Straylight from glistenings in intraocular lenses: an *in vitro* study

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ABSTRACT

Purpose
To assess light scattering from intraocular lenses (IOLs) with different numbers of laboratory-induced glistenings and create a model for predicting glistening effects on straylight.

Methods
Glistenings were induced in 7 Acrysof IOLs using an accelerated aging method. To create different numbers of glistenings, the IOLs were immersed in a balanced salt solution at temperatures ranging from 37°C to 60°C and cooled to room temperature. The glistenings were analyzed with a microscope. Light scattering from the IOLs was assessed using a commercial straylight meter (C-Quant) adapted for in vitro evaluation of IOLs at a 2.5-degree and 7.0-degree scatter angle. A model was proposed relating straylight increase to the total number and surface portion (total number x area) of glistenings. Results were compared to the Mie theory.

Results
The number of induced glistenings ranged from 114 to 12,386 per mm², and the surface portion ranged from 1.4% to 26.9%. At 2.5 degrees, the range in the straylight parameter was 1.49 to 72.49 deg²/steradian (sr); at 7.0 degrees, it was 1.72 to 62.87 deg²/sr. Straylight was proportionally related to the total number of glistenings (0.0046 x total number) \( R^2 = 0.96 \) and the surface portion (217 x surface portion) \( R^2 = 0.97 \). The measurements agreed well with the Mie theory.

Conclusions
Straylight from glistenings in IOLs had an accurate proportional association with their total number and surface portion. The proposed model proved effective in predicting straylight from glistenings. Numerous glistenings are needed to cause significant straylight elevation.
INTRODUCTION

The formation of glistenings (small fluid-filled vacuoles) in intraocular lenses (IOLs) has been a well-recognized phenomenon for more than 2 decades. Although this phenomenon has been studied extensively, the visual implications of the presence of glistenings for the patient remain a subject of debate.1

It is generally understood that glistenings have the effect of increasing light scattering. But how does that affect vision? A decrease in contrast sensitivity2–4 and visual acuity5 has been reported in isolated cases; however, most peer-reviewed studies6–17 have found that glistenings affect contrast sensitivity and visual acuity only slightly or not. The lack of a tangible effect on visual acuity and contrast sensitivity might be counterintuitive, yet it can be simply explained. The situation is somewhat reminiscent of that for laser pits in IOLs (after neodymium:YAG laser posterior capsulotomy), which were also shown to have little effect on visual acuity or contrast sensitivity. The essence of the explanation lies in the fact that the light scattered by the IOL pits or glistenings involves only part of the light entering the eye.18,19 The retinal image then consists of 2 parts; 1 part enters the eye properly, in between the scatterers, forming a proper image, which is superimposed on a background of light originating from the scattered light. In case of a visual acuity or contrast sensitivity test, the main effect of such light scattering is a veil of light projected over the visual acuity or contrast sensitivity target and, consequently, some loss of contrast. However, as a rule, the fraction of light hitting the scatterers is low (a few percent at most). Thus, the loss of contrast is small and does not appreciably influence visual acuity or contrast sensitivity.18 For example, it has been shown that a 5-fold increase in light scattering results in a mere decrease of 0.1 log units in contrast sensitivity and no discernable effect on visual acuity.18 Only in extreme cases can a significant effect on visual acuity and contrast sensitivity be expected. Scattering of light passing through glistenings might, however, give rise to the visual phenomenon called straylight, and thus disability glare.20

The visual phenomenon of straylight corresponds in a precise 1-to-1 fashion with light scattering.19 Scattering has been measured in vitro in IOLs with laboratory-induced glistenings20 and in explanted IOLs with glistenings.21 In vivo straylight studies have, however, given rather inconclusive results. One study reported a lack of association with straylight,12 but another found a significant, albeit small, effect.22 The problem might be the amount of glistenings available in the studies. Proper quantification of glistenings is essential for understanding their importance. Henriksen et al.,22 for example, showed that the total area of microvacuoles in the IOL correlates significantly with ocular straylight.

