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# Retinal layers measurements in healthy eyes and in eyes receiving silicone oil-based endotamponade

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**ABSTRACT.**

***Purpose:*** **To characterize the concordance/symmetry of each retinal layers in individuals without macular pathology and to further characterize the localization of inner retinal thinning in eyes receiving silicone oil-based endotamponade.**

***Methods:*** **Retinal layers of one hundred eyes of 50 individuals without macular pathology were imaged using spectral domain optical coherence tomography (SD-OCT) and manually segmented using ImageJ software (developed by Wayne Rasband, NIH, Bethesda, MD, USA). In the second part of the study, retrospective analysis of 3028 cases of pars plana vitrectomy in University Eye Hospital Cologne, Germany, was conducted, retrieving nine patients with silicone oil-based endotamponade with no macular condition interfering retinal layers measurements. These patients had retinal detachment not involving the macula due to various conditions. In these patients, retinal layer segmentation was performed and compared with the fellow eye.**

***Results:*** **There is a moderate-to-high concordance for all retinal layers between the right and the left eye of the same individual. In eyes receiving silicone oil-based endotamponade, the inner retinal layers become subsequently thinner. Ganglion cell and inner plexiform layers contribute most to this thinning, that is, $0.537 \pm 0.096$ mm$^3$ compared with $0.742 \pm 0.117$ mm$^3$; $p = 0.006$. Outer retinal layers were not affected by silicone oil-based endotamponade ($p = 0.439$ for the differences of calculated outer retinal layers).**

***Conclusion:*** **Ganglion cell and inner retinal layers become subsequently thinner after the use of silicone oil-based endotamponade. This study advocates the use of spectral domain optical coherence tomography for patient management with silicone oil endotamponade to early detect subsequent retinal thinning.**

**Key words:** retinal thickness – silicone oil – spectral domain optical coherence tomography

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## Introduction

In vitreoretinal surgery, silicone oil is a commonly used vitreous substitute in cases, where long-term tamponade is needed. In addition to the complications of the anterior segment such as cataract, keratopathy and glaucoma, there are cases with a visual loss of unknown reason. Christensen and la Cour reported a case series of nine patients receiving silicone oil tamponade with significant visual loss after silicone oil use (Christensen & la Cour 2012). This was correlated with significant thinning of inner retinal layer measured using spectral domain optical coherence tomography (SD-OCT), if compared with eyes receiving perfluoropropane ($C_3F_8$) endotamponade. In healthy individuals, however, there is a variability of retinal thickness (Song et al. 2010; Ooto et al. 2011; Caramoy et al. 2012). While the macular thickness in SD-OCT depends on age, gender and spherical refraction (Caramoy et al. 2012), there is a high concordance between both eyes of the same individual.

The primary aims of this study were the following: (i) to examine the concordance of retinal layer thickness measurements using SD-OCT between healthy fellow eyes and (ii) Subsequently to study changes in retinal layers in patients after silicone oil tamponade.

## Patients and Methods

### Retinal layers concordance in eyes without macular pathologies

We chose 100 eyes of 50 random subjects over 60 years of age with no macular pathologies (epiretinal gliosis, glaucoma, age-related macular degenerations, macular oedema, etc.) and with best-corrected visual acuity better than 20/25 from the European Genetic Database (EuGenDa). It is a German–

**Table 1.** Demographic characteristics of subjects without macular pathologies evaluated in this study.

| Variables | Value (mean ± standard deviation) | |
|---|---|---|
| Age (years) | 67.34 ± 5.04 (range 60–78) | |
| Gender | 21 men, 29 women | |
| Medical history* *n* (%) | | |
| Arterial hypertension | 22 (45.8) | |
| Type 2 diabetes mellitus | 7 (14) | |
| Conditions after stroke | 3 (6) | |
| Coronary heart disease, myocardial infarction, stents or bypass surgery | 2 (4) | |
| Asthma or other allergies | 18 (36) | |
| Hyper- or hypothyreosis | 7 (14) | |
| Oesophageal reflux | 1 (2) | |
| Migraine | 3 (6) | |
| Psoriasis | 1 (2) | |
| Rheumatoid arthritis | 2 (4) | |
| Hypercholesterinaemia | 1 (2) | |
| Kidney stone | 1 (2) | |
| Other malignancies | 3 (6) | |
| Other surgeries | 4 (8) | |
| | Right eye | Left eye |
| Spherical refractive error (dioptre) | 0.80 ± 2.09 (range −5.0 to +6.0) | 0.80 ± 2.30 (range −6.5 to +5.5) |
| BCVA (LogMAR) | 0.02 ± 0.05 (range −0.1 to 0.1) | 0.03 ± 0.06 (range −0.1 to 0.1) |

* Multiple diagnosis for one subject possible.

