Shadowing during intraoperative optical coherence tomography-assisted vitreoretinal surgery: clinical significance and reduction strategy

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Dedication

I dedicate this work to my family: first and foremost, to my wife; without her everlasting love and stern motivation during the countless hours invested in this project, it would have been impossible to achieve. To my parents, thank you for giving me not only the inspiration to chase big dreams but also for teaching me the strength to see them through. Lastly, I would like to thank my brother, my keep-it-real checker.

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1. Introduction

1.1 The human eye: clinical anatomy

The human eye is a sphere-shaped organ dedicated to the sense of vision. It is a remarkably specialized sensory paired organ that serves as a visual receptor. It comprises several intricate components, whose size varies among individuals. On average, it has an approximate axial length, the distance from the front of the cornea to the retina of 24 mm [11]. Its anatomical structure is divided into anterior and posterior segments that intricately interplay to enable the eye to function similarly to a camera, capturing light that can then be interpreted into a visual reality. Figure 1 features the main anatomical structures of the human eye.

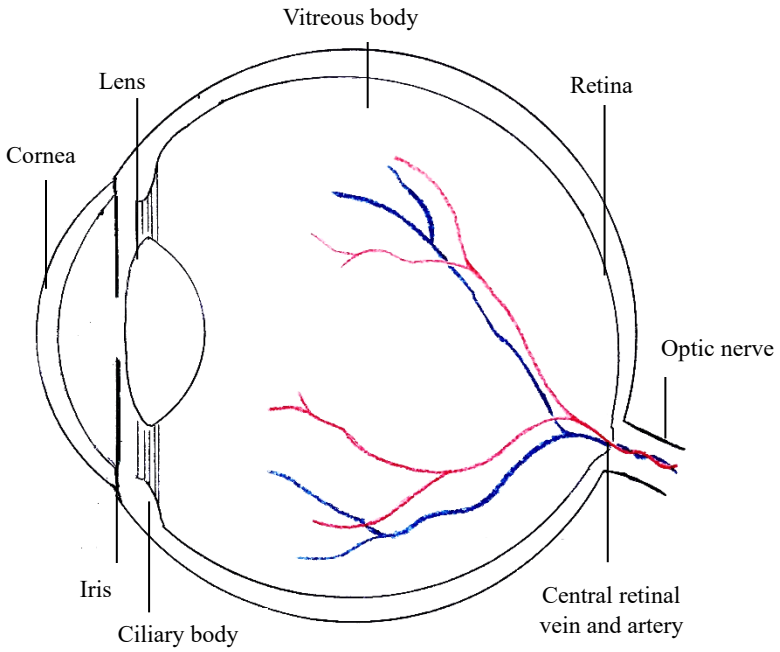


Figure 1. A cross-sectional representation of the human eye. (Original image)

1.1.1 Anterior segment

The anterior segment of the eye is comprised of the following structures: the cornea, the iris, the ciliary body, and the lens. The anterior chamber is situated behind the cornea and is filled with aqueous humor, which is bound by the cornea anteriorly and the iris and lens posteriorly. The iris, a pigmented and vascularized circular tissue imbedded with two muscles, serves as a diaphragm that regulates the amount of light entering the eye through its central orifice, the pupil [3]. Posterior to the iris is the crystalline lens, a clear, and

relatively flexible structure that allows the light entering the eye to be properly focused onto the retina. The process of accommodation, regulated by the ciliary muscle, allows the lens to adjust its shape, enabling clear vision at different distances. The drainage of aqueous humor produced by ciliary body is regulated by the trabecular meshwork and Schlemm's canal, critical components in the homeostasis of intraocular pressure, localized at the circular angle of the anterior chamber, created by the junction of the cornea and the iris [34].

1.1.2 Posterior segment

Comprising the posterior segment of the eye are the vitreous humor, the retina, the choroid, and the optic nerve [3, 11].

The vitreous humor occupies the space between the lens and the retina, maintaining the eye's shape and providing a clear medium for the transmission of light. This gel-like substance also contributes to the structural stability of the eye.

The retina, a highly specialized tissue lining the posterior inner surface of the eye, is essential for converting incoming light into neural signals. Comprising photoreceptor cells, including rods and cones, the retina initiates the complex process of visual transduction [11]. The fovea, located at the center of the macula, is a region of heightened cone density responsible for detailed central vision.

The choroid, situated between the retina and the sclera, is a vascular layer supplying oxygen and nutrients to the outer layers of the retina. Rich in blood vessels, the choroid aids in maintaining the metabolic demands of the highly active retinal cells. The optic nerve, originating at the optic disc, is responsible for transmitting visual information from the retina to the brain. Composed of ganglion cell axons, the optic nerve plays a crucial role in forwarding visual stimuli to the visual cortex for interpretation.

Pathologies affecting the posterior segment, such as age-related macular degeneration, diabetic retinopathy, and retinal detachment, can have profound implications for visual function [34].

1.2 Imaging diagnostics in ophthalmology

Currently, the field of ophthalmology relies heavily on imaging techniques to examine and document the ocular anatomy, pathology, and function. These advanced imaging modalities play a key role in the diagnosis, treatment planning, and monitoring of various ocular conditions [36]. In recent years, technological advancements have propelled the range of applications and quality of ophthalmic imaging, enabling the visualization of ocular tissues

with unprecedented detail. These techniques not only aid in the early detection of ocular disorders but also contribute to a deeper understanding of the physiological processes governing visual function.

The field of ophthalmic imaging technology ranges from traditional techniques such as fundus photography to state-of-the-art innovations like optical coherence tomography (OCT) and optical coherence tomography angiography (OCT-A). From corneal topography to retinal angiography, each imaging technique represents a crucial lens through which we gain insight into of ocular anatomy, pushing the boundaries of our understanding and paving the way for advancements in the care and preservation of vision. Some of the most used imaging techniques to evaluate the posterior segment of the eye are the following [33, 36, 37]:

1) *Fundus photography (FF)*

   As shown in Figure 2, captures detailed images of the posterior segment of the eye, including the retina, optic disc, and blood vessels. Other types of FF, such as fundus autofluorescence (FAF) use retinal fluorescent properties to create light filtered images, as shown in Figure 3.

Figure 2. Funduscopic image of the healthy retina of a left eye. (Device: Zeiss CLARUS® 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

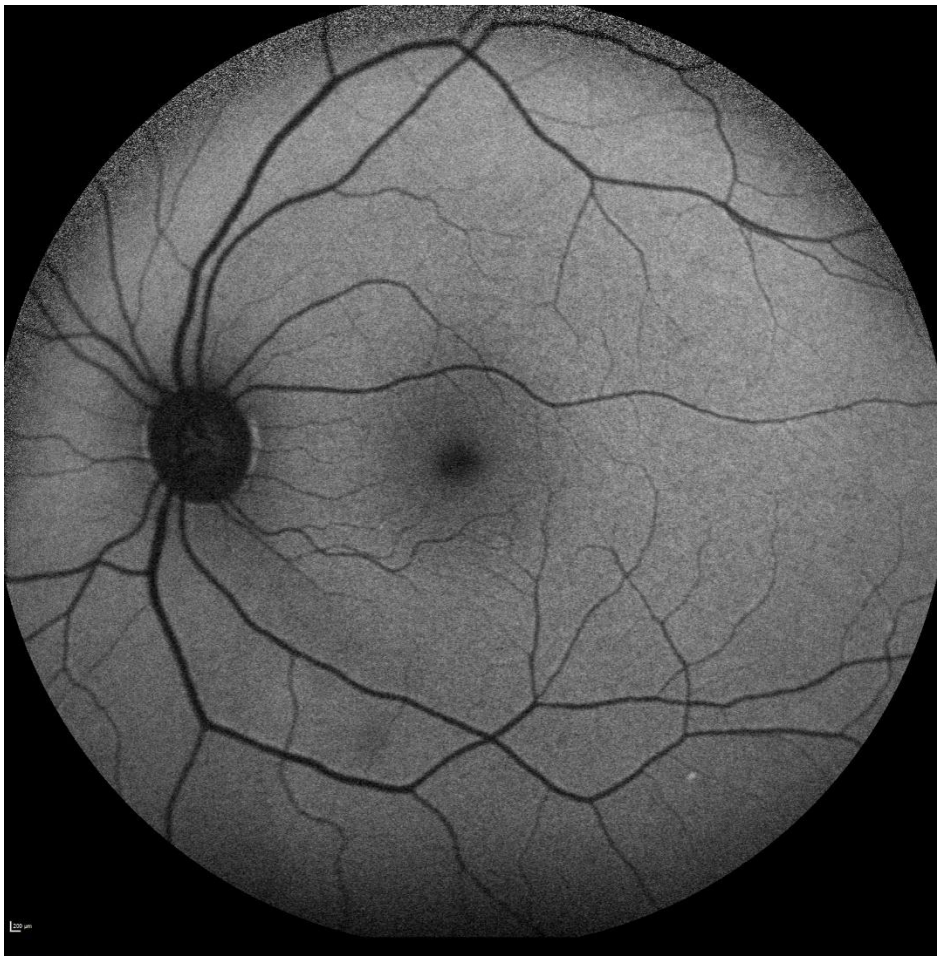


Figure 3. Fundus autofluorescence (FAF) of the healthy retina of a left eye. When subtle abnormal changes in the retina occur, the affected retinal tissue often appears hyperfluorescent during FAF, before these changes become morphologically apparent during regular fundoscopy. (Device: SPECTRALIS®, Heidelberg Engineering, Heidelberg, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

2) *Optical coherence tomography (OCT)*

   A non-invasive imaging technique that provides high-resolution cross-sectional images of ocular structures, as exhibited in Figure 4. It is widely used to assess the layers of the retina, optic nerve head, and anterior segment. OCT has become indispensable in diagnosing and managing conditions like glaucoma, macular edema, and vitreoretinal disorders.

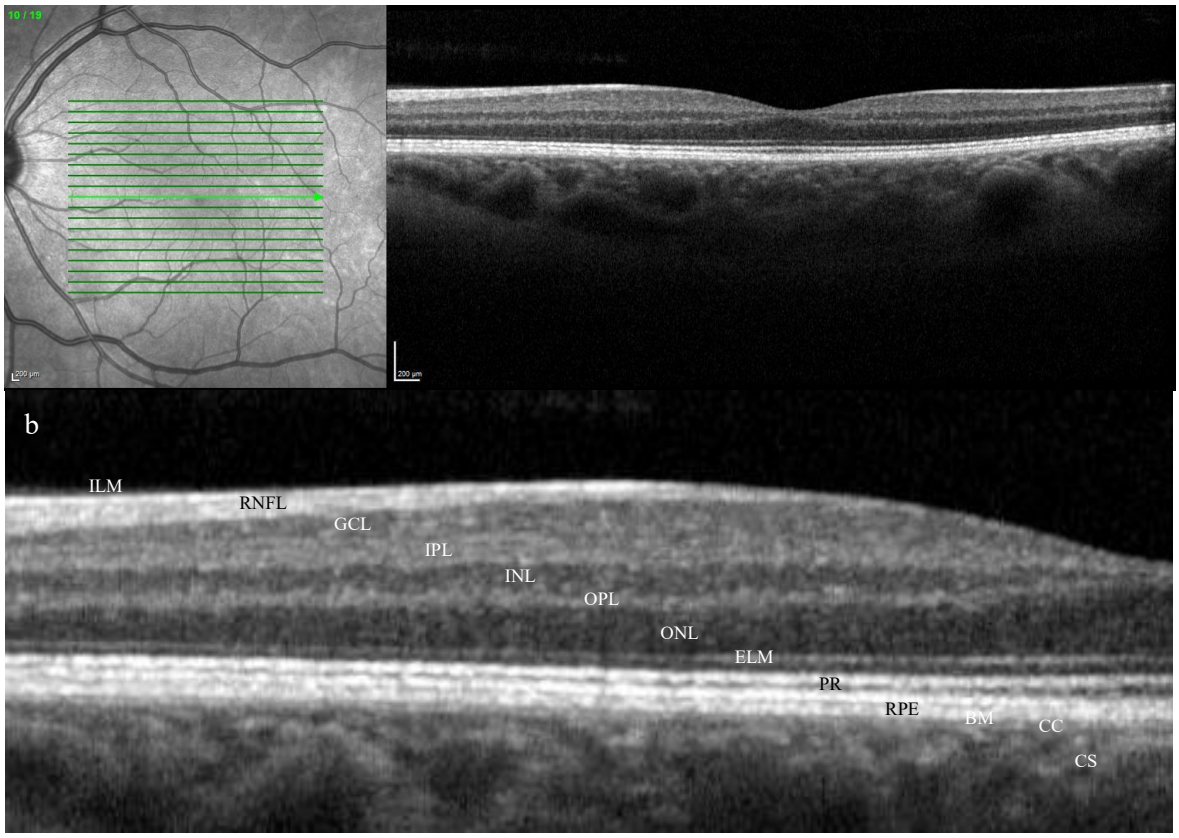


Figure 4. a) Spectral-domain OCT scan of a healthy macula. Left: Infrared image of the macula with overlayed OCT scans. Right: Transverse view of corresponding OCT Scan. b) Zoom-in of retinal layers: internal limiting membrane (ILM), retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), photoreceptor layers (PR), retinal pigment epithelium (RPE), Bruch's membrane (BM); and the choroid: choriocapillaris (CC) and choroidal stroma (CS). (Device: SPECTRALIS®, Heidelberg Engineering, Heidelberg, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

3) *Fluorescein angiography (FA)*

   This examination involves the intravenous injection of fluorescein dye to visualize blood flow in the retina as shown in Figure 5. It is particularly useful in identifying vascular abnormalities, such as choroidal neovascularization in age-related macular degeneration and diabetic retinopathy.

4) *Indocyanine green angiography (ICGA)*

   This study is used to assess choroidal circulation. After intravenous injection of indocyanine green dye, this diagnostic technique obtains the images of the back of the eye and provides valuable information about conditions affecting the choroid, including choroidal neovascularization and inflammatory

choroidopathies. This can be done simultaneously with a FA by mixing the contrast agents and using the adequate image filters as shown in Figure 5.

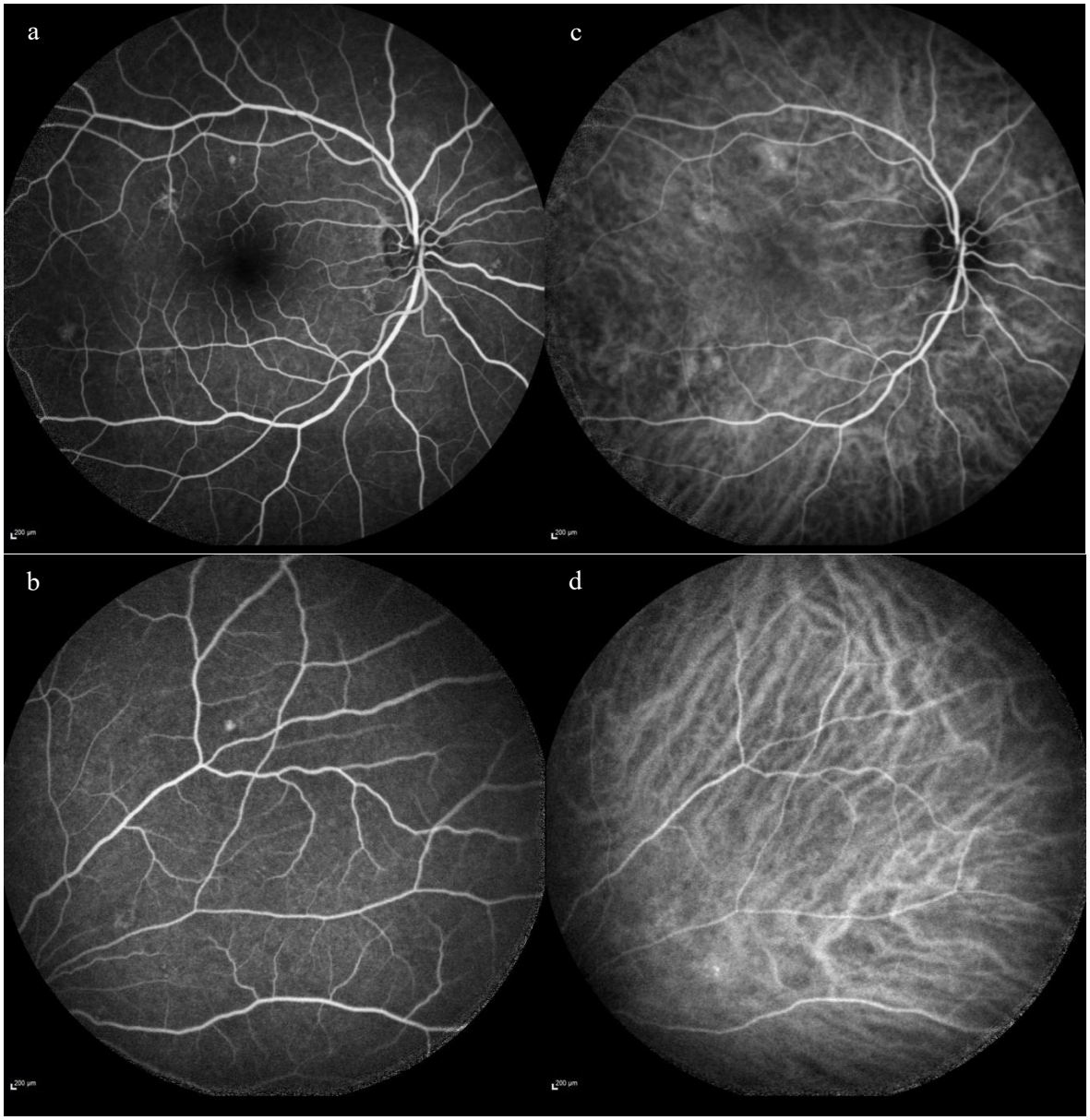


Figure 5. Left: FA images of the a) central and b) peripheral retina of a right eye, with few and small leakage points, with otherwise normal vascular perfusion. Right: simultaneous and corresponding ICGA images of the same eye, emphasizing the choroidal vascular perfusion c) centrally and d) peripherally. (Device: SPECTRALIS®, Heidelberg Engineering, Heidelberg, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

5) *Ultrasound of the eye (Brightness amplitude scan or B-Scan)*

   High-frequency sound waves are used to visualize the entire eyeball. It is especially useful to evaluate the morphology of the posterior segment when

fundoscopy is obstructed due to opaque optical media. An example of this is shown in Figure 6.