In this study, we assessed the relationship between straylight and glistenings in vitro with a clinical straylight meter. A model describing the relationship between straylight and the severity of glistenings was developed. The straylight measurements and the proposed model were compared with theoretically derived scattering based on the Mie theory and with straylight as known from the normal aging population and clinical studies.
MATERIALS AND METHODS

Accelerated Glistenings Formation

This study used 5 SN60WF IOLs and 2 SN60AT IOLs (Alcon Laboratories, Inc.) made of an Acrysof material. The IOLs were removed from their packages and inserted in screw-cap bottles filled with a balanced salt solution. The IOLs were placed separately in a laboratory stove (Tv25u, Memmert GmbH) and subjected to different temperature regimens. Because the primary goal of this study was to evaluate straylight at different stages of glistenings formation, the heating process was performed at different intervals (from 20 minutes to 2 hours) and temperatures (from 37°C to 60°C). The straylight measurements and image recording were performed when the IOLs had cooled to room temperature. For 1 IOL, data for 2 levels of glistenings were included.

Straylight Evaluation

Light scattering from IOLs was evaluated using the C-Quant (Oculus), a clinical device for straylight measurements. It gives straylight expressed as the logarithm of the straylight parameter “s,” log(s). The straylight parameter directly relates to the peripheral part of the functional point spread function (PSF) of the eye. This device was adapted for in vitro straylight analysis of IOLs as described in a recent article. In short, the C-Quant modification (Figure 1) has a plano-convex lens that ensures corresponding angular relations to in vivo measurements of the eye. An IOL is placed in a custom-made holder and inserted into a wet cell filled with a balanced salt solution. During the straylight meter measurement procedure, the plano-convex lens and the IOL project an image of the straylight meter test screen that is partly blocked by a diaphragm. The diaphragm intercepts the rays of the straylight source, while the central bipartite test field can still be seen through a magnifying lens. This enables the straylight meter test to be performed without interference from light scattering in the observer’s own eye; thus, only straylight from the IOL is measured. Light scattering might also take place in the optical components of the setup. Therefore, straylight induced by the IOL \( S_{\text{IOL}} \) was calculated as the straylight value of the setup with the IOL \( \text{Setup + IOL} \) minus the straylight value of the setup without the IOL \( \text{Setup} \) using the following formula:

\[
S_{\text{IOL}} = 10^{\log\text{Setup+IOL} - \log\text{Setup}} \quad [\text{deg}^2/\text{sr}]
\]

where sr stands for steradians.

In addition to the standard straylight meter used in clinics, an elongated straylight meter was also used (Figure 2). The elongation of the straylight meter tube enabled evaluation of light scattering at an angle of 2.5 degrees, instead of 7.0 degrees as applied in the standard straylight meter.

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Image Recording and Analysis

Induced glistenings were recorded using a microscope (Optiphot 2, Nikon Instruments) equipped with a monochromatic camera (model PL-A641, Pixelink). Images were taken from the center of the IOL optic and analyzed with a custom-made software (Image Processing Toolbox, Matlab, Mathworks). Digital image processing involved the following steps: subtraction of background, median filtering, image binarization, morphological operations, and labeling of glistenings. Because some microvoids appeared blurred in microscopic images as a result of the limited depth of field of the system, 2 methods for evaluation of glistenings were used. The size was assessed with analysis of in-focus glistenings solely. The number of glistenings was derived from both in-focus and out-of-focus microvoids. This was achieved by varying the threshold of the binarization process and analyzing the shape of detected objects. The procedure was checked against a ground truth number.
of a manual counting. In cases of intense glistenings, the manual approach alone was applied because overlap between glistenings poses a problem for automatic analysis. The mean frontal surface area per glistening (mean area [mm²]) and the total number per mm² were obtained based on the image analysis. These values were next used to calculate the surface portion of the IOL covered by glistenings as follows:

Surface portion = mean glistenings area x total number of glistenings per mm²

After multiplication by 100, the surface portion is expressed as a percentage. In addition, slit-lamp images were collected using an SL-D 701 slit lamp with reverse illumination and a DC-4 camera system (Topcon Corp.). The slit was set at the maximum width, and x25 magnification was used.