Dutch genetic database studying age-related macular degeneration. Subjects without macular pathologies were included as control patients in the database. The patients chosen for this study are part of a previous project, studying the variability of central retinal thickness (Caramoy et al. 2012). The main inclusion criteria were no macular pathologies as assessed using SD-OCT and visual acuity better than 20/25. Main exclusion criterion was insufficient SD-OCT image quality that might interfere retinal layer segmentation. Demographic characteristics of these patients are shown in Table 1. All eyes examined were phakic. Patients selected for this study were the first 50 patients recruited in the year 2009. The research adhered to the tenets of the Declaration of Helsinki.

#### Eyes with silicone oil-based endotamponade

A retrospective database search of patients receiving silicone oil-based endotamponade was conducted in University Eye Hospital of Cologne, Germany, from the year 2008 (the first use of SD-OCT in our clinic) to 2011. Main inclusion criteria were patients receiving silicone oil-based endotamponade in one eye with subsequent SD-OCT imaging on both eyes. All eyes receiving silicone oil tamponade due to various diseases were included (Table 2). These eyes had retinal detachment not involving the macula. Main exclusion criterion was other condition on the retina affecting retinal layers measurement, for example macular pucker, retinal detachment involving the macula or internal limiting membrane peeling. Patients included in this study were called for a follow-up examinations using SD-OCT protocol described below. SD-OCT measurements were performed after silicone oil removal.

#### Spectral domain optical coherence tomography and measurements using ImageJ

Spectral domain optical coherence tomography was performed using the Spectralis HRA + OCT (Heidelberg Engineering, Heidelberg, Germany). A standard protocol comprising a scan of 37 B-scans in 20°×15° field was used. Each B-scan consisted of 40 000 A-scans.

For the measurement of retinal layers, the segmentation misalignment of the Heidelberg Eye Explorer software (version 1.7.1.0, Heidelberg Engineering, Heidelberg, Germany) was corrected manually. A clinically significant macular edema (CSME) grid (Campbell et al. 2007) was placed on the macula and manually centred on the fovea. This was performed to neutralize the effect of possible eccentric fixation and/or measurements.

The 37 images from the 37 B-scans were downloaded. Using the ImageJ software, these images were configurated into stacks. The volume of each

**Table 2.** Demographic of patients receiving silicone oil-based endotamponade.

| Case no. | Sex/Age | Preop BCVA (LogMAR) | Clock hours detached | Indication for endotamponade | Endotamponade | Tamponade duration (days) | Final BCVA (LogMAR) |
|---|---|---|---|---|---|---|---|
| 1 | F/55 | 0.7 | 4 | Recurrent retinal detachment | 5000 cSt SiO | 93 | 0.6 |
| 2 | M/42 | 2.0 | 2 | IOFB | 5000 cSt SiO | 211 | 1.3 |
| 3 | M/57 | 0 | 5 | Giant retinal tears | 5000 cSt SiO | 311 | 1.0 |
| 4 | F/19 | 0 | 3 | IOFB | 5000 cSt SiO | 127 | 0.5 |
| 5 | M/60 | 0.2 | 6 | PVR | HSO | 65 | 0.4 |
| 6 | M/54 | 0.4 | 6 | Giant retinal tears | 2000 cSt SiO | 74 | 0.2 |
| 7 | F/54 | 0.4 | 4 | Giant retinal tears | 2000 cSt SiO | 99 | 0.5 |
| 8 | F/40 | 0 | 6 | PVR | 2000 cSt SiO | 125 | 0.4 |
| 9 | M/62 | 1.5 | 2 | Multiple tears and vitreous haemorrhage | 2000 cSt SiO | 106 | 0.7 |

F = female, M = male, BCVA = best-corrected visual acuity, LogMAR = logarithm of the minimum angle of resolution, cSt = centistokes, SiO = silicone oil, HSO = heavy silicone oil, IOFB = intraocular foreign body, PVR = proliferative vitreoretinopathy.

retinal layers was calculated using the formula:

$$\text{Volume} \sum_{i=m}^{n} (p_i \cdot x \cdot z \cdot b)$$

where $m$ is the first image and $n$ is the last image showing the marking line in the image stack. The pixel area is represented by p. $x$ and $z$ represent the scaling of the SD-OCT images in the $x$- and $z$-axis. b represents the distance between B-scans. The values of $x$, $z$ and $b$ can be seen in the 'additional information' button in the Heidelberg Eye Explorer software on the upper left-hand side for each SD-OCT image.