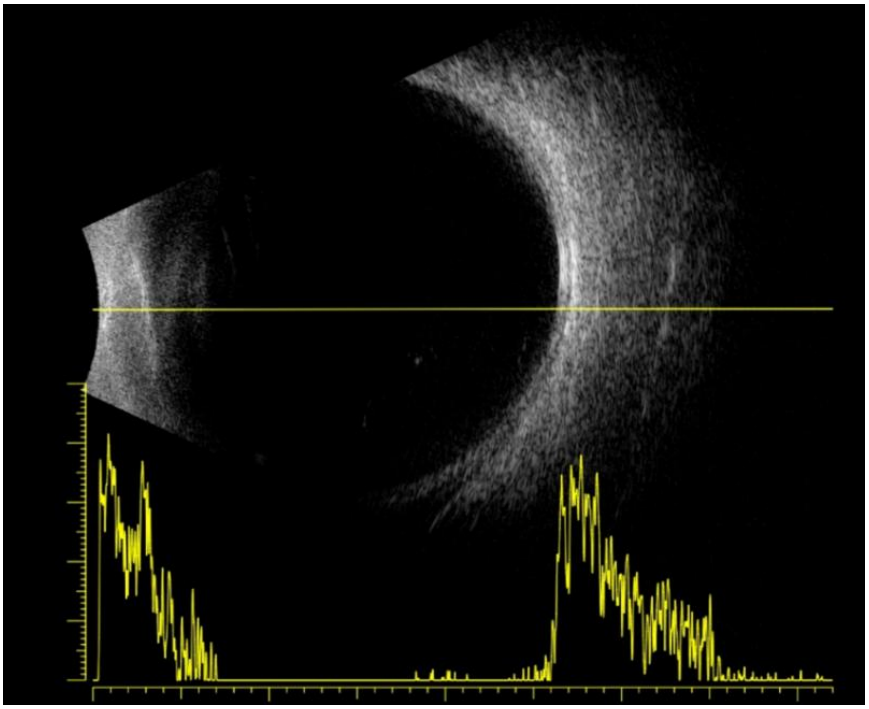


Figure 6. B-Scan ultrasound of a normal eye. (Device: ABSolu®, Quantel Medical, Cournon d'Auvergne Cedex, France) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

These imaging techniques collectively form a comprehensive range of diagnostic imaging methods that allow detailed examinations of ocular structures. Adequate integration of these diagnostic methods aids in determining more precise diagnoses and tailored treatment strategies, ultimately contributing to improved patient outcomes in the field of ophthalmology.

Since this thesis focuses on the application and benefits of intraoperative optical coherence tomography (iOCT) in surgical settings, it is essential to provide a thorough understanding of OCT development and its technological advancements. Therefore, the following section will be dedicated to exploring the evolution of OCT technology, its principles of operation, and the various ways it has revolutionized imaging in ophthalmology and beyond.

1.3 Optical coherence tomography

OCT is a non-invasive imaging technology that employs light waves to produce high-resolution images of biological tissues with the capability to render them in cross-sectional scans. This imaging method provides images that can be correlated with microscopic examinations of living tissue using histologic and histopathologic approach, which has revolutionized the diagnosis and monitoring of eye diseases [15].

The development of OCT technology can be traced back to the 1980s when it emerged as a novel imaging technique with the potential to revolutionize medical diagnostics, particularly in ophthalmology. The principles and early experiments that led to the creation of OCT were rooted in the field of interferometry and low-coherence interferometry [25].

Interferometry, a branch of physics, involves the measurement and analysis of the interference patterns of light waves. The concept of low-coherence interferometry, which underlies OCT, involves the use of a broadband light source that emits a spectrum of wavelengths. This broad spectrum enables high-resolution imaging and the ability to discriminate between different structures based on their reflective properties [29].

The initial OCT systems utilized a time-domain technique, usually referred to as time-domain OCT (TD-OCT). In this approach, a low-coherence light source, such as a luminescent diode, is used to illuminate the eye. The light is split into a sample beam and a reference beam, and the interference between the reflected sample beam and the reference beam is detected to generate cross-sectional images. By altering the reference mirror position and acquiring multiple sequential depth profiles or time-amplitude scans, referred to as A-scans, in this manner a two-dimensional image could be reconstructed. [8, 15], as shown in Figure 7. Building on the success of TD-OCT, newer technologies have further advanced the capabilities and applications of OCT.

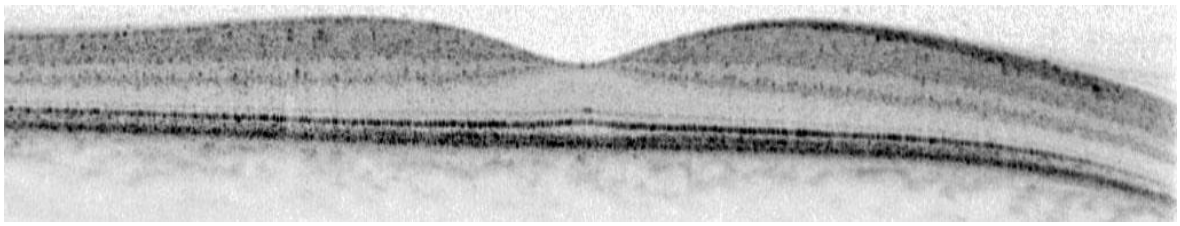


Figure 7. TD-OCT image of a transverse scan of a healthy macula at the level of the fovea. (Device: Zeiss STRATUS OCT™, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

The introduction of this technology into the field of ophthalmology happened in 1991. [25, 29]. This groundbreaking work paved the way for the development and further refinement of OCT technology in subsequent years. One of the unique advantages of OCT is its non-invasive and noncontact imaging modality, which has made it a preferred technology in biomedical applications [51]. Just as the transparent structures of the eye along its axis allow light perception, they also enable easily accessible optical imaging. Ophthalmologists quickly recognized the potential of OCT to capture high-resolution images of ocular structures in vivo, allowing for earlier detection and improved management of a wide range of ophthalmic diseases [2]. Early validation studies have demonstrated the ability of OCT to provide valuable anatomical information, assist in early disease detection, guide treatment decisions, and monitor treatment response [15, 18, 29]. Since then, an ongoing series of research projects has shown OCT's potential in the assessment of retinal disease, which would provide the foundation for its future integration of standard ophthalmologic diagnostic tools [51]. Today, OCT technology is not limited to ophthalmology; it has spread to multiple medical specialties, including dermatology, neurosurgery, oncology and cardiovascular disciplines [45, 47].

While TD-OCT provided valuable insights into retinal structures, it had limitations in terms of imaging speed, resolution, and sensitivity. Chiefly, when the density of the object being analyzed increases, it blocks the OCT signal, impeding appropriate imaging. There the next development strategies aimed to better manage this problem, this culminated with the development of spectral-domain OCT (SD-OCT) in the late 1990s. SD-OCT employed a spectrometer to analyze the interference spectrum generated by the combination of the sample and reference beams. This spectral information enabled the simultaneous acquisition of depth profiles from multiple points within the tissue, greatly enhancing imaging speed and resolution. SD-OCT became the dominant technology in the field, enabling real-time imaging and facilitating clinical applications [9, 58]. An image exampling this is shown previously in Figure 4.

Since then, OCT technology has continued to advance. Swept-source OCT (SS-OCT) was introduced in the early 2000s, utilizing a tunable laser as the light source and a photodetector that rapidly swept through a range of wavelengths. This enabled even faster imaging speeds and enhanced penetration into deeper ocular tissues [1], as shown in Figure 8. Over the years, further refinements have been made to improve image quality, enhance depth resolution, and increase scanning speed, aiding in the diagnosis and management of conditions such as choroidal neovascularization and myopic degeneration [57].

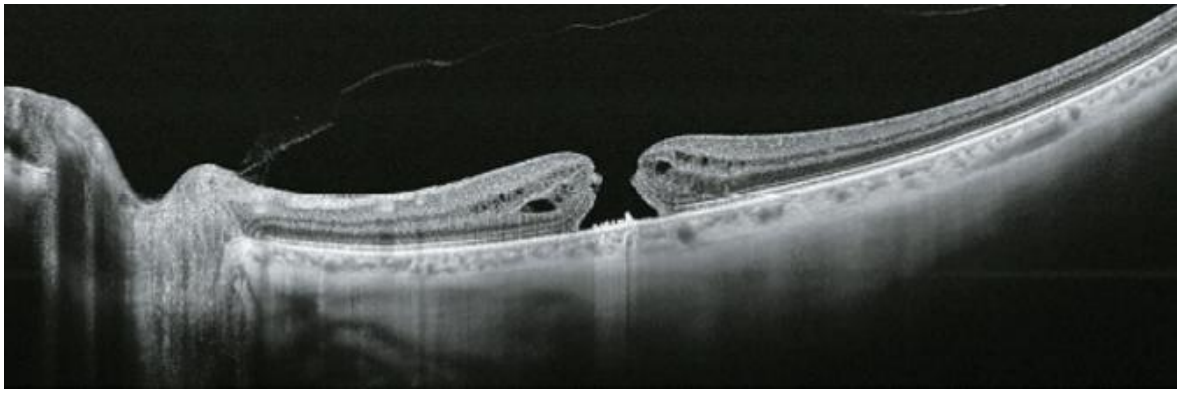


Figure 8. SS-OCT image of a macular hole with detailed visualization of cystoid changes of the retinal layers around the hole. The signal of the SS-OCT has a deeper penetration amplitude that allows the distinction of not only the retinal layers but also between different choroidal layers. (Device: DRI OCT Triton, Topcon Medical Systems, Paramus, USA) (Courtesy of Prof. José María Ruiz Moreno MD, University of Albacete, Spain)

This has paved the way for the development of OCT angiography, which applies the same mechanics to dynamic blood flow imaging within the retina, providing valuable information about vascular structures and perfusion [32]. This concept, just like its intraoperative counterpart, is one of the branches of OCT technology currently being intensively studied and further developed within ophthalmology. However, despite the many advances in OCT technology over the years, its application in ocular surgery is still developing [43, 56].

OCT imaging is based on low-coherence interferometry, which uses long-wavelength infrared light ranging from 840 nm to 1050 nm [7]. The OCT camera utilizes low-coherence light that projects toward the surface being analyzed; this process records detailed scatter patterns as well as intensity information reflected from various depths within the tissue under investigation, generating an accurate cross-sectional image [2].

Since its inception, OCT images have been obtained in a time-domain type system. Time domain scan systems can obtain approximately 400 scans per second using six radial slices, each directed thirty degrees apart, hence obtaining circular image frames with double 180° scans. Because the scans are 30° apart there is unfortunately always the possibility of missing a pathology or other perhaps interesting or significant phenomena between image slices [25]. This has been a constant concern addressed in the continuing developments that have improved OCT imaging.

In contrast to the classic time-domain OCT technology, spectral-domain technology can examine around 20,000 to 40,000 A-scans in the same time span. This higher scanning

speed and volume help decrease the risk of motion artifacts while also improving image resolution accuracy and reducing the chance of missing additional findings between cuts, which could potentially lead to misdiagnosis. Spectral-domain imaging is further enhanced by averaging multiple A-scans concurrently obtained from an identical location to boost the signal-noise ratio. In comparison with traditional time domain systems that have a precision measurement ability of approximately 10-15 microns only, modern spectral domain machines can now reliably render scans of up to three. Instead of capturing just six radial slices like typical time domain devices, spectral domain equipment constantly images over an area measuring 6 mm wide, thereby minimizing the omission of areas [58].

An additional concept that has further fueled the capacity of OCT imaging technology is enhanced depth imaging. It is a modification of the traditional OCT imaging process that aims to improve the visualization of structures located deeper within the tissue, particularly in the posterior segment of the eye [57]. Enhanced depth imaging involves positioning the OCT scanner closer to the eye's surface compared to conventional imaging.

Swept-source technology utilizes longer wavelengths ranging from 1050 nm to 1060 nm, thus eliminating dependence on enhanced depth imaging. This technology fastens acquisition speed reaching up to as high as 1,000,000 to 4,000,000 A-scans every second due to a wavelength-sweeping laser coupled with a dual-balanced photodetector [8]. A summary of the technical differences between these OCT types is exhibited in Table 1.

Table 1. Comparison of technical characteristics of different OCT types [9].

OCT type	Time-domain (TD)	Spectral-domain (SD)	Swept-source (SS)
A-Scans/s	400	27,000 – 70,000	1 – 4 million
Wavelength (nm)	810 nm	840 nm	1050 nm
Axial resolution (µm)	10-15 µm	5-7 µm	5 µm
Transverse resolution (µm)	20 µm	14-20	20

Currently, several studies have included optical coherence tomographic analysis in their standardization efforts, allowing a better understanding of the findings procured by different research groups across diverse medical specialties. This multidisciplinary effort aims to achieve improved diagnosis effectiveness and treatment outcomes [45, 47]. The significant progress made in these technologies has had a profound impact on the way

follow-up assessments are conducted. This enables continuous monitoring throughout different stages of treatment regimens and ultimately enhances patient outcomes. For example, in glaucoma, it has been demonstrated that OCT analysis of the retinal nerve fiber layer and ganglion cell layer effectively detects glaucoma progression along with traditional methods such as visual field testing, intraocular pressure, visual acuity deficit, and optic disc evaluation [6, 26, 49]. The use of OCT in managing retinal disease and glaucoma has now become part of the routine standard of care [27].

Currently, OCT technology continues to advance rapidly. Hand-held OCT devices have also been developed, facilitating imaging in challenging situations, such as with patients with cognitive or corporal disabilities and children. An important example of this innovation, as mentioned previously, is OCT angiography (OCT-A), a non-invasive imaging technique that provides visualization of retinal and choroidal blood vessels, which was introduced, further expanding the applications of OCT in vascular diseases of the retina and choroid [40]. In OCT-A the detection of blood flow employs motion contrast by comparing decorrelation signals between multiple B-scans obtained at each retinal cross-section [32]. This technology relies on the theoretical aspect that only circulating erythrocytes within retinal capillaries should be in motion within the retina or choroid.

More recent advancements have involved the integration of OCT technology directly into surgical microscopes, paving the way for the development of intraoperative optical coherence tomography (iOCT), which enables real-time imaging during surgeries [10, 14].

1.4 Vitreoretinal surgery

Vitreoretinal surgery is a specialized field of ophthalmic surgery focused on diagnosing and treating disorders of the retina, vitreous, and macula. This branch of surgery treats a range of conditions, including retinal detachment, macular holes, epiretinal membranes, diabetic retinopathy, and complications of ocular trauma. Procedures often involve vitrectomy, where the vitreous gel is removed to allow access to the retina, and the use of advanced techniques such as laser photocoagulation, cryotherapy, and the injection of therapeutic agents.

The origins of vitreoretinal surgery can be traced back to the late 19th century when pioneering ophthalmologists began experimenting with rudimentary techniques to address retinal detachments and other vitreoretinal disorders. Early procedures involved the use of indirect ophthalmoscopy and scleral buckling techniques to reattach the detached retina.

However, it wasn't until the mid-20th century that vitrectomy, the cornerstone of modern vitreoretinal surgery, was introduced [31].

Vitrectomy revolutionized the field by allowing surgeons to directly access and manipulate the vitreous cavity, enabling more precise and controlled interventions. The primary goal of vitreoretinal surgery is to restore and preserve vision in patients with a range of vitreoretinal disorders, including retinal detachment, diabetic retinopathy, macular holes, and epiretinal membranes. Additionally, vitreoretinal surgeons aim to relieve symptoms, prevent further deterioration, and enhance the overall quality of life for their patients. Preservation of ocular structures, minimizing complications, and achieving optimal visual outcomes are all central objectives of vitreoretinal surgery.

Vitrectomy was initially performed using large-gauge instruments; the technique evolved with the advent of smaller, more refined instruments and innovative visualization systems. The transition to smaller gauged instruments allowed for more precise surgeries, reduced tissue trauma, faster recovery times, and improved patient outcomes. Techniques such as endolaser photocoagulation, intraocular gas, and silicone oil tamponade, and the development of microincisional vitrectomy systems further expanded this surgical field, enabling surgeons to tackle a wider array of vitreoretinal pathologies [42].

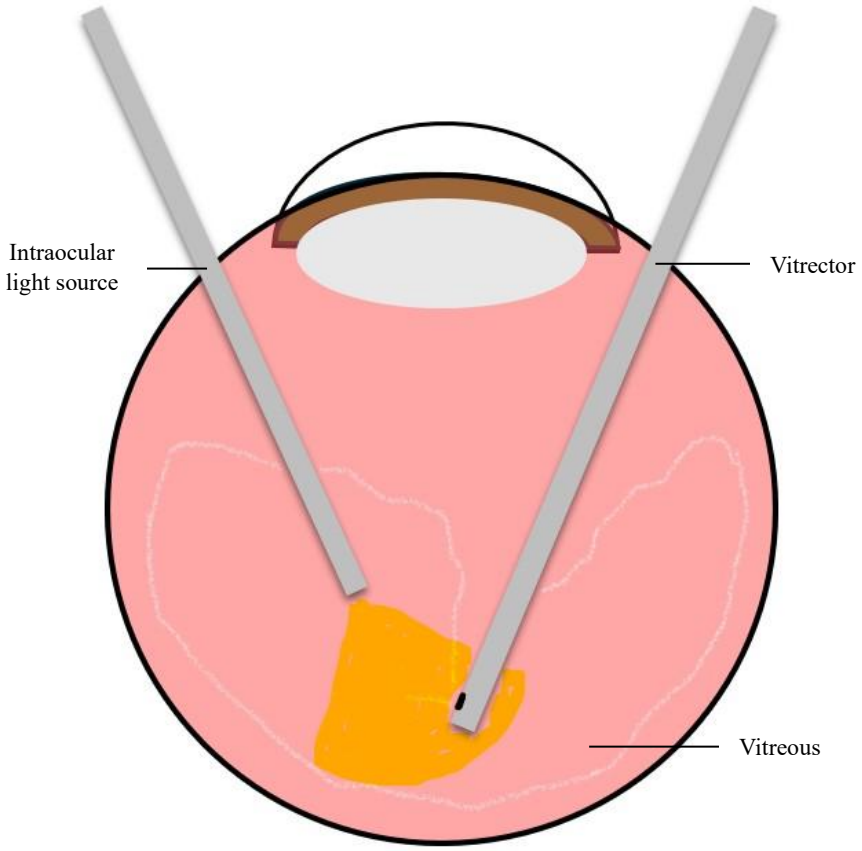


Figure 9. Schematic image of a pars plana vitrectomy (PPV) showing an intraocular light source to allow adequate visibility while performing the removal of the vitreous with a vitrector using suction and cutting maneuvers. (Original image)

Modern vitreoretinal surgery adheres to rigorous standards of patient safety, ethical practice, and technological innovation. High-resolution imaging, such as OCT and fluorescein angiography, aids in accurate preoperative assessment and postoperative monitoring. Minimally invasive techniques, including 23-, 25- and 27-gauge (G) pars plana vitrectomy (PPV) systems, contribute to quicker recovery times and reduced postoperative complications [42]. A schematic representation of this is shown in Figure 9. Additionally, the integration of advanced tools like heads-up 3D visualization systems with intraoperative OCT, as shown in Figure 10, contributes to propel better surgical outcomes [24, 28].

