Reproducibility of the NEI Scheimpflug Cataract Imaging System

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Purpose. The NEI Scheimpflug Cataract Imaging System was developed to allow for easy, accurate and reproducible image analysis of nuclear cataracts. This study was undertaken to determine the reproducibility of densitometric measurements of the lens nucleus using this modified system.

Methods. Replicate Zeiss Scheimpflug images of the lenses in 143 eyes were obtained by one photographer. Normal and cataractous lenses (without central cortical or anterior subcapsular opacities) were sampled. Images were stored after testing for adequacy using immediate exposure checking. Densitometry of the nuclear region was then performed for each image. The interval within which 99% of the differences between repeat measurements may be expected to lie was used as a measure of reproducibility (99% range).

Results. A 99% range of ±0.023 optical density units (odu) was obtained for nuclear densities < 0.30 odu (125 eyes). For lenses with nuclear densities ≥ 0.30 odu (18 eyes), the 99% range was ±0.14 times the first measurement.

Conclusion. This study shows the excellent reproducibility of this Scheimpflug imaging system in the nuclear region and demonstrates its usefulness in studies on nuclear cataracts, particularly for natural history studies and clinical trials of anti-cataract drugs. Invest Ophthalmol Vis Sci. 1994;35:3078-3084.

The Zeiss Scheimpflug Cataract Video Camera (Carl Zeiss, Oberkochen, Germany) was designed to photograph, store, and analyze lens opacities in a semiautomated fashion for cross-sectional and longitudinal studies. The original system had two main components: a slit lamp Newvicon tube camera (Robert Bosch, GmbH, Darmstadt, Germany) and an on-line computer with videotape recorder. The eye is aligned based on the Purkinje image on the lens, and a slit lamp video image of the anterior segment of the eye is captured. Densities of the different lens regions were measured in reflectance units.

An earlier reproducibility study using the original Zeiss system had demonstrated high reproducibility in the different lens regions, particularly in the nucleus.1 The intraclass correlation in the nuclear region was 0.995 with a 95% confidence interval of 0.992 to 0.996, even with the system's limitations at that time. Notable among such limitations were the inability to vary lamp voltages for the same eye, the lack of an algorithm to enable comparability of density measurements with different lamp voltages, and inadequacies in storage, retrieval, and analysis of images.

Numerous modifications in the hardware and software have been made on the system, as will be briefly described below, to correct the deficiencies noted in the older Scheimpflug system and vastly improve the speed, accuracy, and applicability of the present system (now called the National Eye Institute [NEI] Scheimpflug Cataract Imaging System).

Although reproducibility of the Scheimpflug slit lamp camera has already been shown to be highest in the nuclear region,2,4 similar reproducibility experiments are necessary using the present analysis system before its use for precise monitoring of nuclear lens changes over time. Furthermore, we wanted to evaluate the measurement error for the nuclear region, which was not determined previously, so as to define significant changes in densities for longitudinal studies.
METHODS

System Description

Modifications have been made so that this system could employ fast, accurate, and reproducible quantitative densitometric analyses of the different lens regions: New hardware has been developed to link the original Zeiss electronics to a Macintosh Quadra 700 computer (Apple, Cupertino, CA), and appropriate software for imaging and cataract analysis has also been developed on the Macintosh.\(^5\)

Briefly, the new software is designed to produce objective and systematic densitometric analysis of Scheimpflug images useful in both cross-sectional and longitudinal studies of cataract. The software analysis can be summarized into the following series of sequential operations: \(^5\)

1. location of the center of the lens
2. exposure checking
3. detection of the posterior and anterior capsular edges
4. detection of the intralenticular boundaries
5. results calculations
6. output files

The system also allows for operator interaction in determination of the center line and, in edge detection, of the different lens boundaries. Changes in density in different regions of the lens can be monitored separately using this analysis method.

Furthermore, better calibration has been achieved through the use of 14 calibrated neutral density filters to assure comparability of results for the same lens even if lamp voltages are varied at time points. These neutral density filters are positioned in the receiving optical path ahead of the video-camera. Analysis of images of a Zeiss-supplied integrating sphere at different lamp voltages enabled us to produce sets of calibration tables that correlate pixel intensity values (0 to 255) into standardized optical density units (odu).

Procedure

One hundred twenty pairs of repeat video images of one or both lenses of 69 consecutive patients were obtained by one photographer (BVM) using the Zeiss Scheimpflug slit lamp camera. Pairs of repeat images indicate two images taken on the same eye at the same visit. The patients were already part of an ongoing Intramural Review Board approved clinical study at the National Eye Institute, and informed consent had been obtained from them previously. The tenets of the Declaration of Helsinki were strictly followed. The only exclusion was the presence of a central cortical or anterior subcapsular opacity that cast a shadow on the nuclear region.

The pupils were maximally dilated using 1% tropicamide and 2.5% phenylephrine hydrochloride. Lenses were classified using the Lens Opacities Classification System II (LOCS II).\(^6\) All images were taken in a standardized manner.\(^1\) After each photograph was taken, the patient was asked to sit back while the captured image was stored in the optical disc. For the replicate images, the camera was realigned and the patient's head was repositioned.

The fixation coordinates and lamp intensity levels were entered into the patient database. The appropriate lamp intensity for each eye was determined by immediate exposure checking, a new feature of this system, and was maintained for each pair of replicate images. Exposure checking assured that approximately 98% of all the pixels to be within the linear range of the camera.