The retinal layers measured in this study are the following: RNFL = retinal nerve fibre layer, GCLIPL = ganglion cell layer and inner plexiform layer, INL = inner nuclear layer, OPLONLPIS = outer plexiform layer, Henle's fibre layer, outer nuclear layer and inner part of the photoreceptor layer, POSRPEBM = posterior part of the photoreceptor layer, retinal pigment epithelium and Bruch's membrane, MACVOL = macular volume measured as total of the above mentioned retinal layers (Fig. 1).

These layers were chosen, because of their visibility. For example, using the current technology, it is not always possible to differentiate the ganglion cell layer (GCL) and the inner plexiform layer (IPL). Another example is to sum the outer plexiform layer (OPL), the Henle's fibre layer (HFL), the outer nuclear layer (ONL) and the inner part of the photoreceptor layer (PIS) into one single layer OPLONL-PIS, because the thickness of HFL depends on the directionality of the SD-OCT (Lujan et al. 2011).

To test the concurrence validity, the measured MACVOL using this ImageJ algorithm was compared with the measured MACVOL using the Heidelberg Eye Explorer for all patients. Measurement repeatability was tested using 20 eyes of 10 patients. For this purpose, all retinal layers from RNFL to POSR-PEBM were measured twice and compared.

Statistical analysis was performed using SPSS (IBM, version 21.0, Armonk, NY, USA) and MedCalc (Mariakerke, Belgium). Demographic characteristics of the population are described with summary statistics, including frequency and percentage for categorical data. Continuous data are presented with mean ± standard deviation. Testing for normality was carried out either using Shapiro–Wilk test for sample size <50 or using Kolmogorov–Smirnov test for sample size ≥50. Student's $t$-test for dependent variables is used, when comparing the right eye and the left eyes. p-value < 0.05 was deemed as statistically significant. All correlations between two values are presented as Pearson product-moment correlation coefficient ($r$) and concordance correlation coefficient according to Lin ($r_c$) (Lin 1989).

## Results

For the part of the study investigating the effect of silicone oil-based endotamponade, a total of 3028 pars plana vitrectomy from the year 2008 to 2011 were evaluated. Only eyes receiving silicone oil-based endotamponade with subsequent imaging using SD-OCT were included. One hundred 57 eyes were identified. Eyes having other pathologies that might interfere retinal layer measurements (e.g. macular pucker, retinal detachment involving the macula, internal limiting membrane

