DMI).

2.1 Anatomy of the Healthy Human Eye

First, the surroundings of the human eye are briefly discussed for an overall orientation. It is generally known that the human visual system consists of two eyes, located left and right of the nose at the front of the head. Each eye is surrounded by a bone structure, the eye socket, eye lids and periocular fat. The eye is held in place and rotated by a set of muscles, given in figure 2.1 a). The optic nerve leaves the eyeball towards the posterior direction, refer to the yellow structure in figure 2.1 c). It connects each eye to the optic chiasm, passing the optic canal [20]. From there, information is passed to the visual cortex, located in the occipital lobe [20]. Each eyeball's shape is approximately a sphere of 23-24 mm diameter [20].

Moving on to the interior structure of the human eye. The eyeball's outer layer consists of the sclera and the cornea in the anterior part. The sclera is a white, protective layer containing mainly collagen and some elastic fiber. It supports the eye's shape. [23]. The cornea is a transparent structure consisting of collagen, appearing in an ordered arrangement forming multiple layers. The cornea is a curved structure, roughly 500 μm in thickness [20, 24]. The cornea, iris, lens and aqueous humor form the anterior chamber of the eye, visualised in figure 2.2 a).

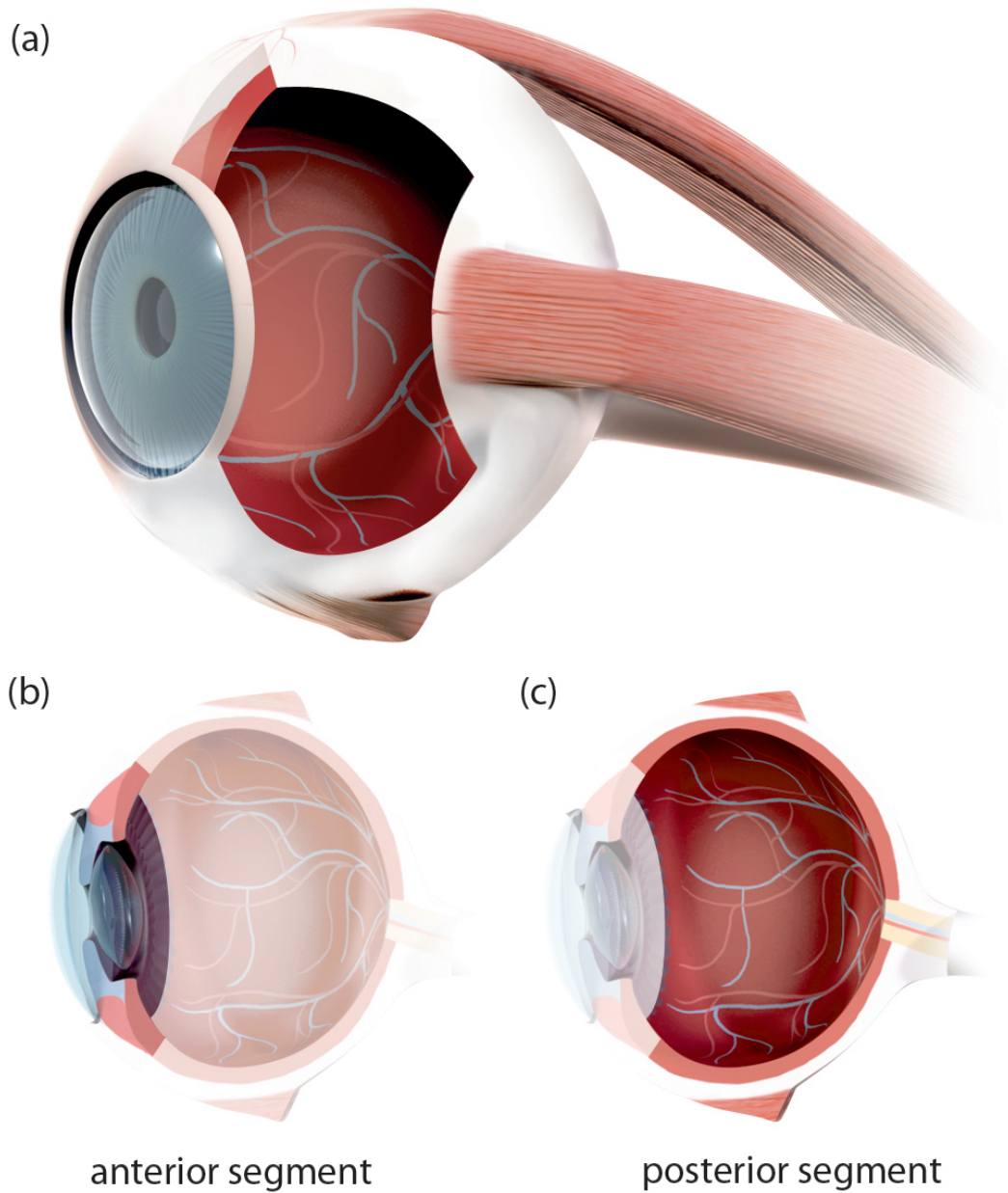


Figure 2.1: The eye is mechanically stabilised and rotated by a set of muscles a). The eyeball consists of an anterior b) and a posterior segment c), divided by the crystalline lens. Reproduced with permission from [22]. The author acknowledges this permission.

Posterior to the crystalline lens, the vitreous body fills the posterior part of the human eye. It is a transparent gel consisting of water and collagen. It provides structural support to the eye and was found to be involved in nourishing the eye [25]. Although various substructures were described [25], it is typically considered a transparent, homogeneous medium in the context of retinal imaging. Imaging of the vitreous itself was demonstrated [26].

The function of the eye and the basics of the vision process are introduced by following the path of the light. The sun emits light, which reaches the earth and illuminates our surroundings. Light is scattered, reflected either in a specular or diffuse manner or transmitted to the human eye. Refraction occurs at the cornea and lens, leading to the formation of an image of the surroundings at the retina plane. The image is reduced in size, vertically and horizontally flipped. The pupil acts as an aperture stop, limiting the total incident power on the retina. The refractive power of the eye is determined by the combination of the cornea radius and shape with its fixed refractive power, the refractive index of the aqueous humor and the crystalline lens in its accommodation status. Accommodation refers to the eye's ability to elongate or relax the lens by relaxing or contracting the ciliary muscle and connected suspensory ligaments [27].

2.2 The Retina

The retina encloses the vitreous body in the posterior chamber. The retina is a multilayered, skin-like, transparent tissue covering the full posterior portion of the eyeball. Its function is the conversion of incoming light stimuli into electrical and chemical signals appropriate for further processing within the retina itself and later on in the visual cortex. Interestingly, the photoreceptor cell layer, the group of cells absorbing incoming light is located at the outside of the globe. Inner layers are made up of various cell types ensuring nutrient supply and information transfer. See figure 2.2 b) for a schematic of the three especially notable structures within the retina. The optic nerve head is the exit point of the optic nerve. The macula and its center, the fovea are specialized regions consisting of only cones. The macula is the center of vision, enabling people to read and see details in general. Looking at its structure, there is a visible notch. For additional details on cell types, functions and signal pathways, the interested reader may refer to [28].

2.3 The Retinal Vascular System

The retina is supplied by a specialised vascular system. Note the vascular system as given in figure 2.2 b). The ophthalmic artery, a branch of the internal carotid artery [29] supplies blood to the posterior eyeball. It bifurcates further and forms the central retinal artery as well as the posterior ciliary artery. The posterior ciliary artery in contrary nourishes the choroid and choriocapillaris. The central retinal artery enters the eyeball within the ocular nerve, branches out immediately and covers the full posterior pole with a dense capillary network. This network is organized in three to four distinct layers as described by Campbell et al. [30]. From the innermost to the outermost layer, those are the radial peripapillary capillary plexus (RPCP), superficial vascular plexus (SVP) including the larger caliber vessels, intermediate capillary plexus (ICP) and deep capillary plexus (DCP) consisting exclusively of capillaries. In a lateral perspective, the fovea comes with a unique characteristic, the foveal avascular

zone (FAZ). It is an approximately round structure free of vasculature. Around the optic nerve head, a complex and dense capillary bed exists. The vascular density is in general higher in the central posterior pole, the region around optic nerve head and macula, compared to the periphery. Looking at vessel sizes, small arteries with diameters of 300-40 μ m branch to arterioles with diameters 40-10 μ m and further to capillaries with diameters of 5-10 μ m. Analogue diameter ranges present on the venular portion of the network [31].

2.4 Diabetic Retinopathy

Diabetes Mellitus is a systemic disease characterised by inadequate blood glucose level control. Elevated blood glucose levels maintained for extended durations are assumed to be the shared root cause of Diabetes Mellitus complications. Multiple types exist. Type I and type II diabetes are commonly known. The diabetes prevalence is rising, as discussed in [32] with a view on Germany and in [33] with a global perspective. Consequently, multiple national governments offer educational material, implement monitoring structures and discuss countermeasures [34]. Diabetes Mellitus creates multiple secondary diseases typically following the malfunction of the microvascular system. Among them, the ocular manifestation of Diabetes Mellitus is named Diabetic Retinopathy.

This introduction covers a brief description of the context before moving on to imaging features. Readers interested in an introduction to Diabetes Mellitus are referred to [35, 36]. Readers interested in Diabetic Retinopathy will find an introduction [37] and an extensive collection of the current understanding in [38], while guidelines contain information on the treatment in the United States [39] and Germany [40]. Those interested in the role of imaging [41] or optical coherence tomography angiography (OCTA) in Diabetic Retinopathy will be interested in specialized reviews [42, 43].

The description of the stages of Diabetic Retinopathy was investigated in a large scale clinical trial, the early treatment diabetic retinopathy study (ETDRS). The stages of the disease are linked to the presentation of the vascular system, as described in ETDRS report number eleven [44].

To date, the classification stage definition differs slightly in the United States compared to Europe [45]. The underlying natural history is incompletely understood. Elevated blood glucose levels over an extended time period were proposed to lead to the death of pericytes, a cell type integral to the structure of capillaries [46]. However, there is an ongoing debate on their role, the timeline as well as the role of neighbouring cell types and mechanisms [47]. Neurovascular coupling is frequently discussed since signs of an altered behaviour were measured before the onset of the disease as defined by vascular signs only [48].

Vascular abnormalities, namely microaneurysms, hemorrhages, venous beading and cotton wool spots occur in the early stages [45]. The more advanced stage, proliferative

diabetic retinopathy, is to be determined once vitreal hemorrhage or neovascularisation is found [45]. diabetic retinopathy (DR) is a slowly progressing disease without the need for urgent intervention. However, once diabetic macular edema (DME) sets on, timely intervention is required. DME is a complication of DR characterised by the accumulation of fluid within the retina. The presence of the fluid accumulation complicates nourishment and waste transport, therefore immediate treatment, typically intravitreal delivery of anti vascular endothelial growth factor (AntiVEGF) or steroids is indicated.

The vascular findings determining the state of the disease make imaging inherently effective when it comes to diagnosis and monitoring. Automated analysis is attractive in this context, the automated detection of microaneurysms from fluorescein angiography was discussed more than 30 years ago [13]. Numerous studies on screening have been revisited [49], notably including the first commercial artificial intelligence applications [50, 51].

2.5 Diabetic Macular Ischemia

The cornerstone of DMI is a reduced capillary density compared to an age-matched healthy control. The process of capillary loss is frequently referred to as capillary dropout. The scientific interest in DMI is rising despite the lack of a generally accepted definition of the term itself. The current interest occurs in parallel to the spread of OCTA. Both aspects, the knowledge on DMI and the knowledge on OCTA evolve in a complex interplay as adding to the understanding of imaging mechanisms leads to an extended methodology for the investigation of DMI and additional knowledge on DMI informs the future development of OCTA. OCTA quantification results were proposed to be applied as a basis for the definition of DMI [8] despite the determination that "OCTA scan quality must be improved" concluded in a study from a major symposium, the DRCR Retina Network [52]. The existence of phenotypes affecting primarily the superficial vascular complex (SVC) or deep vascular complex (DVC) was proposed [8].

Despite a still evolving definition, the following statements will be accepted by many: An enlarged, non-round Foveal Avascular Zone is a sign of DMI. A decline of the number of perfused capillaries over time is a sign of DMI. An increase in inter capillary space over time is a sign of DMI. Increasing number or extent of non-perfused areas is a sign of DMI.

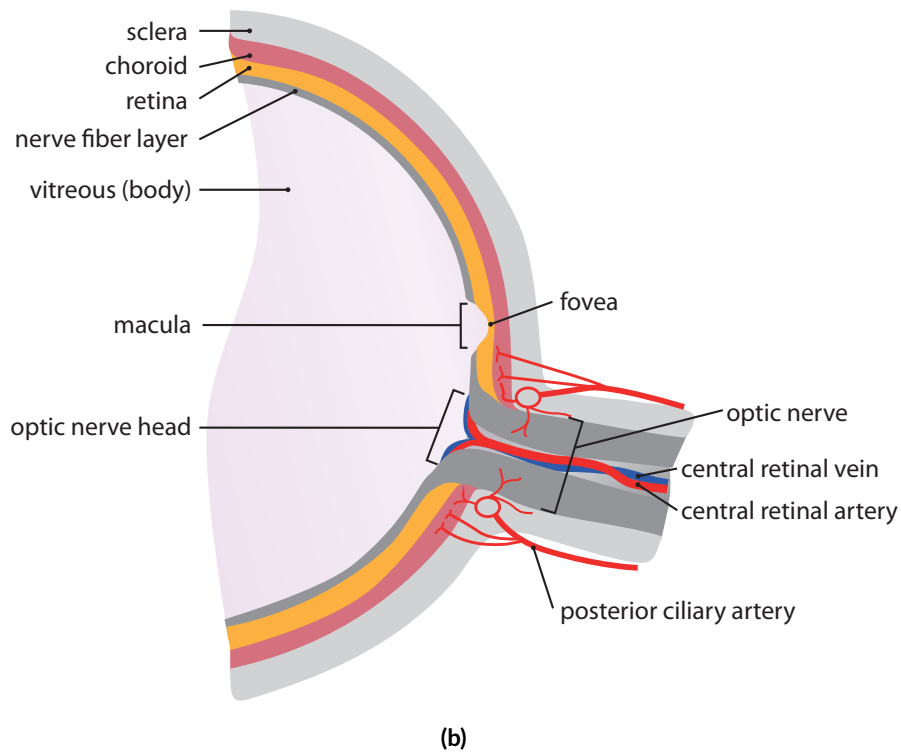
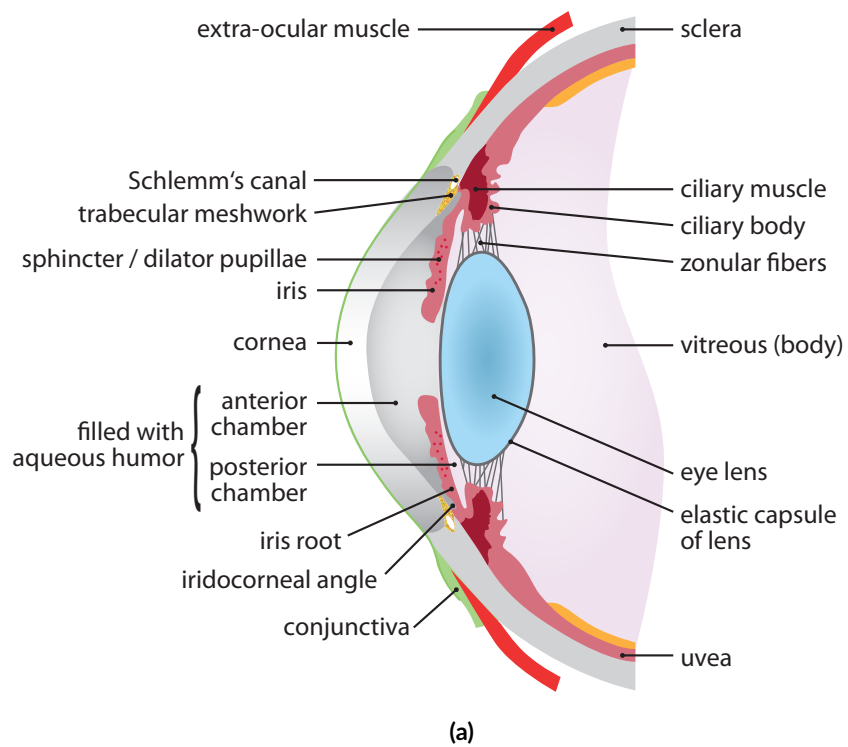


Figure 2.2: Detailed anatomy of the anterior a) an posterior segment b). Reproduced with permission from [22]. The author acknowledges this permission.

Established Eye Examination

Fundamental eye examination techniques are well described in books collecting the basic knowledge. Among them, this publication offers numerous pictures [53], while a comprehensive overview is found in Kaschke et al. [22]. The assessment of manifold aspects of the human eye and its function is made possible by the availability of a rich set of methodology. A brief overview of the established methodology is presented before coherence based imaging technologies, namely optical coherence tomography (OCT) and optical coherence tomography angiography (OCTA) are introduced in more detail.

Several fundamental yet insightful assessment methods are routinely performed without the need for advanced technology. The examiner will ask patients about the symptoms occurring, disease history and systemic diseases. The outer structure of the eye is assessed exercising visual inspection of the outer appearance, possible inflammations, injuries and asymmetries among left and right eye. Intraocular pressure may be measured using one of multiple known measurement strategies. The refractive status of the eye is assessed objectively using an autorefractometer, retinoscopy, sometimes referred to as skiascopy. The refractive status is subjectively assessed as well, using standardized sight testing charts. Results are expressed either in the logarithm of the Minimum Angle of Resolution (log-MAR), Snellen notation or Best Corrected Visual Acuity (BCVA). The assessment in one of the multiple formats may be equivalent when looking at the larger picture, but deviates in details. Not all the assessment charts are equivalent with regard to the assessed measurement range, a fact leading to complications in the discussion of results [54]. Testing the ability to distinguish colors using colored cards containing numbers or letters, the ability to follow a moving target with both eyes in an expected manner and the reaction of the pupil following a bright illumination stimulus further add to the methodology.

3.1 Common Imaging Technologies

Viewing the eye with and without technical support systems is ubiquitously applied, from scientific study setups to routine clinical practice. Simple optical arrangements called ophthalmoscopes allow the user to illuminate the patient's eye and enable usable magnification. In addition, avoiding the need to come very close to each other is another applicative advantage enabled by their use when compared to direct ophthalmoscopy.

The eye's structure is further examined using a slit lamp, offering a magnified view of the anterior chamber under well controlled, adjustable illumination. The findings allow for a check of structural integrity, allow for checking the curvature of the cornea as well as a potential clouding of the lens due to cataract. The posterior structure can be viewed using a slit lamp with additional loupes. Color Fundus Photography is an imaging techniques allowing for the photography of findings.

Although some blood vessels are apparent in the introduced imaging modalitiies. Angiography, the ability to visualize and describe blood vessels, was advanced by the invention of fluorophore-based imaging techniques. Fluorescein angiography (FA) and indocyanine green angiography (ICGA) are available for complimentary assessment of the vascular system. While FA reveals details on the superficial vasculature, deeper structures are revealed in ICGA. Both share the ability to discover leakage.

3.2 Perimetry and Microperimetry

Perimetry is done in order to assess the visual field of the tested patient. Therefore, a light stimulus of defined size and brightness is delivered to the patient's eye. Upon recognising a stimulus, the patient is asked to press a button. A standardised analysis reveals the spatially resolved retinal sensitivity, typically expressed in dB [55]. Variations were introduced in order to keep the number of tested points and therefore the time spent during testing to a minimum [56] and to assess spatially refined sensitivity patterns in and around the macula region with a technique called microperimetry [57]. In order to compensate for involuntary eye motion, the microperimetry device is equipped with a fundus tracking system, allowing to stimulate selected regions precisely [57].

3.3 Optical Coherence Tomography

Starting from the term, the reader is asked to analyze the inherent meaning of the term Optical Coherence Tomography. Starting with tomography, a term referring to the information gained when slices of a specimen were to be cut and viewed from the side. The term originates in greek and refers to cutting. Optical Coherence is exploited to assess information resembling histologic cuts either directly or in several steps. Since multiple implementations

of OCT were realised, differing in setups, components and applied strategies, starting from the objective is advised. However, the name can be interpreted in its original order - OCT is an optical, light-based method exploiting coherence characteristics to generate tomographic images.

Becoming familiar with OCT is associated with a steep learning curve [58]. Multiple concepts need to be known, understood and applicable by the learner. Several introductions to OCT can be found online as well as offline. If you are a starter, please refer to overviews on the history and introductions [59, 60]. Additionally, excellent introductions for the broader public may be found in the format of blog entries, tabular summaries, tutorials and animation-supported explanations in videos.

Advanced introductions for those familiar with OCT basics and those interested in research are found in and recorded lectures [61], books [62], frequent review articles [59, 63] and at leading conferences in the field.

Three different interferometric implementations of OCT were developed until today. There are full field and line field variations of OCT, see [64]. Time Domain OCT, Spectral Domain OCT and Swept Source OCT differ in the choice of the light source and detector type. Light sources are generally high intensity light sources, while the light transmitted to the sample is minimised [64]. While a broadband, low coherence light source like a superluminescent diode (SLD) is used in TD OCT and SD OCT, a rapid swept laser source is used in swept source OCT.

3.3.1 Brief History of Optical Coherence Tomography Development

Historic considerations were applied numerous times to present the complicated, diverse research landscape and application of OCT in an accessible manner. D. Huang was the first covering OCT and coining the term [65]. There was notable prior work, for example [66]. The presented approach was later called Time Domain OCT. A notable characteristic of this publication is the focus on the use case in ophthalmology, especially retinal imaging. This cooperation from the very beginning was reflected upon in [67]. The interplay of laser laboratory experts and ophthalmologists was crucial for the early development of OCT - and is of utmost importance until today [67]. Optical Coherence Tomography was commercially introduced right away with the first product for ophthalmology being released in 1996 [67].

OCT is frequently compared to (medical) ultrasound imaging since both modalities rely on the capture and analysis of reflected waves from structures in the human body. The imaging is aimed at unveiling the reflecting structures from reflection intensity and runtime. In contrast to ultrasound, the runtime of light reflections are captured indirectly, based on interferometry. The speed of light prevents a direct measurement.

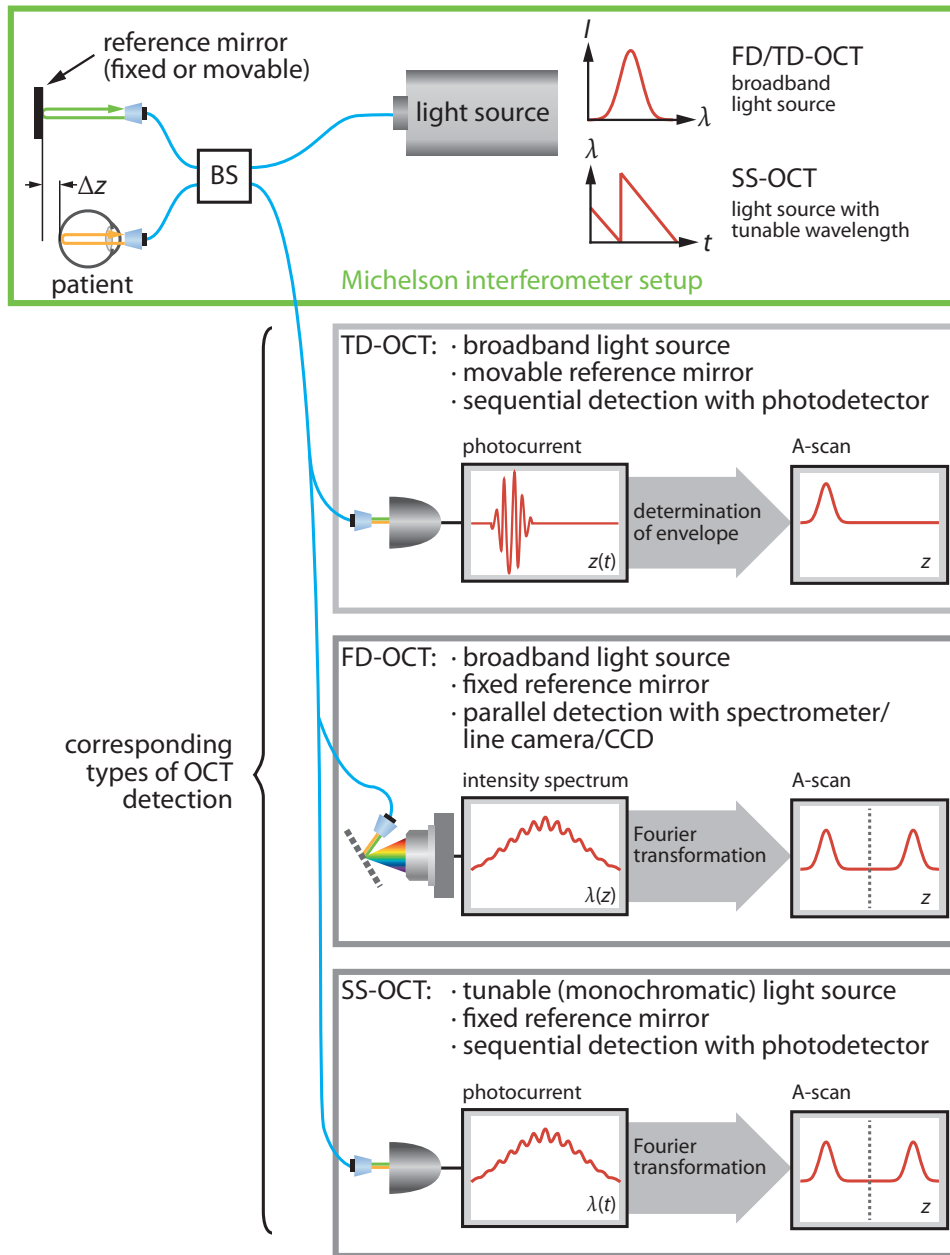


Figure 3.1: Three implementations of OCT were realised. The light source and the detector differ among these techniques. Reproduced with permission from [22]. The author acknowledges this permission.

Aiming for the reconstruction of tomographic information, this first developed strategy to obtain OCT data is rather simplistic. An interferometer is set up and used. Although the physical arrangement is not binding, this introduction will refer to a Michelson setup as schematically drawn in figure 3.1. The light emitted from the source is characterized by a bandwidth in the range of several ten nanometers, resulting in a spatial coherence length of several microns. The light reaches the beam splitter where it will be split accordingly. The

reference portion will travel to a mirror where it is backreflected. The light traveling in the sample arm illuminates an object of interest, typically the human eye. The sample arm spot location is scanned across the sample to generate cross sectional images from consecutive single scans. Backscattered light will be recombined with the reference light, leading to a superposition of the reflections from sample and reference arm at the detector. A part of the energy leaving the source will travel back to the source, since the beam splitter is crossed twice. Adjusting the reference path length by translating the reference mirror will result in detectable interference effects, constructive and destructive interference, when the two beam pathlengths do not differ more than half the coherence length of the light. Therefore, translating the reference mirror and recording the resulting signal allows for an absolute measurement of the z-offset as an optical distance of the sample mirror compared to the reference mirror. By measuring the reference beam path length is measured, an absolute pathlength measurement can be performed. A perfect match of the pathlengths in sample and reference arm is called zero delay. In the case of posterior ophthalmic imaging, the illumination must pass the pupil to hit the retina. Typically, telecentric 4f-scanners are used to enable the optical projection of the scanner plane to the patient's pupil plane [68].