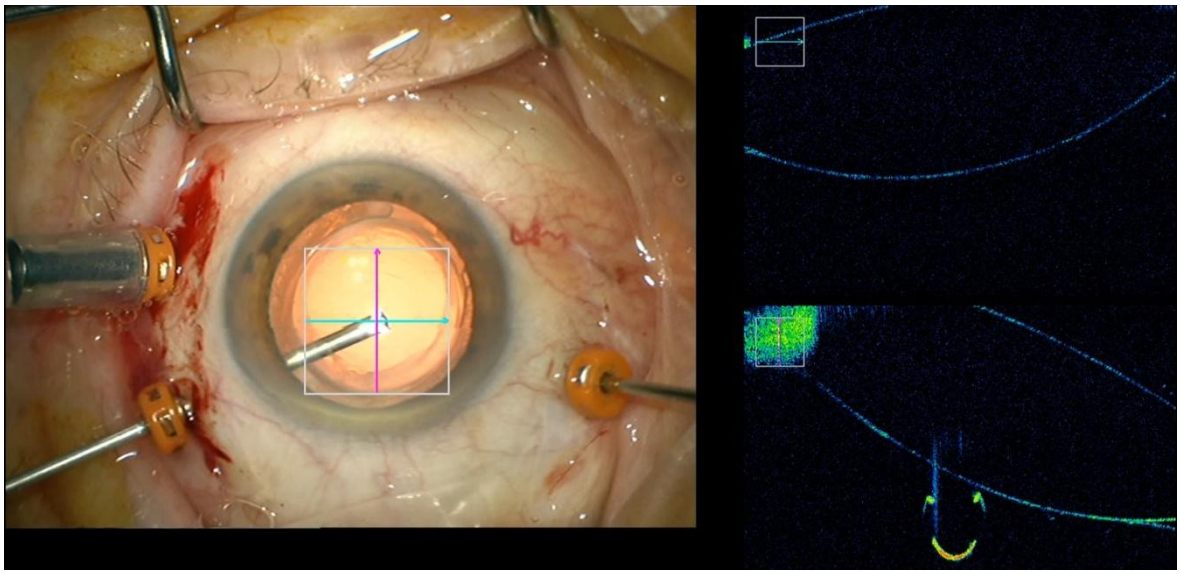


Figure 10. An iOCT-assisted posterior capsulotomy during a small-gauged pars plana vitrectomy (PPV) with an infusion port (superior left) and two instrument ports: a vitrector (inferior left) and an intraocular light source (inferior right). A (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

While vitreoretinal surgery has made remarkable progress, challenges persist. The increasing prevalence of age-related retinal disorders like macular degeneration and those linked to chronic diseases, such as diabetic and hypertensive retinopathies, presents an ongoing clinical burden. Surgeons continue to explore ways to optimize surgical techniques, improve intraoperative visualization, and minimize complications [43, 56]. Additionally, the field is witnessing a greater emphasis on personalized medicine, where genetic and molecular insights inform tailored treatment approaches [53].

Retinal surgery implicates complex and delicate operative techniques that involve the treatment of various disorders affecting the vitreous and retina of the eye. One of the challenges faced during vitreoretinal surgery is the limited visualization of intraocular structures, particularly when delicate maneuvers are required. To address this challenge, Vitreoretinal surgeons have turned to visualization-aiding technologies, such as heads-up 3D display systems and intraoperative OCT to achieve precise outcomes and restore or preserve vision [20].

1.5 Intraoperative optical coherence tomography

Intraoperative optical coherence tomography (iOCT), as its name implies, is the application of OCT imaging technology during surgery. By providing real-time, high-resolution cross-

sectional images of ocular structures, iOCT enables surgeons to visualize tissues with remarkable detail during procedures. The integration of iOCT into the surgical setting allows for enhanced precision and decision-making, facilitating immediate assessment of tissue morphology, verification of surgical maneuvers, and overall improved surgical outcomes [24, 25].

The origin of iOCT was first made possible due to the development of hand-held OCT devices, which arose from the need for imaging in various clinical settings where fixed patient positions were not always possible, such as in pediatric patients, emergencies, and operating rooms [23]. The implementation of these hand-held OCT devices in a surgical setting was the first step towards integrated iOCT surgical ophthalmological microscopes.

The technology facilitates not only diagnosis but also helps clinicians comprehend pathophysiology, enabling a better understanding of how tissues behave under observation. This has paved the way for the use of OCT intraoperatively in most subspecialties within ophthalmology. For example, it can aid to better visualize very thin and transparent tissues, such as the anterior capsule during cataract surgery, as shown in Figure 11, or help confirm correct positioning and wound seal of a transplanted donor cornea during keratoplasty procedures, as shown in Figure 11 [22].

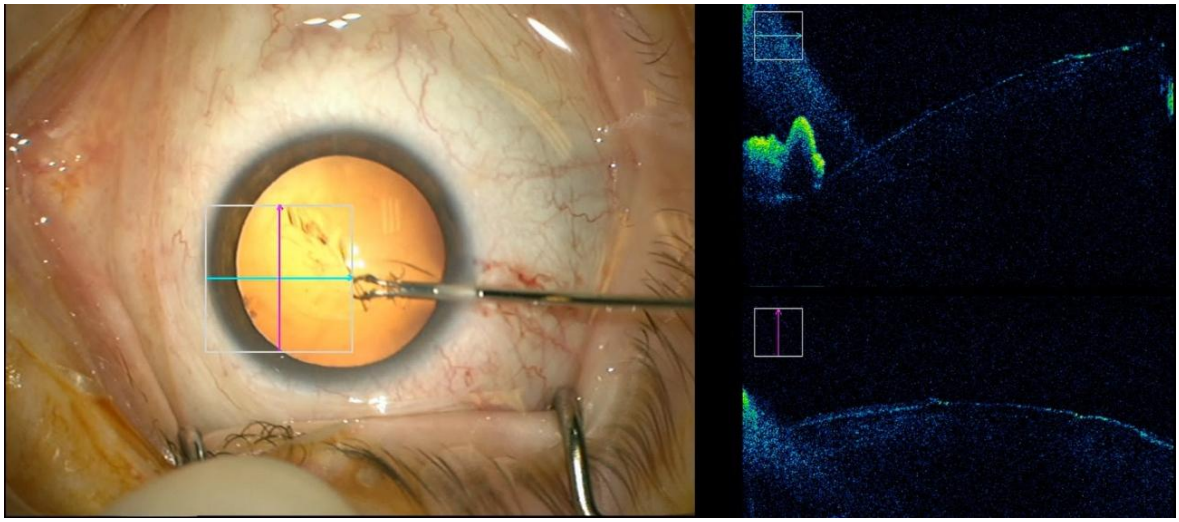


Figure 11. iOCT-assisted anterior capsulorhexis, one of the crucial steps during cataract surgery. Appropriate manipulation of this clear and thin structure can be simultaneously checked with the help of iOCT. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

Intraoperative OCT enables real-time visualization of tissue-instrument interactions that enhance surgical procedure execution through its ability to provide continuous updates on vital information such as anterior chamber angle morphology changes following manipulations [22]. The application of iOCT is used initially most effectively in vitreoretinal surgeries primarily during the assessment of macular holes and epiretinal membranes, but with ongoing advancements in the field, it is possible to expand its usage to other ophthalmic surgeries [30], especially those involving the anterior segment of the eye, as shown in Figures 11 and 12. One such area of ongoing research is the phenomenon of signal shadowing during iOCT-assisted vitreoretinal surgery.

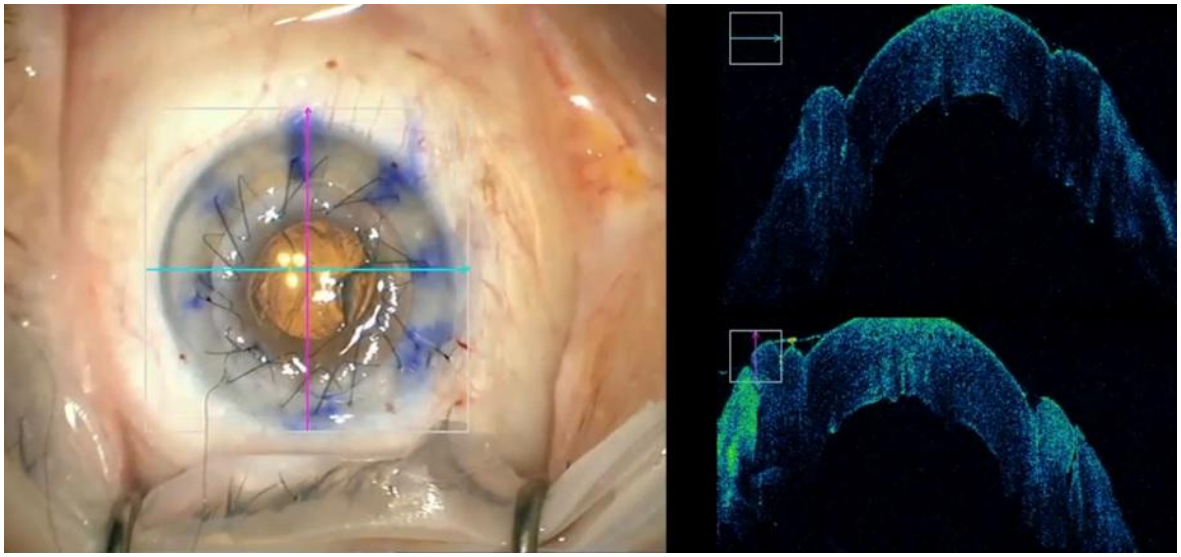


Figure 12. End-of-surgery image of an iOCT-assisted keratoplasty, confirming graft adaptation and proper wound closure. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

In vitreoretinal surgery, iOCT can provide multiple advantages, aside of rendering a histologic-like image of retinal tissue, it also allows an additional viewpoint beyond the traditional downward scope of the microscope, since it can scan transversely as shown in Figure 13. With its unparalleled precision and accuracy, physicians can use this innovative technology to evaluate multiple vitreoretinal pathologies and has proved itself extremely useful during the treatment of macular holes and epiretinal membranes in vitreoretinal surgery [24].

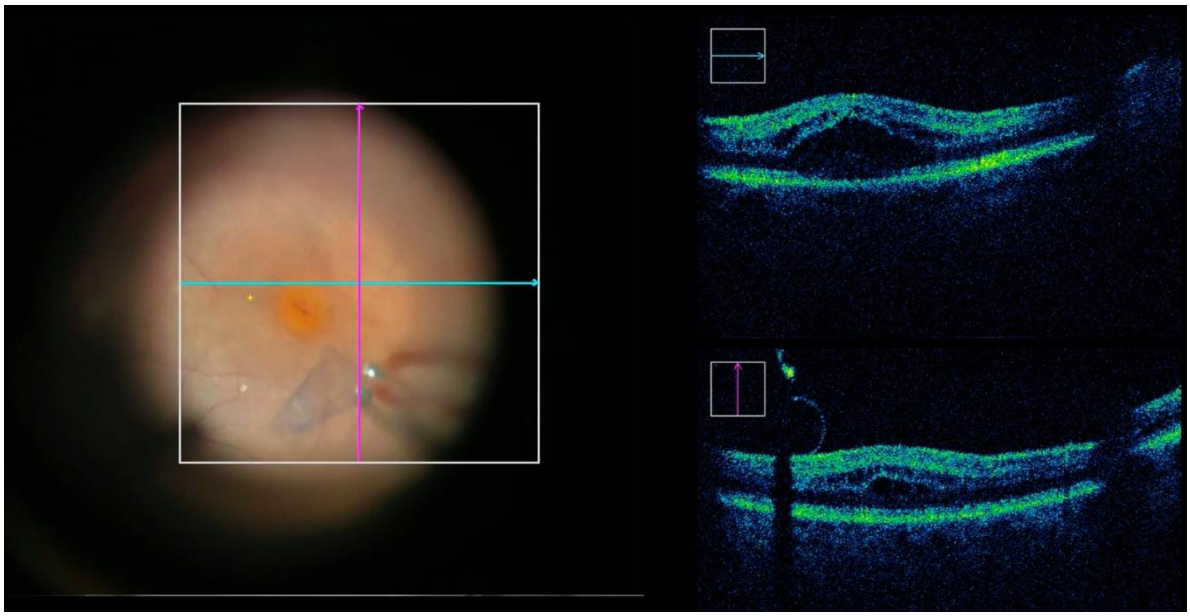


Figure 13. Tissue manipulation whilst performing epiretinal membrane peeling with forceps can be carefully monitored using iOCT during a PPV. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

Apart from aiding pre-operative diagnosis and post-operative evaluation procedures', iOCT provides unique benefits during complex surgeries enabling surgeons to dynamically adjust reconstructions while performing delicate procedures, thereby improving outcomes and significantly leading to reduced complications after surgeries [12]. Therefore, a rise in the use of intraoperative optical coherence tomography has been observed, as it helps surgeons obtain immediate feedback and perform better-informed decisions during vitreoretinal surgeries, prompting an optimization of all available means [17].

These applications represent the benefits of the integration of optical coherence tomography with surgical microscopes, highlighting its potential as a noteworthy advancement in the intraoperative assessment of tissues. This innovative technique allows surgeons to obtain immediate and detailed imaging information during surgery without compromising patient safety or causing any delays [10]. Additionally, iOCT significantly improves visualization by offering clear visual representations even in situations where standard illumination systems may fail to provide reliable results due to limited view angles or the presence of opaque membranes [4]. This combination of OCT and surgical microscopy provides real-time imaging capabilities that enhance the accuracy and precision of various surgical procedures.

1.6 Limitations of iOCT and counteracting strategies

Despite its numerous advantages, there are several limitations that undermine the full potential of OCT imaging intraoperatively. These limitations include limited tissue penetration, a small field of view, and the occurrence of shadowing artifacts [10].

Since iOCT uses light waves to create images [15], this limits its ability to penetrate deeper and non-transparent tissues compared to other imaging modalities like ultrasound, computer tomography of magnetic resonance imaging. This limitation is particularly significant when visualizing structures behind opaque tissues or dense opacities, such as in cases of severe cataracts or dense vitreous hemorrhages.

Another limitation, the small field of view inherent in iOCT systems due to the scope of the camara integrated with the surgical microscope, restricts the amount of the ocular surface that can be captured at one time. This limitation necessitates multiple scans to cover larger areas, which can be time-consuming and may interrupt the flow of surgery.

Signal shadowing is a common challenge encountered during iOCT-assisted vitreoretinal surgery. It refers to the obstruction of the iOCT scan signal by interfering with objects present in the surgical field, leading to compromised image quality and hindrance in evaluating crucial anatomical structures [18, 19]. An example of this is shown in Figure 14.

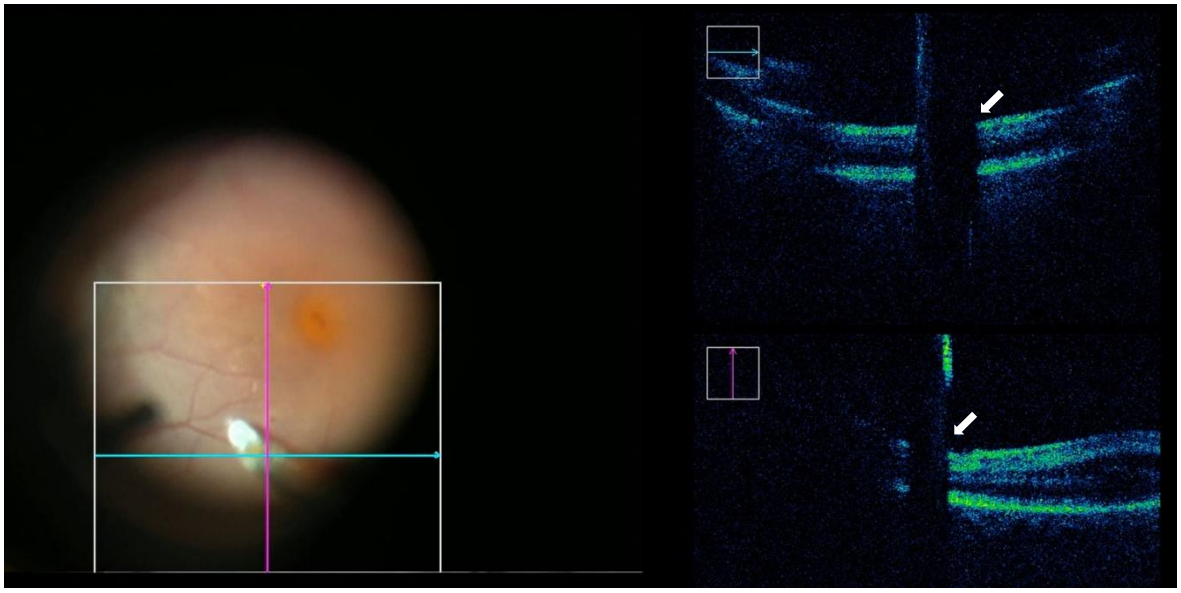


Figure 14. During the same iOCT-assisted epiretinal membrane peeling exhibited in figure 13, a shadow (arrows) casted from the forceps obscures the underlying retinal tissue on the iOCT-scan. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

The investigation into signal shadowing during iOCT-assisted vitreoretinal surgery aims to comprehend its implications on surgical outcomes, which can eventually help develop effective measures to address it. Understanding the causes, characteristics, and consequences of signal shadowing will facilitate improvements in image quality assessment during real-time imaging-guided interventions.

A technique-independent limitation is the current low availability of iOCT, which causes a series of user-dependent problems. Achieving high-quality images while navigating around ocular tissues poses challenges that require optimizing visualization techniques amidst other obstacles faced by surgeons. Some procedures may call for an interruption of the surgical procedure so as not to compromise a clear view while others involve developing specialized instruments capable of providing high-resolution imaging even amid interferences due to fluid dynamics arising from aspirating fluids. Several studies regarding this theme have yet to produce a definitive understanding of the factors contributing to signal shadowing during iOCT-assisted vitreoretinal surgery [20].

It has been shown that iOCT can be feasibly brought into the surgical workflow without major interruption, confirming that its potential can be reached in real-life scenarios [10]. Nonetheless prior findings indicate poor reproducibility concerning OCT measurements leading to varied results depending on examination techniques used by different operators or even inconsistencies over time using identical methodologies [20, 52]. Despite the promising capabilities of iOCT, these practical limitations need to be resolved in order to optimize its use during vitreoretinal surgery [10, 17]. Widespread standardization of iOCT use is pending.