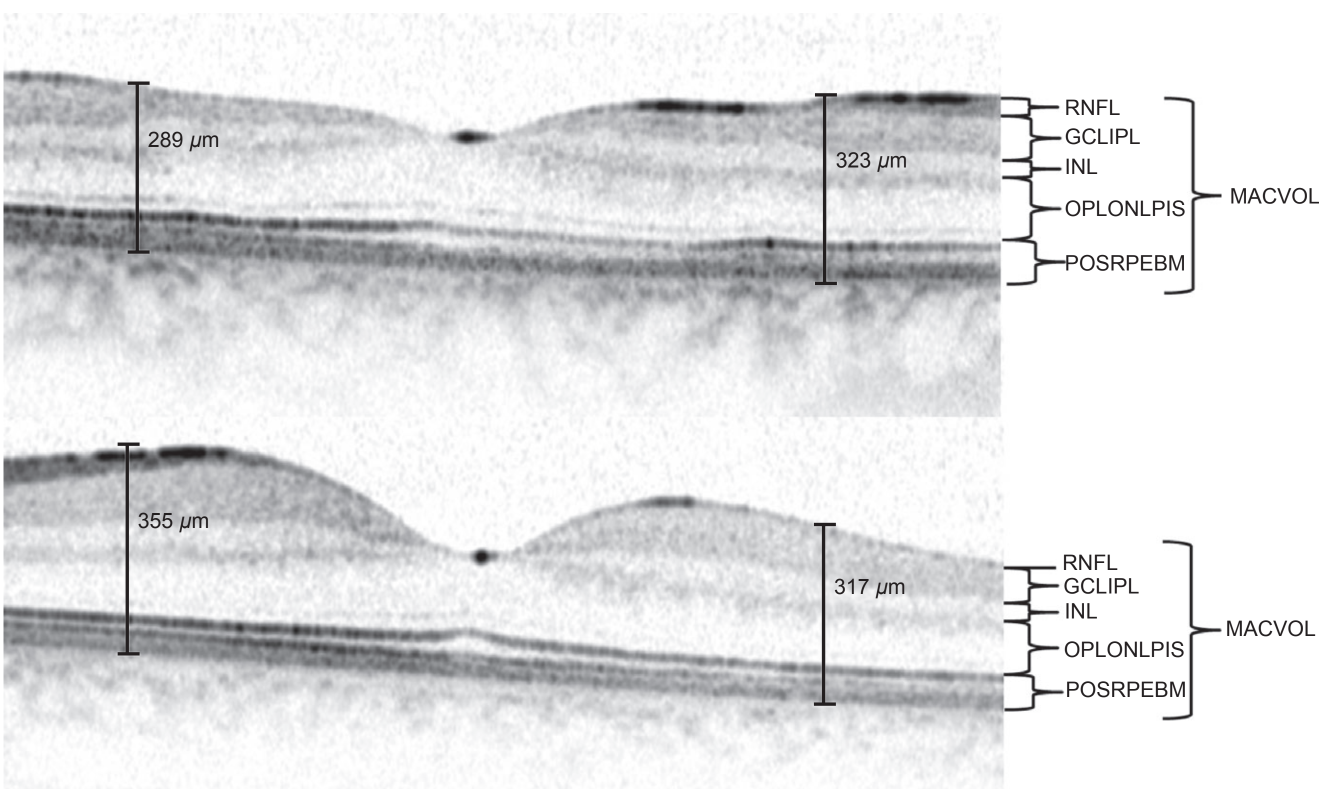


**Fig. 1.** Morphology of the macula (case no. 4) of the silicone oil-filled eye (upper) shows thinning of inner retinal layers in comparison with the fellow eye (bottom). Cf. the methods section for the abbreviation used.

peeling) were excluded. Nine individuals receiving silicone oil-based endotamponade with healthy fellow eyes could be identified (Table 2). Best-corrected visual acuity in these eyes was 0.58 ± 0.72 LogMAR before receiving silicone oil tamponade and was 0.62 ± 0.34 LogMAR after silicone oil removal.