In 1995, Fercher et al. developed and demonstrated the operation of the first known Fourier Domain OCT. They conclude: "As this technique can be very fast and yields the distribution of the object scattering potential along the illuminating light beam it can also be used in partial coherence tomography" [69]. The superior imaging sensitivity, allowing for superior imaging speed was demonstrated in 2003 [70–72]. The increased speed was of importance in the clinical practice. The patient will move his / her eye at some point. A fast imaging device enables to be done before the next inevitable saccade. To date, Time Domain OCT is only applied in specialised applications, while the ophthalmic imaging sector is dominated by Fourier Domain OCT [59].

Fourier Domain OCT employs a broadband light source and a spectrometer. The reference mirror is not translated but fixed. Interference fringes in the spectrometer readout are analysed by remapping linearly to the wavenumber space before a Fourier transform is applied to reconstruct the A-Scan.

In the practical, clinical application, OCT devices are tabletop devices. Two persons are present, the patient and the device operator. The operator cleans the device, asks the patient to sit down in front of the device and to fixate the target. Optical alignment of the device and the patient eye, focusing and adjustments of the reference arm length are done. Then, the actual imaging starts. Within a few seconds, the necessary information is collected without any pain.

Optical Coherence Tomography Angiography (OCTA)

Optical coherence tomography angiography (OCTA) is a term communicating the goal of the named methodology rightaway. Achieving a contrast is the goal. Namely, imaging regions which are part of the vascular network must produce a differentiable signal when compared to imaging regions outside of the vascular network. This goal must be reached applying optical coherence tomography (OCT) imaging. Repeated scans at the same location are generally analyzed for signal fluctuation to generate contrast. This contrast is then attributed to the motion of red blood cells, as the frequently used term "motion contrast" indicates [73]. A number of algorithms was developed, reviewed and classified based on the information included as well as compared among each other [2, 74]. The reader is referred to these reviews and the referenced original literature if details of the specific algorithms or the overall development are of interest. Notably, OCTA algorithms and scan strategies were developed in parallel as manifold combinations are possible. For example, [75] simply employs the algorithm presented in [76] along the slow axis instead of the fast axis or as the authors called it: "The OMAG algorithm is then applied on C-scan direction (elevational direction), rather than B-scan direction (lateral direction) as in the conventional approach." [75]. For the sake of this brief introduction, the existence of various algorithms was clarified. Various algorithms are implemented in clinically applied systems as well. One of the simpler algorithms speckle variance optical coherence tomography angiography (svOCTA), the algorithm employed in this work is will be the only algorithm introduced in more detail.

4.1 Speckle Variance OCTA

As reported by A. Mariampillai et al. in 2008 [77], svOCTA is "a simple and computationally efficient microvascular detection algorithm based on interframe speckle variance processing of structural image intensity". [77], In detail, svOCTA is the result of variance calculation considering multiple revisits of the same location. Mathematically, svOCTA may be given in

the formulation published in their follow-up publication [78]

$$SV_{ijk} = \frac{1}{N} \sum_{i=1}^N \left(I_{ijk} - \frac{1}{N} \sum_{i=1}^N I_{ijk} \right)^2, \quad (4.1)$$

where j, k are referring to the j th transverse and k th axial pixel within the i th frame out of N total frames taken at the same location. Note the absence of any scan pattern assumption as well as the absence of any time dependency in the presented formula. The application potential of svOCTA is therefore versatile.

4.2 OCTA Signal Generation

The details of the OCTA signal generation remain unclear. There is no extensive model describing the signal generation yet. Typically, "motion contrast" is named as the underlying cause of the signal generation [73]. However, several studies were prepared uncovering the importance of scatterer velocity [79, 80] paired with the time between successive scans [80, 81], scatterer concentration [80], and orientation with regard to the probe beam [82]. The combination of scatterer velocity and inter scan time is explored with the goal of flow velocity quantification [83–85].

4.3 OCTA Commercialisation

On the commercial introduction of OCTA, several references report the year 2014 [58, 86, 87], whereas others refer to an FDA approval in 2016 [88]. With the commercialisation, wide availability for the clinical research community was achieved as stated in [2]. Notably, competitors rapidly followed up on the introduction of OCTA, offering variants of the technique. Today, more than ten device types are available. Clinical acceptance of the technique was investigated in an Italian study [89], reporting a decline of fluorophore - based angiography procedures in parallel to the rise of OCTA procedures. The use of OCTA based endpoints for clinical trials was discussed [90]. The introduction of commercially available quantification analysis software, typically as a part of a software package coming with the device followed. Today, several devices come with inbuilt quantification routines. An overview of the available devices for posterior OCTA imaging is presented. The list is to be seen as a plain enumeration without any meaning of the order. Specifications are available from the according suppliers.

- Zeiss CIRRUS / PlexElite
- Heidelberg Engineering Spectralis
- Visionix Optovue Avanti
- Canon OCTA-A1
- TowardPi BMizar
- Topcon DRI Triton
- Intalight DREAM
- Topcon DRI Triton
- Optopol devices
- Moptim Mocean + Vascan module

4.4 Retinal Layer Segmentation and Data Visualisation

OCTA allows for detailed investigations of the human retinal vasculature *in vivo*. Among the various descriptive publications, Campbell et al. [30] is remarkable since it introduces understandable demarcation criteria to differentiate four depth resolved vascular plexi. From the innermost to the outermost vascular plexus: radial peripapillary capillary plexus (RPCP), superficial vascular plexus (SVP), intermediate capillary plexus (ICP) and deep capillary plexus (DCP). The findings are aligned with expectations derived from prior histology studies in macaque monkeys [91, 92].

Following this proposal, all commercial vendors offer a variant of this retina subdivision and variants of the introduced nomenclature. Note however, that the details of plexus demarcation definitions differ among the available devices. Computer vision algorithms were developed, allowing for the automated segmentation of retinal layers and vascular plexi [93, 94]. However, the segmentation is named as a source of artifacts [95]. Once segmented, the layer structure is projected to the en face visualization using either mean or maximum intensity projection [96]. The methodology may be repeated with a view on the various capillary plexi.

4.5 OCTA Quantification

OCTA quantification refers to a set of methodologies translating OCTA imaging results to a set of numbers. The application as well as the methodology itself is diverse. Multiple reviews deal with OCTA quantification, referring to a wealth of original studies each [86, 88, 97]. The rationale of OCTA quantification activities is straightforward: Numbers are automatically generated based on angiographic imaging, therefore supporting an objective, time-efficient analysis workflow [88]. The potential of neural networks and computer aided diagnostics applied in various roles across the data acquisition and analysis was reviewed separately [98].

Original studies on OCTA quantification are manifold, ranging from simplistic technical approaches [99] over more extensive technical considerations [100] to the manifold applications in clinical research [101]. Drawing a complete, coherent picture of the state of the art is impossible since numerous homemade algorithms are developed and applied for various use-cases, tailored to the use with one of various devices and secondary applications. In addition, some of the algorithms remain opaque in terms of their operation scheme. In fact, this combination of various aspects coming with the potential for inconsistency raised criticism in the scientific community. Significant differences among vasculature assessment and quantification results using different devices or device modes were found [9–12]. Calls for standardisation along with a pathway were published [19, 97], transparent quantification

pipelines [102] and open source software projects arose [103–105] during the development time of this work.

Datasets are a valuable resource for any computer vision development project. While the variation among imaging datasets from various modalities is large, the variation of data from identical imaging modalities is limited. Potential variations are simply exhausted at some point. Looking at OCTA, the variability is noteworthy. The several publicly available datasets may serve as a starting point: [106–108]. There are several additional datasets which are a bit harder to find [109], OCTAGON and FOCTAIR. First challenges were realized [110].

Moving on to frequently used algorithms, vasculature segmentation algorithms are applied to distinguish the vasculature, considered the foreground, from background. The technical implementation frequently incorporates thresholding strategies, Gabor and Frangi filtering as well as algorithms relying on optimally oriented flux or neural networks [98, 100, 103]. The use of various segmentation methods was found to lead to various quantification results and conclusions [111]. Skeletonization is commonly applied, creating a one pixel centerline from the binarized images.

A large variety of quantification metrics was proposed, typically occurring as the endpoints of the presented studies. Quantification metrics are used to compare groups among each other, to assess correlations with age [112], disease stage [113] and visual function in glaucoma [114]. Several reviews present overviews of the quantification endpoints [86] and the use of quantification as endpoints of large scale trials was discussed [90]. Standardisation approaches propose unified nomenclature of the quantification results [19, 115] which will be adopted if not explicitly defined differently. A growing number of studies comments on imaging settings like scan speed affecting the quantification outcome [116].

The detailed methodology applied for each quantification study is introduced in the according chapter. For a more detailed introduction to single approaches, the interested reader is referred to the named review articles in combination with the studies themselves. You are advised to limit the scope of your review to a specific algorithm, a disease or a device.

OCTA QUANTIFICATION STUDIES

Optical Coherence Tomography Angiography Quantification is a promising approach to gain in depth knowledge on the state of the retinal vascular network of individual patients in a reasonable time. Comparisons of individual quantification results to a normal range as well as longitudinal trend analysis offer additional insights. Quantification results are especially insightful if the presence of a disease is indicated, if correlation to a disease progression is found and if those findings are undisturbed. This especially refers to being free of technical confounders, sowing the seed of doubt in these studies. The following studies on vasculature morphology markers were conducted with a main interest in Diabetic Macular Ischemia. Quality control measures have been set as an endpoint, but have not been incorporated yet. Instead, the presented studies motivated the parameter space study.

Local ischaemia morphology in diabetics differs from healthy controls

The following chapter was published [117] and will be cited in its entirety.

Aims/Purpose:

To evaluate local macular ischaemia morphology additional to global metrics in diabetic macular ischaemia (DMI) patients and healthy controls.

Methods:

The study was registered: DRKS-ID DRKS00024399. Informed written consent was obtained from all participants. Seven DMI patients and seven patients after cataract surgery were included as healthy controls. 10 by 10 degree Heidelberg Spectralis OCT Angiography was recorded and divided to superficial / deep vascular complexes (SVC / DVC) following the inbuilt segmentation. Further processing was done in MATLAB R2022a. After binarization following Otsu's method for threshold determination, minimum distances to blood vessels were calculated for each pixel applying a sliding window. More than 30 μm distance to the next vessel was considered ischaemic. Morphology analysis of the resulting ischaemic lesions was conducted for SVC and DVC separately. The total ischaemic area; mean ischaemic area per lesion; area and circularity of the second largest ischaemic lesion—avoiding the foveal avascular zone—was extracted. Two-sample Student t-tests were performed testing differences of healthy controls and DMI patients in SVC and DVC for each of the four named metrics. Statistical significance was defined when $p < 0.05$.

Results:

Analysing total ischaemic area, statistically significant differences were found for SVC and DVC data. Mean ischaemic area per lesion was significantly larger in DMI patients in SVC but not in DVC. The area of the largest ischaemic lesion avoiding FAZ was significantly larger in DMI patients in both SVC and DVC. Lesion circularity did not reveal significant differences.

Conclusions:

Supplementing reported global nonperfusion differences, local nonperfusion morphology differs significantly between DMI patients and healthy controls. Findings need to be replicated in a larger trial in the future. Research on structure function correlation in areas of nonperfusion will shed light on clinical implications.

Or in slightly different words: In the given small study, the spatial arrangement of non perfusion areas which we called ischemia morphology, was quantified from OCT Angiography data. Quantification results from a group of diabetic patients was compared to metrics from postoperative cataract patients without Diabetes, considered being healthy controls. Significant differences were found in the superficial and deep vascular complex looking at the second largest ischemia area – avoiding the assessment of the foveal avascular zone –, in the total ischemia area and in the superficial vascular complex looking at the average area of ischemia lesions. Assessing the Deep Vascular Complex however, there was no significant difference to be found in average area. We conclude that OCT Angiography enables morphologic macular ischemia assessment, adding to the frequently reported Perfusion Density, a measure of overall vascularization status. The developed quantification measures confirmed expected differences in ischemia extent comparing diabetes patients in clinical care to healthy controls. As this is a small study, verification of the findings in a larger trial will be needed. Longitudinal progression of ischemia areas as well as structure function correlation remain unclear. Shedding light on progression and structure function correlation will help determining the role of morphologic ischemia analysis in clinical Diabetes monitoring. Let's have a closer look at the methods. Data was recorded using the Superficial and Deep Vascular Complex en face projections as segmented by the Heidelberg Engineering Spectralis device. Scale extraction, appropriate cropping and binarization following Otsu's threshold selection were performed. Ischemia pixels were identified as follows: Each pixel was examined applying a sliding window algorithm. If the closest segmented vessel was further than 30 μm from the given pixel, this pixel was considered ischemic. The 30 μm threshold was chosen based on the reported inter-capillary distance of 30 μm in an extensive review by CMG Cheung on Diabetic Macular Ischemia. We further analyzed the number of pixels forming the second largest ischemia area – the largest one besides the foveal avascular zone –, the overall number of ischemia pixels and the mean number of pixels per ischemia area. Quantifications from the diabetic patient group and healthy controls were visualized in the given boxplots, figure 5.1. Two sample t-tests were performed, resulting p-values are added to the boxplots.

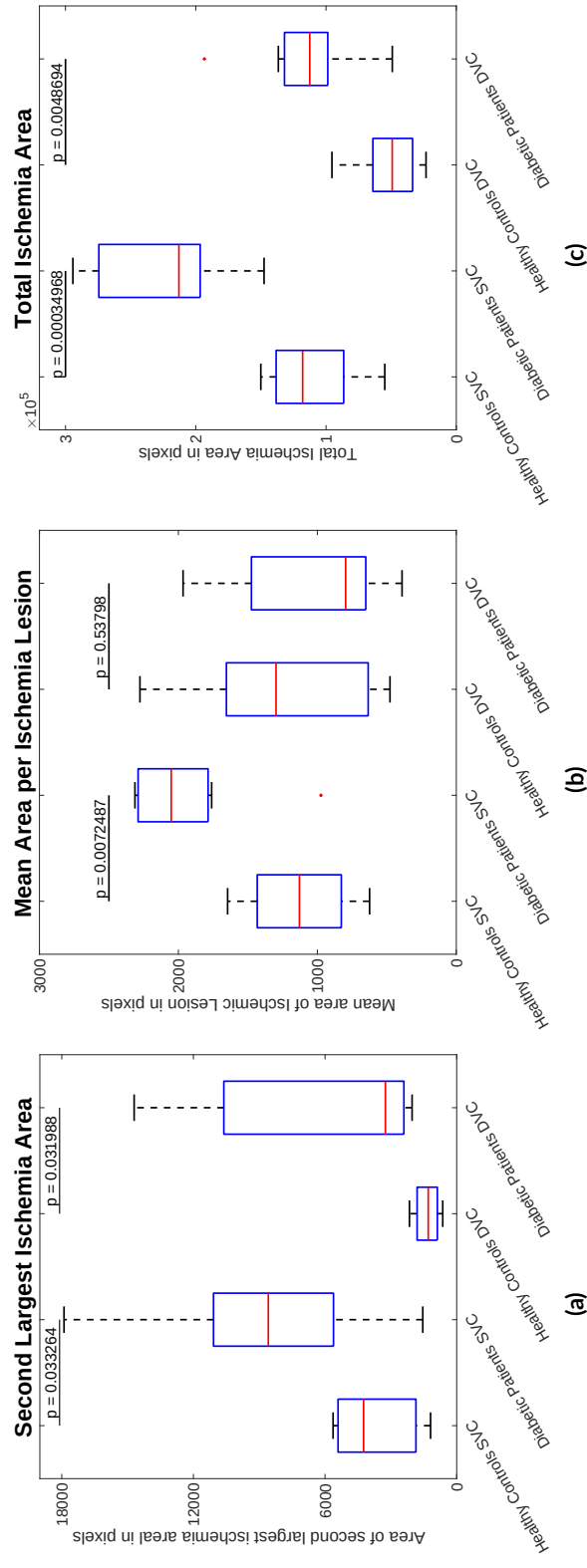


Figure 5.1: Comparison of selected ischemia markers derived from macular OCTA in diabetic retinopathy patients vs. healthy controls

OCT Angiography based Longitudinal Ischemia Morphology Analysis in 42 patients over three years does not reveal evidence supporting Diabetic Macular Ischemia progression phenotypes

The following chapter was presented at FloRetina ICOOR 2023. Unfortunately, there is no abstract book to the best of the author's knowledge.

Purpose:

To screen longitudinal OCT Angiography recordings from a cohort of diabetes patients for evidence supporting Superficial / Deep Vascular Complex Phenotypes in Diabetic Macular Ischemia progression.

Methods:

Written, informed consent was obtained from patients prior to enrolment (DRKS-ID DRKS00024399). Macular 10x10° OCT Angiography scans were recorded using the Spectralis device, follow up mode enabled. Inbuilt Superficial / Deep Vascular Complex Segmentation (SVC / DVC) was followed, images were exported in .tif format. Matlab R2022a was used. OCT Angiography data was cropped. Binarization following the global Otsu method was applied and Perfusion Density was calculated as the percentage of foreground pixels. The earliest dataset forms the 'baseline'. Capillary Dropout rate, defined as the change in perfusion density from follow-up visits compared to baseline data, was analyzed.

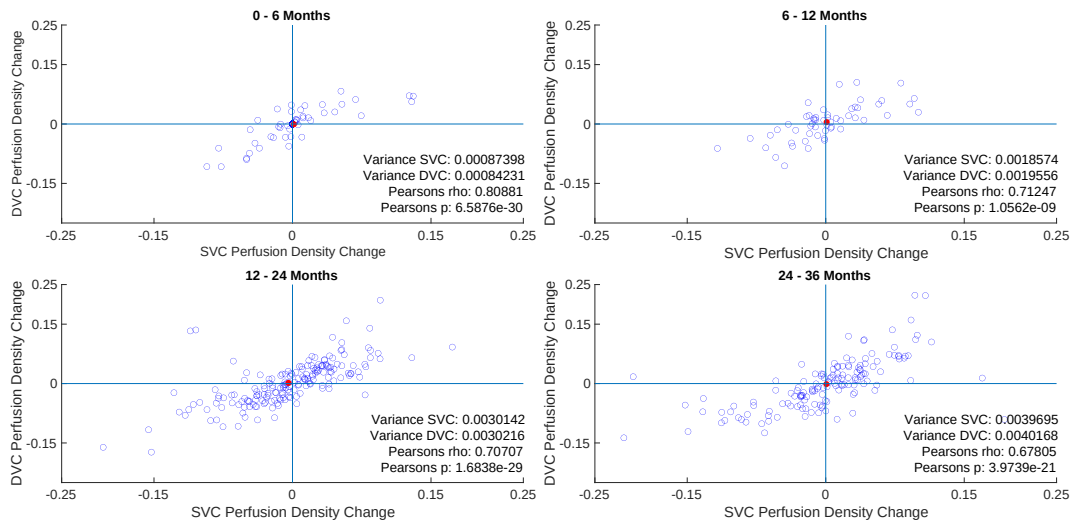


Figure 6.1: Phenotype exploration in a longitudinal imaging study. There are no apparent clusters visible.

Results:

540 records from 78 eyes from 42 patients were included. Based on the time interval from baseline to follow-up, four groups were generated: Follow up at 1-6; 6-12; 12-24; 24-36 months after baseline. SVC and DVC Capillary Dropout Rates were extracted separately and visualized in a scatter plot along with the according mean values (red dots, see Fig 6.1). Mean values remained close to (0/0) for all groups. Correlated variance of capillary dropout rates in SVC and DVC was observed. Pearson's correlation coefficient r for merged groups was 0.6470, $p = 4.6572e-62$.

Conclusions:

Capillary Dropout did not reveal evidence for the existence of SVC / DVC Diabetic Macular Ischemia progression phenotypes. An intuitive visualization was developed. The mean values close to (0/0) in all groups indicate a slow progression rate and showcase the need to further develop imaging and analysis tools.

OCT Angiography Deep Vascular Complex Quantification Correlation to Microperimetry Sensitivity in Diabetic Retinopathy and Healthy Controls

The following chapter was published [118].

Purpose:

The relation of the retinal vasculature morphology and local retinal sensitivity remains poorly understood despite its potential significance in Diabetic Macular Ischemia. Optical Coherence Tomography Angiography (OCTA) Quantification allows to characterize the retinal vasculature. This study explores the correlation among vascular morphology and their relation to retinal sensitivity as assessed with microperimetry.

Methods:

Ethics approval was granted. Trial registration: DRKS00024399. 38 eyes of 31 Diabetic Retinopathy (DR) patients undergoing treatment and 25 eyes of 24 Controls were included. Written informed consent was collected. The Deep Vascular Complex (DVC) vessel morphology was assessed using the OCTA Spectralis module following the inbuilt slab segmentation. DVC projections were exported to tif format and further processed in MATLAB R2024a. Vessel Area Density (VAD), Vessel Length Density (VLD), Vessel Diameter Index (VDI), Total Branchpoint Number and the Mean Distance to the closest Vessel (MD) were extracted. Goldmann III microperimetry data was collected in a standard radial stimulus pattern. Stimuli positioning was registered with OCTA. The correlation among the various DVC quantification parameters was assessed with a correlation matrix, see fig. 7.1. Stepwise linear regression was performed as implemented in 'stepwiselm'. Data from the full cohort including DR and Control data and subgroup analysis was performed.

Results:

Strong correlation was found among VLD, VAD and Branchpoint numbers. VDI and MD did not reveal strong correlations, neither among each other nor to other quantification metrics. The stepwise linear model creation for the full cohort identified the cohort assignment and VLD as significant predictors for retinal sensitivity. The resulting model showed an adjusted R^2 of 0.498. Looking at the DR cohort, VLD was found to be a predictor resulting in an adjusted R^2 of 0.244. None of the chosen vasculature quantification markers correlated significantly with retinal sensitivity in the Control Cohort. See Fig. 7.2 for a visualization.

Conclusions:

In conclusion, retinal sensitivity assessed by microperimetry is correlated to the vascular morphology in DR patients but is not in Controls. Advancements in imaging and the incorporation of additional patient information could contribute to more precise retinal sensitivity prediction.

Layman Abstract: The appearance, form and density of the retinal vessels may be linked to how well a diabetic retina can perceive light, tested with controlled stimuli. Knowing more about this relation could help in understanding how Diabetic Retinopathy works, how it evolves and consequently how to deal with it. Optical Coherence Tomography Angiography, a tool allowing to take high resolution images of the retinal vessels, was combined with a retinal sensitivity test to learn more about this relation. We found that there is an association between the retinal vasculature density and sensitivity in Diabetic Retinopathy patients. No similar relationship was found in a healthy Control cohort.

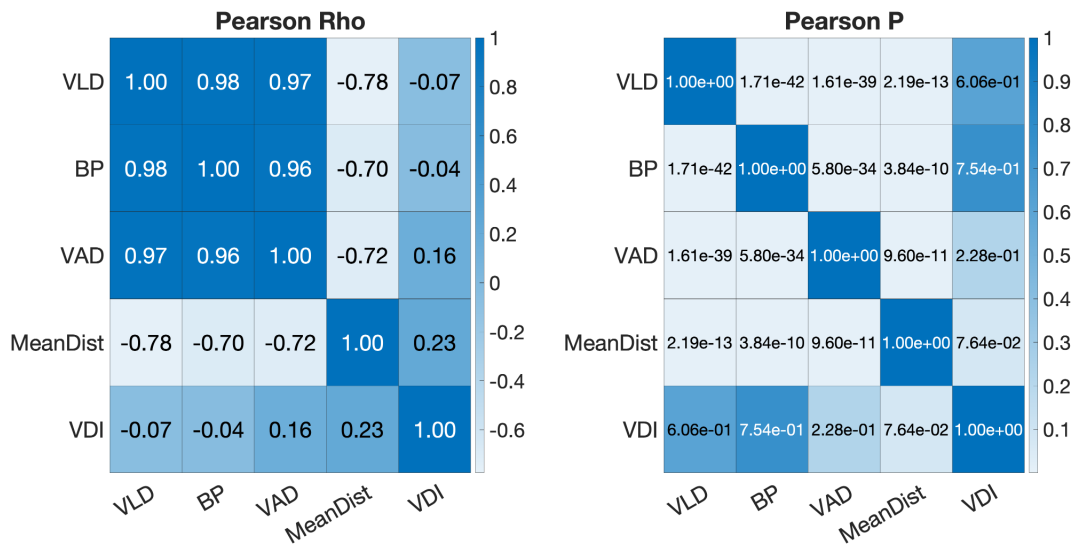


Figure 7.1: Correlation Matrices demonstrating strong correlations with large Pearson's r values in the left heatmap, while significance, p -values are given on the right.

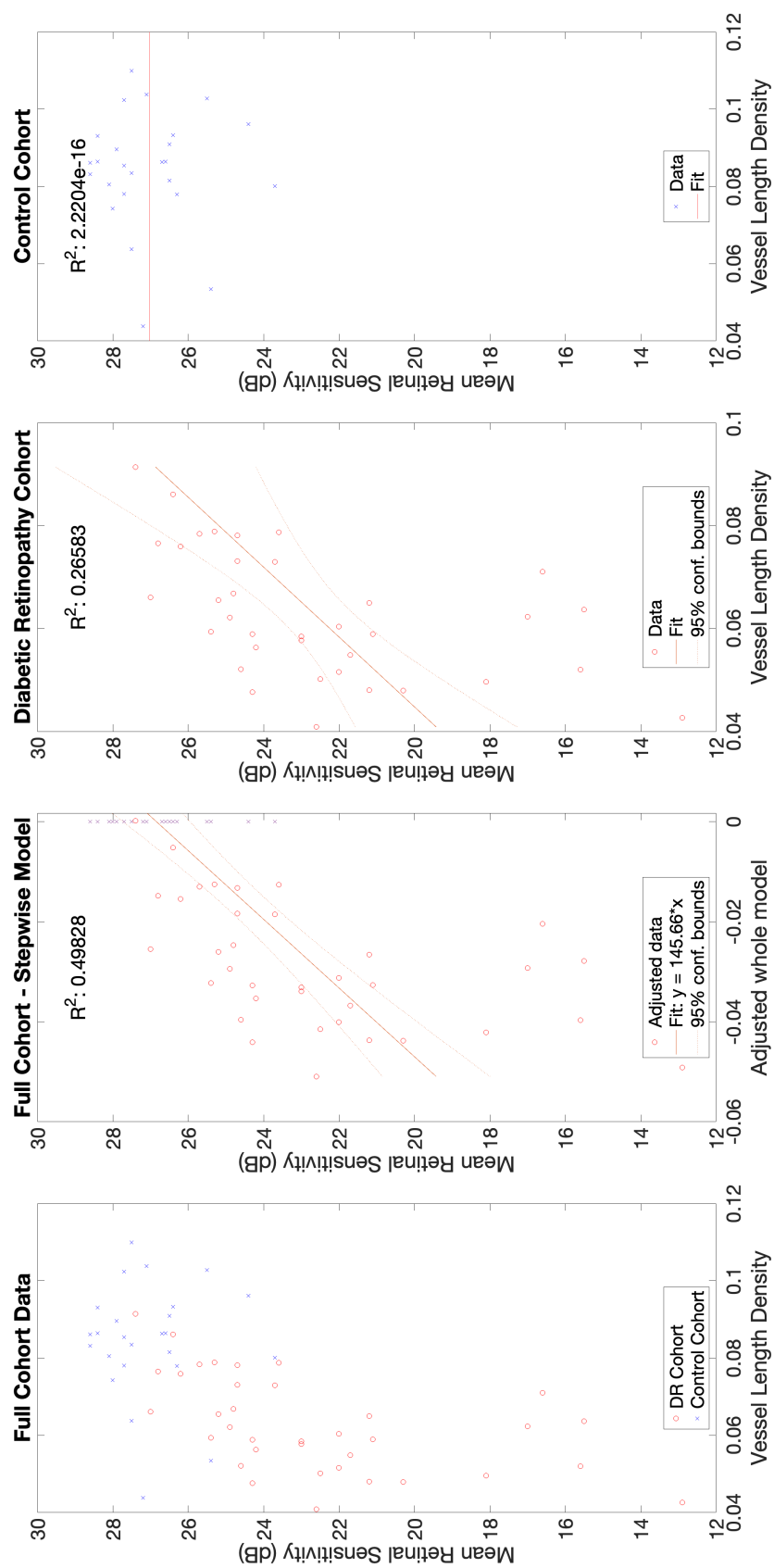


Figure 7.2: Full cohort data on the left. Stepwise regression model result considering the full cohort. Subgroup modelling on the right.