As stated, one of the main challenges associated with iOCT is the occurrence of shadowing artifacts, which can limit the clarity and accuracy of the captured images, as shown in Figure14 and schematically explained in Figure 15. In order to overcome this phenomenon, multiple current strategies are being employed to mitigate shadowing effects during iOCT, highlighting advancements in technology and innovative approaches [21]. Addressing these challenges has been a focus of ongoing research and technological development, particularly through the creation of iOCT-compatible vitreoretinal instruments.

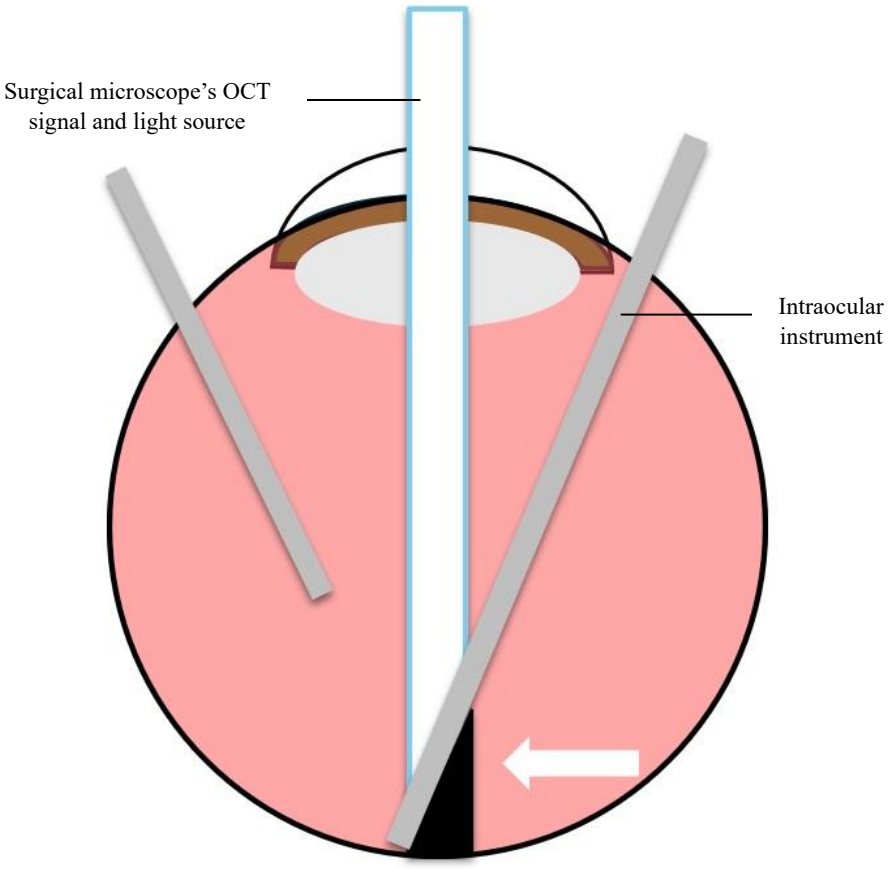


Figure 15. Schematic image exhibiting the shadow (white arrow) created by intraocular instruments during iOCT examination in a PPV. (Original image)

Enhancing the optical design iOCT systems is one of the main methods currently used to minimize shadowing during vitreoretinal surgeries. This involves a meticulous optimization of the arrangement of light sources and detectors, ensuring an efficient and clear imaging process. The evolution of broad-spectrum and high-intensity light sources enhances tissue penetration, thereby reducing shadowing. Implementing dual-channel imaging systems, which capture images using multiple wavelengths simultaneously, is a noteworthy strategy. This approach not only enhances contrast but also mitigates shadowing by incorporating complementary information from different light sources [48]. These technological advances collectively contribute to optimizing iOCT imaging, providing surgeons with clearer and more comprehensive visual data during vitreoretinal procedures.

The integration of sophisticated image processing algorithms plays a vital role in addressing shadowing artifacts. Leveraging machine learning and artificial intelligence methodologies, these algorithms intelligently identify and compensate for shadowing in real-time. This dynamic approach to image processing ensures that the iOCT system can adapt and correct for shadowing effects, further elevating the quality and accuracy of visual

information available to surgeons during vitreoretinal surgeries [5, 13]. In essence, a holistic integration of improved optical design, innovative instrument technology, advanced light sources, and intelligent image processing methodologies collectively contributes to the ongoing refinement of iOCT systems.

Mitigating shadowing in iOCT is a crucial aspect of enhancing its effectiveness as a surgical guidance tool. Ongoing research and technological advancements continue to shape the landscape of strategies to address shadowing artifacts, ensuring that iOCT can provide surgeons with the best quality images during critical procedures. As these strategies evolve, the integration of innovative solutions holds the promise of further improving surgical outcomes and expanding the applicability of iOCT across various medical disciplines.

1.7 Purpose of the study

Until now, the operating surgeon has been solely responsible for determining the degree to which signal shadowing limits the application of iOCT technology. Unfortunately, objective markers for measuring these visual challenges are not well-documented or published. To address this knowledge gap and improve surgical outcomes the following objectives were pursued:

1) This study's primary aim was to quantify the degree of signal shadowing on iOCT imaging caused by intraocular instruments, stains, and vitreous during vitreoretinal surgery and analyze its impact.
2) The secondary objective of this research was to pinpoint the variables that most significantly contribute to the interference with iOCT imaging, affecting the clarity of the visualizations in the crucial surgical field. The goal is not only to identify these factors but also to quantify their impact through systematic data analysis.
3) As a tertiary endpoint, this research project seeks to contribute to standardization efforts by providing objective results for the degree to which signal shadowing hinders iOCT imaging, specifically during vitreoretinal surgeries. Providing an objective measure of the extent to which signal shadowing limits iOCT use during vitreoretinal surgery will help physicians optimize their approach and improve patient outcomes.

The investigation encompassed a comprehensive analysis of various factors, including but not limited to the physical properties of vitreoretinal instruments, their material density, transparency, size, and potential interactions with intraocular tissues. The study involved a meticulous examination of intraoperative scenarios using iOCT, capturing real-time data during vitreoretinal surgeries to assess the specific conditions leading to shadowing effects on the retina.

4) Furthermore, based on the insights gained from the investigative phase, this study aimed to design a novel vitreoretinal instrument that actively counteracts the identified limitations related to shadowing effects. This involved proposing innovative solutions that mitigate or eliminate the interference, utilizing novel instrument designs that can reduce the impact of signal shadowing by intraocular instruments, in order to enhance the quality of iOCT imaging during vitreoretinal surgery, potentially improving surgeons' precision and effectiveness.

2. Methods

2.1 Shadowing during iOCT-assisted vitreoretinal surgery

The following methodology was carried out to objectively determine the usefulness of iOCT and identify the key variables that limit its full potential during the surgical treatment of vitreoretinal diseases.

2.1.1 Ethical statement

The present study is a retrospective, non-randomized observational analysis that involved consecutive cases treated at the Department of Ophthalmology, University Hospital Giessen and Marburg, Campus Giessen of the Justus Liebig University, between 2019 to 2023. The study was conducted with all pertinent approvals from the on-site ethical commission (*Ethik-Kommission des Fachbereichs Medizin der Justus-Liebig-Universität Gießen*) and was registered accordingly under the study number AZ 200/19. The study population consisted of regular patients with vitreoretinal diseases that required and were treated with standard surgical treatment. All enrolled patients bestowed their written informed consent before undergoing the standard-of-care procedures necessary to treat their respective underlying pathologies.

2.1.2 Patients

The imaging data from one hundred seventeen patients who underwent an iOCT-assisted vitrectomy was analyzed to review the potential benefits accrued through this technique in facilitating prompt and effective diagnosis and management of various critical ocular conditions. Patients previously treated with intravitreal implants were excluded from the study due to the implants' potential of impacting iOCT imaging analysis. A previous history of treatment with intravitreal injections was not a criterion for exclusion, as the medication is applied in a transparent liquid vehicle which does not significantly affect iOCT imaging quality as long as the injected substance has diffused properly within the vitreous cavity.

2.1.3 Surgical procedures

All procedures recorded were of pars plana vitrectomies (PPV). In the field of vitreoretinal surgery, a PPV is currently considered to be the standard surgical technique in the field [31]. This approach allows access to the posterior segment of the eye, without damaging the retina, where the removal of emulsified vitreous takes place in order to treat various vitreoretinal

conditions, such as the forementioned diagnosis. Here are the steps involved in a pars plana vitrectomy, as performed on the patient population studied:

1) *Preoperative preparation*
    - The patient underwent a thorough eye examination and the necessity for operative care is determined.
    - The eye was anesthetized using either local anesthesia, typically a retrobulbar block or general anesthesia.
    - The eye and surrounding area were sterilized with an antiseptic solution, and a sterile drape was placed around the eye.
2) *Surgical entry points*
    - For the surgical opening 23-, 25- or 27-gauge incisions on the sclera (sclerotomies) were performed at about 3.5-4 mm posterior to the corneoscleral limbus, through the pars plana, which is the flat part of the ciliary body. The trocars serve as ports of entry for the surgical instruments, whilst providing an artificial seal that helps maintain eye pressure throughout the procedure.
3) *Vitrectomy: removal of the vitreous*
    - Indispensable instruments for the vitrectomies:
        - An infusion line to maintain intraocular pressure and provide a continuous flow of balanced salt solution (BSS).
        - A light pipe to illuminate the interior of the eye.
        - A vitrector, which is a cutting and aspirating pipe-shaped instrument to remove vitreous gel.
    - The vitrector was inserted through one of the sclerotomies. It cuts and aspirates the vitreous, which is then replaced by the infusion fluid (BSS) to maintain the shape and pressure of the eye. The vitreous was removed to gain access to the retina.
4) *Treatment of retinal issues*
    - Depending on the underlying condition being treated, the surgeon may have needed to perform additional procedures that required an array of instruments, intraocular dyes, and various types of vitreous substitutes. While

using these intraocular materials iOCT scans were performed to perform the analysis of this study.

- Epiretinal membranes: The membrane on the retinal surface was peeled off.
- Macular hole: A peeling of the internal limiting membrane (ILM) around the macular hole may be performed to facilitate closure.
- Retinal detachment: Laser or cryotherapy were used to create adhesions around retinal tears. A gas bubble or silicone oil may be injected to hold the retina in place while it heals.
- Vitreous hemorrhage: Any blood present in the vitreous was removed.

5) *Incision closure*

- Main sclerotomies were usually sutured (25-gauge and larger) with resorbable sutures. Very small gauge incisions (27-gauge or smaller) are usually self-sealing.
- The eye is checked for adequate pressure and the absence of leaks from the incisions.

6) *Postoperative care*

- Antibiotic and anti-inflammatory eye drops were then instilled to prevent infection and reduce inflammation.
- The patient may need to maintain a specific head position for a certain period if a gas bubble was used to ensure proper healing.
- In-patient examination and follow-up appointments are scheduled to monitor the healing process and address any complications.

The specifics of each step can vary based on the individual patient's condition and surgical approach taken. All surgical procedures in this research project were performed by an experienced vitreoretinal surgeon who specializes in using iOCT-assisted surgeries, adhering to standard-of-care procedure guidelines.

2.1.4 Intraoperative imaging and data collection

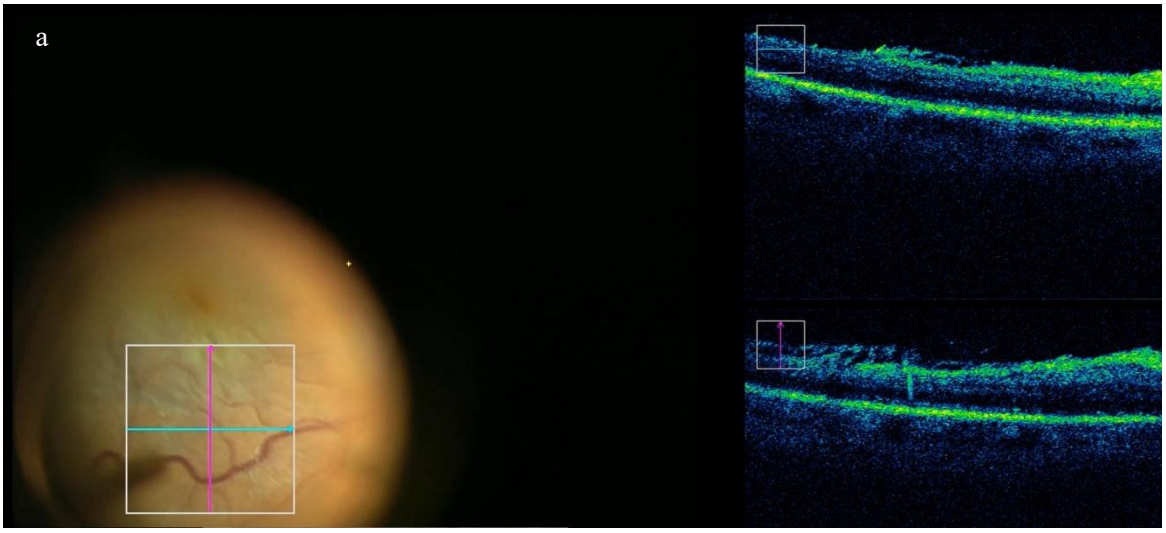
The operating theatres were equipped with iOCT imaging systems that provided real-time visualization of the surgical field during the procedures at the surgeon's discretion. To

achieve a standardized analysis, the video recording was done on all patients using an ophthalmologic surgery microscope with an integrated iOCT camera (Zeiss RESCAN^{TM} 700, Carl Zeiss Meditec AG, Oberkochen, Germany), its technical settings are shown in Table 2.

Table 2. iOCT Settings of the Zeiss RESCAN^{TM} 700 (Carl Zeiss Meditec AG, Oberkochen, Germany) surgical microscope used for this study.

OCT type	Spectral-domain (SD)
A-Scans/s	27,000
Wavelength (nm)	840 nm
Axial resolution (µm)	5.5 µm

The integration between these devices enabled both the in vivo tissue analysis and the iOCT recording of the surgical procedures. After the surgery, the images were thoroughly scrutinized following baseline evaluation criteria. An example of the image selection and analysis is shown in Figure 16. This has been published previously in other works with related topics about the use of OCT and its use during ophthalmologic surgeries [10, 14, 38, 39].



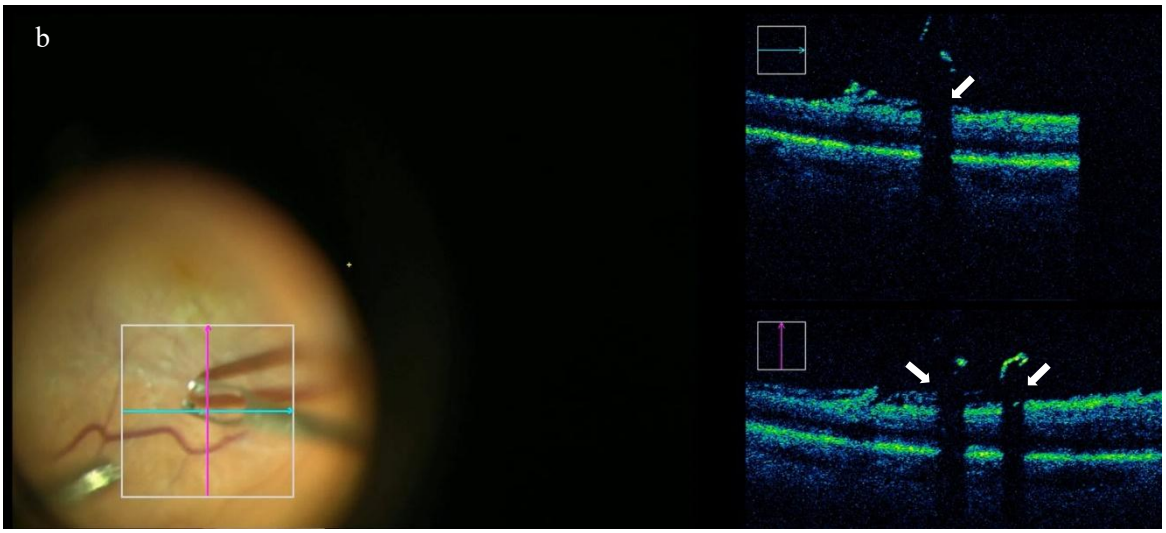


Figure 16. Example of iOCT data collection during surgical procedures: a) an unblocked retinal visualization during iOCT used as control image compared to b) the same area with iOCT signal shadowing (arrows). (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

2.1.5 Study groups

According to their prospective generative shadowing effect, the iOCT image data obtained from these vitreoretinal procedures were assigned to three main groups: vitreoretinal instruments, intraocular dyes, and vitreous substitutes. Each category was comprised of a series of specific items that were further analyzed as exhibited in Table 3.

1) *Intraocular instruments*

   This group encompassed various surgical instruments essential to vitreoretinal procedures, including standard vitrector, internal limiting membrane (ILM) forceps, silicone-tipped cannula (D.O.R.C. International BV, Zuidland, Netherlands), Finesse® Flex Loop cannula (Alcon Laboratories Inc., Fort-Worth, USA), and Tano Diamond Dusted Membrane Scraper (Synergetics®, Bausch + Lomb, Rochester, USA).

2) *Intraocular dyes or stains*

   The second category was comprised intraocular dyes: indocyanine green, brilliant blue dye (D.O.R.C. International BV, Zuidland, The Netherlands), triamcinolone acetonide, and a lutein-based dye VITREODYNE™ (Kemin Pharma, Barcarena, Portugal).

3) *Vitreous substitutes*

The final group was dedicated to vitreous substitutes utilized in vitreoretinal surgery. This assortment consisted of air, sulfur hexafluoride (SF_6), octafluoropropane (C_3F_8), perfluorocarbon liquid (PFCL), and silicone oil. These substitutes serve various purposes, such as tamponade for the retina or maintaining the structural integrity of the eye. iOCT scans of balanced salt solution (BSS) filled eyes were used for the paired image controls.

Table 3. Study groups divided accordingly into vitreoretinal instruments, intraocular dyes and vitreous substitutes.