Mie Theory
A theoretical prediction of light scattering by glistenings was calculated based on Mie theory. The analysis was done for particles with an average diameter of 5 mm, 10 mm, and 15 mm and a Gaussian distribution with a standard deviation (SD) of 1.25, 2.50, and 3.75. A wavelength of 555 nm in air (the peak of the visual spectrum) was chosen, and an assumption was made that the refractive index of the medium (IOL material) is $n_m=1.55$ and that of glistenings was $n_p=1.336$. Because the straylight meter assesses straylight over 5- to 10-degree scatter angle (effective average 7.0 degrees), the results were averaged from 5 degrees to 10 degrees.

Statistical Analysis
Descriptive statistics and data analysis were performed using Excel software (2013, Microsoft Corp.). The mean area and size of the glistenings and their total number were derived from 3 regions close to the center of the IOL. The data were averaged and presented as the median ±SD and range.

The straylight value was based on the mean of 2 measurements. Straylight of the studied IOLs was compared with the level of a normal crystalline lens at age 20 years and 70 years and to that of a cataractous lens. For this comparison, the Commission Internationale de l’Éclairage (CIE) standard function for the PSF was used, which includes straylight and has age as a parameter. Straylight of the eye includes contributions from ocular components other than the lens, and this is estimated to be two thirds for a young healthy eye. Correcting for this, the 20-year-old lens is estimated to have a log(s) value of 0.39 and the 70-year-old lens, 1.05. The age parameter of the CIE standard function for the PSF can also be used to model an eye with cataract. To this end, the mean straylight level for a cataractous lens as found in a cataract population study was used as follows: log(s) = 1.52. The equivalent age corresponding to the CIE’s PSF model for this level is 95 years. For comparison, norm values for the young healthy eye (log[s] = 0.9) and for early cataract extraction (log[s] = 1.4) are given.
RESULTS

The median size of the laboratory-induced glistenings in the IOLs was 5.4 mm ±2.7 (SD) (range 4.6 to 12.5 μm), with total numbers varying from 114 to 12 386 per mm² between IOLs. The surface portion varied between 1.4% and 26.9% within the studied IOLs.

The straylight parameter measured at an angle of 2.5 degrees and 7.0 degrees ranged from 1.49 to 72.49 deg²/sr and from 1.72 to 62.87 deg²/sr, respectively. Figure 3 shows the straylight parameter as a function of angle for different surface portion values; for comparison, it also shows straylight levels of the human crystalline lens for different conditions as explained above. A surface portion of 1.4% showed straylight well below the level of that for a young crystalline lens. Surface portion values from 3.9% to 7.7% resulted in functionally significant straylight values that were close to the level of the aged (70-year-old) lens. For extreme glistenings, such as when the surface portion equaled 19.6% and 26.9%, the amount of scattered light was comparable to the effect of cataract. Figures 4 and 5 show a nearly proportional relationship between straylight (at 7.0 degrees) and the amount of glistenings (R² = 0.96) and the surface portion (R² = 0.97). Thus, the glistenings effect on the straylight parameter can be modeled using the total number of glistenings or the surface portion value as follows:

Straylight parameter = 0.0046 x number of glistenings per mm² [deg²/sr]
Straylight parameter = 215 x surface portion [deg²/sr]

![Graph showing straylight parameter for different surface portions and angles.](image)

**Figure 3.** Straylight parameter for 2 angles for different grades of glistenings expressed by the surface portion. The surface portion indicates the IOL area covered by glistenings per mm². The dashed lines refer to straylight of a model crystalline lens at the age of 20 years (green) and years 70 (red) and to a model cataractous lens (black) (SP = surface portion; sr = steradians).
In Figure 6, the theoretical analysis based on Mie theory was compared with the proposed model and to the measured straylight values at 7.0 degrees (expressed in log units). The Mie calculation for 5 mm glistenings corresponded well with the individual data points and the model (i.e., the yellow curve nearly overlaps the green one). A clear dependency of straylight on the glistenings size was found, with larger microvacuoles yielding more straylight.