Impact of Lens Opacity on OCTA Metrics

The following chapter was published [119] and the majority of the text will be cited. For figures and tables, please refer to the original publication.

Introduction:

Microvascular complications in patients with retinal diseases are common. It can range from diabetic retinal changes and the associated diabetic macular ischemia (DMI) to hypertensive retinopathy or vascular occlusions. The development of optical coherence tomography angiography (OCTA) facilitated the quantification of perfusion metrics and therefore provided a better insight in the microvasculature of the eye. Recently, OCTA has been discussed as a tool to monitor microvascular pathologies such as DMI that has not been well understood and has no treatment so far. The ischemic changes to the macula are difficult to detect in biomicroscopy. Before the introduction of optical coherence tomography angiography (OCTA) one needed fluorescein angiography (FA) for grading and follow-up.[120] OCTA has emerged as a promising three-dimensional imaging technique for the fast visualization of the eye's microvasculature. Compared to fluorescein angiography, there is a shorter acquisition time, it is non-invasive, and allows visualization of deeper vessels such as the choroid. In addition, the detailed projection of flow signals in both superficial and deep vascular complex is independent of the presence of vascular leakage, which enables a better discrimination of macular vasculature parameters for their quantification; [121] whereas the FA hyperfluorescent signal due to leakage can interfere with the visibility of the surrounding microvasculature in FA. Several studies have shown OCTA to be superior to FA in detecting pathologies in the macula, such as non-perfused areas and FAZ parameters in both healthy subjects and diabetic patients.[122, 123] These results suggested that OCTA metrics can be applied for disease staging in DMI. [124–126] Consequently, including OCTA metrics as an ancillary anatomical endpoint in diabetic retinopathy and DMI trials has been frequently discussed in the literature.[127] However, the reliability of the quantification of vessel parameters when using OCTA presents a significant challenge. Kamalipou et al. reported

poor quality in roughly 33% of 649 OCTA images that they acquired in glaucoma patients, glaucoma suspects and healthy individuals - mainly due to artifacts such as eye movement, blinking, defocus, media opacity and shadows or segmentation errors.[128] For therapy guidance in clinical settings, however, microvasculature metrics derived from OCTA must be reproducible, and reliable for subsequent comparisons. As cataracts are among the earliest complications of diabetes mellitus, it is important to know the impact of different artifacts, such as media opacity, on the signal strength and quantitative analysis, before OCTA metrics can be employed as endpoints in clinical DMI studies.[129] Lens opacity can induce signal strength reduction, which leads to alteration in OCTA metrics [130, 131] and the impression that vessel density increases over time after cataract surgery.[132] While experimenting with neutral density filters in healthy subjects, Yu et al. demonstrated that vessel density measurements, defined as the ratio of white pixels to all pixels, can be affected by signal strength variation.[130] Following these findings, OCTA data in patients suffering from cataract might be distorted. Image processing algorithms aiming to quantify information on the retinal microvasculature based on OCTA can only provide an automated interpretation based on high-quality imaging data. Therefore, the quality of obtained imaging data is crucial, and the influence from cataract needs to be assessed. Putting our focus on clinical application, we aimed to clarify what influence cataract has in OCTA by making a quantitative description based on comparing pre-operative and post-operative datasets obtained with quantitative Scheimpflug tomography measurements of lens opacity. In contrast to previous reports, this study not only aimed to describe the changes of vessel metrics before and after cataract surgery, but to provide an analysis of the underlying changes of image properties such as local contrast that led to those postoperative differences and the role of different segmentation algorithm that might impact the results. One objective of this study was also to provide a threshold of vessel caliber that was reproducible even in cataract patients for guidance in clinical assessment of OCTA images.

Methods:

This study adhered to the tenets of the Declaration of Helsinki, had the approval of the local Research Ethics Committee, and was entered on the German Clinical Trial Register (registration number: DRKS00024399). We obtained written informed consent from all patients.

Study cohort

Sample-size calculation was done with G Power version 3.1.9.7.14 One-sided inference of mean analysis resulted in a sample size requirement of 27 patients for an effect size of 0.5 (alpha error 0.05 and power 0.8). In total, we recruited 35 patients to account for a dropout rate of 25%. Inclusion criteria were age older than 18 years, no evidence of retinal diseases such as diabetic macular edema or neovascular age-related macular degeneration, no advanced or uncontrolled glaucoma, no central corneal opacity and refractive error of less than six diopters spherical equivalent. We excluded eyes with a history of intraocular surgery within 6 months or any refractive surgery before recruitment. Thirty-five patients

who were referred to our clinic for cataract surgery and consented to attend a follow-up visit 3 months after surgery were eligible for study inclusion. After inclusion, two patients canceled their surgery appointment for other reasons, and another two did not complete the follow-up visit. Therefore, in total, data from 31 study patients were included in this study and they underwent cataract surgery between February and November 2021. All patients underwent a comprehensive ophthalmic investigation before and after surgery, including measurements of best corrected visual acuity (BCVA) assessed with Snellen chart, SD-OCT of the macula and optic nerve and 3 by 3 mm^2 OCTA of the macula (Spectralis 2, Heidelberg Engineering GmbH, Heidelberg, Germany).

Image acquisition and analysis

We measured lens opacity using the Scheimpflug tomography images that were acquired in mydriasis with Pentacam AXL, (Oculus GmbH, Wetzlar, Germany) following the protocols of Lim et al. and Grewal et al. [133, 134] Briefly, all 25 Scheimpflug images from each patient were exported to ImageJ (US National Institutes of Health, Bethesda, US. <https://imagej.net/software/fiji/>) for quantitative analysis. Average lens density (ALD) was defined as the area within the circumference of the lens edges and nuclear lens density (NLD) was defined as the area of the lens nucleus encompassed by an elliptical mask. The density of the selected area was measured in pixel intensity units on a scale of 0 to 255. Light-reflectance artifacts were excluded from the region of interest. ALD and NLD were defined in all Scheimpflug images and averaged. Using the Heidelberg Engineering Spectralis OCT angiography module we obtained 3 by 3 mm^2 OCT angiography images before cataract surgery (18.94 ± 12.22 days) and 3 months after surgery (111 ± 23.45 days). The inbuilt projection artifact removal mode was applied. Patient data representing the superficial vascular complex (SVC) and deep vascular complex (DVC) was extracted following the inbuilt processing and layer segmentation. Datasets were exported as .png files. For quantification, datasets were imported to MATLAB (MathWorks, Inc., Natick, USA). Binarization following the Otsu method and thinning to a single pixel centerline was done to determine a global threshold and skeletonization. The FAZ was segmented following three approaches: Region Growing, Active Contours and a reimplementation of the Kanno Saitana Makro (KSM).[135–137] Assessing the segmentation performance, the FAZ was labeled manually by two expert raters and automated versus manual annotation was compared focusing on FAZ area. The automated alternative performing closest to manual annotation was considered valid. A set of metrics was calculated representing various aspects of the information contained in OCTA en face projections. Perfusion density was defined as the ratio of “foreground” pixels in the binarization related to the overall number of pixels. Vice versa, the ratio of background pixels related to overall were defined as flow deficit. The vessel diameter index was defined as the number of “foreground pixels” divided by the number of centreline pixels. FAZ area was defined as the avascular zone corresponding to the foveal pit in transversal OCT encompassed by capillaries.

Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics software, version 27.0 (IBM Corp., Armonk, NY, USA). Data distribution was tested with Kolmogorov–Smirnov test and Q-Q plot. The paired t-test or the Wilcoxon rank-sum test was applied to test for differences between groups depending on the data distribution. The relationship between the signal strength index and lens opacity was assessed by univariate regression analysis and expressed by Pearson’s correlation coefficient r . Two-sided $p < .05$ was considered statistically significant, if not otherwise stated. p Value from multiple testing was adjusted with the Bonferroni method. We used Bland-Altman plots to visualize agreement in FAZ quantification between rater 1 and rater 2 or between automated algorithms versus rater 1.

Results:

Study cohort

The mean age of the patients was 67.55 ± 12.16 years ranging from 41 to 85. Twenty men (64%) and 11 women were included in this study. In 17 patients, the right eye was the study eye (54%). The mean follow-up time was 111 ± 23.45 days (range 85–210) after cataract surgery. All patients underwent uncomplicated cataract surgery. Three patients developed a postsurgical cystoid macular edema, which resolved before the follow-up examination with no sign of enduring intraocular inflammation, thus we did not exclude these patients.

Verification of automated FAZ segmentation

FAZ from SVC and DVC from both time points were manually segmented by two masked ophthalmologists using MATLAB. Manually outlined FAZ area in SVC and DVC was compared with the automated generated FAZ area measurement in MATLAB. Kanno Saitama Macro, Active Contours method and Region Growing were applied, varying their parameters: namely, the number of dilations and erosions run in the KSM varied from 6 to 40 in steps of 2, the active Contour method’s iteration number varied from 100 to 1000 in steps of 100, the smoothness factor was varied from 0.5 to 3 in steps of 0.5. The threshold for inclusion for the region growing method varied from 1% to 10% of maximum minus minimum gray value. Bland Altman plots were created for each variation. Rater 1 versus rater 2 (Figure 1(A)) and rater 1 versus Region Growing (Figure 1(B)) are shown. Figure 1(C) shows the corresponding en face projection of DVC of an outlier. Rater 1 and Rater 2 showed large agreement, while the comparison between the automated algorithm and Rater 1 showed a few outlier, mainly due to larger FAZ segmentation in the automated algorithm that was induced by areas with low contrasts.

Image contrast of microvasculature is affected by cataract

We first determined the relationship between cataract and the distribution of pixel values in OCTA images. In particular, the image properties of the smaller capillaries were assessed. We compared image contrast with histograms before and after cataract surgery. In preoperative OCTA images, the histogram revealed that most pixels accumulated around the lower gray values (0: black, 255: white), thus resulting in an image with low contrast. After

cataract surgery, image contrast was enhanced, and the histogram became more uniformly distributed (Figure 2). Significant changes in OCTA metrics before and after cataract surgery

Quantification of imaging data in metrics

Table 1 shows Baseline and postoperative clinical and OCT parameters. BCVA significantly improved after cataract extraction, as well as signal strength index in OCTA images (Cohen's $d = 0.87$, $p < .001$). All OCTA metrics significantly changed after surgery, except for vessel-diameter index in the superior vascular complex (SVC) and the area of foveal avascular zone (FAZ). At baseline, signal strength index (SSI) was inversely correlated to average lens density (ALD) (Pearson's $r = -.40$, $p = .027$), flow deficit (SVC and DVC: $r = -.70$, $p < .001$) (Figure 3) and FAZ in both SVC and DVC (SVC: $r = -.43$, $p = .015$; DVC: $r = -.45$, $p = .01$). Perfusion density in each complex was also significantly correlated to the SSI (SVC and DVC: $r = .70$, $p < .001$), while only the vessel-diameter index in the DVC but not in the SVC was significantly correlated ($r = .55$, $p < .001$).

Pixel caliber threshold for vessel detection

The investigation of image contrast indicated that the visualization of capillaries is especially affected by lens opacity due to cataract (see Figure 2). Stepwise suppression of flow signal according to their pixel caliber might allow deriving a caliber threshold from which vessels are reliably visualized and quantified. Based on binarized en face projections, erosion and dilation were applied multiple times to suppress small caliber vessel visualization in en face projections (Figure 4(A)). Note that one erosion step reduces the vessel caliber by two pixels, one from each side of the vessel. Therefore, a dataset suppressing calibers of two, four, six and eight pixels was created, leaving structures of minimum three, five, seven, and nine pixels caliber. Perfusion density of SVC and DVC was derived and tested for comparable means at baseline and 3 months after uncomplicated cataract surgery (Figure 4(B,C)). Datasets suppressing vessel caliber of six and fewer pixels did not present significant variation pre-, and post-operatively; therefore, we concluded that in this cohort, with OCTA vessel caliber of seven pixels (20–30 μm) can reliably visualized.

Discussion

This study showed the impact of image acquisition and processing in the analytic chain on the interpretation of OCTA images. There is a lack of standardization, leading to a variety of quantification approaches and processing routines enabling the extraction of these metrics. The FAZ detection verification presented here clearly demonstrated that wherever image processing is applied, the application introduces assumptions— for example by assuming an undisrupted round circumference of the FAZ. In this case, lens opacity related contrast reduction may lead to incorrect segmentation. Furthermore, since the ground truth of the microvasculature is inaccessible, quantification must be conducted and interpreted with appropriate care. Early studies on the reliability of OCT in the presence of cataract focused on retina thickness estimations. It was found that cataract indeed influences OCT data, but

thickness estimations appeared to be comparable before and after cataract extraction.[138–140]

In contrast, OCTA metrics seem to be severely affected which might be due to the influence of cataract on lateral and axial resolution of imaging. Several studies demonstrated that lens opacity significantly influences quantification of vessel metrics.[130, 132, 141, 142] In most reports, vessel metrics increased after cataract surgery and FAZ area appeared to be smaller. Different reasons and factors for this observation have been proposed. Some authors suggested that the increased blood flow signal might be a part of a postsurgical inflammation or induced by increased light fall on the retina, which enhances retinal metabolism and consequently triggers higher blood flow.[132] In contrast, Yu et al. and Lee et al. by using neutral density filters of varying intensity to attenuate signal strength in OCTA images, demonstrated a linear relationship between signal strength and quantitative flow measurements. Both research groups concluded that a higher signal strength correlates to increased values of vessel metrics, such as perfusion density. Our results are consistent with those previous reports, and we showed a significant correlation between signal strength and most vessel parameters and FAZ area. Neutral density filters cause a homogenous reduction in signal across the entire scan field and therefore may not sufficiently simulate the impact of natural media opacity. Hence, our study demonstrated that signal strength correlates to the average lens density quantified in Scheimpflug images. Furthermore, we showed that the contrast in areas of small vessels increased after cataract surgery, which presumably led to a better detection of blood flow and consequently increased most vessel metrics. Despite the difference being statistically significant for most OCTA metrics, it is important to note that the average difference before and after cataract surgery in perfusion density and flow deficit measured was between 3 to 6%. The clinical significance of these differences must be evaluated critically. Normative OCTA databases are scarce and therefore, it is difficult to determine the significance of temporal changes. We compared our results to the cross-sectional dataset from Fernandez-Vigo et al. and our postoperative perfusion density is similar to the reported range of $22 \pm 5\%$ for SVC and $19 \pm 6\%$ for DVC. [143] It is noteworthy, that extraction of vessel metrics is device dependent. Therefore, we further compared our results with previous OCTA studies conducted with the Spectralis device. Hosari et al. reported a perfusion density of 30–33% in SVC, 23–27% in DVC and a mean FAZ area of $0.43 \pm 0.16 \text{ mm}^2$ in SVC and $0.44 \pm 0.12 \text{ mm}^2$ in DVC. [144] Similarly, Corvi et al. reported a perfusion density of $32 \pm 3\%$ in SVC and $26 \pm 5\%$ in DVC. [145] The standard deviation ranged from 3 to 6%, which was comparable to the measurement errors induced by cataract in our study. Media opacity and artifacts can interfere with the calculation of vessel metrics by beam attenuation, aberrations, or alteration of tissue reflectivity. Any optical opacity or macular edema in the optical path can attenuate the beam and its reflected signal, thus making the OCT image less contrasted.[146] The contrast of an image is partly expressed by the signal strength index that can vary among devices due to different proprietary software. The Spectralis device used in this study employs a sum projection method based on the column sum of OCTA signals at every single region divided by the local slab thickness. A single OCTA pixel value in the column can be between zero for static tissue and one for

blood flow signal.[147] The contrast threshold can be set by the interpreter to favor either larger or smaller vessels in the en-face projection. In our study, we used a fixed contrast setting of 1:4, which means that a sum value of one (i.e. smaller vessels) would be 25% (1:4) white and a sum value of four (i.e. larger vessels) would be 100% (4:4) white, to account for both larger and small vessels as global OCTA parameters were evaluated in this study. For sequel studies with OCTA, it is crucial to understand the impact of image contrast on blood flow visualization in OCTA as contrast reduction by progressing media opacity will likely result in subsequent lower sum values and compromises the visualization of smaller vessels, which can lead to the misinterpretation that the microvasculature reduced over time and could be misunderstood as progression of the underlying disease such as diabetic retinopathy or macular ischemia. Not only the visualization in the inbuilt device itself can be influenced by lower contrast, but all subsequent image processing steps will be affected as well. The choice of binarization method and quantification will also affect the results. One major limitation of this study and most other OCTA studies is that, in clinical routine, there is no alternative approach to verify the OCTA capillary metrics. In fluorescein angiography, capillary vessels are hard to quantify, and the DVC is not sufficiently represented in the two-dimensional image. Hence, in this study, it remains unclear whether the differences between pre- and post-surgical changes of OCTA metrics might be partially explained by postsurgical vascular changes. To limit the effect of postoperative inflammation, which can alter retinal vascularization, we chose to examine patients at 3 to 4 months because the onset of acute and most chronic postsurgical inflammation (such as pseudophakic macular edema) is usually within 12 weeks after surgery. By choosing to examine patients after this critical period we expect that we reduced the likelihood of underlying perioperative inflammation that could cause transient vascular alterations. The strength of our study lies in its prospective nature with a rather long follow-up time of 3–4 months compared to previous studies and the inclusion of several OCTA metrics. Yu J et al. compared only perfusion density in healthy subjects before and after the application of a neutral density filter and defocus.[130] Yu S et al. conducted a similar prospective study with thirteen patients but followed them for only 1 week after cataract surgery.[148] Using a flare meter, the group ensured that there was no inflammatory sign in the anterior chamber at 1 week, but it should be noted that flare meter cannot measure mild inflammation in the posterior segment that might occur after cataract surgery. Furthermore, this study investigated the causes behind the observed changes in vessel metrics by evaluating the applied algorithm and image properties that were not done in previous reports. In summary, to enable OCTA metrics to become meaningful and reliable parameters for clinical trials and everyday clinical decision-making, the mechanism of signal attenuation should be considered when interpreting OCTA images. Diabetic and older patients with retinal diseases are at risk for cataract development and therefore, assessing vessel metrics should be done with caution in phakic patients with cataract, especially when evaluating capillaries. In addition to signal strength indices from proprietary devices, image quality assessment can include the evaluation of vessel calibers as we performed in our study.

Conclusion

When longitudinal studies are conducted in phakic patients with cataracts, the interpretation of OCTA parameters requires a cautious approach. Contrast enhancement in smaller capillaries contributes to differences in vessel parameters as demonstrated in stepwise suppression of smaller caliber vessels. To clarify whether postoperative changes of OCTA parameters are due to anatomical changes or enhanced signal, further investigation differentiating between influences of phakic lens opacities and potential anatomic changes in a controlled model is needed. Reliable OCTA metrics are crucial for the management of patients with retinal diseases who are affected by both lens opacity and microvasculature changes. Vessels with >6 pixels (20–30 μm) in caliber seem to be reproducible in perfusion density quantification.

Focus variance effects in longitudinal Optical Coherence Tomography Angiography

The following chapter was published, abstract number PSa03-03 in [149]

Optical Coherence Tomography Angiography (OCTA) imaging with vasculature quantification is attractive for longitudinal monitoring of the retinal vasculature. The focus must be adjusted manually. The effect of focus variance in a longitudinal dataset is analysed.

OCTA data was collected in a cohort of Diabetic Retinopathy patients undergoing regular treatment from August 2019 to September 2023. 81 Eyes of 43 patients were imaged using the Heidelberg Spectralis device. The number of follow up sessions was 2 to 14. Focusing was done based on a subjective sharpness evaluation of the infrared fundus image. The resulting device focus and OCTA Quality were extracted from the dataset meta-information. Focus deviation was calculated, quantifying the absolute difference to the eye-specific mean focus. Superficial (SVC) and Deep Vascular Complex (DVC) en face projections were exported following the inbuilt segmentation. Images were binarized with Otsu's global method and the centreline was extracted. Vessel Area Density (VAD, number of white pixels divided by total pixel number), Vessel Length Density (VLD, number of centreline pixels divided by total pixel number) and Vessel Diameter Index (VDI, VAD divided by VLD) were calculated. Like the focus deviation, quantification metric deviations were calculated as the difference to the plexus-specific mean. Linear regression was used to investigate the relation of focus deviation with OCTA Quality, Quantification metrics and Quantification metrics deviation. Significance was assumed when $p < 0.05$.

Significant negative correlations were found among focus deviation and VAD in SVC ($p < 0.01$), DVC ($p < 0.0002$); focus deviation and VLD in SVC ($p < 0.01$) and DVC ($p < 0.001$) and focus deviation and VDI in DVC ($p < 0.05$), but not in SVC. Significant negative correlations were found correlating the focus deviation and VAD deviation in SVC and DVC, focus

deviation and VLD deviation in SVC and DVC (all $p < 0.0001$) focus deviation and VDI deviation in DVC ($p < 0.05$) but not in SVC. Focus deviation and OCTA Quality revealed a significant negative correlation ($p < 0.0001$). R^2 was below 0.1 in each model.

The study revealed significant correlations among focus deviation and downstream vasculature quantification. Despite the inability to explain the full variation, the role and acceptance ranges of defocus in OCTA need to be characterised. Focus quality assurance and control tools will be needed to enable even more informative longitudinal studies.

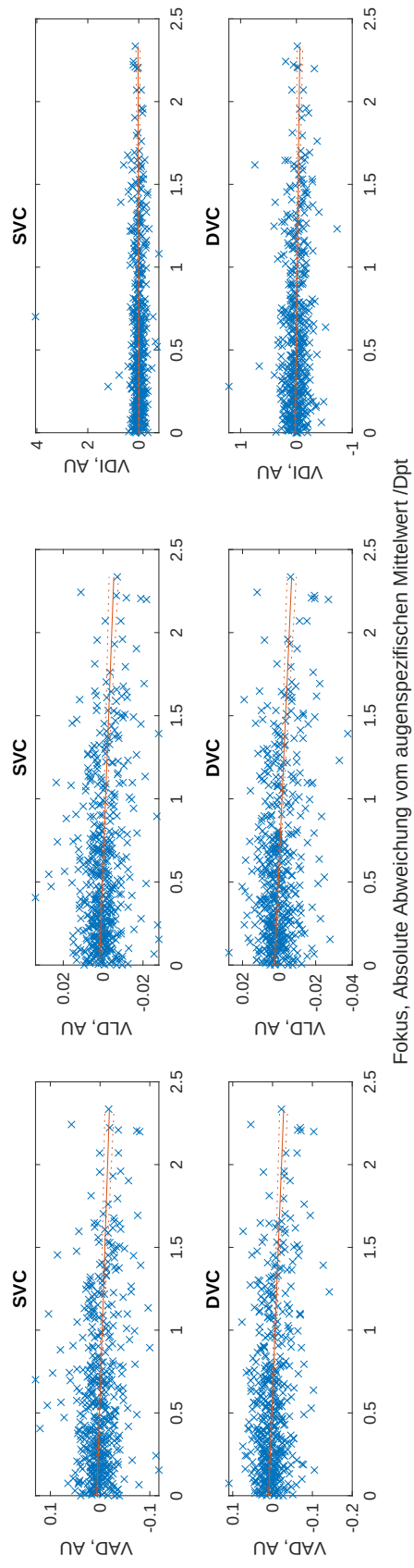
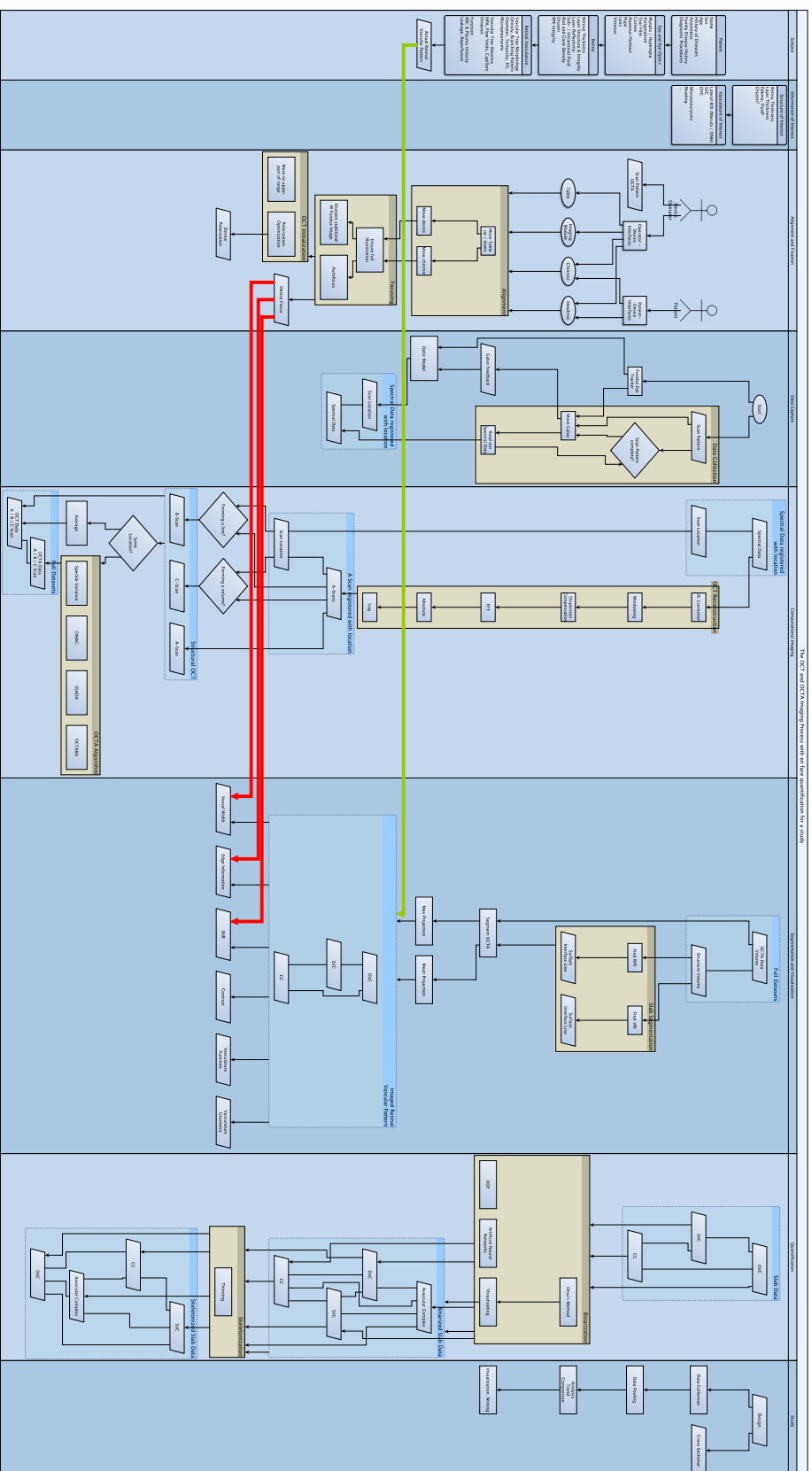


Figure 9.1: Scatter plots with correlations among normalized focus value and normalised vasculature quantification result.