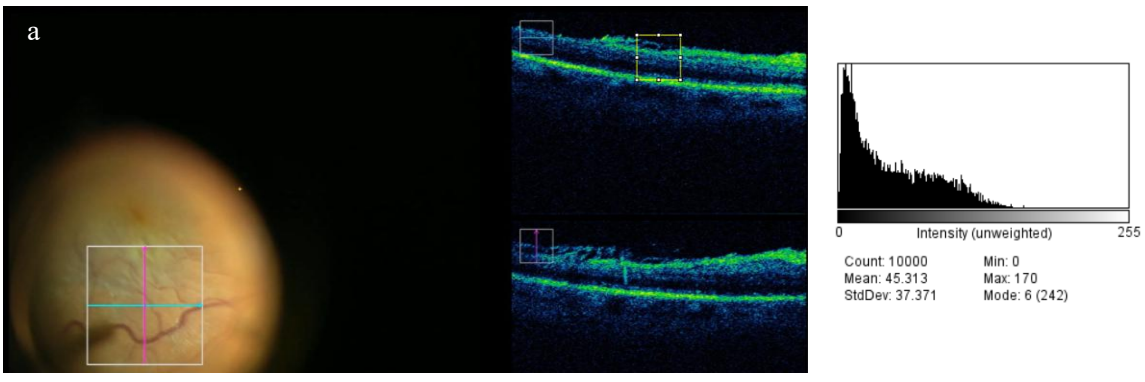
Intraocular instruments
Vitrector
ILM forceps
Silicone-tipped cannula
Looped cannula
Tano Diamond Dusted Membrane Scraper
Intraocular dyes
Indocyanine green
Brilliant blue
Triamcinolone acetonide
VITREODYNE™ lutein-based intraocular dye
Vitreous substitutes
Balanced salt solution (BSS)
Air
Sulfur hexafluoride (SF_6)
Octafluoropropane (C_3F_8)
Perfluorocarbon liquid (PFCL)
Silicone oil

As per standard procedure during a PPV, the vitreous cavity is filled with balanced salt solution (BSS) as the vitreous is removed. BSS filled eyes without any type of signal shadowing were used for the paired image controls in every category. This means every eye studied was first filled with BSS after the initial vitrectomy as per standard procedure, and

at this stage of the surgery a control iOCT scan was performed. Every iOCT scan analyzed during which an intraocular material was used was then compared to its original paired control scan of the same eye and same surgical procedure. This meticulous categorization and detailed delineation allowed for a comprehensive analysis of the iOCT data, enabling a more granular examination of each material type and its specific applications within vitreoretinal surgical procedures.

2.1.6 Post-processing and statistical analysis

The data underwent post-processing using the open-source graphic software ImageJ from the developer LOCI of the University of Wisconsin, Madison, USA. Specifically, the histogram function in the ImageJ software was used to visualize and analyze the distribution of pixel values within the study images. It provided a graphical representation of how frequently different pixel values occur in the image along an axis. The histogram x-axis represents the range of pixel values, typically from 0 to 255 for 8-bit images, while the y-axis shows the frequency or count of pixels with each value. By examining the histogram, it is possible to gather quantifiable data regarding various aspects of the image, such as its contrast, brightness, and the presence of distinct regions with specific pixel values. Peaks in the histogram indicate common pixel values, and the spread of values can provide information about the image's overall tonal range. The histogram function is also used to set thresholds for image segmentation, enabling the isolation of specific areas or objects of interest [54, 55]. An example of the histogram analysis feature used on the study images is shown in Figure 17.



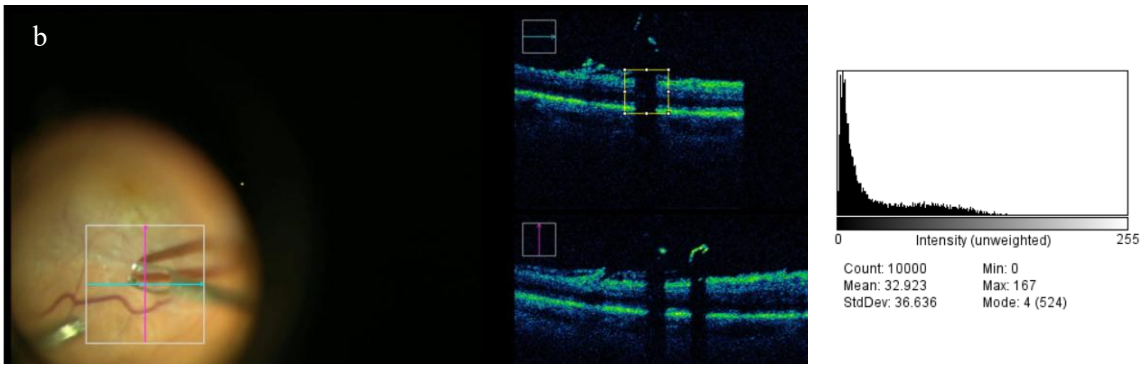


Figure 17. A 100 x 100 pixel area (yellow square) of a) an unblocked iOCT-scanned retinal control image was chosen and analyzed using a graphic software (Image J. LOCI of the University of Wisconsin, Madison, USA), rendering the graphical statistics exhibited to the right of the image. The same process was carried out on b) a paired study image where a study item blocked the iOCT signal corresponding to the area of the control image. Multiple control-study image series for each study item were performed and the obtained graphical data was then statistically compared. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

The purpose was to measure the degree of picture quality distortion via a histogram analysis and determine average gray level interference. Gray level image quality refers to the measure of how well the details and information in a grayscale image are preserved and accurately represented. The procured iOCT scans exhibited a spectrum of intensities ranging from the darkest shade, symbolized as black, to the lightest shade, represented as white. Each pixel within such images was assigned a specific intensity value, encapsulating an aspect of the visual information. Gray level image quality scrutinizes the effectiveness of this assignment, assessing how faithfully the image mirrors the real object being scanned. In most cases, a high gray level would indicate minimal distortion and a faithful representation of the object, while a low gray level would suggest significant distortion and potential loss of information in the image. Several exceptions arose during the analysis of materials that could be detected on iOCT scans without a relevant reduction of the scope of vision of the underlying tissue, which resulted in a net gray level gain on the histogram-pixel analysis.

In picture frames where a visually significant interference was detected, the average gray level of the shadowed area was determined and then compared it with paired image controls that did not show any clinically perceptive interference. To conduct the analysis, recorded sequences were collected for each group item in multiple square 100 by 100 pixel frames along with their respective paired controls. In some instances, where aberrant artifacts resulted in gray level distortions, individually sized smaller pixel frames were analyzed separately.

Finally, a statistical analysis was performed using Microsoft Excel spreadsheet software to ascertain individual scores and draw relevant conclusions about trends or patterns visible throughout the dataset. The study utilized a quantitative approach, utilizing both descriptive statistics and multivariate analyses for data analysis.

2.2 iOCT-compatible vitreoretinal instrument prototypes

This project aimed to contribute to the ongoing research effort to comprehend, measure, and objectify the limitations of iOCT by analyzing and characterizing the patterns and causes of signal shadowing in order to help devise strategies to mitigate its impact on image quality and subsequently optimize surgical outcomes. In this manner, this study seeks to contribute to the ongoing research effort that propels OCT innovation in ophthalmology.

Based on the preliminary results of this work, a secondary project was set in motion that designed novel vitreoretinal instruments with the purpose of creating iOCT-compatible prototypes that were better adept at avoiding signal shadowing. This secondary experimental branch was conducted as a continuation of the base study, paralleling its methodology: using the same ophthalmologic surgical microscope with an integrated OCT camera for data collection, and the same graphic software for image analysis.

The forementioned shadowing effect on iOCT refers to an obstruction of the OCT signal between the surgical microscope and the tissue being examined, usually caused by interfering intraocular surgical materials. Previous studies have noted the detrimental impact that signal shadowing has on the quality of iOCT, diminishing its full potential usefulness, as exemplified in Figure 14 and schematically shown in Figure 15.

With this project, we have aimed to create novel vitreoretinal prototypes that address these problems, not only by reducing the potential shadowing effect compared to standard vitreoretinal instruments, but also by considering the crucial balance between rigidity and transparency that would facilitate their introduction into real-world surgical employment. To achieve these three OCT-compatible vitreoretinal instrument prototypes with 25-gauge sheaths: an internal limiting membrane forceps, a 41-gauge tipped subretinal cannula, and a retractable aspiration cannula were designed to include two slit openings, strategically positioned on opposing shaft walls, 2 mm long, close the instrument's tip, to mitigate potential signal shadowing while maintaining structural integrity. The original schematic design idea is shown in Figure 18. These prototypes were then tested in experimental conditions determining their gray level interference and compared to the baseline findings, using the same methodology as the base study.

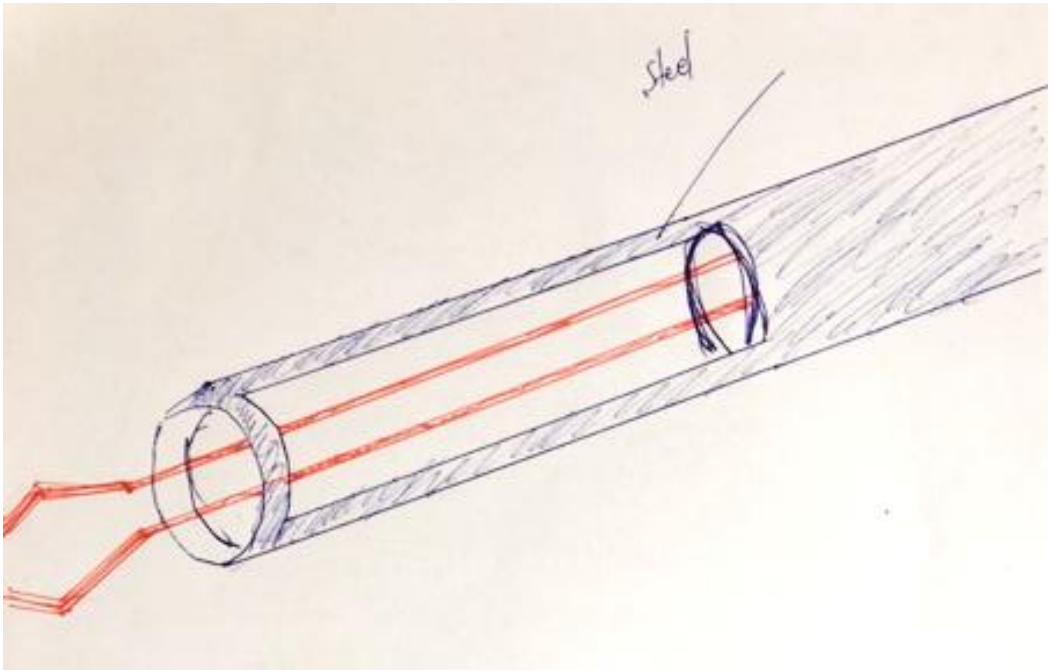


Figure 18. Original hand-drawn design of ILM forceps prototype with double-sided sheath openings were made on opposing shaft walls near the instrument's tip. (Original image and design from PD Dr. k.m.n. Lyubomyr Lytvynchuk PhD)

3. Results

3.1 Demographic analysis

As shown in Table 4 of the 117 individuals that participated in the study, 63% were men while almost half (45%) were sixty years or older. The procedures performed on fourteen individuals involved reoperations. Epiretinal membranes with positive metamorphopsia and visual deterioration were the main working diagnosis that implicated a surgical intervention in fifty-two participants, followed by macular holes in forty-one cases. Retinal detachment was diagnosed in twenty-seven patients and eight patients were diagnosed with persistent vitreous hemorrhage, requiring surgery. These pathologies represent some of the most common indications necessitating surgical intervention within ophthalmology. Some overlapping of concomitant diagnosis occurred, particularly between cases of epiretinal membranes and macular holes.

Table 4. Study population demographics and diagnosis.

Demographics		(n)	(%)
Population	Total	117	100
Sex	Male	74	63.24
	Female	43	36.75
Age	< 50 years	18	15.38
	50 – 64 years	46	39.31
	≥ 65 years	53	45.29
Diagnosis	Retinal membranes	52*	44.44
	Macular holes	41	35.04
	Retinal detachment	27	23.07
	Vitreous hemorrhage	8	6.83
*11 retinal membrane cases overlap with other diagnoses: macular holes (8), retinal detachment (1), and vitreous hemorrhage (2).			

3.2 Influence of intraocular materials during iOCT imaging

Through the histogram analysis of the images studied, it was possible to obtain a series of gray levels to create a corresponding dataset of each material, therefore objectively

quantifying the image quality and determining the extent of signal shadowing in iOCT-assisted vitreoretinal surgery caused by the analyzed materials used intraoperatively.

3.2.1 Instruments

The study revealed that all instruments caused significant shadowing and interference. After accounting for shadow size and image artifacts, it was observed that the intraocular portion of all vitreoretinal instruments led to a significantly higher average gray level interference when compared to controls. These results are summarized in Table 5. The vitrector yielded the highest level of interference with a 68.51% gray level reduction. Forceps exhibited a 36.21% lower gray level (25.47 ± 7.34 vs. 39.94 ± 6.57), while silicone-tipped cannula demonstrated a 43.96% lower gray level (22.89 ± 6.01 vs. 40.85 ± 2.26). Notably, both forceps and cannulas cast extensive and dense shadows that obscured the underlying tissue being analyzed entirely. However, instruments with smaller tips such as looped cannulas resulted in minimal perceived shadowing effects, allowing for better discernment of the underlying tissue. Additionally, the study found that the size of the shadow cast by the instruments had a significant impact on the level of interference.

Table 5. Gray level of different vitreoretinal surgery instruments.

Instrument	Mean gray level	Standard deviation	Offset from control	p-value (Significance <0.05)
Vitrector	12.57	2.05	-68.51%	<.001
Forceps	25.47	7.34	-36.21%	<.001
Silicone tipped cannula	22.89	6.01	-43.96%	<.001
Looped cannula	51.43	2.86	13.75%	<.001
Looped cannula (shadow adjusted)	30.4	7.2	-32.75%	<.001
Tano sweeper	40.7	0.36	6.18%	<.001
Tano sweeper (shadow adjusted)	18.43	9.48	-51.92%	<.001

Other instruments consistently produced hyperreflectivity artifacts during iOCT recordings, resulting in gray levels that were similar to or higher than the controls on the

histogram analysis. This was observed with the Tano sweepers (6.18% average increase in gray level; 40.7 ± 0.36 vs. 38.33 ± 0.12) and looped cannulas (13.75% increase in gray level; 851.43 ± 2.86 compared to 45.21 ± 0.65). These instruments had either non-metallic or wire-thin tips. Furthermore, smaller shadow-specific pixel frames were measured for these cases, revealing a pronounced shadow effect in localized areas that decreased the perceived gray level similarly to other intraocular instrumentation. Nevertheless, in these cases, the perceived signal block was small enough that it was deemed less clinically relevant since the outline of the underlying tissue could still be visualized. Images exemplifying this analysis are shown in Figure 19.

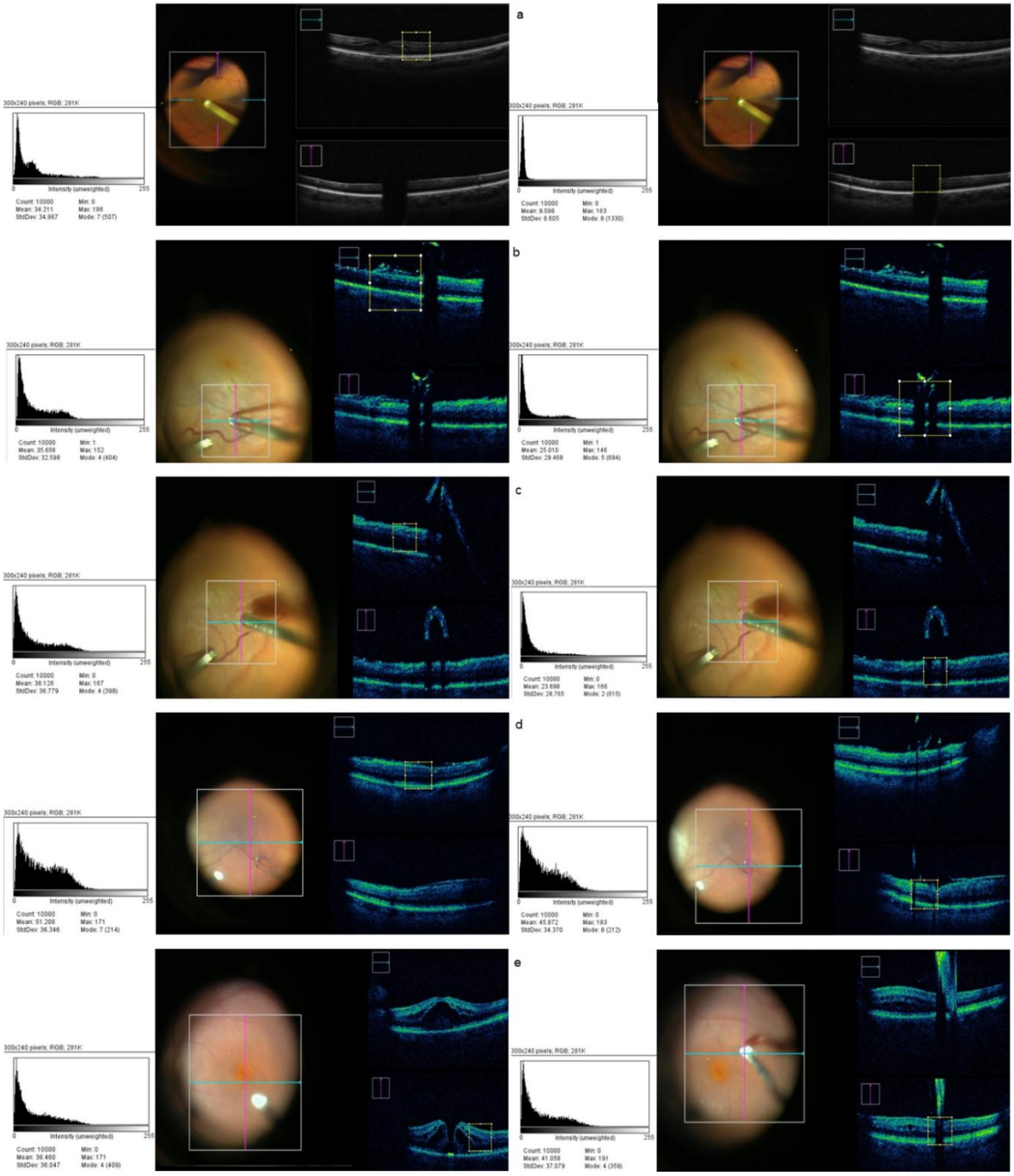


Figure 19. Histogram analysis comparison examples between controls (left) and study images (right) with noticeable shadowing effect of the analyzed tools: a) Vitrector. b) Forceps. c) Silicone tipped cannula. d) Looped cannula. e) Tano sweeper. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

3.2.2 Intraocular dyes

The stains analyzed demonstrated a wide range of image interference behaviors on iOCT as shown in Table 6. Indocyanine green (75.12% lower gray level; 8.34±1.37 vs. 33.52±10.48) and triamcinolone acetonide (26.13% lower gray level; 13.9±10.0 vs. 18.81±0.84) showed a

high to medium shadowing effect of the underlying tissue when compared to controls. Triamcinolone particularly demonstrated a dose-dependent interference effect, more substance equaled more interference, which directly correlates to the wide standard deviation observed. On average the lutein-based dye VITREODYNE™ demonstrated a significantly higher gray level (49.3%; 31.84±8.37 vs. 21.32±5.16) as controls because the substance itself was detected by the iOCT camera whilst casting no significant shadow over the underlying tissue. A similar effect with no visually perceptible interference was observed with brilliant blue, but with only marginally higher (15.06%) gray levels than controls (47.75±8.96 vs. 41.5±7.24), exhibiting no significant distortion. Examples of the image analysis are shown in Figure 20.