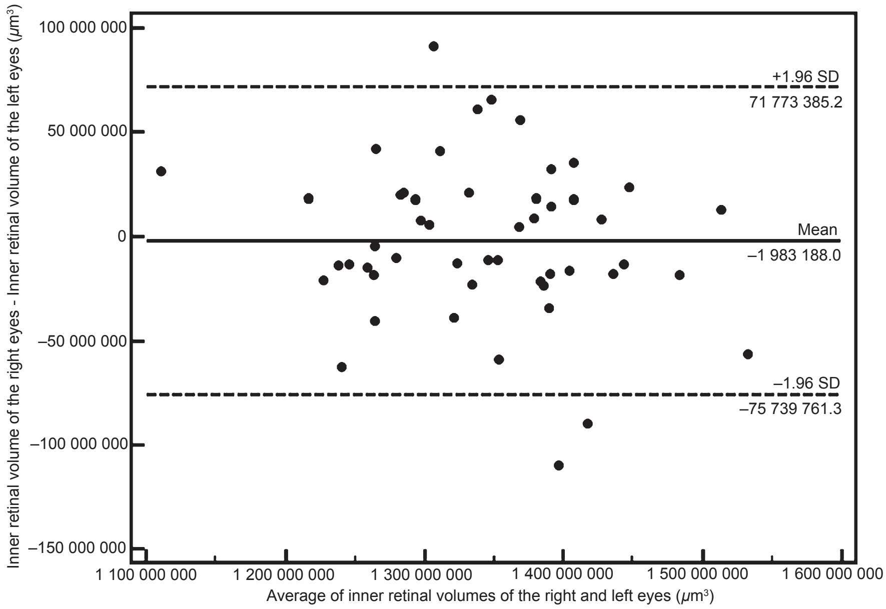


**Fig. 2.** Bland-Altmann plot of inner retinal volume of the right and left eyes of patients without macular pathology.

#### Concurrence validity of the retinal segmentation algorithm

The macular volume measured using ImageJ in the ophthalmological healthy individuals is highly correlated with the macular volume measured using Heidelberg Eye Explorer (for the right eye $r = 0.961$, $p < 0.001$; for the left eye $r = 0.969$, $p < 0.001$). If analysed using Bland-Altmann plots, no obvious bias can be seen. The mean difference of MACVOL between the two methods was 0.03 $mm^3$ (95% CI from 0.02 to 0.04 $mm^3$) for the right eyes and 0.03 $mm^3$ (95% CI from 0.02 to 0.04 $mm^3$) for the left eyes. The Bland-Altmann plot of inner retinal layer volume of the right and left eyes is depicted in Fig. 2. Overall, no obvious bias can be seen.

**Table 3.** Measurement repeatability, normative values and concordance of retinal layer volumes in eyes without macular pathology.

| | Measurement repeatability | | Retinal layer volumes ($mm^3$) Mean ± SD | | | |
|---|---|---|---|---|---|---|
| Variables | Right eye | Left eye | Right eye | Left eye | Concordance between right and left eyes | ICC<br>CCC |
| MACVOL | $r = 0.989$<br>$p < 0.001$ | $r = 0.988$<br>$p < 0.001$ | 3.101 ± 0.127 | 3.100 ± 0.136 | $r = 0.921$<br>$p < 0.001$<br>$r_c = 0.919$ | 0.992<br>0.984 |
| RNFL | $r = 0.905$<br>$p < 0.001$ | $r = 0.931$<br>$p < 0.001$ | 0.291 ± 0.023 | 0.291 ± 0.027 | $r = 0.734$<br>$p < 0.001$<br>$r_c = 0.724$ | 0.949<br>0.725 |
| GCLIPL | $r = 0.966$<br>$p < 0.001$ | $r = 0.952$<br>$p < 0.001$ | 0.745 ± 0.056 | 0.740 ± 0.060 | $r = 0.918$<br>$p < 0.001$<br>$r_c = 0.913$ | 0.975<br>0.942 |
| INL | $r = 0.808$<br>$p = 0.005$ | $r = 0.649$<br>$p = 0.042$ | 0.307 ± 0.025 | 0.313 ± 0.026 | $r = 0.759$<br>$p < 0.001$<br>$r_c = 0.736$ | 0.892<br>0.509 |
| Calculated inner retinal layers | $r = 0.914$<br>$p < 0.001$ | $r = 0.846$<br>$p = 0.002$ | 1.342 ± 0.081 | 1.344 ± 0.086 | $r = 0.901$<br>$p < 0.001$<br>$r_c = 0.899$ | 0.935<br>0.825 |
| OPLONLPIS | $r = 0.999$<br>$p < 0.001$ | $r = 0.996$<br>$p < 0.001$ | 1.102 ± 0.071 | 1.108 ± 0.070 | $r = 0.946$<br>$p < 0.001$<br>$r_c = 0.942$ | 0.999<br>0.994 |
| POSRPEBM | $r = 0.870$<br>$p = 0.001$ | $r = 0.930$<br>$p < 0.001$ | 0.644 ± 0.030 | 0.650 ± 0.029 | $r = 0.855$<br>$p < 0.001$<br>$r_c = 0.841$ | 0.914<br>0.856 |
| Calculated outer retinal layers | $r = 0.987$<br>$p < 0.001$ | $r = 0.982$<br>$p < 0.001$ | 1.747 ± 0.082 | 1.758 ± 0.078 | $r = 0.934$<br>$p < 0.001$<br>$r_c = 0.924$ | 0.989<br>0.978 |

Confer the Methods section for the abbreviation used. $r$ = Pearson's product-moment correlation coefficient, $r_c$ = concordance correlation coefficient according to Lin. ICC = Intra class correlation coefficient for intra-examiner reproducibility, CCC = concordance correlation coefficient for interexaminer reproducibility, MACVOL = macular volume, RNFL = retinal nerve fibre layer, GCLIPL = ganglion cell layer and inner plexiform layer, INL = inner nuclear layer, OPLONLPIS = outer plexiform layer, Henle's fibre layer, outer nuclear layer and inner part of the photoreceptor layer, POSRPEBM = posterior part of the photoreceptor layer, retinal pigment epithelium and Bruch's membrane.
Calculated Inner Retinal Layers = RNFL + GCLIPL + INL, Calculated Outer Retinal Layers = OPLONLPIS + POSRPEBM.