FROM THE DIAGNOSTIC CHAIN TO THE OCTA PARAMETER SPACE

The following part introduces two constructs, two lines of reasoning. The first one is an observation, an overarching line of reasoning characteristic of most vasculature morphology studies. The name "The Diagnostic Chain" is assigned to this line of reasoning. The concept and according observations are introduced in chapter 10.

The second one, a complementary line of reasoning was developed by the author of this work. It is called "OCTA Parameter Space". The conception directly leads to a systematic approach to the exploration of the underlying principles in optical coherence tomography angiography (OCTA) imaging by the structured variation of single parameters in controlled setups. It therefore allows for the derivation of such a experimental setup. Although a complete study of the relevant parameters is beyond the scope of this work, one implementation was developed and applied to study one particular parameter of interest, the role of focus precision.



The Diagnostic Chain

The presented studies showcase potential applications of vasculature quantification from OCTA to explore diabetic macular ischemia. The studies shed light on the differences among healthy controls and diabetic macular ischemia patients, on the lack of postulated phenotypes in vasculature quantification progression, the association of vascular and micro-perimetric assessment of retinal function, the influence of cataract presence and the impact of defocus on quantification.

One observation is noteworthy. All presented studies share a common aspect: The argumentation line is a sequential, step by step approach. Shared with the large majority of published research works on optical coherence tomography angiography (OCTA) quantification, an overarching general approach was determined by the author of this dissertation. This approach is referred to as unidirectional diagnostic chain. A visualisation is presented in figure 10.1.

Starting from the present retinal vasculature anatomy of a given patient, a digital image is created. OCTA en face projections are frequently considered. Segmentation, skeletonization and metric calculation allow for a nuanced analysis of the taken images. Metrics are frequently tested for their ability to indicate disease states [150], progression [113] and treatment responses [151]. The quantification result is assumed to be representative of the present vascular status.

In a secondary step, the image is already taken and assumed to fully represent the patient's vasculature. The task for the researcher is the development of an optimal quantification software or even software pipeline, leading to one of the existing or a novel study endpoint. The inherent liberty is typically pruned by some sort of verification like the comparison to a manual annotation.

Although the diagnostic chain appears to be straightforward and the assumption of vascular state representation is plausible, a more complete visualisation is presented in figure 9.2. There is a large number of technical parameters set along the diagnostic chain. The choices made along the way, setting the large number of parameters is not trivial. Variations

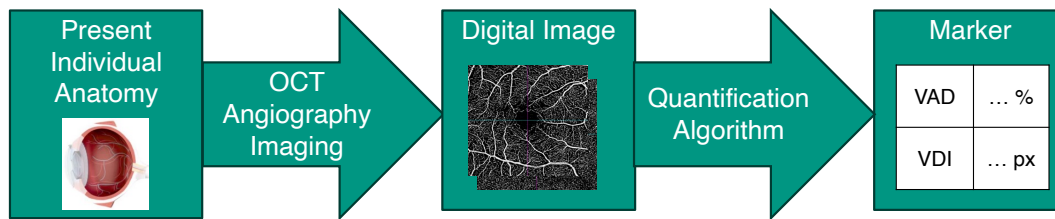


Figure 10.1: Visual presentation of the unidirectional diagnostic chain.

might occur, looking at the large number of implicitly included steps and technical details. Any OCTA quantification result should therefore be seen as a preliminary result due to the ongoing maturation of the technology itself.

The lack of reliable ground truth data complicates the development of algorithms suitable for the given patient cohort and imaging situation. Influences besides the velocity of blood flow have been identified, namely the presence of cataract and the variation of focus precision. Although these explorative studies are worthwhile, the vasculature quantification methodology will only reveal the information contained in the en face projection in the first place. Vessel segments missed by the imaging procedure will not be part of the quantification. Clearly, the quantification of retinal vessels is not solely an algorithm development task, but an algorithm development task that needs to be solved in combination with the upstream OCTA imaging behaviour.

Parameter Space Introduction

The following paragraph is a prosaic description of the experience of an exemplary clinical researcher interested in optical coherence tomography angiography (OCTA) quantification. This introduction will lead to the concept of the OCTA parameter space, serve as a basis to derive a research strategy and an adequate experimental setup.

There is a strong interest in the application of OCTA quantification in basic medical as well as clinical research. Numerous studies discussed findings across various OCTA devices and various diseases looking for insight on the disease mechanisms with and without medical intervention. Exemplary studies were introduced in chapter 6 along with the concept of the diagnostic chain. OCTA quantification comes with the promise of increased comparability and objectivity. Quantitative assessments are typically considered repeatable, precise and well developed.

The exemplary researcher got interested in OCTA quantification, too. A choice on the instrument employed for the study has to be made. Several commercially available quantification modules exist as software packages coming with one among multiple devices. The imaging modality is called OCTA throughout. The field is rapidly evolving, leading to frequent technical changes, nomenclature imperfection and software updates. The exemplary researcher is interested in medical or clinical insights, not in technical details. But the complex nature of the imaging modality leads to the need for cumbersome analysis and the obvious pathway towards the already available, most accessible or most affordable device choice appears tempting to the researcher. Simply assume the insignificance or even negligibility of details. Since quantitative numbers are wanted, the inbuilt algorithms will be applied as is or a custom algorithm will be prepared. In the worst case scenario, the findings are limited to the one single device type and scan pattern used, which will still be accessible for the exemplary researcher to conduct follow-up studies.

Although reasonable, the inherent problem woven in this approach becomes visible looking at the first published meta-analysis [97]. Heterogeneous vasculature assessment methodologies complicate the comparison of vascular findings. The authors pointed out "massive heterogeneity in the methods employed to assess retinal perfusion, supporting calls for standardisation of methodology." [97]. Reproducibility requirements in the context of retinal vascular characteristic and longitudinal development research are fulfilled once reliable and

comparable results are acquired routinely, no matter the device details. However, the failure to fully characterise or standardise the detailed diagnostic chain (refer to figure 9.2) led to the current confusing knowledge landscape complicating meta-analyses, data pooling and multi-centric studies. Lacking comparability among the vasculature quantification outcomes from various commercially available devices was found and criticised by the scientific community [10, 11, 19, 152, 153]. While significant differences in absolute quantification values were found among devices, the repeatability using the same device, imaging mode and software was reported to be acceptable. The choice of the image processing method further influences downstream conclusion as highlighted by [111]. A major review was published, highlighting the importance and even detailing pathways towards a standardised OCTA quantification routine, for retinal vasculature assessments, approaching enhanced comparability and overcoming the current confusing research landscape [19]. However, the impact of the technical details making the difference among devices and modes on the downstream quantification result is unknown. The term parameter will be used for these technical details. The following definition is fundamental for the introduction of the concept of the OCTA parameter space:

The term parameter refers to any characteristic secondary to the red blood cell velocity specifying or influencing the signal generation in OCTA imaging procedures.

OCTA imaging can not be realised without defining a set of parameters. Each device comes with parameters hidden in the device design and inbuilt algorithm specifics. The set of all possible parameter choices is named parameter space. Device specifications are considered a working point within this parameter space. The following thesis is formulated: The inconsistent choice of technical details, inconsistent parameter choices among devices is the root cause of the inconsistent vasculature quantification outcomes. The equivalence of parameter sets explains the found intra-mode repeatability, whereas differences of parameter sets among devices or modes explain the lacking inter-mode repeatability. In order to explore the parameter space concept, the following question will lead the experimental design:

How to systematically investigate the OCTA imaging behaviour following the variation of imaging parameters?

The systematic investigation of single parameter influences is done by determining the parameter of interest as a variable. Variation of this variable within the full parameter space extent, while controlling, fixating all other parameters. The isolated, controlled variation of a single parameter will reveal its influence on downstream signal characteristics and vasculature quantification. Clinical studies will not allow to fulfill the high level of control needed. Numerous parameters are involved, therefore a collection and ranking of the most significant candidates is done. The significance is assessed based on reports in the literature and the impact in clinical application. The complete analysis of all the parameters potentially involved is beyond the scope of this work. However, the development of the experimental setup and the analysis of a first influencing parameter are significant steps. Replication or justified adaptation of the presented setup will be straightforward.

OCTA Parameter Identification

A full specification of an optical coherence tomography angiography (OCTA) imaging process must include details on the sample being imaged, dynamics occurring in bulk and locally within the sample, the imaging specifications and analysis characteristics. Starting with the sample characteristics, scatterer (red blood cell) size, shape and orientation, vessel size and optical characteristics of the tissue are identified. Sample dynamics make up the second group, importantly covering the cell velocity and parallelism of motion direction, differentiating collective flow from diffusion processes. Following the clinical context of imaging an unknown, vascularized sample, the parameters describing the sample characteristics and dynamics must be accepted as these will be given. Setting and varying these parameters in a controlled setup is promising for performance testing in one or multiple defined test scenarios. Moving on to imaging and analysis parameters. The parameters given in these groups may be chosen based on technical feasibility and match the design choices to be made when developing an OCTA system.

Imaging hardware parameters:

1. Spectral characteristics of the Illumination
2. Sample illumination power
3. Sample arm optics
4. Reference arm optics and power
5. Spectrometer resolution
6. Spectrometer readout speed

Imaging software parameters:

1. Dispersion compensation
2. Signal drop off correction
3. k-linearisation
4. OCTA Algorithm
5. Scan pattern, determining the spatial sampling and the interscan time

Imaging patient interaction parameters:

1. Chinrest, patient head fixation
2. Alignment
3. Patient eye fixation stability
4. Focus precision achieved

Once the imaging is completed, the patient's anatomy and the occurring dynamics will be imaged in a manner determined by the imaging parameter set. Angiography reconstruction and vasculature quantification are executed sequentially, starting from the imaging results. The analysis routine introduces additional parameters:

Vasculature contrasting and quantification parameters:

1. OCTA algorithm employed
2. Vascular plexus definition
3. Vascular plexus segmentation
4. En face projection method
5. Vasculature segmentation
6. Vasculature skeletonisation
7. Metric calculation rules

Identified parameters potentially influence the optical coherence tomography (OCT) imaging behaviour. Characterising the resulting behaviour is worthwhile. Looking at literature introductions, motion contrast is typically named as the phenomenon underlying the OCTA signal generation. Its actual origins were studied but are not understood as indicated in various papers [79, 80]. However, the involvement of the scatterer velocity as a fundamental driver of the signal generation is ubiquitously assumed as formulated below: The OCTA signal is a response to the collective movement of scatterers like blood cells travelling in a uniform direction with a given velocity. The velocity magnitude is assumed to be the main driver of the OCTA signal response, specified in a "Velocity Response Curve". Any secondary parameter must be tested for the influence on the velocity response curve.

Investigating parameter effects looking at a set of velocities is imperative since imaging blood flow in capillaries as well as larger vessels must be sensitive to the various mean velocities present in the various vessels. The rationale for this approach is the following. "Motion Contrast" [58] is the description of the OCTA signal generation. "OCTA signal" is the description proposed by an expert panel [115]. Although both may appear similar, the imprecise terminology reveals a lack of understanding of the signal generation principles in OCTA. Is the signal a result of the velocity measured in meter per second, the flow rate measured in litre per second or flux, the product of mean flow velocity and scatterer concentration? Working towards the goal of flow quantification, several studies investigated the effect of the flow velocity on the OCTA. While Tokayer et al. noted an almost linear relation of blood flow speed and signal for a limited flow speed ranges [79], Choi et al. argued that flux, the product of flow speed and scatterer concentration, is the signal driver [80].

The Parameter of Interest: Focus Precision

Among the large number of parameters identified in the prior chapter, focus precision takes a prominent role. Focusing optical devices is a ubiquitous premise enabling the user to capture high resolution, high contrast images of a subject of interest. optical coherence tomography angiography (OCTA) is no exception. Each and every scan taken needs to be taken in focus. The realisation of the focusing activity differs among the various devices. While automated focusing units are available in some devices, these units are likely to differ among one another. In addition, there are devices operating without such a focusing unit. Surprisingly, there is little information on the influence of focus precision and device behaviour in defocus available. Tomlinson highlighted the need for precise focusing in an image series taken in focus and various degrees of defocus [154]. Yu took advantage of signal strength attenuation in defocus conditions in order to investigate the effects of signal strength on quantification metrics [155].

However, both studies were conducted *in vivo* and therefore the defocus degree and secondary variables cannot be fully controlled. Therefore, investigating defocus effects in a controlled environment is worthwhile. The experimental observations will simplify the recognition of defocus effects *in vivo*. Guidelines on OCTA imaging do not cover defocus [95], despite it being found frequently.

The ubiquitous requirement to focus OCTA devices before the image capture starts, paired with the absence of well controlled experimental observation, guideline coverage and performance tests makes defocus assessment an interesting and impactful contribution towards a routine data collection.

Experimental Setup

The following experimental setup is a phantom, a simplified model of a biological subject. Phantoms are tools, serving a goal. In this case, the phantom was developed with the primary goal of parameter control. Secondary, a versatile setup will allow for diverse investigations. Instead of accepting the inevitable parameter set for a given optical coherence tomography angiography (OCTA) implementation, single parameters will be varied to understand their impact. The secondary goal of anatomic validity is based on the following interpretation of the anatomic processes in retinal microvasculature:

First, on the fluid used. Blood cells within blood plasma move along the vessel geometry. Cells and plasma are combined in a suspension. The differences in the material composition of blood cells and plasma lead to heterogeneity in the optical properties in blood cells and plasma. This heterogeneity, paired with motion, is assumed to be the root cause for OCTA signal generation. Optically heterogeneous suspensions must therefore be used to allow for OCTA imaging. Several options have been applied before: Intralipid solution [80], milk-water mixtures [84] as well as blood [79]. A Milk-water mixture, consisting of 50 ml full fat milk, 3.5 % fat, mixed with 500 ml distilled water was chosen as a default fluid due to ubiquitous availability, low cost and simplicity. The development of a custom blood model including hydrogel beads was presented to the public, but discontinued [156].

The vessel centreline may be described mathematically as a curve with constant diameter. Changes in diameter are assumed to occur at bifurcations only. The curve tangent is equivalent to the tangential cell motion direction. Piecewise linear tangential approximation may be used as a description of the suspension motion in constant velocity along the vessel, therefore the knowledge on motion in a straight line is satisfactory. Following this interpretation, a detailed understanding of motion along a straight line allows for the approximation of more complicated movements along curved trajectories.

Straight channel microfluidic chips with given dimensions, known material properties and practicable fluid connectors are used as a vasculature model. The cross section is quadratic instead of approximately round, as expected in the retinal vasculature. But the analysis of data taken within a quadratic cross section channel is much simpler. Microfluidic 156, produced by microfluidicChipShop, made of Topas, including channels of 200x200µm

dimensions were chosen as a default model.

Volume flow control was realized using a programmable Harvard Apparatus Pump 11 Elite syringe pump. Connecting the syringe pump to the microfluidic chip was done using infusion sets with a cut off drip chamber. These come with integrated luer connectors allowing for convenient interfacing and importantly the included roller clamp. Filling of the tubes was needed before attachment to the microfluidic chip was done, since the small channel dimension prevents ejection of significant trapped air volume.

The collective motion of the optically heterogeneous solution along a straight line is then imaged repeatedly with OCT scans, resulting in OCTA image data. In order to investigate the impact of single imaging parameters and their interplay, the single parameter choices must be controllable by the operator. The commercially available Ganymede GAN612C1/(M) OCT system produced by Thorlabs was specifically designed for laboratory applications and therefore offers extensive control on the physical arrangement and imaging settings. The system operates at a center wavelength of 880 nm, offering an axial resolution of $6.0\mu\text{m}$ in air / $4.5\mu\text{m}$ in water. Up to 1024 pixels cover up to 3.4 mm in air axially. Multiple scan lenses are available, offering a variety of working distances, effective focal length and numerical aperture. The scan head is mounted to a stand allowing to move it in vertical direction. Importantly, this movement can be done using one of the adjustment knobs, allowing for repeatable, characterised translation.

The role of defocus will be characterized in the following chapter. Each single imaging session will be based on the focusing done by the device operator. Technical support systems supporting precise focus adjustment are integrated with some devices, but differ qualitatively. The influence on downstream quantification results is not understood. Although the OCTA parameter space concept is comprehensive and may be extended beyond the characterization of defocus by including additional parameters, the scope of this work is limited to an understanding of the defocus implications.

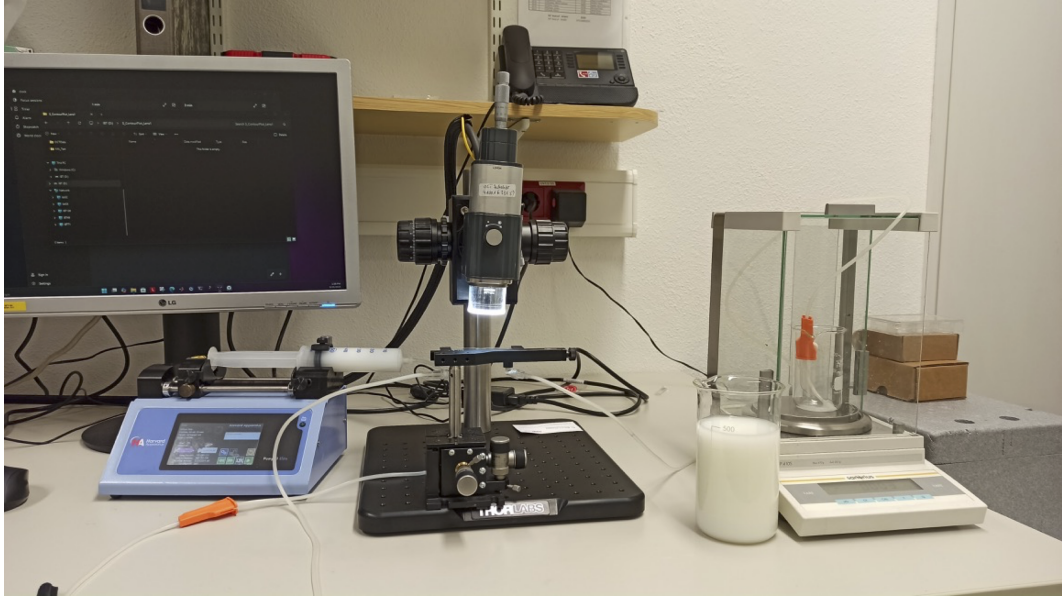


Figure 14.1: The developed experimental setup.



Figure 14.2: Close up of a microfluidic chip.

PART IV

DEFOCUS CONSEQUENCES FOR THE OCTA SIGNAL AND IMAGE

Defocus impacts Signal Strength

An impact of defocus on signal strength is known. It was even used to incorporate signal strength loss in a clinical investigation [155]. The impact of defocus on signal strength is expected to be symmetric around the focal position. The relation is assessed using a diffuse reflectance target as well as a microfluidic chip.

Diffuse Reflectance Target Assessment

Defocus effects are typically assessed using laterally extended targets like mirrors. A mirror was found to be unsuitable since signal saturation prevented an undistorted analysis. A diffuse reflective target (Labsphere model SRT-99-100, serial number 99AA03-0719-2681) was used to assess the effects of defocus.

The target was put in the imaging area of the Ganymede GAN612C1(/M) introduced in chapter 14. The scan head was equipped with the lens OCT-LK4-BB and translated in the vertical direction by a full rotation of the fine positioning knob. One full rotation is equivalent to 225 μm travel. 33 vertical positions were included. There was no adjustment of the reference arm length or iris. At each position, a three dimensional dataset was recorded, sampling a field of view of 0.5mm x 2mm in 250 B-Scans. Each B-Scan is made up of 1000 individual A-Scans, each consisting of 1024 axial pixels. The scan rate was set at 248 kHz. The mean intensity recorded at each position was calculated and plotted against the depth position.

The results are visualized in figure 15.1. The mean signal level recorded at various distances between scan head and target varied with the distance. The shape of the curve is reminiscent of a gaussian function. It was expected to be symmetric around the maximum signal level. However, there is a prominent indent visible around 3500 μm relative distance change. Nevertheless, the overall variation magnitude is limited. The presence of the target is detected throughout the depth positions.

Two B-Scans taken at relative distance changes of 2925 μm (a) and 3600 μm (b) are given in figure 15.2. The origin of the reduced signal level registered, leading to the visible indent in figure 15.1, originates from the location of the target relative to the zero delay position of the imaging system. The target does not appear as a single surface, but as a three dimensional

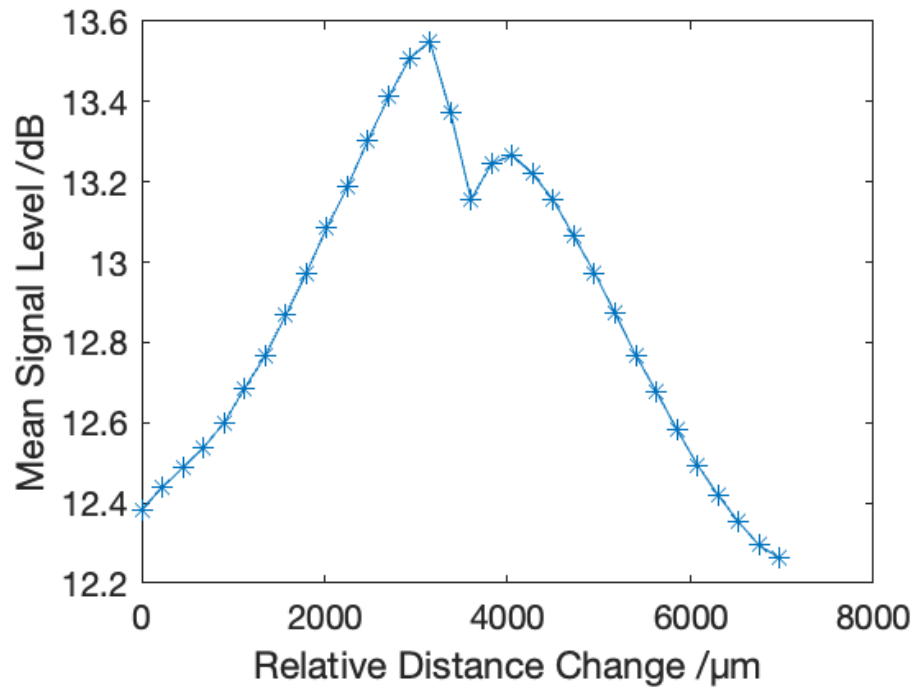


Figure 15.1: Signal strength reduction with defocus referring to a diffuse reflectance target.

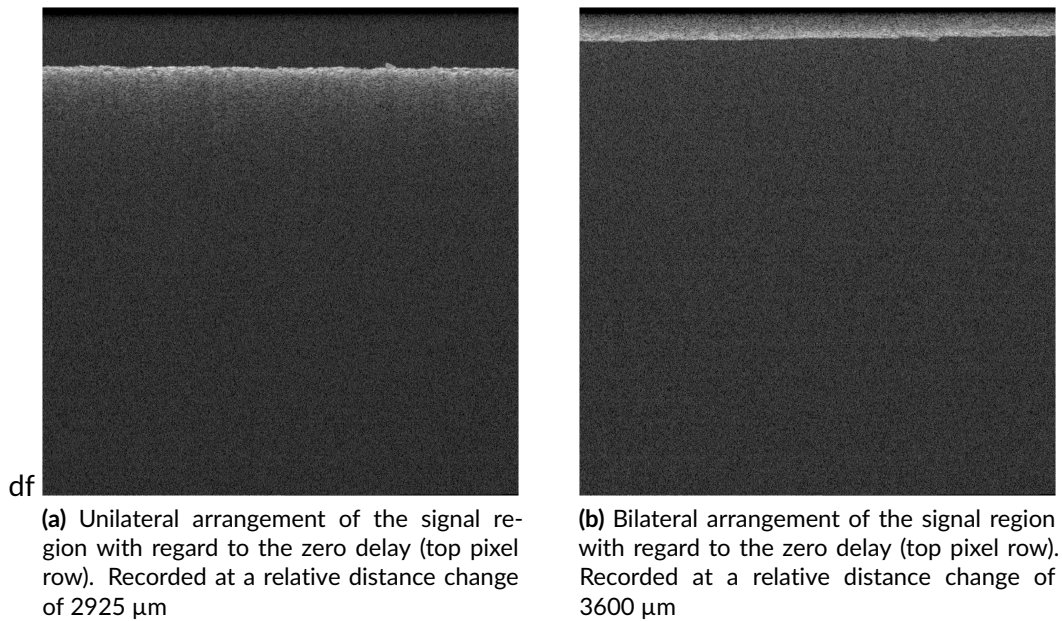


Figure 15.2: Data captures of the diffuse reflectance target. A penetration depth is determined, which is located unilateral (a) or bilateral (b) with regard to the zero delay.

structure presenting with a recognisable penetration depth. The signal is mirrored when the zero delay position is crossed, leading to the visualisation of the upper and lower signal portions on top of each other. Consequently, the signal is elevated in a specific region, but the mean of all pixels is reduced.

In conclusion, mirrors are unsuitable targets due to the resulting signal saturation. The lateral extended diffuse reflection target was used to verify a variation of the signal level with focus precision. However, the nonzero penetration depth leads to pixels carrying information from both sides of the zero delay position. This leads to an apparently reduced signal in the full scan average. A single surface of a non scattering material coming with a limited reflectivity should be investigated for defocus signal strength characterisation.