Table 6. Gray level of different intraocular dyes.

Dye	Mean gray level	Standard deviation	Offset from control	p-value (Significance <0.05)
Indocyanine green	8.34	1.37	-75.12%	<.001
Brilliant blue	47.75	8.96	15.06%	<.001
Triamcinolone acetonide	13.9	10.0	-2.61%	<.001
Lutein-based dye VITREODYNE™	31.84	8.37	49.3%	<.001

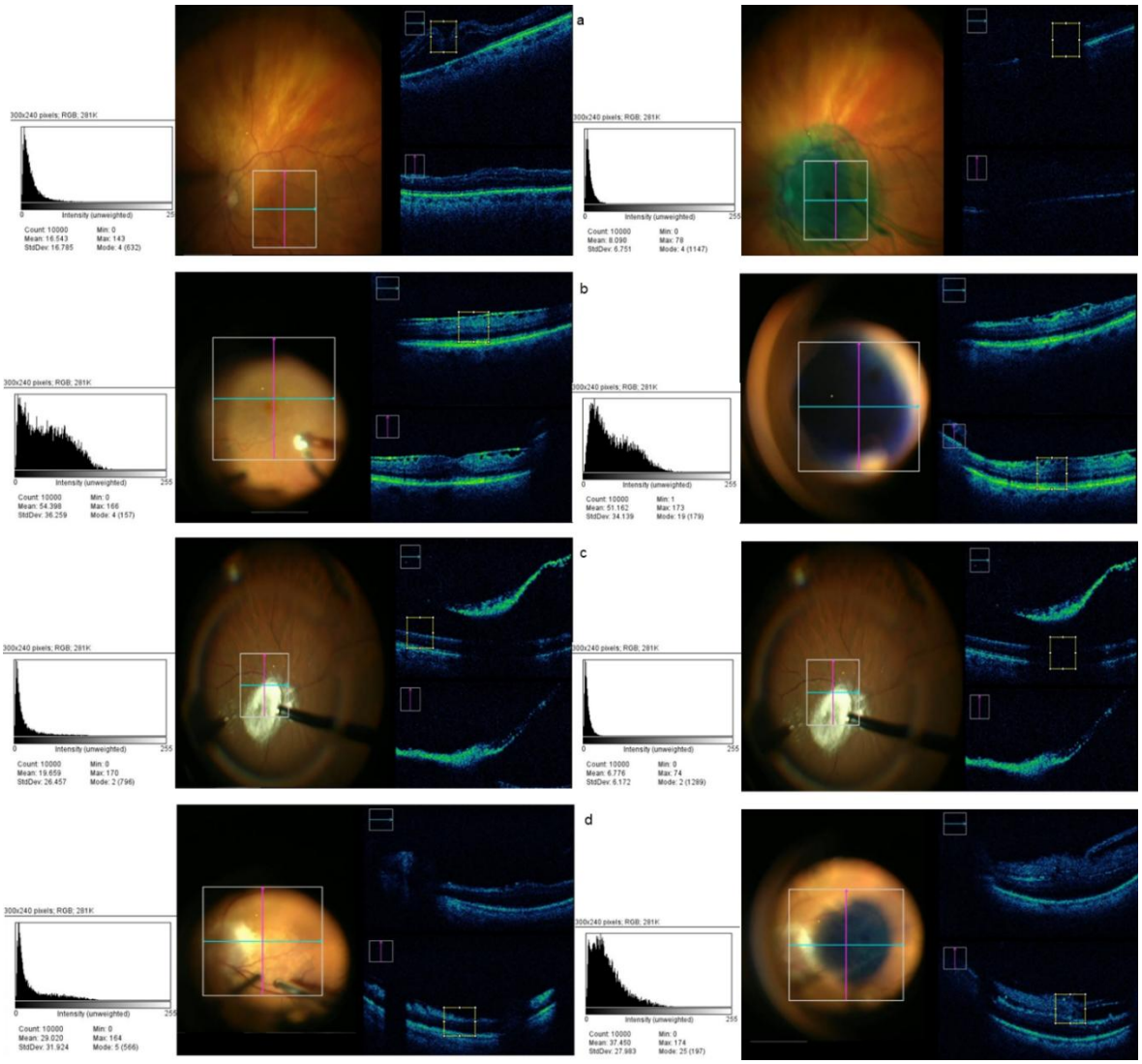


Figure 20. Histogram analysis comparison examples between controls (left) and study images (right) of the analyzed stains: a) Indocyanine green. b) Brilliant blue. c) Triamcinolone acetonide. d) Lutein-based dye VITREODYNE™. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

3.2.3 Vitreous substitutes

After analyzing various vitreous substitutes, it was observed that all of them had minimal to insignificant impact on the iOCT shadowing effect when compared to control substances, which was a balanced salt solution (BSS), widespread in ophthalmologic surgery procedures. Air demonstrated a minimal gray level decrease of 0.29% (p-value: 0.97), SF_6 showed a 2.6% gray level decrease (p-value: 0.77), and similarly, C_3F_8 exhibited a 5.52% gray level reduction (p-value: 0.56). The summary of these results is shown in Table 7. Images exhibiting examples of this analysis are shown in Figure 21. The fluid vitreous substitutes analyzed also showed insignificant levels of gray level interference, but opposite to the gas

substitutes studied, the fluids produced slight gray level increases. In the case of PFCL, it was 1.6% (p-value: 0.83) and silicone oil exhibited the highest overall deviation from controls with a 9.24% increase, which was nonetheless statistically insignificant (p-value: 0.32).

Table 7. Gray level of different vitreous substitutes.

Vitreous substitute	Mean gray level	Standard deviation	Offset from control (BSS)	p-value (Significance <0.05)
Air	39.82	5.7	-0.29%	0.97
SF_6	20.77	4.92	-2.6%	0.77
C_3F_8	20.15	0.77	-5.52%	0.56
PFCL	40.58	4.8	1.6%	0.83
Silicone oil	43.63	2.21	9.24%	0.32

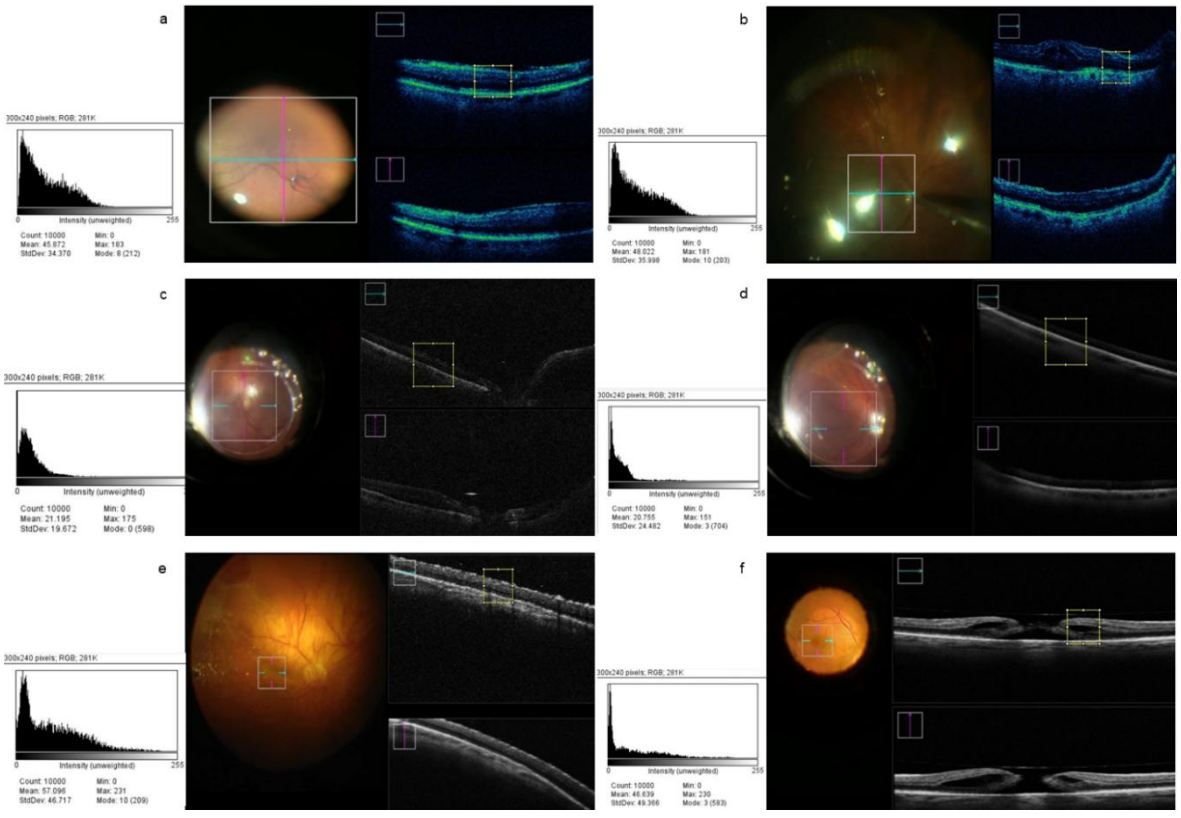


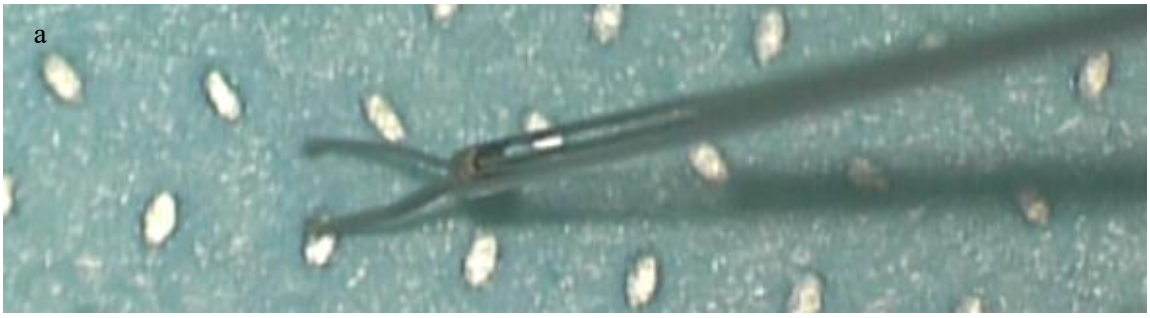
Figure 21. Histogram analysis examples of the analyzed vitreous substitutes: a) BSS (control). b) Air. c) SF6. d) C3F8. e) PFCL. f) Silicone oil. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

3.3. iOCT-compatible vitreoretinal instrument prototypes

Based on the preliminary results of this work, a secondary project was set in motion that designed novel vitreoretinal instruments with the purpose of creating iOCT-compatible prototypes that were better adept at avoiding signal shadowing. This secondary experimental branch was conducted as a continuation of the base study, paralleling its methodology: using the same ophthalmologic surgical microscope with an integrated iOCT source for data collection, and the same graphic software for image analysis.

Under experimental conditions, the designed shown in Figure 18 with two slit openings, on opposing shaft walls, close the instrument's tip, to reduce potential signal shadowing was produced, its original model was an internal limiting membrane forceps, as shown in Figure 22. Thereafter three prototypes of different intraocular instruments for vitreoretinal surgery with 25-gauge sized shafts were created with two slit openings 2 mm long and tested. These included an internal limiting membrane forceps, a 41-gauge tipped subretinal cannula, and a retractable aspiration cannula as shown in Figure 23.

Figure 22. Internal limiting membrane forceps showcasing a 2 mm-long slit opening design aimed to increase iOCT signal transparency. (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)



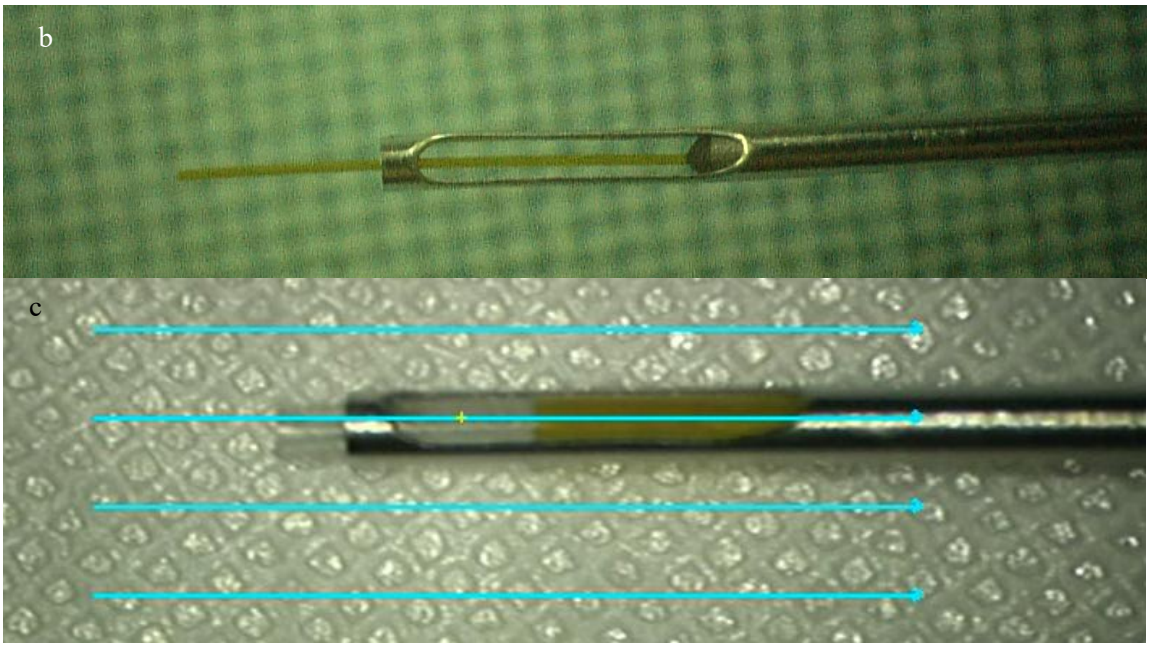


Figure 23. Novel iOCT-compatible 25-gauge vitreoretinal instrument prototypes: a) internal limiting membrane forceps, b) a 41-gauge tipped subretinal cannula, and c) a retractable aspiration cannula. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

To investigate signal disruptions caused by shadow effects, the same iOCT device RESCAN™ 700 (Carl Zeiss Meditec AG, Oberkochen, Germany) was used, just as the base study. Seventeen (17) iOCT sequences were analyzed. The background field used during these experimental recordings was a surgical cloth. The iOCT image data obtained from this experimental study branch were post-processed using the graphic software ImageJ to measure the degree of distortion in image quality as shown in Figure 24. The obtained results were compared to corresponding control images from the same experimental video sequences without clinically perceptible disturbances, where no instruments are in the iOCT signal's path where, as mentioned, a surgical cloth served as the background field during these recordings. These preliminary findings were not directly compared to the base surgical analysis performed.

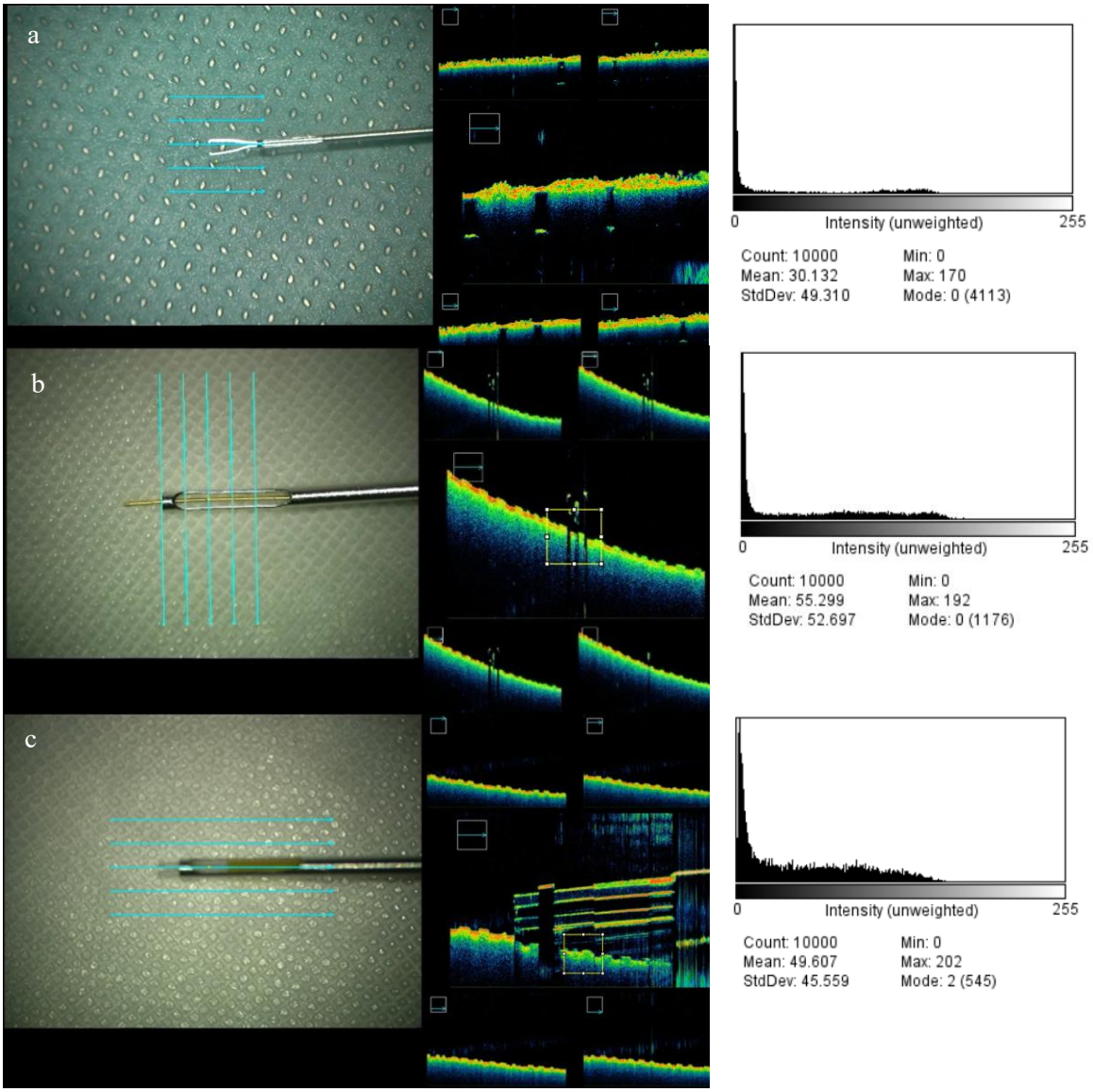


Figure 24. Histogram analysis examples of the analyzed vitreous substitutes: a) internal limiting membrane forceps, b) a 41-gauge tipped subretinal cannula, and c) a retractable aspiration cannula. (Device: Zeiss RESCAN™ 700, Carl Zeiss Meditec AG, Oberkochen, Germany) (Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany)

The experimental branch of the study with novel iOCT-compatible instrument prototypes yielded the following preliminary results. Instruments with clear materials, such as silicon-tipped and polyamide vitreoretinal instruments, demonstrated minimal and insignificant shadowing effects. Interestingly, even small-sized instrument tips, such as the forceps' tips, irrespective of their density, caused clinically irrelevant shadowing.