**DISCUSSION**

The most important result that we found is a proportional relationship between the number of glistenings and their functional effect on straylight. Because straylight contributes to the functional PSF, potential effects on visual function, such as disability glare and loss of contrast sensitivity, can be derived from these data. Moreover, the present result can be used to understand the seemingly variable results reported in the literature.

The accelerated aging method of the IOLs proved effective, showing that it can be used to induce a wide range of severity of glistenings in IOLs. The correlation parameters were high for both the relationship between straylight and the number of glistenings and the relationship between straylight and the surface portion. A slightly better alignment can be seen for the surface portion in Figure 5, presumably because this parameter also accounts for glistening size.
Figure 5. Straylight parameter (7-degree scatter angle) versus surface portion. The surface portion indicates the IOL area covered by glistenings per mm². Three demonstration slit-lamp images show IOLs with glistenings that are connected (dashed lines) to the measured straylight levels. The solid line shows a directly proportional relationship between straylight and the surface portion (sr = steradians).

Figure 6. Logarithm of straylight as function of glistenings numbers for 5 mm, 10 mm, and 15 mm of size derived from the Mie theory. The black crosses indicate measured straylight for different glistening numbers. The dashed lines refer to straylight of a model crystalline lens at the age of 20 years (green) and 70 years (red) and to a model cataractous lens (black).
Table 1. Review of published used in clinical studies on glistenings.

<table>
<thead>
<tr>
<th>First Author (Year)</th>
<th>Follow-up [Mo]</th>
<th>IOL Model</th>
<th>Grade [№]</th>
<th>Total [№]</th>
<th>GS</th>
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<tbody>
<tr>
<td>Dhaliwal2 [1996]</td>
<td>NA</td>
<td>Undisclosed AcrySof model (Alcon, Inc.)</td>
<td>2 2 6 0</td>
<td>10* I</td>
<td></td>
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<tr>
<td>Christiansen5 [2001]</td>
<td>29</td>
<td>AcrySof MA60BM and MA30BA (both from Alcon, Inc.)</td>
<td>27 5 5 3 2</td>
<td>42 II</td>
<td></td>
</tr>
<tr>
<td>Gunenc3 [2001]</td>
<td>13</td>
<td>AcrySof MA30BA, MA60BA (both from Alcon, Inc.)</td>
<td>57 5 8 11 10</td>
<td>91 III</td>
<td></td>
</tr>
<tr>
<td>Miyata29 [2001]</td>
<td>13</td>
<td>AcrySof MA60BM and MA30BA (both from Alcon, Inc.)</td>
<td>21 28 IOLs with grade ≥1/1+</td>
<td>49 IV</td>
<td></td>
</tr>
<tr>
<td>Wilkins6 [2001]</td>
<td>98</td>
<td>Surgidev B20/20 (Surgidev Corp.)</td>
<td>8 30 34 1 -</td>
<td>73 V</td>
<td></td>
</tr>
<tr>
<td>Tognetto7 [2002]</td>
<td>24</td>
<td>CeeOn Edge 911A (Pharmacia &amp; UpjohnCo); ACR6D (Corneal); AcrySof (Alcon, Inc.); Hydroview H60M (Storz); Sensar and SI-40NB (both from Abbott Medical Optics); Stabibag (Ioltech)</td>
<td>Glistenings were found in all studied lenses</td>
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<td>Moreno-Montañes8 [2003]</td>
<td>21</td>
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<td>Cisneros-Lanuza9 [2007]</td>
<td>27</td>
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<td>0 2 5 6 13</td>
<td>26 III</td>
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<tr>
<td>Colin11 [2009]</td>
<td>&lt;24**</td>
<td>AcrySof SN60AT, SN60WF and MA-type (all from Alcon, Inc.)</td>
<td>40 40 19 - -</td>
<td>99 VI</td>
<td></td>
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<tr>
<td>Colin12 [2011]</td>
<td>&gt;24**</td>
<td>AcrySof MA-type, SA60AT, SN60AT, SN60WF (all from Alcon, Inc.)</td>
<td>63 47 51 - -</td>
<td>161 VI</td>
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<tr>
<td>Mönestam13 [2011]</td>
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<td>Colin14 [2012]</td>
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<td>111 VI</td>
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<td>Chang15 [2013]</td>
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<td>AcrySof SA60AT (Alcon, Inc.)</td>
<td>6 12 6 5 -</td>
<td>29 I</td>
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<td>Schweitzer16 [2014]</td>
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<td>AcrySof SA60AT and SN60AT (both from Alcon, Inc.)</td>
<td>4 9 12</td>
<td>25 VII</td>
<td></td>
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<td>30 30 30 30 -</td>
<td>120 I</td>
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<td>First Author (Year)</td>
<td>Follow-up [Mo]</td>
<td>IOL Model</td>
<td>Grade (№)</td>
<td>Total (№)</td>
<td>GS</td>
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<td>Biwer31 (2015)</td>
<td>43</td>
<td>Akreos-Adapt (Bausch &amp; Lomb), Lentis L-307 (Oculentis), undisclosed AcrySof models (Alcon, Inc.), AR40e and Clrlflxc (both from Abbott Medical Optics)</td>
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<td>8</td>
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<tr>
<td>Chang17 (2015)</td>
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<td>AcrySof SA60AT (Alcon, Inc.)</td>
<td>12</td>
<td>7</td>
<td>9</td>
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<tr>
<td>Kahraman32 (2015)</td>
<td>36</td>
<td>BL27 (Bausch &amp; Lomb, Inc.)</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