Impact of Defocus on OCT Signal Strength - Surface Model Versus Particle Model

The following chapter was contributed to the European Conference on Biomedical Optics (ECBO) 2025 taking place in Munich, Germany and published [157]. The contribution content is cited in its entirety since it fully describes the study. Start of the article:

Optical Coherence Tomography (OCT) is well established in the context of ophthalmology. Building upon OCT signals, Optical Coherence Tomography Angiography (OCTA) gains traction in the clinical use case. Consequently, the number of procedures is growing, a trend likely to be continued [89]. The impact of non-optimal imaging settings needs to be clarified to inform the scientific community and medical professionals. Detailed knowledge on their impact will help in assessing the importance of prevention, detection and potentially correction. Proper focusing was identified being a prerequisite for reliable inspection in OCTA [154]. Defocus leads to a decrease in signal strength [158]. Defocus was even used to intentionally decrease the signal strength [155]. This decrease is typically characterized based on a laterally extended mirror, which is reflected in well accepted publications [158, 159]. The transferability of this finding when imaging spherical scatterers like fat droplets in water emulsion is not straightforward. However, a detailed understanding of this relation will be needed to properly develop a detailed description of defocus effects and artefacts in OCT as well as OCTA. Therefore, we aim to characterize and compare the defocus effects looking at the laterally extended surface of a microfluidic chip as well as the signal from the channel filled with spherical scattering particles in diluted homogenized milk.

Methods:

A microfluidic chip (Microfluidic ChipShop, Fluidic 156) was used, offering channels with a rectangular cross section. Channel dimensions were 200µm in width and 200µm in height. Homogenized full fat milk was mixed with distilled water in a volume ratio 1:10. A syringe pump (Harvard Apparatus Pump Elite 11) was used to pump the mixture along the microfluidic channel. The perfusion rate was kept constant for all data acquisition positions at 100 µl per minute. The channel was imaged using a commercial SD-OCT System (Ganymede GAN612C1, Thorlabs, lens OCT-LK4-BB) operating at 248 kHz and 880 nm. The inbuilt

speckle variance mode was used. Pixel size was fixed at $6\mu\text{m}$. The field of view was set to 6 mm in x and 0.12 mm in y, leading to $1024 \times 1000 \times 20$ pixels. The distance between the microfluidic chip surface and channel to the scan head was varied by moving the scan head. The commercially available adjustable stand including focus block was used. In a first step, the channel was focused, looking at the subjectively assessed strongest signal. Then, the scan head was moved towards the chip until the upper surface was just visible in the ghost image. A speckle variance dataset was captured. The scan head was moved away from the chip turning the fine adjustment of the focus block for a full rotation, equivalent to $225\mu\text{m}$ of travel. Another dataset was captured. This modulation was repeated until the upper surface could not be detected anymore due to the maximum imaging depth. For each position, captured intensity values in decibel (dB) representing the structural OCT in one exemplary B-Scan were further analyzed. For each distance three Regions of Interest (ROI) were manually selected. First, a ROI containing the background signal only sized 100×100 pixels was extracted and the mean intensity within this ROI was calculated. The signal strength from the interface was assessed by fitting a ROI containing the upper surface of the chip, referred to as ROI Edge and picking the maximum value within this ROI. The mean value of the background ROI was subtracted. The signal strength coming from the channel perfused with diluted milk was extracted selecting a ROI within the channel, referred to as ROI Channel. The size of the ROI was kept constant at 30×30 pixels. The mean intensity was calculated. The mean value of the background ROI was subtracted. Based on the signal strength from interface and channel from each depth, a model was fit to applying the Matlab R2024b 'fit' function making with the 'gauss2' option. Although the fit model was not assumed to be purely gaussian, an approximate full width at half maximum (FWHM) was extracted to compare the two datasets picking the closest interpolated fit values.

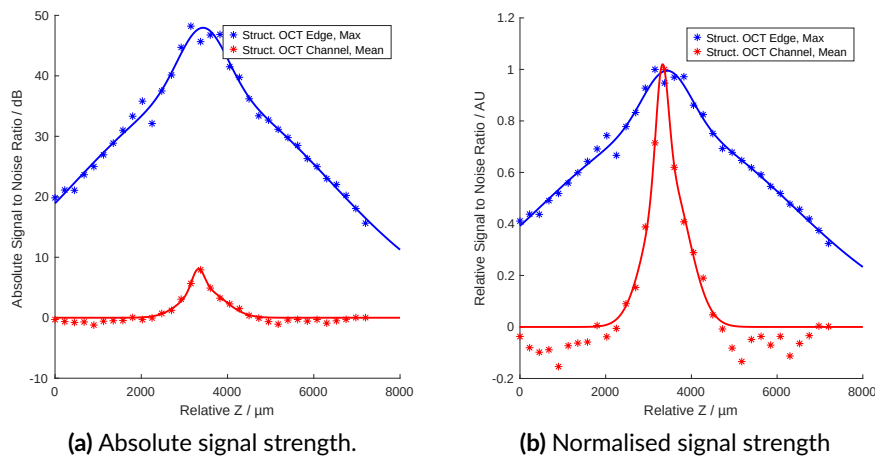


Figure 15.3: Absolute (a) and normalized (b) Signal Strength from the ROI Edge and the ROI Channel show a strong dependency on the (relative) distance to the OCT scan head.

Results:

The absolute as well as normalized signal strength for each position is visualized in figure 15.3. The signal strength level detected at the microfluidic chip surface is six-fold as high as the signal strength level detected from the diluted milk solution. The FWHM looking at the edge was approximated at 5488 μm . The FWHM looking at the Channel was approximated at 688 μm , leading to a factor 8 differentiating the two estimates.

Discussion:

The large difference in absolute signal strength is plausible due to the prominent difference of the refractive indices at the interface of air to the polymer microfluidic chip. In comparison, the refractive index difference among fat droplets and water is much smaller. The decay in signal strength is approximately symmetric for positive and negative deviations from the strongest signal position. This was found for the ROI Edge data as well as the ROI Channel data. Symmetric findings seem plausible due to the symmetric geometry of the converging / diverging beam around the focus. The signal strength decay with defocus based on the ROI Channel data is not a scaled version of the decay looking at the ROI edge data. On the contrary, Figure 15.4 reveals a much steeper decay when looking at the ROI Channel data as compared to the ROI edge data. The smaller FWHM based on ROI Channel becomes clear in Figure 15.4, right. We hypothesize that this observation is to be explained looking at the lateral extension of scatterers. Due to the limited size of the fat droplets, only parts of the incident OCT illumination power will hit the droplet and therefore be scattered. Consequently, the backscattered intensity will be lowered with increasing defocus and beam diameter. Looking at the laterally extended chip surface however, the illumination power will hit the surface. Consequently, the signal decrease with increasing defocus will be more pronounced looking at fat droplets than the chip surface. A theoretical derivation of this hypothesis as well as an analysis of the impact on variance / OCTA data will further detail the impact of defocus on signal strength.

Conclusion:

A sharper decrease in signal strength with smaller FWHM was observed when imaging particles compared to a laterally extended surface. This finding highlights the importance of focus in OCT and OCTA. The imaging strategy in OCTA is based on imaging particles, in contrast to structural OCT of layered retinal structures. Imaging with small defocus settings will be less impactful in structural OCT compared to OCTA. **End of the ECBO contribution.**

The defocus effect on optical coherence tomography angiography (OCTA) was analysed analogously. The result is visualised in figure 15.4. The OCTA signal strength is strongly affected by defocus, apparently equivalent to the optical coherence tomography (OCT) intensity from the channel ROI. The assessment of defocus effects on OCT signal strength using laterally extended targets must not be directly transferred to estimate the effects on OCTA signal strength. The OCTA signal is inherently caused by optically heterogeneous, laterally restricted, moving scatterers and is therefore subject to stricter focus precision

requirements compared to the visualisation of laterally extended surfaces. This finding is especially relevant in combined OCT and OCTA imaging strategies, since the retinal layer interfaces represent laterally extended surfaces while the red blood cell motion assessed through OCTA refers to laterally restricted targets. Consequential, a high signal strength in the OCT signal is necessary to obtain high signal strength in OCTA. However, the signal strength decrease in OCTA with defocus is less forgiving than the signal strength decrease in OCT. Focusing must be performed more precisely in OCTA.

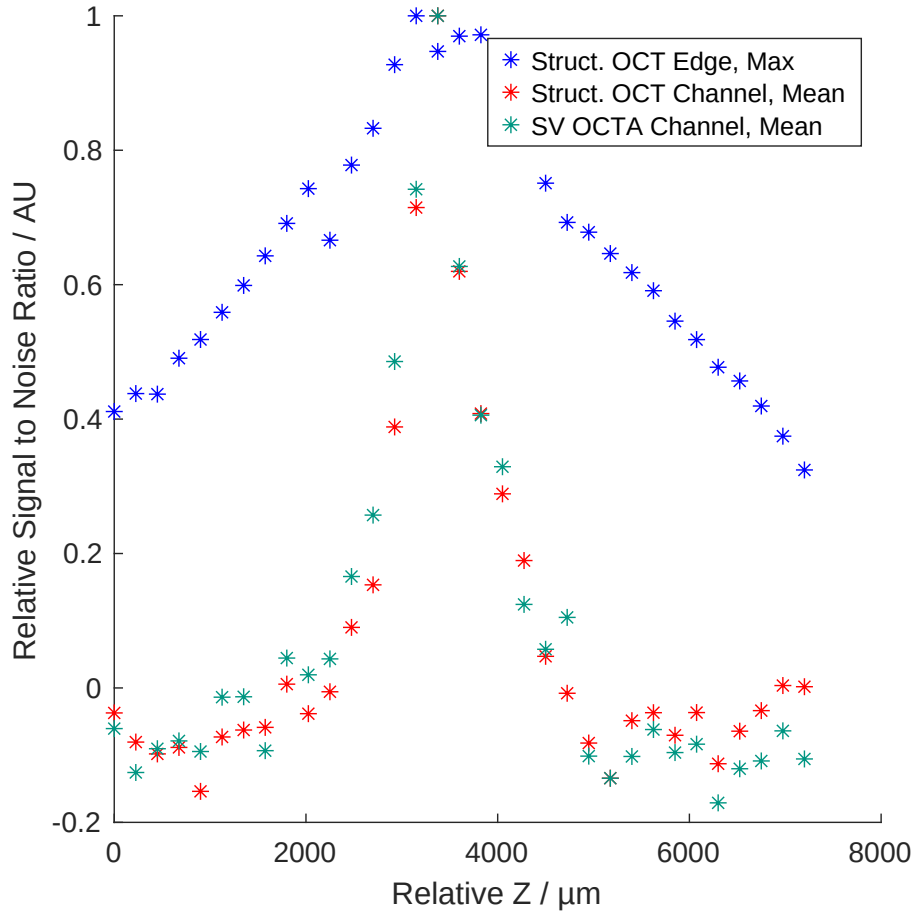


Figure 15.4: Normalized Signal Strength from Edge ROI (blue), OCT intensity from channel ROI (red) and speckle variance optical coherence tomography angiography (svOCTA) intensity from channel ROI (green) taken in varying distance between scan head and microfluidic chip.

Velocity Response Curve Parametrization by Defocus

The velocity response of optical coherence tomography angiography (OCTA) may be altered in defocus conditions compared to in focus positions. Multiple studies building on top of each other were conducted. Several velocities were set, covering a range of velocities that may occur in ophthalmic retinal vessels.

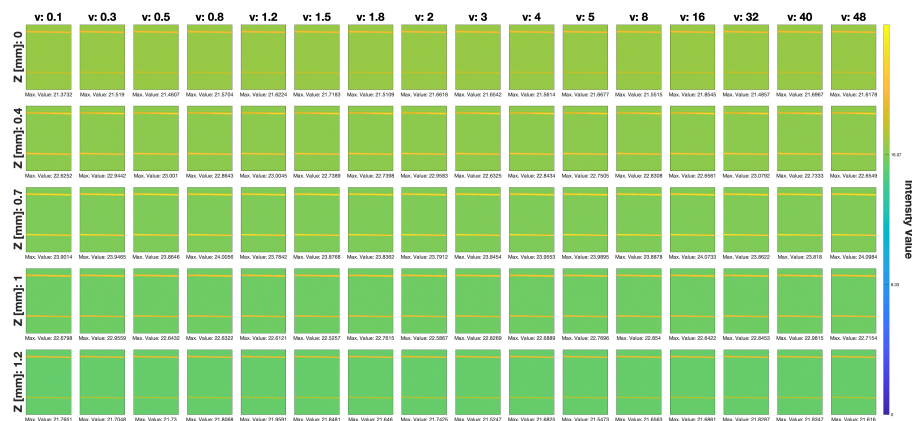


Figure 16.1: Structural OCT intensity values, en face projection resulting from mean projection covering the channel region of the microfluidic chip.

Speckle Variance Optical Coherence Tomography Angiography Depends on Distance to Focus Position

The following chapter was contributed to FloRetina ICOOR 2024 and may be found as abstract 421 of ICOOR 2024, Abstract Book available at https://floretina.com/storage/abstractsbook/floretina_2024_abstract_book.pdf.

Purpose

Optical Coherence Tomography Angiography (OCTA) gains more and more attention in re-

search and clinical practice. Interscan time was identified as an influence on OCTA intensity, potentially allowing for flow velocity quantification. This study demonstrates the additional influence of the distance to optimal focus on speckle variance intensity.

Methods

A 248 kHz OCT system was used. (Ganymede GAN612C1, Thorlabs, lens OCT-LK4-BB) A microfluidic chip (Microfluidic 156, Microfluidic Chipshop) was perfused with a mixture of homogenized milk (3,5% fat) and distilled water in a volume ratio 1:10. Mounted to a stage, the perfused channel was placed at five different distances to the OCT scanner. A syringe pump (Harvard Apparatus Pump Elite 11) was used to perfuse the channel. Speckle variance datasets were taken and mean projection was performed along Z in a slab with fixed thickness and manually set position. The full channel height was included.

Results

Setting the closest position to the scanner as a reference, included positions were located at $Z = 0; 0.4; 0.7; 1.0; 1.2$ mm. 16 flow velocities (v in mm/s) were set as indicated in figure 16.1. The upper channel was filled with air, the lower was perfused. Speckle Variance values increased slightly with increasing flow speed when in focus, as expected. Note the high scan rate. Additionally, speckle variance values depend on the distance to the optimal focus position. Larger distances were associated with lower speckle variance values. Despite this variation, the structural OCT did not change remarkably.

Conclusions

OCTA intensity values are related to the focus position with regard to the vessel despite plausible structural OCT data. Careful focussing will be necessary to ensure maximum contrast. Implications for vasculature quantification remain to be clarified. The role of vessel caliber and scatterer size is unclear.

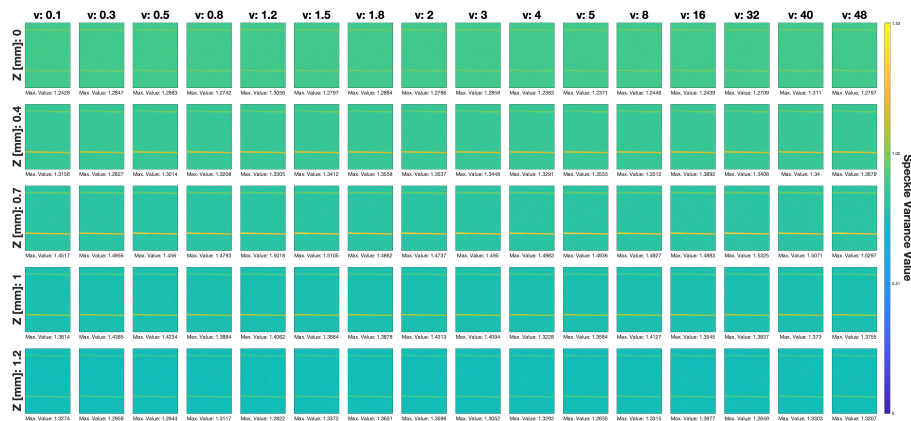


Figure 16.2: OCTA speckle variance values, en face projection resulting from mean projection covering the channel region of the microfluidic chip.

Extension of the ICOOR Study:

The previous study sparked interest. Apparently, there is a maximum defocus level allowable, beyond which the ability to observe correlated speckle variance optical coherence tomography angiography (svOCTA) values and set velocities. But: How does this relation present within the acceptance window, if densely sampled?

The presented setup was reused. However, densely sampled z positions were included and additional velocity settings were included. The analysis method was slightly updated. For the following results, regions of interest were defined within the channel, at the channel edge and covering the background signal. Two runs were conducted, covering slightly different parameter values for focus and scatterer speed.

Run One:

The methodology is equivalent to the methodology presented for Floretina ICOOR except for the different sampling density. A set of flow velocities was set for the milk-water mixture passing the $200\mu\text{m} \times 200\mu\text{m}$ rectangular cross section channel in the microfluidic chip. The passage was imaged applying the described optical coherence tomography (OCT) system. The scan head was mounted in various distances from the microfluidic chip. The relative translation is given in the legend of figure 16.3. Obtained svOCTA values are plotted against the set flow speed. A drop in overall signal level is observed, expressing in the offset of the single curves in figure 16.3. More severe defocus is associated with reduced overall signal levels.

In addition to this observation, the svOCTA response to slow flow velocities is a low signal, faster flow velocities result in larger values. A normalisation of the curves visualised in figure 16.4 with regard to the value observed when the fastest flow is set highlights the variable slope occurring as a response to setting faster flow velocities. The slope is apparently inconsistent, which was unexpected. This behaviour is named slope variation in the scope of this work.

The slope variation was further assessed by fitting linear models to the interplay of set velocity and svOCTA response, resulting in one linear model per relative position of the scan head. The resulting linear models are plotted in figure 16.5 on the left handside. Slopes found in the modelling step are visualized in figure 16.5 on the right handside along with the p-values obtained during modelling. A continuous variation of the slope with the focus position was found. The p values resulting from the modeling activity are low when the slope is relatively large, but grow once the slope is small. The acceptance range found before was therefore reproduced.

Run Two:

For run two, an even denser sampling strategy was applied. Additionally, a threefold redundancy was introduced to check for the repeatability of findings. For each scan head position and each set velocity value, three datasets were taken. The variability among the three takes was found to be negligible, therefore the following report will focus on one of the

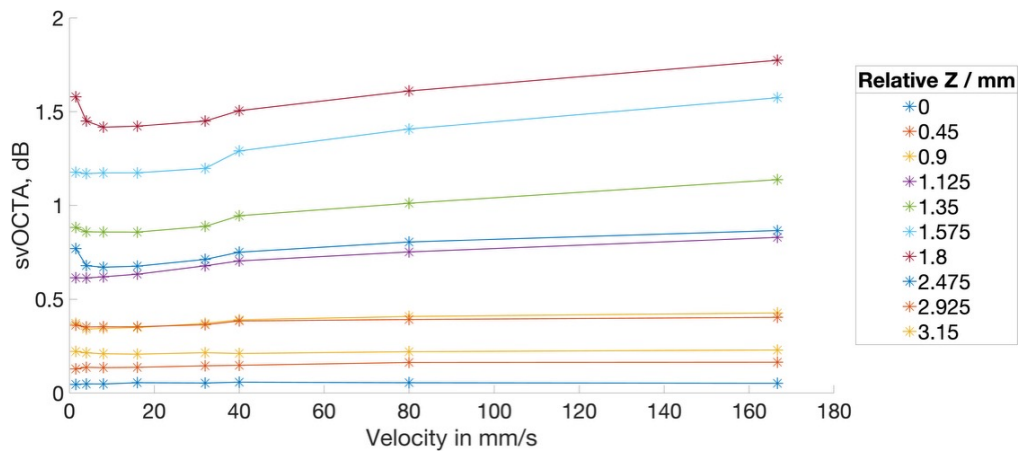


Figure 16.3: OCTA speckle variance values, taken at various positions, depend on the set flow velocity.

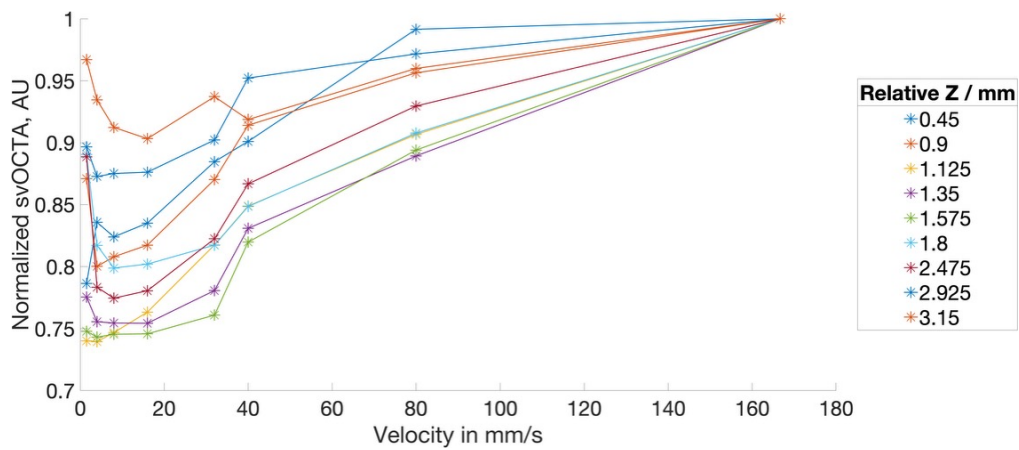


Figure 16.4: OCTA speckle variance values sensitivity to the set flow velocity varies with the relative distance to the scan head.

takes only. The visualisation was updated, too. The svOCTA response is a function of two independent variables, defocus, see figure 16.6 and flow velocity, see figure 16.7.

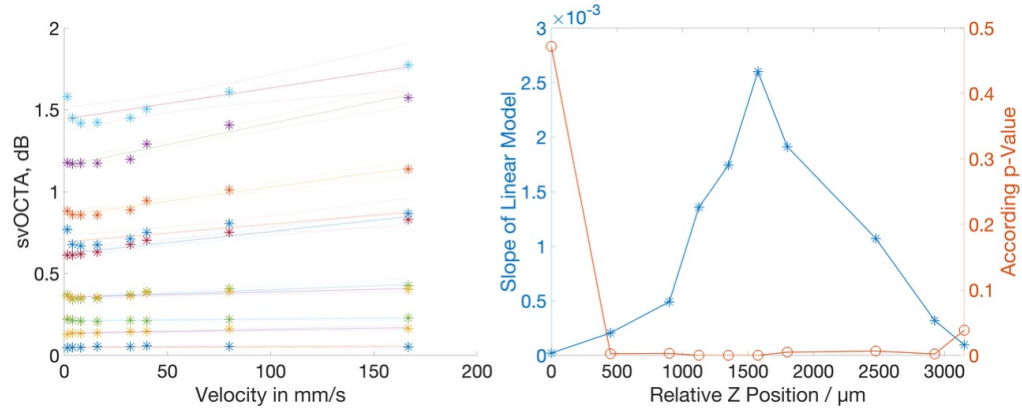


Figure 16.5: OCTA speckle variance values with linear regression modelling results, left. Calculated slope values vary with the relative distance to the scan head.

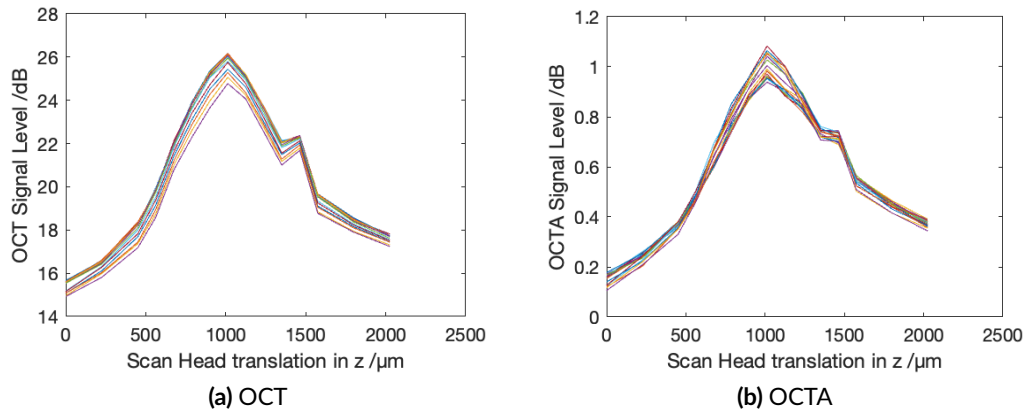


Figure 16.6: Run 2: OCT (a) and OCTA (b) Signal Strength shows a dependency on the (relative) distance to the OCT scan head.

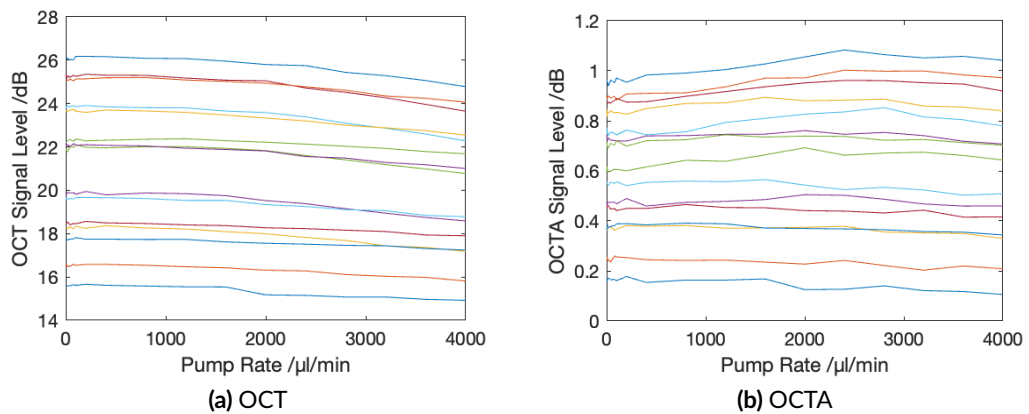


Figure 16.7: Run 2: OCT (a) and OCTA (b) Signal Strength shows a dependency on the pump rate, which is proportional to the flow velocity in the chip geometry.

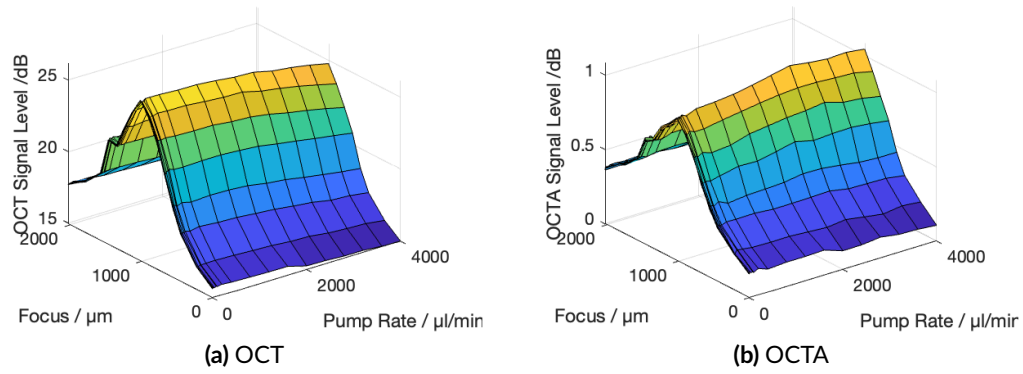


Figure 16.8: Run 2: OCT (a) and OCTA (b) Signal Strength show dependencies on defocus and pump rate.