As displayed in Table 8, a general quantitative analysis of all prototypes revealed that all instruments showed a reduction of gray level compared to control images without any

perceivable shadowing effect. The average gray level reduction of 9.01% ± 9.13. Considering the designed instruments specifically, the gray level reduction was 7.89% ± 12.26 for the internal limiting membrane forceps, 18.44% ± 4.09 for the retractable aspiration cannula, and 0.99% ± 0.32 for the 41-guage tipped subretinal cannula. These results indicate that the choice of material and design significantly influences the extent of shadowing effects, with clear materials and smaller instrument tips exhibiting superior iOCT performance.

Table 8. Gray level of the novel iOCT-compatible instrument prototypes.

Instrument prototype	Mean gray level	Standard deviation	Offset from control	p-value (Significance <0.05)
Internal limiting membrane forceps	46.24	12.26	-7.89%	0.74
41-guage tipped subretinal cannula	49.71	0.32	-0.99%	0.96
Retractable aspiration cannula	40.95	4.09	-18.4%	0.42

4. Discussion

4.1 Study relevance

The future of ophthalmic surgery implicates a continuously increasing use of accurate and effective intraoperative imaging tools, such as iOCT, as it provides real-time visualization and improves accuracy during surgical procedures. The feasibility of this diagnostic technique, applied in real-time during surgeries has been previously explored and published in the pioneer study by Binder et al. [10], highlighting the advantages of iOCT application. However, the presence of shadowing caused by surgical instruments and other ophthalmic medical devices used during standard vitreoretinal surgery can limit both the microscope and iOCT visualization of the underlying tissue, obstructing the view of the area of interest and significantly limiting the potential of this technology.

Therefore, this study aimed to analyze the phenomenon of signal shadowing during iOCT-assisted vitreoretinal surgery and identify potential factors that contribute to this shadowing effect. To achieve this, the influence on the iOCT imaging of the most frequently used materials in vitreoretinal surgery, including instrumentation, intraocular dyes, and vitreous substitutes, was analyzed.

If a clear iOCT view cannot be achieved during surgery, this diagnostic tool loses its potential to aid in the decision-making process, precisely when detailed tissue visualization is required to effectively perform the intended surgical intervention. This can be particularly crucial in the treatment of macular diseases, such as macula holes, macular pucker, and macular hemorrhages, as previously carried out studies have found [14, 19, 20, 24]. Its utility is nonetheless not restricted to macular pathology, it has also been successfully employed for retinal detachment treatments [39], gene- and cell-based therapy of retinal degenerative diseases, as well as in novel experimental procedures with retinal prosthetics [38]. OCT carried out during vitreoretinal surgery has also proved to be a valuable tool for visualizing tissue-instrument interactions and surgical dynamics, especially analyzing subretinal fluid [19].

Nevertheless, very few studies have addressed the limitations regarding OCT and its surgical application in ophthalmology [56]. The lack of evidence on this subject, in my opinion, is the result of a number of issues with iOCT usage and dissemination that eventually prevent its broad use and, consequently, the possibility for research development. Firstly, the limited availability of fully integrated iOCT surgical ophthalmological microscopes is by itself the main limiting issue. This indirectly produces a vicious cycle of

insufficient training accessibility, mainly linked to the previously mentioned capped diffusion of this technology, signaling that iOCT is mostly a novel tool in the centers where it is applied [17]. Due to these contextual problems, even when technical issues arise during the application of iOCT, such as image shadowing, surgeons are discouraged from utilizing this tool and nevertheless continue with the procedure in order to provide an uncompromised surgical treatment. This point has been brought up by different research projects, reflecting the need to improve the technology's vantage points to render it more user-friendly and truly maximize its aiding potential during surgical treatments [10, 14].

Currently, very few studies have addressed the impact of signal loss experienced during interventional OCT due to materials involved in the procedure [18, 52]. Some examples from the field of cardiology, during stent and coil placement procedures, illustrate that OCT plays a crucial role in guiding the precise deployment of the implant [45]. Stents are small mesh-like devices that are used to prop open narrowed or blocked arteries, restoring blood flow. By using OCT, an accurate stent placement can be ensured by assessing factors such as the stent's expansion against the vessel wall, its positioning, and any potential dissections or complications that might arise from the procedure. This real-time visual feedback helps optimize the outcome of the stent placement and reduces the risk of complications. These studies have addressed a similar issue of signal shadowing during interventional OCT procedures. Instruments used during coronary angiographies like the guide wire produce a significant shadowing effect, limiting the visibility of the vessel wall and potential complications during stent placement. One of their proposed solutions has been the use of semi-transparent, OCT-compatible instruments or image-processing techniques to compensate for the shadowing effect caused by surgical instruments such as coils [45].

Similarly, these findings in the field of vitreoretinal surgery identified the same general base problem when dealing with shadow artifacts on OCT: dense materials cast dense shadows that block the view of the underlying tissue [18]. This simplified paradigm proves true for most analyzed substances, especially with solid instrumentation that is classically made from metal alloys. Yet, most interestingly, in vitreoretinal surgery, gases and fluids are very frequently used on par with solid instrumentation, some of which are transparent or possess varying degrees of mass concentration and are used at different ranges of volume. All these points imply a wide range of physical factors that play a role in the light beam diffusion that allows the surgical view. Additionally, a wide range of translucid materials such as glass and plastic components are also employed, which also prove to behave differently under iOCT image scrutiny [18, 20].

In order to properly grasp the often subjective and multiple variables that play a role in the interpretation of the shadowing effect observed during iOCT-assisted vitreoretinal surgery, strategies were developed throughout the analytical process of this investigation, aiming toward objectifying the observed signal shadowing block during iOCT in a standardized fashion. To our knowledge, such standardized scaled image analysis has not been previously systematically attempted, nor has a comprehensive review been executed, examining the impact that the shadowing effect during iOCT has on the technique's usefulness and overall application in vitreoretinal surgery. As a secondary endpoint, the study strived to identify potential solutions or strategies that can minimize or compensate for this effect in the future.

The research project encompassed a substantial cohort, comprising one hundred and seventeen eyes that underwent vitrectomy procedures, all meticulously recorded through iOCT. Throughout these surgical interventions, a diverse range of vitreous substitutes, intraocular instrumentation, and intraocular dyes were systematically employed in real surgical scenarios, during patient treatment.

The subsequent data analysis phase involved post-processing techniques utilizing graphic software. One of the key parameters investigated as previously mentioned was the average gray level of the recorded signals, determined through a comprehensive pixel-histogram analysis. This approach permitted for a standardized evaluation of the iOCT recordings, allowing the identification of variable signal intensities across different circumstances and conditions within the surgical field. Each image cohort was compared to paired image controls to provide a standardized evaluation. This meticulous analysis aimed to provide a comparative perspective and reveal the specific image distortion behavior of vitreoretinal instruments, intraocular dyes, and vitreous substitutes to the observed signal shadowing phenomenon.

Importantly, the research setting was a tertiary-level university hospital with a significant caseload, emphasizing the practical relevance and applicability of the study's outcomes and ensuring a diverse and representative sample, thereby enhancing the generalizability of the study's findings to broader clinical contexts.

It is noteworthy that, to the best of our knowledge, this investigation represents the first systematic attempt to quantify and statistically examine the intricacies of signal shadowing during intraoperative optical coherence tomography in the context of vitreoretinal surgery. The significance of this study lies in its potential to contribute valuable

insights that can inform and optimize surgical practices, ultimately improving patient outcomes in the rapidly evolving field of ophthalmic surgery.

4.2 Result interpretations

The comprehensive statistical comparative analysis of the study images and their corresponding controls yielded insights into the intricate dynamics of signal shadowing during iOCT in vitreoretinal surgery. The findings of this investigation can be distilled into the following generalizations:

1) *Insignificant shadowing by clear materials*
   The examination revealed that clear materials induced an insignificant or, at most, a very low-grade shadowing effect. This observation underscores that the impact on iOCT image quality of such materials through signal shadowing is minimal.
2) *Intrinsic correlation between material density and shadowing effect*
   A discernible correlation emerged between material density and the shadowing effect produced. Specifically, less concentrated materials were associated with a lower level of shadowing, while highly concentrated materials exhibited a more pronounced shadowing effect. This correlation suggests that the density of materials plays a pivotal role in influencing the intensity of signal shadows observed during iOCT scans.
3) *Small instruments produce shadowing of limited clinical relevance*
   Irrespective of their density, small instruments were found to have less impact on the overall gray level reduction, objectively maintaining better image quality. Also, these instruments induced mostly clinically irrelevant perceived shadowing. This phenomenon was attributed to the correspondingly small size of the shadows cast by these items over the analyzed areas.

However, it is important to note that this generalization may not universally apply, as several cases demonstrated complex interference behavior. Such cases revealed distinct variables that contributed to the shadowing effect observed on iOCT, indicating the need for a case-specific approach to further the understanding of the diverse variables that can influence signal shadowing in specific surgical contexts.

4.3 Critical analysis of specific signal shadowing behavior

4.3.1 Small shadow areas

As previously noted, a general observation was that larger items equipped with dense metallic hulls consistently generated substantial interference, both perceptively and objectively. This phenomenon was particularly evident in most of the studied cannulas and forceps. Conversely, certain instruments, such as looped cannulas, displayed visually thin, low-grade shadowing produced by their tips. This visual characteristic allowed for the differentiation of underlying tissue on iOCT. On the initial 100 x 100 pixel histogram analysis, these instruments exhibited a mild gray level reduction. The further analyze the intensity of the shadow effect in these smaller size shadowed areas, in these cases, a 50 x 50 pixel field, half the size of the standard area used in the general analysis, was additionally evaluated. This revealed a significant reduction in gray levels compared to paired controls. This reduction was then similar to the levels observed by instruments that cast large shadows, albeit the overall frame image remained visible.

During these cases, the small dimension of the shadowed areas was considered clinically irrelevant, emphasizing that the outline of the underlying tissue remained easily identifiable. This interpretation aligns with the approach taken by several other studies that have developed similar analytical frameworks or adopted such benchmarks in their study designs. However, the recognition of this apparent discrepancy raises a critical concern, necessitating the establishment of objective criteria to define what constitutes clinically relevant interference. Such criteria would not only ensure the reproducibility of analyses but also enhance the compatibility of future references to the data.

The need for standardized criteria in determining clinically relevant interference has been indirectly acknowledged by previous studies, as highlighted in the literature [20, 36]. Addressing this imperative would contribute to the establishment of a unitary interpretation, fostering analysis reproducibility across studies and facilitating a more cohesive understanding of interference phenomena in iOCT. This underscores the importance of ongoing efforts within the research community to establish robust benchmarks and criteria, ultimately advancing the standardization and comparability of data interpretation in the rapidly evolving field of intraoperative optical coherence tomography.

4.3.2 Hyperreflective artifacts

In certain scenarios, the use of intraocular instrumentation and stains in vitreoretinal surgery has led to the emergence of hyperreflective artifacts iOCT recordings. These artifacts exhibited a dual impact, causing a notable spike in the measured gray level during histographic analysis and concurrently obscuring the visualization of underlying tissue structures. This variable can hold significant clinical implications for the perceived interference as compared to the measured interference. Therefore, special attention went into analyzing the behavior between instrumentation characteristics and interference patterns recorded on iOCT. In response to this challenge, a shadow-specific analysis was performed, similar to the small shadow area cases, to ensure a more accurate representation of the interference caused by these artifacts. Similar findings have been published by previous studies, but a thorough measurement and analysis of its impact was, to our knowledge, yet to be made [10, 17].

As shown in table 3, the results of the shadow-specific analysis mirrored the patterns observed in previous instances. Size-specific pixel analysis consistently revealed a significant reduction in gray level. However, the clinical relevance of this reduction was dependent upon the size of the recorded artifact generated by the utilized material. Specifically, the reduction in gray level was deemed clinically relevant only when the size of the artifact was sufficiently large to obscure the visualization of the underlying tissue. This criterion for clinical relevance highlights the importance of not only considering the magnitude of gray level reduction but also evaluating its impact on the practical interpretation of iOCT images in a surgical context.

It is noteworthy that similar challenges and considerations have been observed in earlier studies, emphasizing the recurrent nature of these issues within the field [29]. This further highlights the need for a standardized approach to addressing artifacts and their potential interference with iOCT data. The incorporation of shadow-specific analyses, as described in this study, represents a step forward in refining methodologies to ensure a more accurate and clinically meaningful interpretation of iOCT recordings in the presence of hyperreflective artifacts. Continued efforts to address and standardize these challenges will contribute to the ongoing evolution and optimization of intraoperative imaging techniques in vitreoretinal surgery.

4.3.3 Complex shadowing effects of intraocular dyes

The examination of various dyes using iOCT revealed a spectrum of interference behaviors. Notably, indocyanine green and triamcinolone acetonide demonstrated high to medium shadowing effects on the underlying tissue when compared to controls. Of particular interest was the dose-dependent interference effect exhibited by triamcinolone, where higher concentrations of the substance correlated with a more pronounced interference. In these instances, a gradual reduction in gray level was observed, aligning with the clinically perceived interference. This phenomenon had previously been described in a pioneer study [18], though no objective measurement of the shadowing effect was carried out. Intriguingly, even with minimal substance concentrations, a statistically significant (-2.61%; p-value: <.001) GL reduction was noted when adjusting for shadow size and hyperreflectivity artifacts. This highlights the sensitivity of iOCT in detecting subtle variations in interference, even at lower concentrations of interfering substances.

On the other hand, VITREODYNE™ (Kemin Pharma, Barcarena, Portugal), a lutein-based dye, presented a distinctive profile, displaying significantly higher gray levels than controls. Notably, this elevation in gray level was not accompanied by significant shadowing, either in measured or perceived terms. This unique characteristic is attributed to the relatively high infrared light transparency of VITREODYNE™, as detected by the iOCT camera [40, 50]. The absence of perceptible interference despite the higher gray level suggests that interference caused by the VITREODYNE™ dye, if any, might manifest in a manner not easily discernible through visual inspection alone. This observation underlines the importance of considering not only the gray level values but also the specific characteristics of interference when evaluating the impact of dyes on iOCT recordings.

Brilliant blue, possessing physical properties similar to VITREODYNE™ [50], presented an interesting shadow behavior. It exhibited no visually perceptible interference and only marginally higher gray levels than controls, indicating no significant distortion of the iOCT images. This makes it an ideal material for iOCT-assisted vitreoretinal surgeries. This finding highlights the specificity of the interference behavior associated with different dyes, even when sharing common physical attributes. Further investigation into the physical properties of brilliant blue should be taken into consideration when developing new iOCT-friendly materials for ophthalmologic surgery.

These results appropriately capture the diversity in interference behaviors among dyes used in vitreoretinal surgery, emphasizing the need for a critical and substance-specific analysis. The study's detailed exploration of the impact of substance concentration on

interference, along with the consideration of infrared light transparency, contributes to a more comprehensive understanding of how dyes influence iOCT imaging. These findings pave the way for refined methodologies in evaluating and selecting dyes for optimal intraoperative imaging outcomes in vitreoretinal surgery.

4.3.4 Vitreous substitutes and transparency

The study of vitreous substitutes unveiled notable patterns in their interference effects on iOCT. Specifically, both air and PFCL demonstrated an irrelevant shadowing effect on iOCT recordings, with similar gray levels when compared with controls. This outcome implies that these vitreous substitutes exert a minimal impact on iOCT imaging, preserving clarity and facilitating effective visualization during vitreoretinal surgeries.

Interestingly, gases exhibited a tendency to induce a reduction in gray level, while highly viscous liquids such as silicon oil yielded a relatively higher gray level [7]. This observation aligns with the perceived distortion, which, in every instance, was minimal and statistically not significant. The correspondence between the observed reduction in gray level and the perceived distortion exhibits the sensitivity of iOCT in detecting subtle variations in the interference caused by different vitreous substitutes.

These findings collectively suggest that the choice of vitreous substitutes in vitreoretinal surgeries can be made with confidence in maintaining optimal iOCT imaging conditions. The minimal interference observed with all substitutes analyzed supports their utility in maintaining clear visualization on iOCT scans during surgical procedures. The understanding of the interference patterns associated with gases and liquids contributes valuable insights to the optimization of iOCT imaging in the context of vitreous substitutes. As such, this study aids in refining the selection and application of vitreous substitutes, ultimately enhancing the precision and clarity of iOCT-guided vitreoretinal surgeries.

4.3.5 Influence of physical properties and iOCT

The study's findings support the overarching conclusion that, beyond considerations of size and transparency, the principal determinants influencing the shadow-generating effect on iOCT of an instrument are the physical state of the interfering item and the material density or concentration. This insight highlights the multifactorial nature of the interference phenomena, emphasizing the need for a comprehensive understanding of the physical properties that play a role in the interaction between instruments and iOCT imaging scans.

Additionally, the study suggests that other physical properties, including but not limited to polarization, fluid-lipid affinity, and refractive index, could serve as critical variables in discerning materials that are optimally suited for use in clinical settings during iOCT-assisted vitrectomies. Taking these properties into consideration could provide a more refined understanding of how different materials interact with the iOCT system, facilitating informed choices in the selection of instruments for vitreoretinal procedures.

It is crucial to highlight that the characteristics of the camera system also play a central role in potential image distortions arising from various signal interrupters. Recognizing this interaction is essential for optimizing the integration of iOCT into vitreoretinal surgical workflows and ensuring accurate and reliable imaging outcomes.

In light of these considerations, the study advocates for the ideal scenario where materials employed intraocularly during vitreoretinal procedures should produce minimal to no shadow effect. In cases where some level of interference is unavoidable, preference should be given to thin instrumentation, when the procedure being performed allows for such accommodation, which visually generates only partial interference. This strategic approach aligns with the study's findings, emphasizing the practical benefits of employing thin-gauged instruments in iOCT-assisted vitreoretinal surgery. By extrapolating these insights, clinicians can make informed decisions regarding instrument selection, contributing to enhanced imaging precision and minimized interference during the dynamic context of vitreoretinal surgeries guided by iOCT.