I: Trace/0 (absence); 1/1+ (trace/countable vacuoles); 2/2+ (moderate); 3/3+ (severe).
II: Trace (<10); 1+ (10-20); 2+ (20-30); 3+ (30-40); 4+ (>40).
III: 0 (absence); 1 (a few); 2 (minor); 3 (moderate); 4 (pronounced).
IV: 0 (absence); 1 (50); 2 (100); 3 (200).
V: 0 (absence); 1+ (<10); 2+ (10-50); 3+ (>50).
VI: 0 (absence); 1 (moderate); 2 (dense).
VII: 0 (<50); 1 (50-150); 2 (>150).
* Only subjects with an AcrySof lens in one eye and a silicone lens in the fellow eye.
** Mean follow-up = 33 months.
Figure 6 shows that larger glistenings result in more straylight. DeHoog and Doraiswamy\textsuperscript{29} simulated the effects of glistenings on the quality of vision with optical software. They concluded that smaller glistenings (i.e., 2 mm) have a greater impact on visual function than larger glistenings (i.e., 20 mm). This seeming discrepancy with our results can be explained when realizing that DeHoog and Doraiswamy\textsuperscript{29} compared, for example, 135,000 glistenings of 2 μm with 135 glistenings of 20 μm to keep the total volume that the glistenings occupied constant (i.e., they assumed an equal volume fraction). We, however, looked at a cross-section of glistenings (expressed by the surface portion), which correlates more closely to what is seen at the slit lamp and also might be a more relevant parameter for determining the amount of light scattered. To relate their result to our findings, one can calculate the surface portion based on the given number of glistenings. This shows that when assuming an equal volume fraction, a surface portion of an IOL with larger glistenings (i.e., 20 μm) is 10 times lower than of the IOL with smaller glistenings (i.e., 2 μm). The reason is that the number of smaller glistenings is much higher than that of larger glistenings (i.e., 135,000 versus 135) in cases of equal-volume fractions. Thus, for such comparison, smaller glistenings are more important. In practice, however, the opposite is the case; that is, larger glistenings are more important.

The best quantitative comparison of the present results can be made with a recent in vivo study by Henriksen et al.\textsuperscript{22} They also found a significant correlation between surface portion (for which they used the term severity index) and ocular straylight. Looking at their plot of straylight versus surface portion (i.e., severity index), we can deduce that they found, on average, 1.03 log(s) for a glistenings-free IOL (severity index = 0) and 1.26 log(s) for an IOL with glistenings with a surface portion of 3.5%. After conversion to the straylight parameter(s), the comparison of these values (1.03 log(s) versus 1.26 log(s)) results in a difference of s = 8 deg\textsuperscript{2}/sr. When surface portion = 3.5% is entered in our model for predicting straylight from glistenings, a value of 0.035 x 215 = 8 deg\textsuperscript{2}/sr is predicted, showing that our model agrees very well with their results.