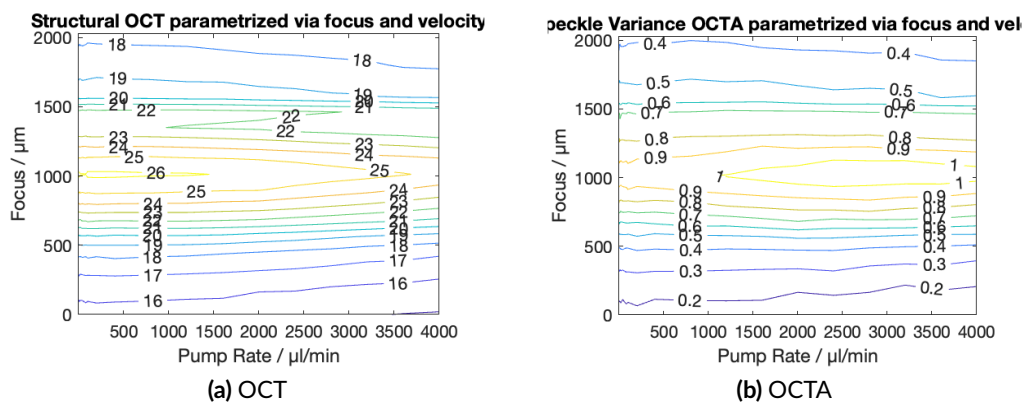


Figure 16.9: Run 2: OCT (a) and OCTA (b) Signal Strength show dependencies on defocus and pump rate.

The Role of the Scan Lens

The purpose of run two and three is ensuring visual comparability following on the equivalent structure of the underlying datasets. Run three is recorded using the scan lens OCT-LK2-BB, offering a numerical aperture of 0.148 when combined with the OCT system instead of the previously used lens OCT-LK4-BB coming with a numerical aperture of 0.05. Consequently, the lateral resolution is better by a factor of three, $4\mu\text{m}$ instead of $12\mu\text{m}$. Due to the even more limited Depth of focus, the distance of the relative positions among each other was further reduced. The set of positions are presented in the following result section. Results of run three are directly compared to result from run two.

OCTA findings comparing lens OCT-LK4-BB (left) and OCT-LK2-BB (right)

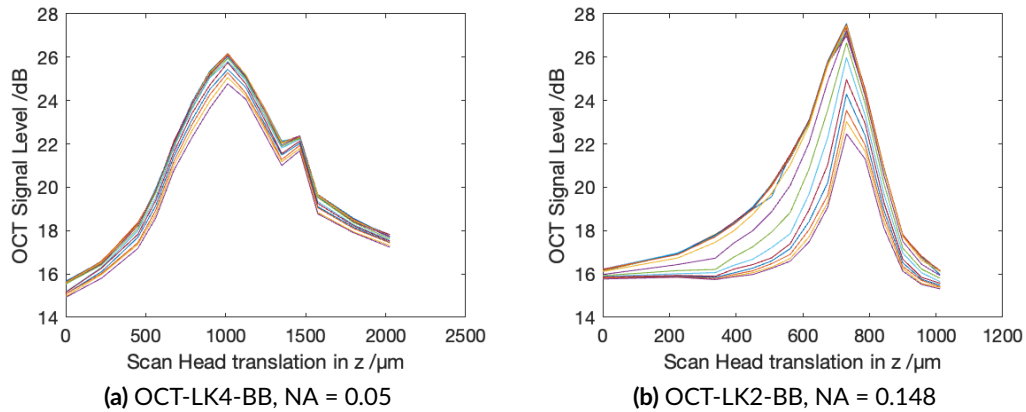


Figure 17.1: Data recorded with (a) OCT-LK4-BB, (b) OCT-LK2-BB: OCT Signal Strength shows a dependency on the (relative) distance to the OCT scan head.

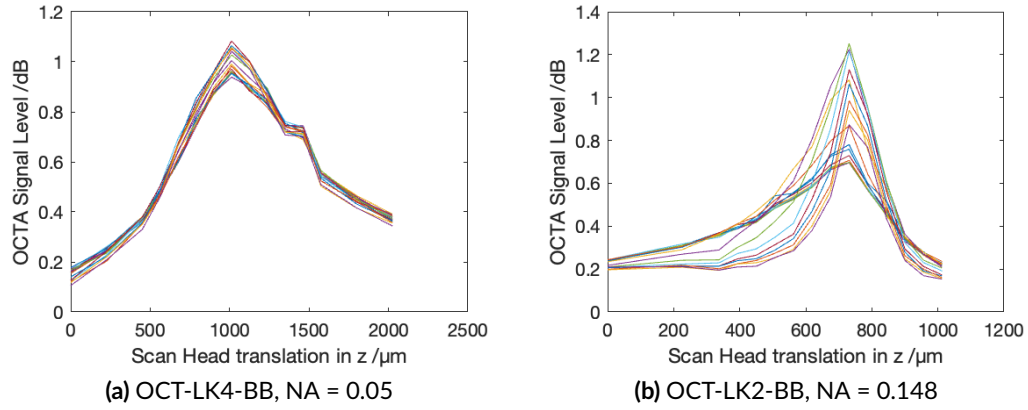


Figure 17.2: Data recorded with (a) OCT-LK4-BB, (b) OCT-LK2-BB: OCTA Signal Strength shows a dependency on the (relative) distance to the OCT scan head.

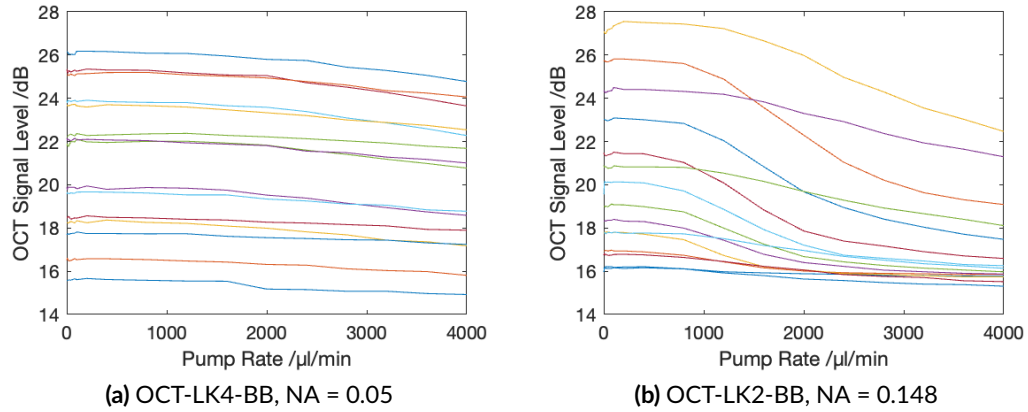


Figure 17.3: Data recorded with (a) OCT-LK4-BB, (b) OCT-LK2-BB: OCT Signal Strength shows a dependency on the pump rate, which is proportional to the flow velocity in the chip geometry.

Discussion

Qualitative differences in the velocity response were found comparing the two lenses. With a larger numerical aperture, the optical coherence tomography (OCT) signal attenuation appears to be stronger and asymmetric as seen in figure 17.1, where the signal decay with defocus is steeper. In parallel, the velocity dependence appears to be stronger, expressing in the larger spread among the single velocity curves in b) compared to a). Looking at optical coherence tomography angiography (OCTA), the defocus parametrisation presents more complex, apparently due to an interplay of velocity response and defocus. While defocus is the main factor in 17.2 a), verified through the equivalent graph shapes, graphs are much less equivalent among each other in b).

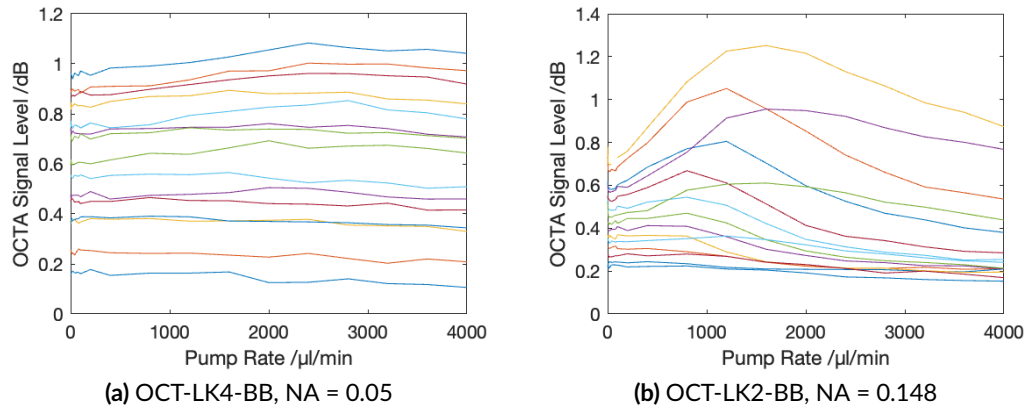


Figure 17.4: Data recorded with (a) OCT-LK4-BB, (b) OCT-LK2-BB: OCTA Signal Strength shows a dependency on the pump rate, which is proportional to the flow velocity in the chip geometry.

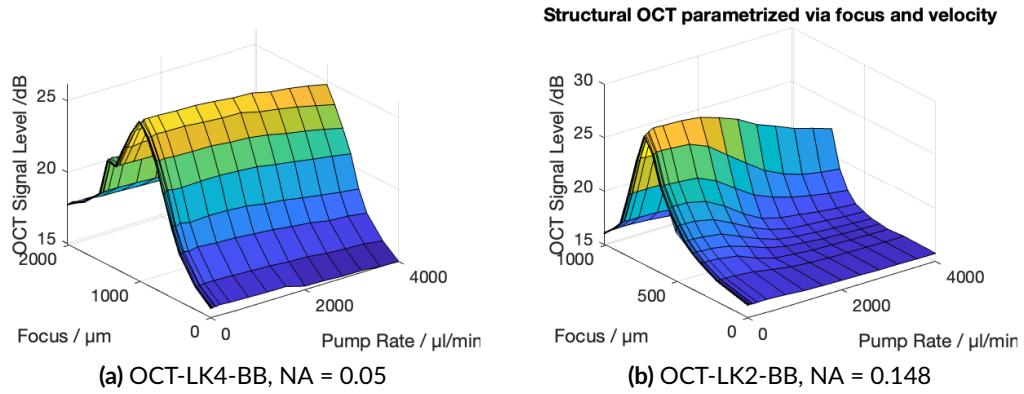


Figure 17.5: Data recorded with (a) OCT-LK4-BB, (b) OCT-LK2-BB: OCT Signal Strength show dependencies on defocus and pump rate.

The OCT signal strength is reduced with faster flows in both cases, compare figure 17.3. The reduction is more pronounced when imaging is done with the larger numerical aperture lens. Consistently, the OCTA velocity response is more pronounced, see figure 17.4.

The interplay is visualized in figures 17.5, 17.6, 17.7, 17.8

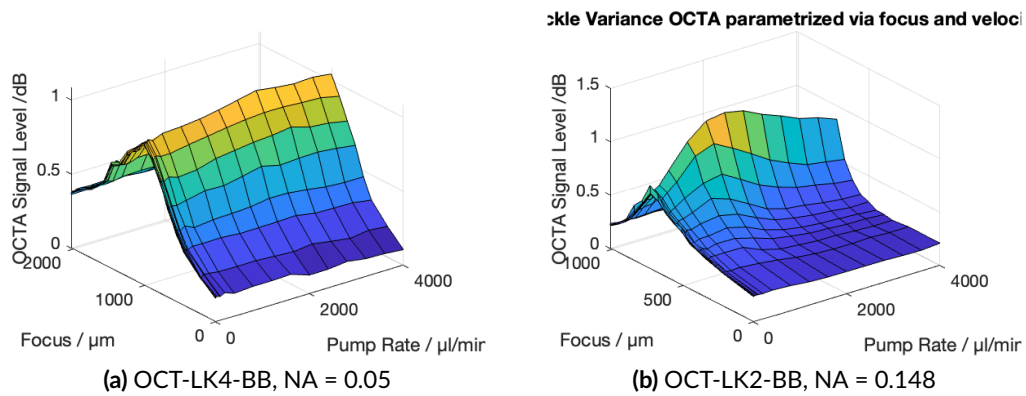


Figure 17.6: Data recorded with (a) OCT-LK4-BB, (b) OCT-LK2-BB: OCTA Signal Strength show dependencies on defocus and pump rate.

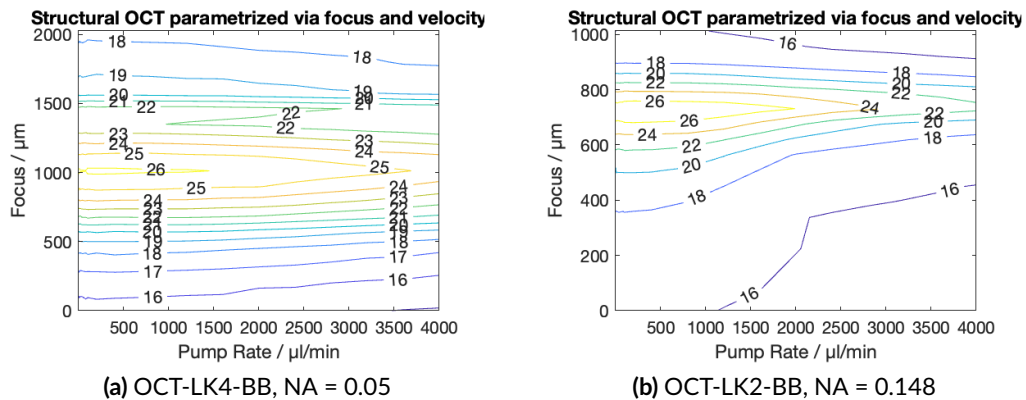


Figure 17.7: Data recorded with (a) OCT-LK4-BB, (b) OCT-LK2-BB: OCT Signal Strength show dependencies on defocus and pump rate.

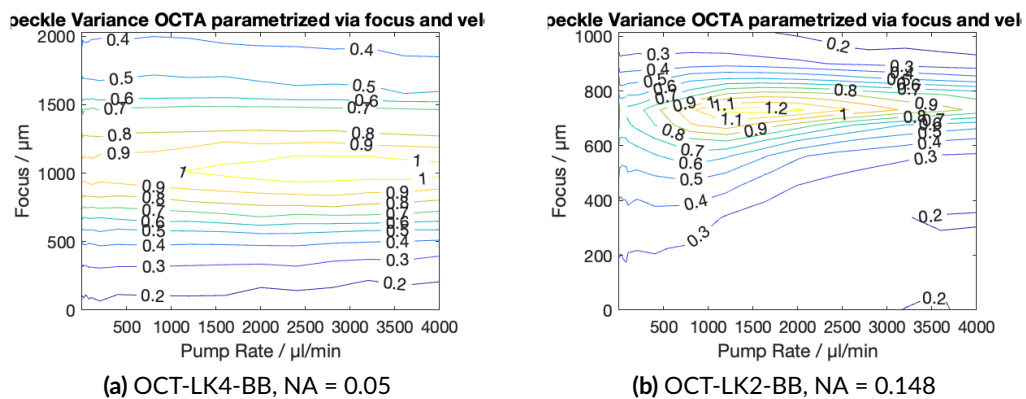


Figure 17.8: Data recorded with (a) OCT-LK4-BB, (b) OCT-LK2-BB: OCTA Signal Strength show dependencies on defocus and pump rate.

Defocus Impact on En Face Projections

Defocus effects on en face projections are of particular interest since the majority of quantification algorithms is built as en face projection analysis. The impact of defocus on the en face projection is therefore of direct relevance to everyone interested in the conduction of vasculature quantification studies, but also for those aiming to draw conclusions from a multitude of studies. The following investigation does neither allow to claim completeness nor exhaustive precision. However, qualitative trends and tendencies will become obvious and offer a base for the planning of more extensive follow-up studies.

Defocus impacts on the signal at single scan locations were described in the previous chapters. How does this behaviour translate to the en face projection appearance? Several expectations and hypotheses were formulated. First, with growing defocus level, the en face projection will become gradually darker. In addition, the signal from single vessels will become gradually darker.

Second, single vessels will appear broadened. This appearance shift will be quadratic with defocus, since the scan points just outside to both sides when in focus will capture the vessel once the defocus is more severe.

Third, small vessels, presenting slow flow velocities will not be visualised anymore.

Fourth, the contrast will be reduced. While the signal from moving particles is expected to decrease quickly, the signal and signal fluctuation from interfaces will decrease more slowly. This combination leads to an overall contrast reduction with growing defocus.

Methods

En face projections in various degrees of defocus were taken from one healthy volunteer. The volunteer was myopic at -4.5 dpt. The Heidelberg Spectralis device was used. It offers feedback on the focus setting for the device operator. In addition, the set focus values were exported from the device along with the en face projections of superficial vascular complex (SVC) and deep vascular complex (DVC). The inbuilt segmentation routine was followed

after segmentation results were checked for plausibility. Further processing was done in Matlab R2024b. The optical coherence tomography angiography (OCTA) portion of the exported en face projections was cropped. The outermost pixel lines were cropped as well to prevent downstream issues with contrast calculations. The cropped region of interest was binarised by thresholding, applying Otsu's method for the threshold determination. The binary image was further skeletonised to obtain a single pixel centreline.

Mean and median were calculated for each original en face projection. The Michelson contrast within the OCTA information was extracted. Vessel Area Density, Vessel Length Density and Vessel Diameter Index were calculated.

$$MichelsonContrast = \frac{MaximumGrayValue - MinimumGrayValue}{MaximumGrayValue + MinimumGrayValue}, \quad (18.1)$$

$$VesselAreaDensity(VAD) = \frac{NumberForegroundPixels}{TotalPixelNumber}, \quad (18.2)$$

$$VesselLengthDensity(VLD) = \frac{NumberCentrelinePixels}{TotalPixelNumber}, \quad (18.3)$$

$$VesselDiameterIndex(VDI) = \frac{VAD}{VLD}, \quad (18.4)$$

Results

The en face projections recorded from the SVC are presented in figure 18.1, those from the DVC are presented in figure 18.2. The dependency of quantification metrics in the SVC on defocus is demonstrated in figure 18.3 with linear and logarithmic scaling. Equivalent results for the DVC are shown in figure 18.4

VAD, VLD, mean and median values are affected by defocus. Michelson Contrast and VDI appear robust against defocus. Find the visualisation in figure 18.3 and 18.4.

Discussion

Capillaries are easily overlooked in defocus conditions, while the larger vessels remain visible. The overall brightness decreases, as is expected from the phantom learnings. The disappearance of the capillaries is speculated to be due to the smaller velocity within capillaries as compared to larger vessels. Vasculature density values, VAD and VLD are reduced with increasing defocus, in accordance with the potential to simply miss vessels when imaging under defocus condition. While the severe cases appear visibly implausible, overlooking capillaries presents in a subtle way. The flawed imaging is hard to identify, defocus cases might therefore go unnoticed. Interestingly, the contrast was found to be robust against defocus. This behaviour might be explainable considering the proprietary reconstruction algorithm, since the imaging task is considered a classification [160]. In conclusion, defocus was identified being a control variable if vasculature quantification is planned.

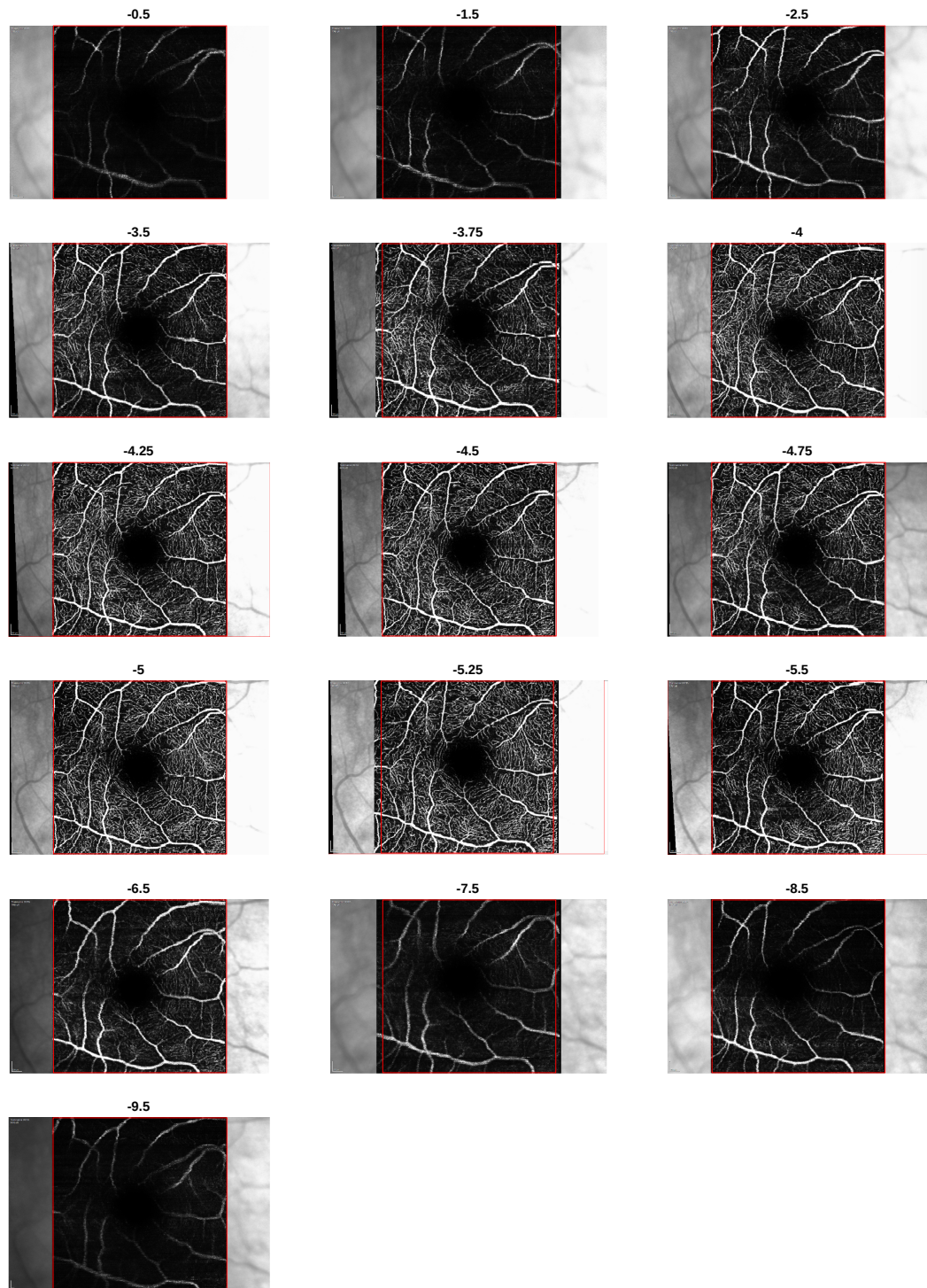


Figure 18.1: Collage of SVC en face projections taken in more or less severe defocus conditions. Titles refer to the focus setting in dpt. The volunteer was myopic at -4.5 dpt.

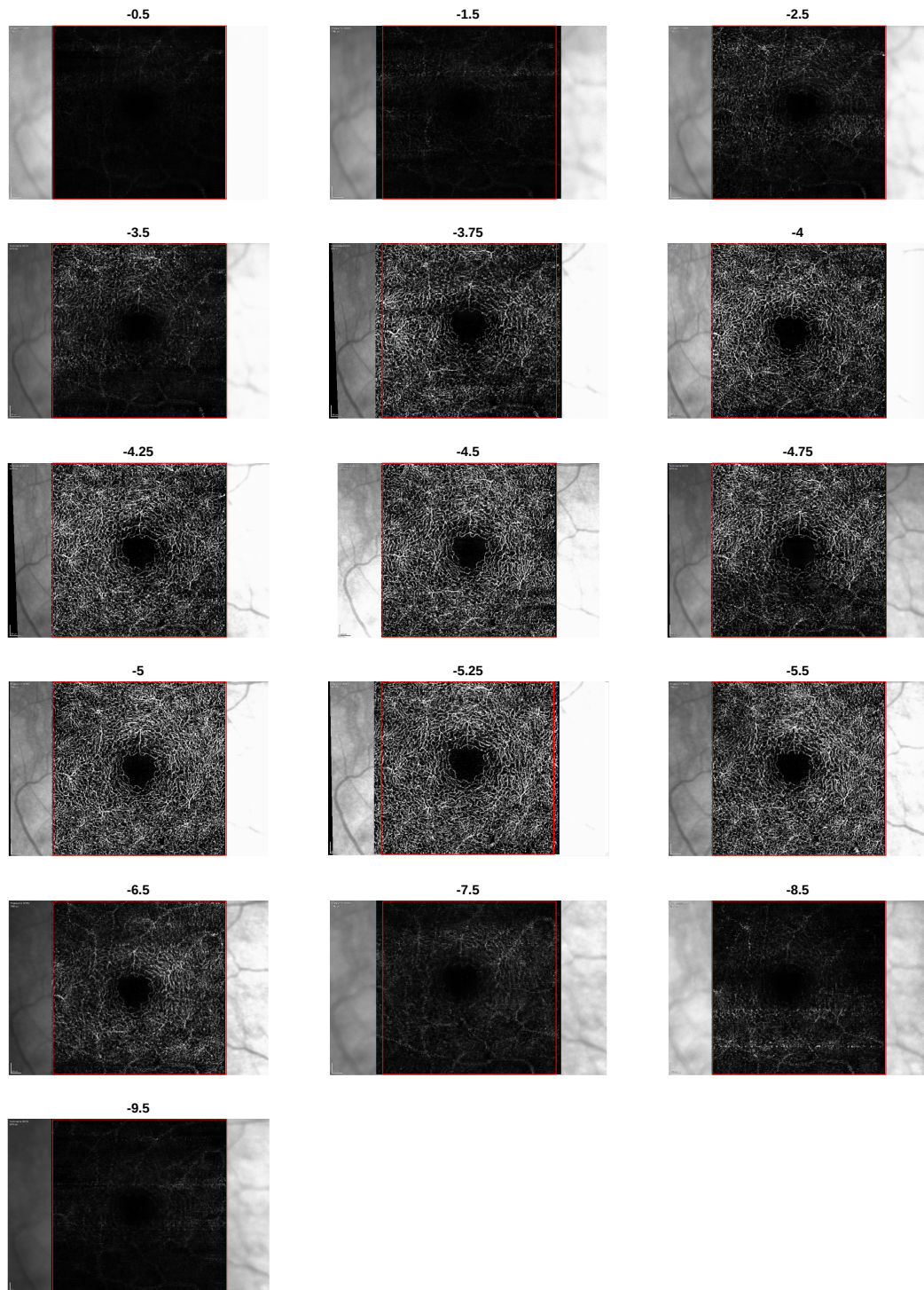
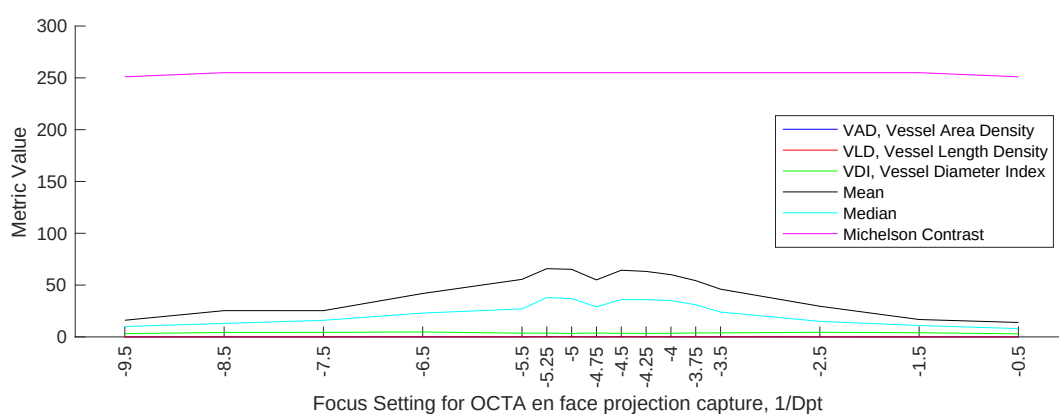
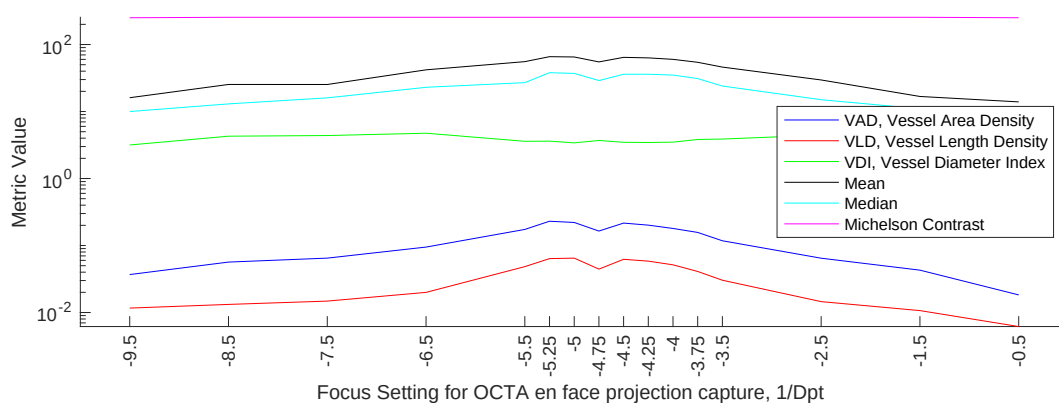


Figure 18.2: Collage of DVC en face projections taken in more or less severe defocus conditions. Titles refer to the focus setting in dpt. The volunteer was myopic at -4.5 dpt.