**Measurement repeatability**

In the repeatability testing, there was a moderate-to-high correlation between the measured values at two different time-points (Table 3). If analysed using Bland-Altmann plot, the mean differences of two measurements for all retinal layers were relatively small (ranging from −0.0003 to +0.012 $\mu m^3$).

**Table 4.** Retinal layer volumes of eyes receiving silicone oil-based endotamponade and their concordance to normal fellow eyes.

| Variables | Retinal layer volumes ($mm^3$) Mean ± SD: Eyes receiving silicone oil-based endotamponade | Fellow eyes | p-value* | Concordance between both eyes |
|---|---|---|---|---|
| MACVOL | 2.931 ± 0.205 | 3.126 ± 0.179 | 0.031 | $r$ = 0.330<br>p = 0.385<br>$r_c$ = 0.208 |
| RNFL | 0.277 ± 0.078 | 0.320 ± 0.031 | 0.121 | $r$ = 0.318<br>p = 0.405<br>$r_c$ = 0.167 |
| GCLIPL | 0.537 ± 0.096 | 0.742 ± 0.117 | 0.006 | $r$ = −0.209<br>p = 0.589<br>$r_c$ = −0.067 |
| INL | 0.312 ± 0.061 | 0.301 ± 0.019 | 0.499 | $r$ = 0.730<br>p = 0.026<br>$r_c$ = 0.410 |
| Calculated inner retinal layers | 1.127 ± 0.160 | 1.363 ± 0.150 | 0.012 | $r$ = −0.010<br>p = 0.980<br>$r_c$ = −0.004 |
| OPLONLPIS | 1.153 ± 0.203 | 1.103 ± 0.103 | 0.234 | $r$ = 0.920<br>p < 0.001<br>$r_c$ = 0.704 |
| POSRPEBM | 0.653 ± 0.072 | 0.663 ± 0.046 | 0.744 | $r$ = −0.031<br>p = 0.936<br>$r_c$ = −0.028 |
| Calculated outer retinal layers | 1.806 ± 0.258 | 1.766 ± 0.126 | 0.439 | $r$ = 0.938<br>p < 0.001<br>$r_c$ = 0.722 |

$r$ = Pearson's product-moment correlation coefficient. $r_c$ = concordance correlation coefficient according to Lin.
* Paired $t$-test after testing for normality using Shapiro–Wilk test.

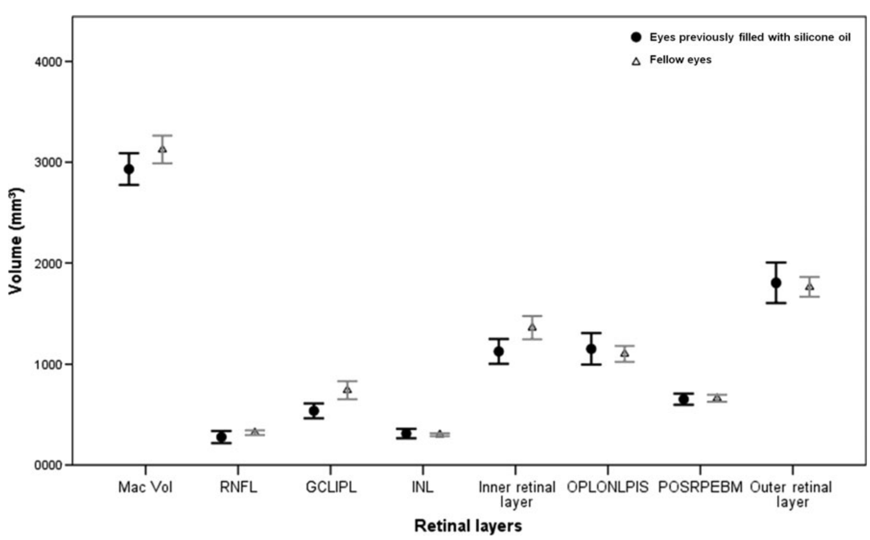


**Fig. 3.** Retinal layer volumes in eyes previously received silicone oil tamponade ($n$ = 9) are reduced in comparison with the fellow eyes.

**Normative values**

Retinal layer volumes measurement data are presented in Table 3. If the right eye is compared with the left eye, a moderate-to-high concordance can be seen.