4.4 Study limitations

A limitation of this study is the fact that all surgeries analyzed, and all recordings scanned were exclusively performed with only one type of ophthalmologic surgical microscope Zeiss RESCAN^{TM} 700 (Carl Zeiss Meditec AG, Oberkochen, Germany) paired with the house-brand ZEISS CALLISTO eye® iOCT software. This approach introduces the possibility of subtle variations that might arise when comparing these samples to those obtained using microscopes equipped with different iOCT software versions. However, despite this limitation, this study delineated discernible and statistically significant signal shadowing relations between the control and study groups. This observation sustains a wide applicability of the validity of these findings and their generalizability within the scope of the instrumentation utilized.

Another limitation of the study is associated with the specific instruments, intraocular dyes, and vitreous substitutes sourced exclusively from certain companies. The exclusive

use of products from particular manufacturers could imply that similar items from alternative companies might yield different results in the signal shadowing analysis. Variations in manufacturing processes, material compositions, or design intricacies among different product lines could introduce subtle yet real discrepancies in the interference patterns observed. As a result, even though standardized criteria of material characteristics and quality are widespread among mainstream ophthalmologic material suppliers, the generalizability of these findings to procedures employing alternative products calls for consideration.

Despite these limitations, the study contributes valuable insights into the dynamics of signal shadowing during intraoperative optical coherence tomography-assisted vitreoretinal surgery. Acknowledging the constraints of the chosen equipment and materials, future research projects could explore broader instrument and equipment variability to refine our understanding of the relationship between different surgical tools and iOCT imaging outcomes. This, in turn, could contribute to the establishment of more comprehensive guidelines for optimizing iOCT-assisted vitreoretinal surgeries across diverse clinical settings and equipment configurations.

4.5 Development of iOCT-compatible vitreoretinal instruments

The identification of substantial interference caused by conventional instruments and materials during ocular surgeries has incited efforts toward the development of novel instruments optimized for iOCT. One strategy involves the exploration of iOCT- compatible materials, with a predominant focus on non-metallic substances such as silicone and polycarbonate. These materials hold promise for minimizing signal shadowing during iOCT-assisted vitreoretinal surgery, particularly in procedures targeting the vitreoretinal interface. The incorporation of such materials into the design of surgical instruments aims to enhance their compatibility with iOCT scans, facilitating clearer and more precise imaging. Nevertheless, many of the published models exhibit a critical drawback: their reduced rigidity [20, 21]. This compromise undermines the efficacy and versatility of these innovative instruments in the context of vitreoretinal surgery, limiting their real-life applicability.

Beyond material considerations, there is a concurrent emphasis on adapting instrument design to enhance light transparency specifically for iOCT scans. The proposed modifications in design seek to optimize the visualization of intraocular structures, acknowledging the unique imaging requirements posed by iOCT. This strategic adjustment

in design principles reflects a proactive approach to mitigate signal shadowing challenges and the interdisciplinary collaboration between surgeons and engineers in the pursuit of innovative solutions [20]. However, the inherent complexity of this task, especially in the context of delicate surgical maneuvers where precise visualization is paramount, still requires extensive development and scrutiny. Further development dedicated to refining and testing these techniques must be performed to ensure their accuracy and reliability in the setting of interventional procedures.

As a consequence of this research project, the Eye Clinic, University Hospital Giessen and Marburg GmbH, Campus Giessen, Germany is actively engaged in formulating several innovative configurations and designs aimed at facilitating superior visual scanning during iOCT. The preliminary findings of this ongoing project have been exhibited here. This will be subsequently analyzed and published in the future, with the ultimate goal of enhancing surgical precision and patient outcomes. These results indicate that the choice of material and design significantly influences the extent of shadowing effects, with clear materials and smaller instrument tips exhibiting superior iOCT performance.

The newly designed instruments showcased a more favorable gray level profile in comparison to standard instrumentation. This improvement was attributed to the innovative design, allowing the iOCT signal to pass through the double-slitted distal tubing, enabling clearer imaging of the area of interest. Importantly, the shadow effect produced by these newly designed instruments was clinically irrelevant, suggesting a potential breakthrough in mitigating the interference associated with traditional instruments.

These findings not only highlight the limitations of standard vitreoretinal instruments but also emphasize the potential of innovative designs in minimizing shadowing effects during iOCT-assisted vitreoretinal surgery. The study's outcomes support the notion that optimizing materials and configurations employed on vitreoretinal instruments can substantially enhance the quality of iOCT imaging, thereby improving the precision and safety of vitreoretinal surgical procedures. The next crucial step involves subjecting these newly designed prototypes to rigorous testing under actual clinical conditions to further their potential and perhaps discover new variables that could influence iOCT compatibility. Clinical trials and real-world applications will help validate the performance, safety, and practicality of novel instruments. This phase of evaluation will provide invaluable data on how these instruments perform in diverse surgical scenarios, further informing their potential integration into routine vitreoretinal procedures.

In conclusion, the systematic quantification of iOCT image quality, coupled with the development of new vitreoretinal prototypes, presents a promising way to advance the field of vitreoretinal surgery. The observed reduction in shadowing effects suggests an improvement in visualizing the area of interest, potentially revolutionizing surgical practices and contributing to the overall safety and efficacy of iOCT-assisted vitreoretinal procedures.

5. Conclusion

In recent years, intraoperative optical coherence tomography has emerged as a powerful tool in ophthalmic surgery, providing real-time, high-resolution imaging of ocular structures during surgical interventions. iOCT allows surgeons to visualize the structures of the anterior eye segment as well as the retina, the vitreous cavity, and other critical anatomical features, enabling precise decision-making and enhancing surgical outcomes. However, despite its numerous advantages, iOCT imaging can be affected by signal shadowing caused by intraocular instruments, stains, and vitreous substitutes. Understanding the impact of these factors on iOCT imaging is crucial for optimizing the use of this technology and improving surgical precision.

To better comprehend this problem, this study was devised in order to examine the impact of signal shadowing during iOCT-assisted vitreoretinal surgeries caused by intraocular instruments, stains, and vitreous substitutes. By quantitatively assessing the influence of these factors on iOCT imaging, the study aimed to provide valuable insights into the significance of different forms of image interference and shadowing effects. To achieve the objective, a comprehensive investigation was conducted using a combination of real-time and postproduction image analysis techniques and clinical observations to study different intraocular instruments, commonly used tissue stains, and vitreous substitutes to evaluate their impact on iOCT imaging.

The findings of this study shed light on the importance of considering signal shadowing in iOCT-assisted vitreoretinal surgeries. The findings from this study illuminate critical aspects of signal shadowing in the context of intraoperative optical coherence tomography-assisted vitreoretinal surgeries. The results revealed varying degrees of interference caused by different intraocular instruments and stains, while also highlighting the negligible impact of vitreous substitutes on iOCT imaging. The comprehensive investigation into the impact of signal shadowing stemming from intraocular instruments, dyes, and vitreous substitutes contributes valuable insights that extend beyond the confines of this specific study, informing future research directions and potentially revolutionizing the landscape of vitreoretinal surgery.

Firstly, the study identified that certain intraocular instruments, such as silicone-tipped cannulas, looped cannulas, and retinal sweepers, produced noticeable shadowing artifacts, leading to reduced visibility of the underlying tissues. The magnitude of shadowing varied depending on the design and material composition of the instruments. These findings

highlight the importance of instrument selection for iOCT-guided surgeries, as certain materials may hinder the surgeon's ability to obtain a clear view and accurate iOCT images.

Secondly, the research explored how commonly used stains in vitreoretinal surgeries affect iOCT imaging. The presence of stains was found to create complex shadowing effects, impacting iOCT image quality in different ways. A significant observation is the dose-dependent interference effect observed with triamcinolone acetonide, highlighting the importance of carefully managing its concentration during surgical procedures. This emphasizes the potential for improving the use of substances that can cause interference, with surgeons adjusting the moment where certain materials are used to minimize negative effects on iOCT imaging.

Moreover, the study's finding that the interference caused by small items is clinically irrelevant prompts a reassessment of the perceived impact of small instruments in surgery. Surgeons can now approach these smaller items with an understanding that their interference is minimal, potentially influencing their decisions in procedures where space and maneuverability are critical while using iOCT technology. This consideration becomes crucial for surgeons when selecting stains for iOCT-assisted procedures, as it can affect the image quality and, consequently, impact the decisions made during surgery.

Interestingly, the study revealed that vitreous substitutes had a negligible impact on iOCT imaging. This finding suggests that the choice of vitreous substitutes, such as gas or silicone oil, does not significantly contribute to signal shadowing. However, it is important to note that other factors related to the use of vitreous substitutes, such as optical clarity and refractive index matching, may still influence the overall quality of iOCT images.

The implications of these findings are substantial for surgeons utilizing iOCT technology in vitreoretinal surgery. The ability to accurately assess the impact of different intraocular instruments and stains on iOCT imaging enables surgeons to make informed decisions during the surgical procedure. By selecting instruments with minimal shadowing effects and carefully considering the use of stains, surgeons can optimize the clarity and quality of iOCT images, facilitating precise surgical maneuvers and improving patient outcomes.

This study has provided valuable insights into the impact of signal shadowing during iOCT-assisted vitreoretinal surgeries. The findings emphasize the importance of considering various factors contributing to image interference and shadowing effects, particularly intraocular instruments, and stains. By quantitatively assessing the influence of these factors, surgeons can make informed decisions regarding instrument selection and stain usage,

ultimately enhancing the accuracy and efficacy of iOCT-guided procedures in vitreoretinal surgery.

While this study significantly advances our understanding of signal shadowing in iOCT-assisted vitreoretinal surgeries, there are several avenues for future research as exhibited by the experimental branch of the study. A systematic quantification of iOCT image quality showed that all vitreoretinal instruments caused significant shadowing of the area of interest, while the shadowing effect caused by newly designed vitreoretinal instruments was clinically irrelevant. The use of new iOCT-compatible vitreoretinal instruments may enhance the visualization of tissue behavior during sensitive tissue-manipulating maneuvers, hence improving its safety and efficacy. Further investigations could focus on developing strategies to minimize signal shadowing and optimize iOCT imaging. Novel instrument designs, coatings, or modifications may be explored to reduce shadowing artifacts, thereby enhancing the clarity of iOCT images. Additionally, the research could pave the way to the development of stain formulations with reduced shadowing effects, enabling improved visualization without compromising surgical safety.

By addressing these problems and embarking further into research pathways, future advancements in iOCT technology can maximize its potential for improved surgical outcomes, ultimately benefiting patients undergoing ophthalmologic surgeries. With a comprehensive understanding of signal shadowing and its implications, surgeons can harness the full capabilities of iOCT, enhancing precision, safety, and success rates in the field of vitreoretinal surgery.

6. Abstract

6.1 English

Purpose: To analyze the clinical significance of signal shadowing during intraoperative optical coherence tomography (iOCT)-assisted vitreoretinal surgery caused by intraocular instruments, intraocular dyes, and vitreous substitutes, and to objectively quantify its impact on iOCT imaging in order to produce strategies to mitigate its negative effects.

Methods: This is a retrospective observational study of postoperative image analysis from one hundred seventeen (117) patients who underwent iOCT-assisted vitrectomy. The image data were assigned to three groups: intraocular instruments, intraocular dyes, and vitreous substitutes. The data was then processed using graphic software to measure the grade of picture quality distortion and compared to paired image controls without clinically perceptive interference; consequently, analyzed statistically. Newly designed vitreoretinal instrument prototypes aimed at reducing baseline interference were also similarly tested.

Results: The intraocular portion of all studied vitreoretinal instruments caused a high average gray level interference compared to controls ranging from 32% to 68% reduction, obscuring the area of interest significantly. The tips of the instruments produced low-grade shadowing, allowing the underlying tissue to be distinguished. The analyzed dyes demonstrated a wide interference range: ICG (-75.12%), and triamcinolone (-26.13%) showed dose-dependent high shadowing, while VITREODYNE™ (49.3%) and brilliant blue (15.0%) exhibited no perceived distortions whilst increasing average gray levels. All analyzed vitreous substitutes (air, SF_6, C_3F_8, PFCL, and silicone oil) showed an insignificant shadowing effect on iOCT. The novel instrument prototypes showed an average gray level reduction of only 9.01%.

Conclusions: Certain dyes and vitreous substitutes produce a negligible shadowing effect compared to controls and other dyes, giving an advantage during real-time iOCT imaging. All analyzed intraocular instruments showed a significant interference that should prompt the development of new imaging techniques or the implementation of materials with low-grade interference to overcome a clinically relevant shadowing effect on iOCT, maximizing the technology's visual accuracy and in vivo surgical diagnostic aid proficiency.

6.2 Deutsch

Zweck: Analyse der klinischen Bedeutung von Signalabschattungen während der intraoperativen optischen Kohärenztomographie (iOCT)-gestützten vitreoretinalen Chirurgie, verursacht durch intraokulare Instrumente, Farbstoffe und Glaskörperersatzstoffe, sowie die objektive Quantifizierung ihres Einflusses auf die iOCT-Bildgebung, um Strategien zur Abschwächung der negativen Auswirkungen zu entwickeln.

Methoden: Eine retrospektive Beobachtungsstudie zur postoperativen Bildanalyse von einhundertsiebzehn (117) Patienten, die eine iOCT-gestützte Vitrektomie durchgemacht haben. Die Bilddaten wurden in drei Gruppen unterteilt: intraokulare Instrumente, Gewebefarbstoffe und Glaskörperersatzstoffe. Die Daten wurden mit einer Grafiksoftware verarbeitet und mit gepaarten Bildkontrollen ohne klinisch wahrnehmbare Störungen vergleichen. Neu entwickelte Prototypen von vitreoretinalen Instrumenten, die darauf abzielen, die Grundinterferenz zu reduzieren, wurden ebenfalls ähnlich getestet.

Ergebnisse: Der intraokulare Teil aller untersuchten vitreoretinalen Instrumente verursachte eine hohe durchschnittliche Grauwertstörung-Reduktion von 32% bis 68%, wodurch der interessierende Bereich signifikant verdeckt wurde. Die Instrumentenspitzen erzeugten eine geringgradige Abschattung, die es ermöglichte, das darunterliegende Gewebe zu unterscheiden. Die analysierten Farbstoffe zeigten eine breite Störfaktorenrange: ICG (-75,12%) und Triamcinolon (-26,13%) zeigten eine dosisabhängige starke Schattierung, während VITREODYNE™ (49,3%) und Brilliant Blue (15,0%) keine wahrnehmbaren Verzerrungen zeigten und gleichzeitig die durchschnittlichen Grauwerte erhöhten. Alle analysierten Glaskörperersatzstoffe (Luft, SF_6, C_3F_8, PFCL und Silikonöl) zeigten einen irrelevanten Schatteneffekt auf iOCT. Die neuartigen Instrumentenprototypen zeigten eine durchschnittliche Reduzierung des Grauwertniveaus von nur 9,01%.

Schlussfolgerungen: Bestimmte Farbstoffe und Glaskörperersatz erzeugen im Vergleich zu Kontrollen und anderen Farbstoffen einen vernachlässigbaren Schatteneffekt, was während der echtzeitigen iOCT-Bildgebung einen Vorteil bringt. Alle analysierten intraokularen Instrumente zeigten eine signifikante Störung, die die Entwicklung neuer Bildgebungstechniken oder die Implementierung von Materialien mit geringgradiger Störung erfordert.

7. References

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8. Related publications

8.1 Peer-reviewed published article

I. Carlos Reyna, E., Öztek, M., Petrovski, G., Binder, S., Stieger, K., & Lytvynchuk, L. (2024). Clinical significance of signal shadowing during intraoperative optical coherence tomography-assisted vitreoretinal surgery. *Scientific reports*, *14*(1), 5393. https://doi.org/10.1038/s41598-024-56125-y

8.2 Abstracts

II. Carlos Reyna, E., Öztek, M., Binder, S., Stieger, K., Lytvynchuk, L. Signalverschattungen wahrend der intraoperativen optischen Koharenztomographie-gestutzten vitreoretinalen Chirurgie. *Ophthalmologie* 2022. 119 (Suppl 3):S157–S344. https:// doi.org/ 10.1007/ s00347- 022- 01723-2

III. Öztek, M., Carlos Reyna, E., Binder, S., Stieger, K., Lytvynchuk, L. Anwendung der intraoperativen optischen Koharenztomographie (iOCT) bei modernen Katarakt-Operationen. *Ophthalmologie* 2022. 119 (Suppl 3):S157–S344. https:// doi.org/ 10.1007/ s00347- 022- 01723-2

IV. Carlos Reyna, E., Öztek, M., Binder, S., Stieger, K., Lytvynchuk, L. Abstracts from the 2022 European Association for Vision and Eye Research Festival, 13-15 October 2022, Valencia. Signal shadowing during intraoperative optical coherence tomography assisted vitreoretinal surgery: A systemic analysis. *Acta Ophthalmologica*. 20 December 2022. https://doi.org/10.1111/j.1755-3768.2022.0025

V. Öztek, M., Carlos Reyna, E., Binder, S., Stieger, K., Lytvynchuk, L. Abstracts from the 2022 European Association for Vision and Eye Research Festival, 13-15 October 2022, Valencia. The role and clinical significance of intraoperative optical coherence tomography for cataract surgery. *Acta Ophthalmologica*. 20 December 2022. https://doi.org/10.1111/j.1755-3768.2022.0451

VI. Carlos Reyna, E., Binder, S. Stieger, K., Lytvynchuk, L. Neue Instrumente fur die iOCT-gestutzte vitreoretinale Chirurgie. *Ophthalmologie* 2023. 120 (Suppl 2):S71–S253. https:// doi.org/ 10.1007/ s00347- 023- 01901-w

VII. Öztek, M., Carlos Reyna, E., Binder, S., Stieger, K., Lytvynchuk, L. Intraoperative optische Koharenztomografie (iOCT) assistierte Lens-in-Bag Implantationstechnik: Erleichterte Operationsmoglichkeit durch begleitende Bildgebung. *Ophthalmologie* 2023. 120 (Suppl 2):S71–S253. https:// doi.org/ 10.1007/ s00347- 023- 01901-w

VIII. Carlos Reyna, E., Binder, S. Stieger, K., Lytvynchuk, L. Neue Instrumente für intraoperative optische Kohärenz-tomographie-assistierte (iOCT) vitreoretinale Chirurgie. *96. Versammlung des Rhein-Mainischer Augenärzte e.V*. 2023.

IX. Carlos Reyna, E., Stieger, K., Ansari Shahrezaei, S., Binder, S., Lytvynchuk, L. Evaluation of newly designed vitreoretinal instruments aiming to prevent shadowing effects during intraoperative optical coherence tomography assisted vitreoretinal surgery. *ARVO Annual Meeting Abstract*. June 2024.

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10. Pledge of honor / Ehrenwörtliche Erklärung

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