(a) SVC, Linear Scale



(b) SVC, Logarithmic Scale

Figure 18.3: SVC en face projection quantification results with regard to defocus.

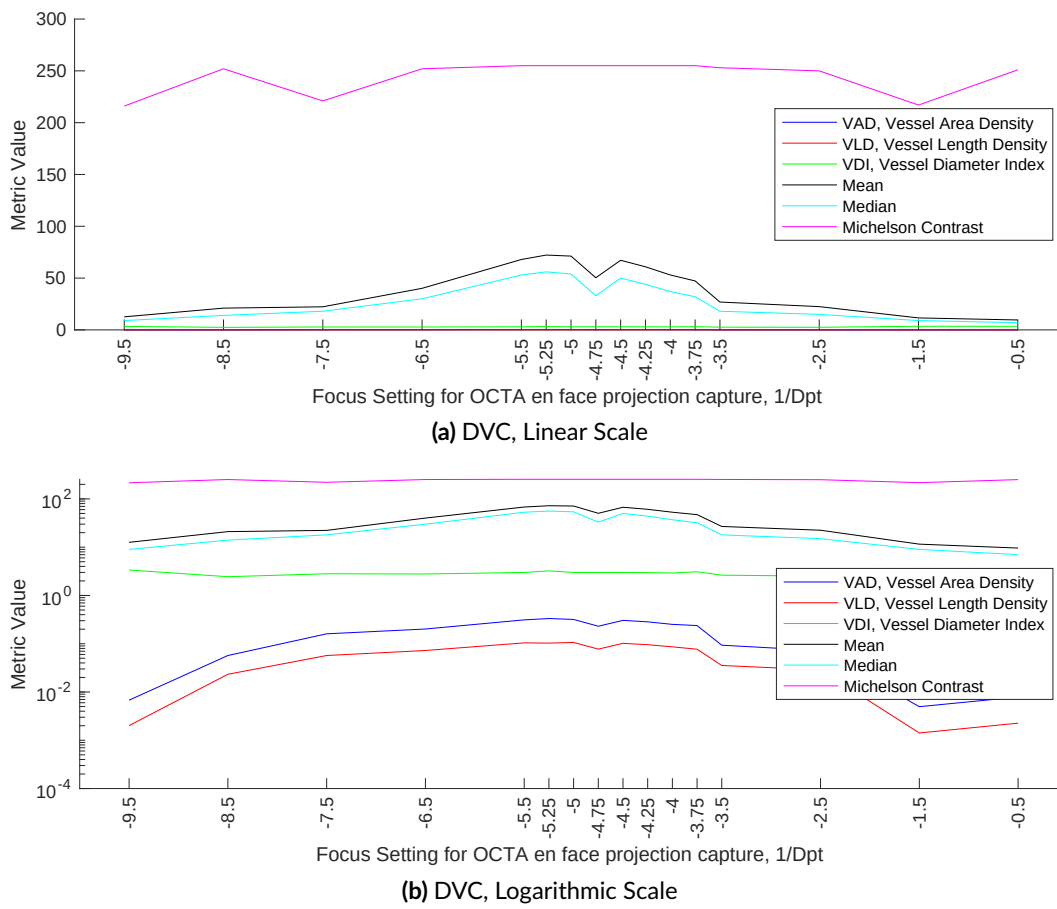


Figure 18.4: DVC en face projection quantification results with regard to defocus.

DISCUSSION AND CLOSING REMARKS

The presented work contributes additional quantification studies on diabetic macular ischemia (DMI). However, the main contribution of this work is the recognition of the overarching line of reasoning, the diagnostic chain. However, quantification studies typically limit themselves to algorithm development and the clinically relevant analysis. The impact of imaging settings is only occasionally studied, despite being interwoven in the images analysed. The phantom setup presented in this work allows for a systematic investigation of these imaging parameters. Retinal vasculature quantification must include imaging and downstream analysis, at least until the imaging is comprehensively characterised, well understood and repeatable. In order to move towards this direction, a few thoughts on an early quality management in the context of OCTA are discussed in the upcoming sections.

First, on insights on the vascular presentation of DMI, an understudied vascular complication secondary to Diabetic Retinopathy and Diabetes Mellitus. Nonperfusion areas, which we called ischemia areas differ significantly comparing healthy controls and diabetic retinopathy patients, appearing more frequent and in larger size in diabetics. The postulated existence of DMI phenotypes was not confirmed at the first sight. However, there is a lot of room for exploration left. The vascular density is associated with retinal sensitivity as assessed with microperimetry in Diabetics when a sufficiently large field of view is assessed, 10 degree by 10 degree in our case. When it comes to a smaller scale local association of vascularity and sensitivity, no correlation was found. Similarly, this relation was not found in healthy persons. Apparently, there is more to the disease progression than a sole explanation of the sensitivity drop due to reduced perfusion. Caution is advised when imaging patients suffering from cataract despite the good reproducibility of retinal thickness measurements [161]. Last but not least, focus precision was determined as a significant predictor of quantification results. Despite the inability to explain the full variability, its role must be better understood.

The presented study highlights an interesting aspect on phantoms. A phantom is designed with a goal in mind. However, it often times is a starting point of a thought process. It may be used to understand the technology, the parameter space thoroughly. This is the use case of a the phantom developed in and for this work. A good training phantom allows for an easy, straightforward start into the matter. A verification phantom on the other hand is used in a different scenario: It is used to demonstrate and document quality assessment and may be implemented to test manufactured and / or installed devices. This phantom is used

to answer questions like "What is the resolution", "Is a certain benchmark hit", "Does this device comply with regulatory / internal quality demands?".

In a simplified setup, several key aspects of defocus impact on OCTA signals were found. There is a signal loss scaling with the severity of defocus. Assessing this signal loss using laterally extended surfaces is misleading if the imaging behaviour with small moving particles is of interest. The OCTA velocity response is altered with defocus. Surprisingly, a strong influence of the scan lens, equipped with a larger numerical aperture was found. The velocity response presented notably steeper in this case. In addition, the symmetric appearance of the signal drop was lost.

A Defocus Quality Control Ecosystem

The aim of optical coherence tomography angiography (OCTA) quantification is the provision of a noninvasive, objective, precise retinal vasculature assessment tool. Therefore, the technical aspects must be traceable but should stay in the background. However, their visibility in the calls for standardisation document an underdeveloped technology maturity and the need to establish quality control methodology and ecosystem [97].

The knowledge collected in the previous parts of this dissertation is analysed with regard to the development of an initial version of a quality control system for the focusing step. This contribution to the setup of a defocus control system is a necessary yet early step in the development of a comprehensive quality management strategy for vasculature quantification using OCTA. Establishing a full quality control system is beyond the scope of the presented research project. Looking at defocus exclusively, several options and cornerstones are clearly sketched out already.

The scientific contribution coming with the development of a quality management strategy for vasculature quantification is twofold. On the one hand, a characterisation of the imaging physics is needed to come up with suitable control mechanisms. On the other hand, the application of developed quality control systems enables the quantification of the retinal vasculature in a comparable manner across use cases, devices and device modes. Comparability includes the case of explainable deviations among devices and modes.

Multiple aspects contributing to a quality-aware OCTA quantification ecosystem are discussed. First, knowledge on defocus effects must be documented in an accessible manner. The potential to avoid defocus implications in the first place is discussed. Methods aiming at the detection of defocus in en face projections are developed and must be further developed in the future. Lastly, the potential of computational defocus correction is discussed.

Become and stay aware of defocus effects!

Although imaging under defocus conditions is typically unwanted, its occurrence is inevitable. Instead of condemning the presence of defocus, awareness and a conscious defocus handling strategy must be developed. The first step is the development of comprehensive accessible information on defocus implications. While Tomlinson et al. [154] commented on the disturbance introduced by defocus in quantitative OCTA analysis, the according guidelines neither mention defocus effects nor appropriate focusing strategies [95]. However, many studies report on the importance of signal strength and a minimum signal strength is increasingly introduced as a quality control step in study methodology. Factors affecting signal strength [162] and the consequences of reduced signal strength were explored [155], should be further explored and supplemented with the data. Studies on defocus prevalence exist [163]. Quality control systems must be further developed [52].

Similarly, there are no established OCTA reporting guidelines yet and focus management strategies are rarely reported despite being part of the imaging methodology. This circumstance complicates meta-analyses [97]. The focusing strategy and the use of potential technical support tools should be routinely reported. Reporting may further be simplified once metadata on focus settings is ubiquitously available as part of the device readouts. The presented work contributes additional perspectives on the subject. However, the interplay of defocus and additional parameters remains unknown. Future studies may contribute to this knowledge gap.

Despite the documentation of knowledge among the scientific community, OCTA quantification is primarily an enabling technology for basic medical and clinical research. Topics discussed in the more technical journals and conferences do not necessarily reach this group. Consequently, spreading the acquired knowledge among clinical scientists and device operators is pivotal for the development of an objective OCTA quantification methodology and research landscape.

Emerging scientists, newcomers, typically struggle with the amount of novel concepts and methods. Preparing accessible information for this group simplifies their training and lessens the burden of erroneous study protocols.

Avoid defocus!

OCTA quantification building on a dataset taken under defocus conditions is disadvantageous. Therefore, defocus should be avoided wherever possible. On the one hand, reducing the numerical aperture involved is an option. Reducing the numerical aperture is equivalent to a less pronounced convergence of the probe beam. Despite its potential to push back the prevalence of defocus imaging sessions, the decreased lateral resolution makes this approach appear unattractive.

On the other hand, simplifying and clarifying the current focus precision during the imaging procedure may be realised. Several indicators may be used. Among them, a qualitative evaluation of the sharpness of fine vessels surrounding the foveal avascular zone in the

infrared fundus image is recommended in various manufacturer trainings.

Dynamic focusing was discussed but was not routinely implemented until now [164]. Further developments on sharpness assurance and indicators analysing various datasets in multimodal imaging systems will be needed. The development must be tailored to the device due to the variable optic specifications and imaging strategies.

Find and flag defocus!

Awareness will lead to a direct follow up question: How to realise that defocus does play a role in a dataset that was already taken? There are some straightforward options: Add the device focus settings to the metadata. Come up with a convenient image exchange format allowing to contain information on the patient, the device and the image capturing process.

However, looking at the existing datasets taken with subjective focus precision management without bookkeeping, image-based heuristics and quality scores may help. Although defocus was found as a predictor of quantification results, no model was found so far allowing to predict defocus from quantification results. This approach is promising if relative defocus values in images from the same patient are of interest. However, this will rarely be the case in clinical studies.

Correct for defocus!

Computation defocus correction was developed for point scanning as well as full field optical coherence tomography (OCT) implementations as early as 2006 [165]. A detailed overview on the various approaches is found in reference [166]. Despite the consistent scientific interest, developed methods are not without drawbacks. Increased sensitivity to motion was found, currently preventing the translation to the clinical practice [166]. Moreover, dense lateral sampling was identified as a premise of computational refocusing ability [167, 168]. However, dense sampling increases the imaging time and therefore increases the likelihood of occurring motion artefacts. Computational defocus correction is an interesting pathway deserving scientific attention. Incorporation of computational refocusing methods in a quality control ecosystem is attractive once technical feasibility is reached.

Outlook - Parameter Space Exploration beyond Defocus

The root cause analysis on defocus effects presented in the given scientific body of work may serve as a role model for upcoming scientific as well as educational projects. Further details may be added to the defocus impact characterisation. Additional parameters need urgent attention to enhance the reliability in vasculature quantification beyond the state of the art. The presented phantom offers a starting point for further investigations. Phantoms will continue to play an interesting role in research and science, clarifying the fundamentals of imaging physics. In addition, reproducible phantoms may be interesting in the routine verification of imaging performance in actively used devices. Operator education and training will benefit from accessible and well-built phantoms since training can be performed without the presence of a second person.

Several parameters and associated questions were collected. Scientists interested in the field are encouraged to tackle these issues step by step.

Sample - Anterior and Vitreous Impacts

Question: How does the anterior chamber and vitreous passage influence the signal?

Thesis:

- A) There is magnification.
- B) There is image distortion.

Hypothesis:

- A) With knowledge on the magnification from autorefractor, magnification effects may be undone, leading to more consistent findings in longitudinal studies in patients with progressing myopia.
- B) The findings from lab setups are to be seen as a best case scenario and upper performance limit.

Potential Outcome:

- A) Consistent measurements of structures of a stable size despite refractive change.
- B) Resolution is worsened once random refractive media is inserted.

Sample - Dispersion Necessity - Signal from a single, homogeneous fluid?

Question: Is velocity the only driver of OCTA signal or is there more to it?

Thesis: Heterogeneity is needed, too!

Hypothesis: There is no OCTA signal from moving saline solution and stained water.

Potential Outcome: In focus velocity set imaging. Little OCTA signal throughout. A problem: How little is little?

Sample - Dispersive Phase Concentration

Question: How does scatterer concentration influence the OCTA signal?

Thesis: Most back and forth will lead to the strongest OCTA signal.

Hypothesis: There is a maximum signal level at 50 percent volume.

Potential Outcome: x: Concentration, y: Velocity, z: OCTA signal.

Sample - Dispersive Phase Droplet / Particle Size

Question: How does scatterer particle size influence the OCTA signal?

Thesis: There is an interplay explainable via NA, Mie theory and particle size.

Hypothesis: Detail out the theory for this. This is likely allowing for a mathematical model.

Potential Outcome: x: Particle size, y: Velocity, z: OCTA signal.

Sample - Dispersive Phase Reflectivity in all combinations

Question: How does the dispersive phase reflectivity influence the OCTA signal?

Assumption: Reflectivity is a diffuse material issue only. There is no specular reflective contribution.

Thesis: It is on contrast. OCTA signal strength scales with reflectivity contrast.

Hypothesis: Swapping materials in phase and solvent does not influence OCTA signal levels if the concentration is set to 50 % Vol. Playing with the contrast by exchanging one or both materials does.

Potential Outcome: Signal level for various material combinations. Precondition: Suspension must be realised.

Sample - Vessel Caliber

Question: Is there an influence of the vessel caliber on the OCTA signal?

Thesis: Multiple scattering effectively enlarges the focal volume beyond the calculated one.

Hypothesis: There is a slightly increased signal level from large caliber vessels.

Potential Outcome: Large vs. small channel, same velocity set, comparison of levels.

Sample Dynamics - Velocity at zero:

Question: Is (collimated) velocity the only driver of OCTA signal or is there more to it?

Thesis: Brownian motion contributes.

Hypothesis: There is an OCTA signal at room temperature. This contribution increases once the sample is heated and decreases with cooling.

Sample Dynamics - Velocity

Question: How does velocity influence the OCTA signal?

Thesis: There are multiple regimes to this behaviour.

Hypothesis: After an initial rise in signal with rising velocity, there is a turning point beyond which this proportional relation fades.

Potential Outcome: Velocity Response Curve

Imaging - Lateral Sampling Density

Question: At what point is increasing the lateral sampling density becoming ineffective?

Thesis: Beyond half the spotsize, as of Nyquist. Partial overlap at edges is sampled very precisely.

Hypothesis: At a single edge, denser sampling does not lead to more details being imaged beyond the Nyquist limit.

Potential Outcome: x: Scan location, y: stepsize. z: OCT

Imaging - Wavelength center and bandwidth

Question: How do OCT systems with various wavelength ranges performing in comparison to one another?

Thesis: Penetration depth differs.

Hypothesis: The knowledge on resolution and penetration depths fully explains the behaviour.

Analysis - Amplitude / Phase / Complex based OCTA

Question: How do the OCTA algorithms compare against each other?

Thesis: Different algorithms highlight different aspects in differing weighings.

Hypothesis: There are sensitivity differences expressing in various Velocity Response Curves.

Potential Outcome: Velocity Response Curves per Algorithm.

Isohyps theory and Equivalencies

Question: How are the OCT parameters connected among each other?

Thesis: There are parameter pairs balancing out each other's variation.

Hypotheses:

- a) Enlarged illumination spot size and enlarged effective backscattering cross section balance out each other.
- b) Enlarged particle velocity and faster scan rate balance out each other.
- b) Enlarged particle velocity and faster scan rate balance out each other.

Conclusion

Although there is a lot of room for future improvement in the field of blood flow imaging using optical coherence tomography angiography (OCTA) as discussed in the outlook section, this work contributes several aspects to the body of knowledge.

First, a theory on the origin of inter device inconsistencies in vasculature quantification was developed. This theory, supplemented with an experimental setup suitable for the systematic exploration of the postulated parameter space is publicly available from now on and remains to be verified or falsified.

Second, a versatile experimental setup was developed, allowing for the assessment of isolated parameters in a controlled environment, therefore allowing for a systematic investigation of the isolated parameter's effects.

Third, the signal strength reduction effects of defocus were characterised in a controlled setup. Besides the expected verification of signal loss looking at surfaces, a steeper decrease was found when particles were imaged instead of surfaces. This finding is especially relevant when combined optical coherence tomography (OCT) and OCTA imaging is done, since sufficiently strong OCT signals do not necessarily indicate strong OCTA signals.

Fourth, the OCTA velocity response is parametrised by defocus. Comparing two scan lenses with two distinct numerical apertures, a parametrisation role of the scan lens was found. This finding highlights the importance of sample arm optics and its potential role in limiting comparability.

Fifth, several quantification studies were contributed to the field of diabetic macular ischemia (DMI) research.

The knowledge base on OCTA continues to grow. Precise, controlled focusing must be met as a premise for precise and reliable downstream vasculature quantification. The early approach called diagnostic chain, as introduced in chapter 10 needs to be complemented with the identification of premises rooted in the existence of the OCTA parameter space. Its characterisation will allow for an estimation of downstream effects, enabling informed decision making and the development of appropriate quality control during imaging. The updated version of the diagnostic chain is visualised in figure 21.1. OCTA is increasingly

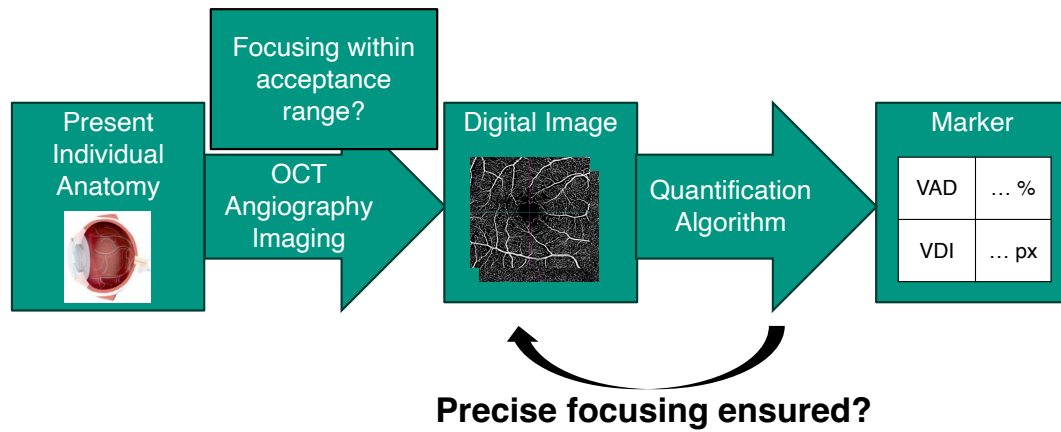


Figure 21.1: Visual presentation of the diagnostic chain including the premise of precise focusing and a potential quality control system.

accepted in clinical research and is therefore forced to evolve from a research tool operated by imaging enthusiasts with great caution towards a broadly applied tool for clinical practice and research. This work contributed fundamental information on the role of defocus in this transition and in downstream quantification.

Collection of Poster Presentations contributed to Conferences

Poster contributions for the following conference attendances are attached:

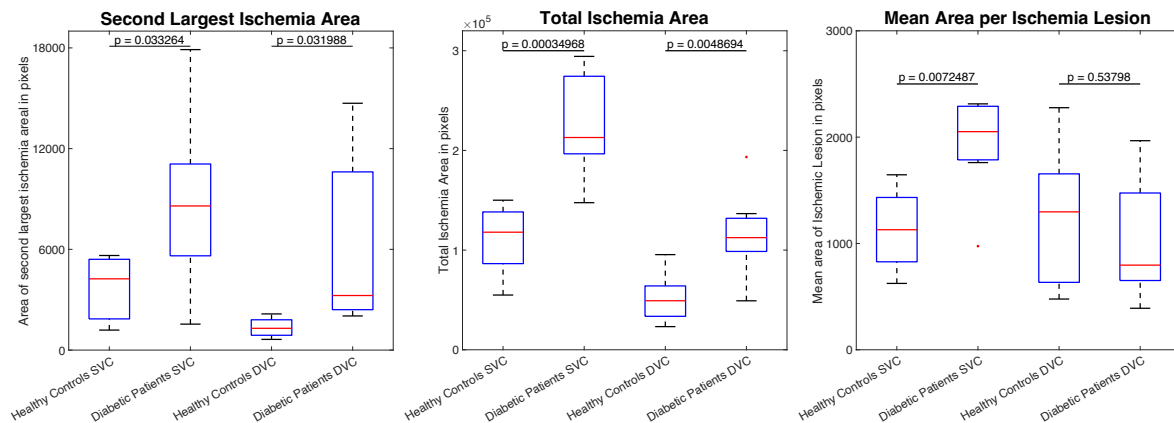
- EVER Congress 2023, Valencia
- ARVO 2025, Salt Lake City
- SPIE Summer School, Hven
- ECBO 2025, Munich
- DOG Congress 2025, Berlin

Local Ischemia Morphology in Diabetics Differs from Healthy Controls

Simon Hoffmann¹, Lucy J Kessler², Ramin Khoramnia² and Werner Nahm¹

¹: Institute of Biomedical Engineering, Karlsruhe Institute of Technology, Karlsruhe, Germany

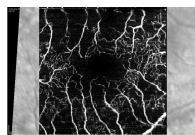
²: Ophthalmology Department, University Clinic Heidelberg, Heidelberg, Germany



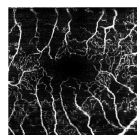
Conclusion:

- Optical Coherence Tomography Angiography (OCTA) analysis enables morphologic macular ischemia assessment.
- Developed ischemia morphology markers from OCTA differ in diabetic patients compared to healthy controls.
- Future studies will be needed to verify results and to develop quantification tailored to clinical monitoring.
- Longitudinal development and structure-function correlation remain unclear.

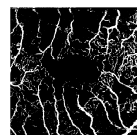
Methods – Image Processing:



Superficial (SVC) and Deep Vascular Complex (DVC) en face projection



Scale extracted
Cropped



Binarized (Otsu)
Sliding Window



Local Ischemia
Morphology

- Second Largest Ischemia Area
- Total Ischemia Area
- Mean Area per Ischemia Lesion

Methods – Patients and Tests:

- Patients underwent OCT Angiography imaging secondary to routine visits to the clinic.
- Small Study. Demonstrate image processing, create box plot visualization and test using two Sample t-test.

Results:

- Small Study: 7 diabetic patients vs. 7 Healthy Controls – postoperative cataract patients without Diabetes.
- Second Largest Ischemia Area was significantly larger in diabetic patients than healthy controls in SVC and DVC.
- Total Ischemia Area was significantly larger in diabetic patients compared to healthy controls in SVC and DVC.
- Mean Area per Ischemia Lesion was significantly larger in diabetic patients in SVC but not in DVC.

Discussion:

- Developed quantification measures confirm expected differences in ischemia extent.
- Morphologic ischemia analysis adds to overall perfusion status assessment and reveals detailed insight.
- Image Processing and visualizations for each processing step were developed and demonstrated.
- The small trial size is a limitation. Future verification will be needed based on a larger study group.

Disclosures:

RK reports payment for lectures and presentations from Zeiss, Heidelberg Engineering, Roche, Alimera Bayer, Novartis and Allergan. Other Authors: None.

Interested in Image Processing and OCT Angiography? Get in touch:

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Purpose

The relation of the retinal vasculature morphology and local retinal sensitivity remains poorly understood despite its potential significance in Diabetic Macular Ischemia. Optical Coherence Tomography Angiography (OCTA) Quantification allows to characterize the retinal vasculature. This study explores the correlation among vascular morphology and their relation to retinal sensitivity as assessed with microperimetry.