To relate our findings to previously reported clinical results, a literature survey was performed. This showed that glistening severity is usually clinically quantified using a grading system. Seven grading systems have been published (Table 1).\textsuperscript{2–9,11–17,30–33} The 3 most commonly used grading scales involve subjective grading (Table 1; grading scales [GS]: I, III, and VI). The subjective grades use terms such as minor, moderate, pronounced, and severe and make comparison with quantitative data difficult. The other 4 grading scales apply counting (Table 1; GS: II, IV, V, and VII), which might be considered a more reproducible and objective approach. However, Biwer et al.\textsuperscript{32} showed only moderate agreement between rates of glistenings counted with a slit lamp versus subsequent assessment of slit-lamp images. It was concluded that the reliability of counting at the slit lamp can be limited by the examiner’s experience, but they also pointed to difficulties in reproducibility of slit-lamp illumination.\textsuperscript{32}
As knowledge of the quantitative amount of glistenings is clearly relevant, what does Table 1 tell us? In the study by Miyata et al.,30 up to 200 microvacuoles per mm² in IOLs were counted with a slit lamp. Based on our model for predicting straylight from glistenings based on the number of glistenings, the expected straylight parameter in such a case would be approximately 1 (log[s] = 0), which is very small compared with normal values in eyes of 10 (log[s] = 1) or more. It seems clear that straylight elevation will be significant only in cases with (much) larger numbers. The literature review showed that 19% of evaluated IOLs (limited to grading systems based on counting) fall into the highest grade, specified as having more than 40 to 200 glistenings per mm². Actual counting numbers are not given but would be needed for straylight estimation using our model. In the study by Colin and Orignac,12 the highest reported number of microvacuoles per mm² was 597. The expected corresponding straylight value calculated with our model would be 3 deg²/sr. If this value were added to the value 1.2 log(s) for their group with no glistenings (i.e., grade 0), straylight would increase to 1.28 log(s). This is close to the straylight level of their most severe grade of glistenings severity (i.e., grade 2) of 1.3 log(s). Note that in terms of importance for quality of vision, a 0.1 log unit increase in ocular straylight is comparable to a loss of 1 line on the logMAR visual acuity test.34 In the study of Colin and Orignac,12 intense glistenings (grade 2) were found in 27 of 97 IOLs; however, the authors did not consider any of them to be extremely dense. This might partly explain why visual difficulties from glistenings have been observed in very few cases.4,21,35

Thus far, 2 studies of in vitro scatter evaluation of glistenings have been published.20,21 In an experimental setting with off-the-shelf IOLs, glistenings were induced in the Acrysof material.20 The mean glistening number was 1800 per mm³; however, a direct comparison with our results cannot be made because the mean scatter level was not published in that study. The mean size of the microvacuoles (6.2 μm) reported by van der Mooren et al.20 is close to the 5.4 μm (median) we found. In a case report of an exchange of a multifocal IOL because of glistenings, an in vitro light scatter analysis of IOLs (after several years in the eye) was also presented.21 In the 2 cases described straylight was found to be higher than that of the 20-year-old crystalline lens, and this was considered the major contributor to the patient’s visual complaints. Although a quantitative analysis of microvacuoles was not given and the analysis was limited to 2.5 degrees of scattering angle, a comparable effect can be found in our IOLs with a glistenings surface portion of 3.9% or 4.5% (Figure 3).

In conclusion, we found a proportional relationship between the number of glistenings and straylight. The relationship proved to agree with data in the literature. A large number of glistenings is needed to cause an increase in straylight that is clinically relevant to the patient. The relationship also clarifies the variable results published on (the lack of) functional effects of glistenings. Although effects on visual acuity or contrast sensitivity are improbable, effects on straylight and thus disability glare seem possible.
REFERENCES