*Silicone oil-filled eyes*

Retinal layer volumes of eyes receiving silicone oil-based endotamponade in relation to their fellow eyes are presented in Table 4 and in Fig. 3. Overall, MACVOL, GCLIPL and calculated inner retinal layers of eyes receiving silicone oil-based endotamponade are significantly thinner than their fellow eyes (p = 0.031, p = 0.006, and p = 0.012, respectively). The reduction was not seen in other retinal layer volumes. As seen in Table 4, concordance between both eyes of the same individual was sustained for INL, OPLONLPIS and calculated outer retinal layers.

If the central retinal thickness (CRT) between silicone oil eyes and fellow eyes was compared, no statistical significant differences can be seen (p = 0.536).

# Discussion

Silicone oil-based endotamponade is a common substance used as a long-term tamponade. Due to its hydrophobic nature, it provides compartments in the eyes, allowing retinal tears to be sealed. Although silicone oils have been used for years and is relatively safe, there are cases of visual loss after the use of silicone oil (la Cour et al. 2010). Christensen and la Cour reported a case series of nine patients with significant retinal thinning after the use of silicone oil, compared with patients receiving $C_3F_8$ tamponade. In healthy individuals, however, there is a considerable variability of macular volume and thickness (Song et al. 2010; Ooto et al. 2011). Hence, in a study with small sample size, there might be a chance of overestimating macular measurements due to this interindividual variability. In a previous study, we found a high concordance of macular thickness and volume of the same individual (Caramoy et al. 2012); therefore, it is logical to assume that the fellow eye of the same individual will suit better as a control group. In this study, we analysed patients received silicone oil tamponade due to various diseases not involving the macula. Previous studies have shown that macular thickness was not compromised in diseases without macular involvement (Christensen et al. 2007; Lee et al. 2012).

The measurements taken in our study show that there is a considerable concordance of all retinal layers between fellow eyes of the same individual. In eyes receiving silicone oil-based endotamponade, there is a subsequent thinning of inner retinal layers, which concurs to findings reported by Christensen and la Cour (Christensen & la Cour 2012). Accordingly, we are able to further determine which retinal layer contributes most to this thinning, that is, in the ganglion cell layer and inner plexiform layer (GCLIPL).

Because of its hydrophobic nature, silicone oil displaces the aqueous milieu above the retina. This is desirable in cases of proliferative vitreoretinopathy (PVR) or retinal holes. Due to this mechanism, silicone oil displaces the inflammatory milieu responsible for PVR development, and in case of retinal hole, silicone oil seals off the retinal hole, preventing further progression of retinal detachment. However, the displacement of aqueous milieu is undesirable for the healthy retina. A study by Winter et al. (2000) suggested that silicone oil toxicity may be caused by the failure of potassium siphoning by Müller cells. Due to the displacement of vitreous fluid, intraretinal accumulation of potassium occurs and leads to a subsequent neuronal degeneration. This would explain the inner retinal thinning and visual loss after silicone oil use.

Studies in rabbit, guinea-pig, mouse, owl monkey, cat and tiger salamander show that if the potassium siphoning into the vitreous does not work, the subretinal space might be used as an alternative sink (Newman 1987; Newman & Reichenbach 1996). Long-lasting potassium accumulation causes subsequent retinal degeneration; in our study, however, only inner retinal degeneration was encountered, possibly because the outer retinal layers are still protected by means of alternative siphoning mentioned above (Chang et al. 1987; Stolba et al. 1997).

In this study, we evaluate retinal layer thickness with SD-OCT after the use of silicone oil. The values measured provide an objective parameter other than visual acuity alone.

The best time for silicone oil removal is unknown. The decision when to remove silicone oil should be also based on the measurements of retinal layer by SD-OCT as it might serve as a good tool to detect early retinal thinning.

In conclusion, ganglion cell and inner plexiform layer is reduced subsequently in eyes receiving silicone oil-based endotamponade. It might be prudent to integrate SD-OCT in the management of patients with silicone oil tamponade.

## Author Contributions

AC, SF, BK are responsible for the study conception and design AC, KMD, SF are responsible for data acquisition AC, SF, BK are responsible for the analysis and interpretation of the data. AC, KMD, BK, SF are responsible for drafting and revising the article. AC, KMD, BK, SF did the final approval for the manuscript.

## Financial Disclosure

AC received financial support for studies involving silicone oils from Bausch and Lomb, Alamedics GmbH and Fluoron GmbH. KMD (None), BK (None), SF (None).

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