Methods

Ethics approval was granted. Trial registration: DRKS00024399. 38 eyes of 31 Diabetic Retinopathy (DR) patients undergoing treatment and 25 eyes of 24 Controls were included. Written informed consent was collected.

The Deep Vascular Complex (DVC) vessel morphology was assessed using the OCTA Spectralis module following the inbuilt slab segmentation. DVC projections were exported to tif format and further processed in MATLAB R2024a. Vessel Area Density (VAD), Vessel Length Density (VLD), Vessel Diameter Index (VDI), Total Branchpoint Number and the Mean Distance to the closest Vessel (MD) were extracted. Goldmann III microperimetry data was collected in a standard radial stimulus pattern. Stimuli positioning was registered with OCTA.

The correlation among the various DVC quantification parameters was assessed with a correlation matrix. Stepwise linear regression was performed as implemented in 'stepwiselm'. Data from the full cohort including DR and Control data and subgroup analysis was performed.

Results

Pearson Rho					
VLD	1.00	0.98	0.97	-0.78	-0.07
BP	0.98	1.00	0.96	-0.70	-0.04
VAD	0.97	0.96	1.00	-0.72	0.16
MeanDist	-0.78	-0.70	-0.72	1.00	0.23
VDI	-0.07	-0.04	0.16	0.23	1.00

Pearson P					
VLD	1.00e+00	1.71e-42	1.61e-39	2.19e-13	8.06e-01
BP	1.71e-42	1.00e+00	5.80e-34	3.84e-10	7.54e-01
VAD	1.61e-39	5.80e-34	1.00e+00	9.60e-11	2.28e-01
MeanDist	2.19e-13	3.84e-10	9.60e-11	1.00e+00	7.64e-02
VDI	8.06e-01	7.54e-01	2.28e-01	7.64e-02	1.00e+00

Discussion and Conclusion

Vessel Length Density and Cohort assignment were found to be significant predictors for retinal sensitivity, in line with previous works. In conclusion, retinal sensitivity assessed by microperimetry is correlated to the vascular morphology in DR patients but is not in Controls. Advancements in imaging and the incorporation of additional patient information will contribute to more precise retinal sensitivity prediction.



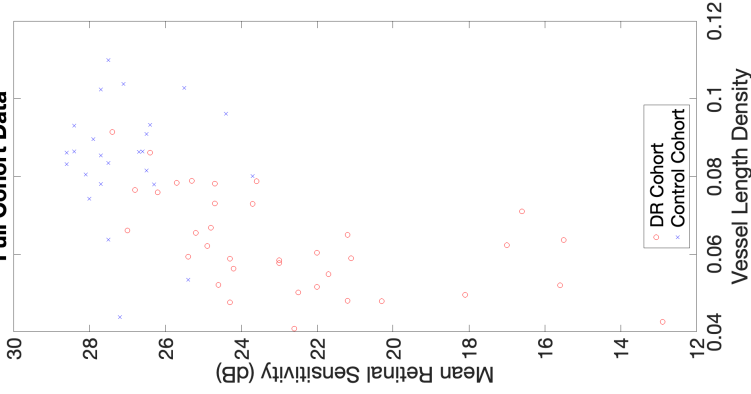
About the Author

Disclosures: None.
Research Interests:
OCTA Imaging Physics
OCTA Quantification
Mail: Simon.Hoffmann2@kit.edu

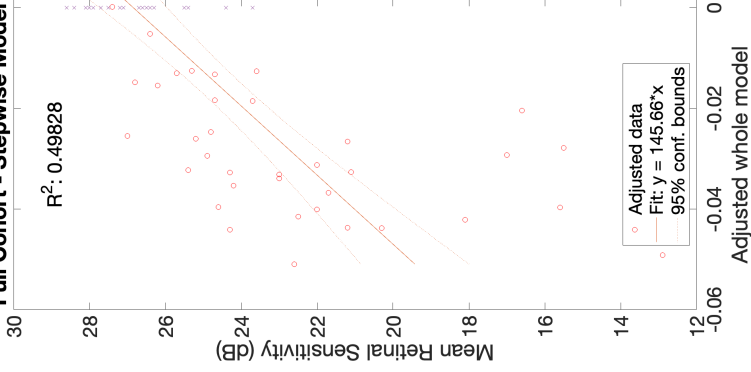


PHOTOGRAPHY
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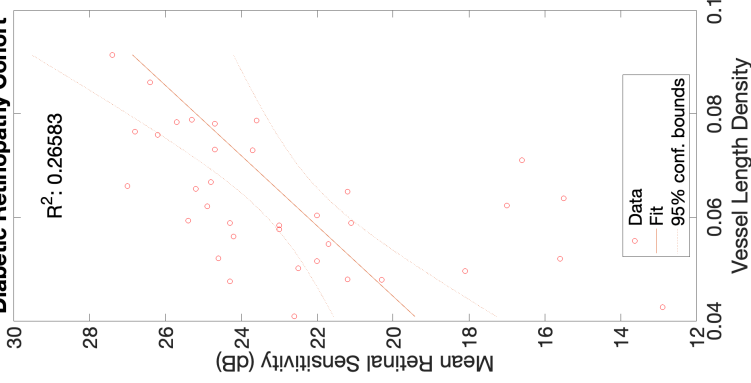
Full Cohort Data



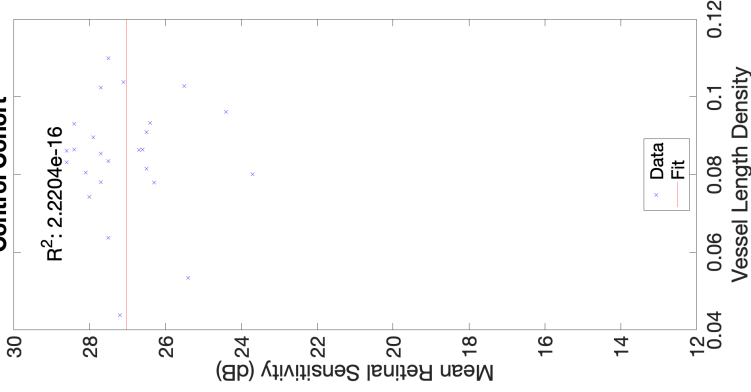
Full Cohort - Stepwise Model



Diabetic Retinopathy Cohort



Control Cohort

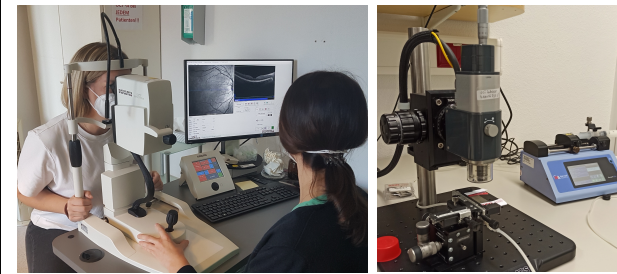
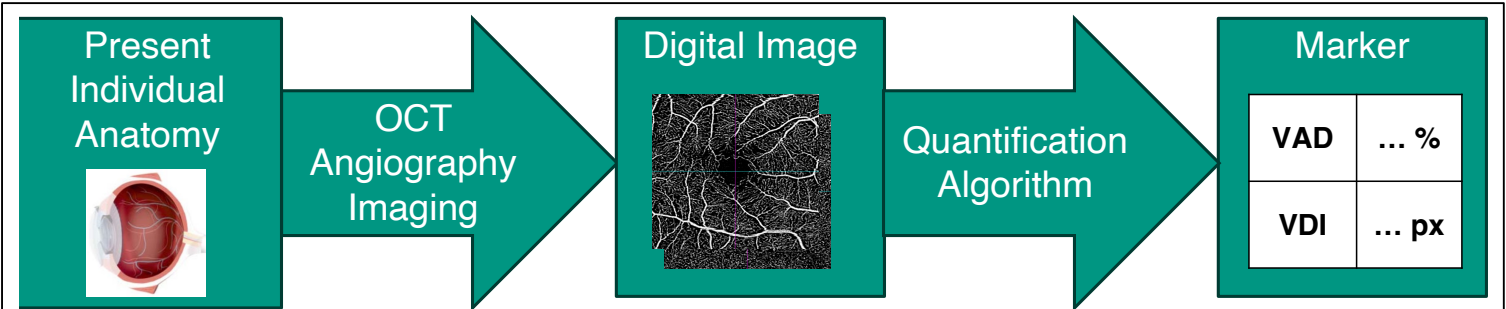


Towards Quality Assurance in OCT Angiography

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Introduction



Optical Coherence Tomography Angiography (OCTA) is an imaging technique applied in retina research and daily clinical practice. OCTA quantification attracts interest, promising objective data for cross-sectional and longitudinal comparisons. The assumed comparability requires the use of a standardized methodology or extensive knowledge on the impact of the methodology details on results. However, OCTA imaging and quantification are far from being standardized. The development of standards is important but complicated due to lacking knowledge on signal and image formation. The impact of focus precision along the quantification pipeline is studied applying phantoms.

Methods

Vasculature Model:

- Microfluidic Chip, square channel cross section
- Sizes [μm^2]: 20x20 / 50x50 / 100x100 / 200x200



Imaging Device:

- Central wavelength 880 nm
- A-scan rates: 5 / 10 / 25 / 50 / 100 / 200 / 248 kHz
- Resolution axial (Air): 6 μm

Optics Interface:

- Telecentric Lens, resolution Lateral: 4 / 12 μm
- Numerical Aperture: 0,148 / 0,050
- Focusing by moving the Chip or Scan Head.

Blood Model and Flow Control:

- Full fat milk mixed with distilled water, 1:10 Vol.
- Syringe Pump Harvard Apparatus Pump Elite 11

Results

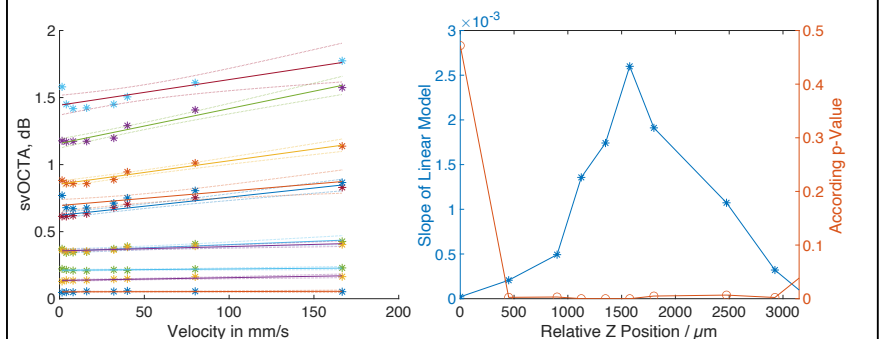
Impact on Signal Strength:

$$\text{RelSignalStrength}(z) \propto 1 - |z - z_{\text{Focus}}| * c_{\text{conf}} * \frac{\text{CrossSection}_{\text{Reflector}}}{\text{Spotsize}}$$

Impact on Lateral Appearance:

$$\text{ApparentWidth}(z) \propto \text{Width} * (1 + \text{NA}) * |z - z_{\text{Focus}}|$$

Parametrizing Role in Velocity Quantification:



Discussion & Conclusion

While the existence of defocus implications was expected, this study contributes findings on their nature. Defocus consequences regarding Signal Strength, lateral appearance of structures and on the potential for velocity quantification were characterized in a well controlled laboratory setup. Findings will simplify the recognition of similar patterns in clinical imaging, the discussion on acceptance intervals, defocus detection and management during imaging. Findings will enable the development and implementation of Quality Control / Quality Assurance systems for retinal OCTA imaging and quantification. The presented line of argumentation and laboratory methodology is simplistic and may be adapted to influencing factors besides defocus.

The author would like to thank HEIKA Research Bridges and the Karlsruhe School of Optics and Photonics for their support.

Impact of Defocus on OCT Signal Strength – Surface Model Versus Particle Model

Hoffmann, Simon¹ and Nahm, Werner¹

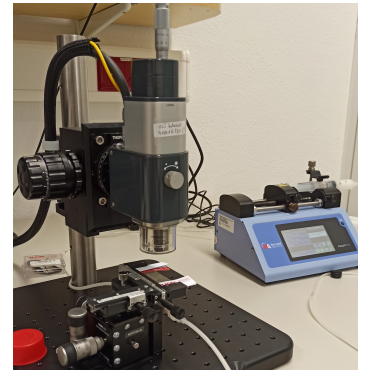
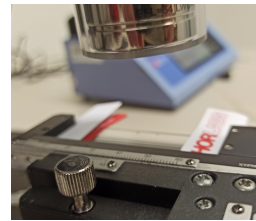
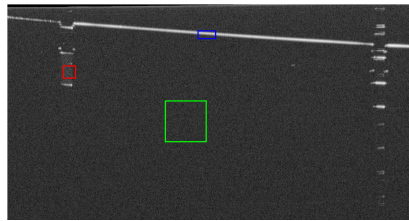
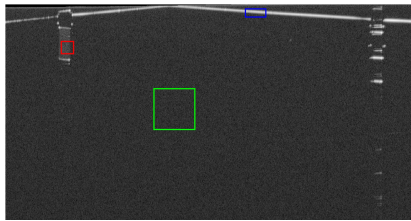
¹Institute of Biomedical Engineering, Karlsruhe Institute of Technology, Karlsruhe, Germany

Introduction

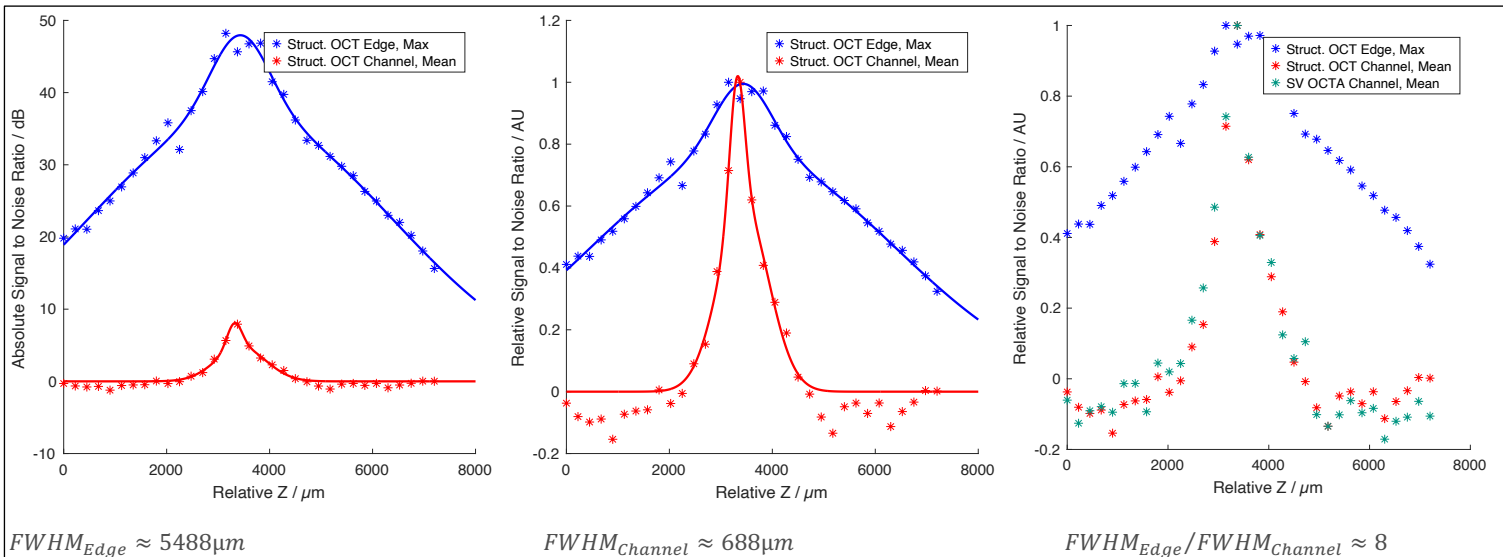
Accompanying the adoption of Optical Coherence Tomography (OCT) Angiography (OCTA), progress in quality control is needed. The impact of defocus on signal strength is characterized and compared among an extended surface and particle-like fat droplets.

Methods

A channel with square cross section sized $200\mu\text{m} \times 200\mu\text{m}$ was perfused with a milk-water mixture (1:10 volume ratio). The flow rate was set at $100\mu\text{l}$ per minute using Harvard Apparatus Pump Elite 11, causing $\sim 42\text{ mm/s}$ mean velocity. The chip was mounted. The channel was imaged using a commercial SD-OCT System (Ganymede GAN612C1, Thorlabs, lens OCT-LK4-BB) operating at 248 kHz, 880 nm, speckle variance mode. Then, the scan head was translated in the vertical direction for $225\mu\text{m}$, imaging was repeated. This process was repeated as far as the imaging depth allowed for, resulting in 33 speckle variance datasets. Data was analysed looking at three Regions of Interest, identified and presented in cross-sectional visualizations. The maximum value of the interface of chip and air, the mean signals from the channel region and a background region were extracted for each set. Background values were subtracted from the edge and channel values. Results were plotted over position and Full Width at half Maximum (FWHM) was estimated with “fit”, “gauss2” as given in Matlab R2024b.



Results



Discussion & Conclusion

Robustness to defocus when imaging laterally extended structures does not justify the assumption of a robust behaviour in defocus when imaging particles or their motion. This finding is relevant in OCT and OCTA: A robust behaviour in the case of retinal layer imaging with OCT may lead to assuming a robust behaviour with defocus in OCTA. The focus precision requirement in OCTA is expected to be stricter compared to structural OCT. Lacking precision in focusing and various focusing aids available in the various devices may contribute to the found ambiguities in downstream vasculature quantification.

Fokusvariationseffekte in Optischer Kohärenztomographie Angiographie

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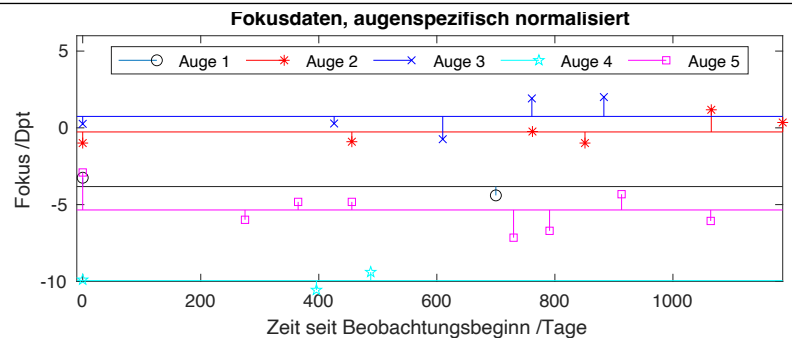
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Einleitung

Die kommerzielle Einführung der Optischen Kohärenztomographie Angiographie fügt der retinalen Bildgebung eine weitere Methodik hinzu. Kombiniert mit einer Quantifizierung der Gefäßmorphologie ist sie insbesondere für longitudinale Erhebungen interessant. Die Fokussierung ist manuell einzustellen. Der Einfluss von Varianz dieser Einstellung in Quantifizierungsergebnissen wird erhoben.

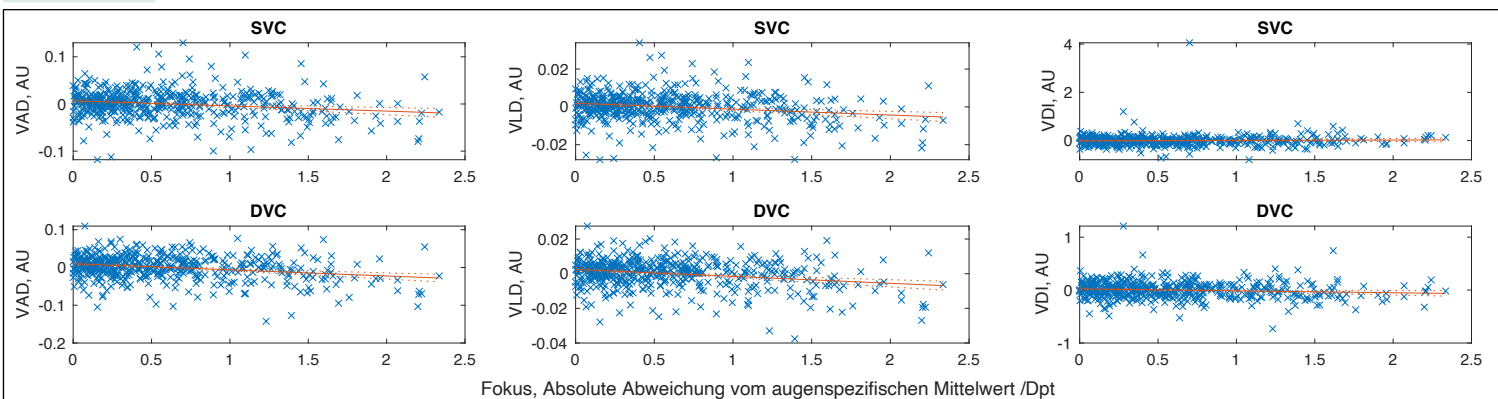
Methodik

Datum	TT.MM.JJ	TT.MM.JJ	TT.MM.JJ
Fokus	... Dpt	... Dpt	... Dpt
Qualität	... dB	... dB	... dB
SVC	VAD: ... VLD: ... VDI: ...	VAD: ... VLD: ... VDI: ...	VAD: ... VLD: ... VDI: ...
DVC	VAD: ... VLD: ... VDI: ...	VAD: ... VLD: ... VDI: ...	VAD: ... VLD: ... VDI: ...



Die Studie ist registriert. (DRKS00024399) Die Bildgebung erfolgte an 81 Augen von 43 Patienten (Diabetische Retinopathie, späte Stadien) mit deren schriftlichem Einverständnis sekundär zu den regelmäßigen Terminen. Das Gerät "Spectralis", OCTA-Modul wurden verwendet. Die geräteeigene Segmentierung wurde auf Plausibilität geprüft. Plausibel erscheinende En Face Projektionen der „Slabs“ „Superficial Vascular Complex“ SVC, und „Deep Vascular Complex“, DVC, wurden nach .tif exportiert. Zusätzlich wurden die gerätegegebene „OCTA Qualität“ in dB und der Fokuswert extrahiert. Binarisierung unter Nutzung des globalen Schwellwertverfahrens nach Otsu und Skelettierung wurden in Matlab R2024b durchgeführt. Die Metriken Vessel Area Density (VAD, Anzahl Vordergrundpixel / Anzahl aller Pixel), Vessel Length Density (VLD, , Anzahl Mittellinienpixel / Anzahl aller Pixel) und Vessel Diameter Index (VDI, VAD / VLD) wurden berechnet. Die Fokuswerte wurden zur absoluten Abweichung vom augenspezifischen Mittelwert normalisiert (Länge der vertikalen Abstände). Die Metriken wurden als vorzeichenrichtige Abweichung vom slabspezifischen Mittelwert normalisiert. Lineare Regression wurde angewandt, um Einflüsse des Fokus auf Metriken und OCTA Qualität zu klären. Signifikanz wurde bei $p < 0,05$ angenommen.

Ergebnisse



	VAD ~ Fokus	VLD ~ Fokus	VDI ~ Fokus
SVC:	Steigung: -0,0109, $p < 10^{-4}$, $R^2: 0.034$	Steigung: -0,0031, $p < 10^{-5}$, $R^2: 0.042$	Steigung: 0,0165, $p = 0,458$, $R^2: 0.001$
DVC:	Steigung: -0,0162, $p < 10^{-7}$, $R^2: 0.062$	Steigung: -0,0040, $p < 10^{-7}$, $R^2: 0.059$	Steigung: -0,0361, $p = 0,013$, $R^2: 0.012$
Qualität	Steigung: -1,1508, $p < 10^{-5}$, $R^2: 0.038$		

Diskussion & Schlussfolgerung

Die Präzision des Fokus wurde als signifikanter Prädiktor des Quantifizierungsergebnis identifiziert. Die Auswirkungen von Defokus sind unter kontrollierten Bedingungen zu ermitteln. Die Überlagerung von tatsächlicher anatomischer Veränderung mit Effekten der unvollständig kontrollierten Bildgebung erschwert die klinische Diskussion. Die Kontrolle oder Berücksichtigung der bildgebungsbedingten Variablen ist ein Schritt zur Erschließung vergleichbar quantitativ ausgewerteter OCTA und damit noch aussagekräftigeren longitudinalen Daten.

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List of Publications and Supervised Theses

Journal Articles

- LJ Kessler, **S Hoffmann**, W Nahm, D Bagautdinov, GU Auffarth, G Labuz, R Khoramnia, *Impact of Lens Opacity on Optical Coherence Tomography Angiography Metrics*, Current Eye Research, 2023 Oct;48(10):965-972.
- LJ Kessler, **S Hoffmann**, W Nahm, GU Auffarth, M Dugas, M Pham, Y Eo, R Khoramnia, *Clinical and optical coherence tomography angiography-based risk factors for reduced retinal sensitivity in diabetic macular edema*, under review.

Refereed Conference Articles

- **S Hoffmann**, W Nahm, *Impact of Defocus on OCT Signal Strength - Surface Model Versus Particle Model*, Proceedings: European Conferences on Biomedical Optics 2025 2025.W3A.22
- G Hentschel, M Weiß, **S Hoffmann**, W Nahm, B Glasmacher, *Using Hydrogel Beads as a Blood Model in Optical Coherence Tomography*, Current Directions in Biomedical Engineering 2023;9(1): 579-582

Refereed Conference Abstracts

- **S Hoffmann**, LJ Kessler, R Khoramnia, W Nahm, *OCT Angiography Deep Vascular Complex Quantification Correlation to Microperimetry Sensitivity in Diabetic Retinopathy and Healthy Controls*, Investigative Ophthalmology and Visual Science June 2025, Vol.66, 77.
- **S Hoffmann**, LJ Kessler, R Khoramnia, W Nahm, *Local ischaemia morphology in diabetics differs from healthy controls*, Abstracts From The 2023 European Association For Vision And Eye Research Festival, 26-28 October 2023, Valencia

- **S Hoffmann**, W Nahm, *Speckle Variance Optical Coherence Tomography Angiography Depends on distance to focus position*, Abstract 421 of ICOOR 2024, Abstract Book available at https://floretina.com/storage/abstractsbook/floretina_2024_abstract_book.pdf.
- **S Hoffmann**, LJ Kessler, R Khoramnia, W Nahum, *OCT Angiography based Longitudinal Ischemia Morphology Analysis in 42 patients over three years does not reveal evidence supporting Diabetic Macular Ischemia progression phenotypes*, ICOOR 2024, not available anymore.
- **S Hoffmann**, BKJ Viertel, LJ Kessler, R Khoramnia, W Nahm, *Effect of white line artifacts in OCTA images on perfusion density estimation*, EURETINA 2022, not available anymore.

Supervised Student Theses

- J Breitling, *Algorithm Benchmarking for Foveal Avascular Zone Quantification from OCT Angiography*, Bachelor Thesis, Institute of Biomedical Engineering, Karlsruhe Institute of Technology (KIT), 2024
- L Lyu, *Analysis on effect of Incident Angle on OCT Signal Intensity*, Research Project, Institute of Biomedical Engineering, Karlsruhe Institute of Technology (KIT), 2025

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