Paired images of each eye were then analyzed in a masked fashion using the developed software. Multilinear microdensitometry was automatically performed for the posterior cortical, nuclear, and anterior cortical regions along the axial center of the lens using a rectangular region of interest (ROI) 0.41 mm (32 pixels) in height, although only the nuclear region was evaluated for this reproducibility study. Repeat testing disclosed that the 0.41 mm height (1/16 of the total image height) matched the outer lens curvature along the axial center and avoided the external pixels, which substantially decrease the average density within the ROI.\(^5\) The width of the ROI varies depending on the thickness of the nucleus. Figure 1 shows an example of an analyzed Scheimpflug image of a patient with a pure nuclear cataract with a LOCS II grade of +2 for opalescence.

In addition to the 120 pairs of repeat images taken at baseline, for a subgroup of 23 eyes (15 patients) another pair of repeat images was obtained at a second visit 4 to 12 months later. The difference between these two repeat measurements obtained at a second visit was independent of the difference between two repeat measurements at baseline. This allowed us to increase the number of pairs of repeat measurements for images in the higher densities because patients with advanced cataracts were not numerous.

One hundred forty-three pairs of repeat measurements were used to assess the reproducibility of nuclear density measurements. Some persons contributed both eyes, whereas in others another pair of repeat measurements was obtained in the same eye at a second visit 4 to 12 months later. Possible correlation between fellow eyes, or possible progression (or regression) between baseline and second visit, was not important (even though such an event may have occurred) because we evaluated only the difference be-
tween repeat measurements at the same visit, which was the measurement error. The measurement errors were assumed to be independent for fellow eyes or for the same eye at different visits.

**Statistical Analysis**

To quantify reproducibility, we used the 99% range\(^7\text{--}^9\) for the difference between two repeat measurements, that is, the interval within which 99% of the differences between two repeat measurements may be expected to lie.

An important assumption in the assessment of the 99% range is that the standard deviation of the difference between two repeat measurements is not associated with the size of the measurements. To see if the standard deviation of the differences was homogenous, a scatterplot of the absolute differences between two repeat measurements against the average of the two measurements was initially performed (Fig. 2). The scatter of the differences was mainly homogenous for average densities \(< 0.30 \text{ odu}\). These densities are approximately \(\leq\) grade 2 opalescence (Fig. 4), denoting clear lenses and lenses with early to moderately advanced nuclear opacification. Lenses with densities below 0.30 odu are of primary interest for clinical trials because this includes normal lenses and lenses with early nuclear opacification in which some intervention would be more likely to be successful.

Our first option then was to restrict ourselves to this density interval and to estimate the 99% range only for densities \(< 0.30 \text{ odu}\). One can assume that the differences of density measurements follow a normal distribution. Then the 99% range is \(\pm 2.58 \text{ std(D)}\), the same as was described above.

Because densities above 0.30 odu may be of interest in natural history studies, we also tried to find a way to estimate the 99% range for the whole interval of densities (0.01 to 0.69 odu). Figure 2 shows that for the average densities above 0.30 odu, the scatter of the differences increased with the size of the measurements. A formal test for independence across the whole interval of densities showed that there was a significant relationship \((P < 0.0001)\) between the absolute difference of repeat measurements and the magnitude of the measurements.

A logarithmic transformation of the measurements was attempted to see whether it would make the differences homogenous across the entire density interval. However, the logarithmic transformation was unsuccessful: The plot of the differences \([\log X_2 - \log X_1]\) of the logarithms of two repeat measurements \(X_2\) and \(X_1\) against the average of the measurements showed a strong relationship between the two values (Fig. 3). When only the higher densities (0.30 to 0.69 odu) were considered, the scatter of the differences \([\log X_2 - \log X_1]\) in this interval was observed to be homogenous. This observation was used in assessing the 99% range for the higher density measurements (see last paragraph of the Appendix).

This finding precluded having the same 99% range for all eyes across the entire density interval. To obtain the 99% range for the whole density interval, we developed a model that allowed us to evaluate the 99% range separately for the two density groups (see Appendix). In this model, for the lower density group \((< 0.30 \text{ odu})\), the 99% range for the difference \(D = X_2 - X_1\) of two measurements does not depend on the magnitude of measurements and is \(\pm 2.58 \text{ std(D)}\), the same as was described above.

![FIGURE 2. A scatterplot of the absolute differences between two repeat density measurements against the average of the two measurements in the nuclear region. The bubble size indicates the point frequency.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933405/ on 06/23/2017)
RESULTS

The distribution of opacities at baseline was as follows: 14 nuclear, 6 cortical, 7 posterior subcapsular, 71 mixed (predominantly nuclear cataracts), and 22 normal lenses. LOCS II gradings for nuclear opalescence in the 120 eyes were distributed as: NO = 39, NI = 46, NII = 23, NIII = 9, and NIV = 3.

The average nuclear densities in the entire sample of 143 eyes (including the 23 additional pairs of repeat images taken on a second visit) ranged from 0.01 to 0.69 odu. Figure 4 shows the association between the nuclear LOCS II grades obtained at the slit lamp and the measured nuclear densities (odu). The measurements were divided into two groups based on the scatter of the differences (Fig. 2): a low density group consisting of lenses with average nuclear densities <0.30 odu, and a high density group consisting of lenses with average nuclear densities ≥ 0.30 odu. Nuclear cataracts with densities < 0.30 odu would approximately be ≤ grade 2 opalescence.

For nuclear densities below 0.30 odu, the standard deviation of the difference of two repeat measurements was 0.009, and the 99% range for the difference (X2 - X1) was ± 0.14X1. Figure 6 shows how the 99% range extends for nuclear densities over 0.30 odu. The widening part of the funnel is formed by the lines X2 = 1.14X1 and X2 = 0.86X1 and contains all pairs of measurements (X1, X2) for which |X2 - X1| ≤ 0.14X1, or similarly, |(X2/X1) - 1| ≤ 0.14 (see Appendix).

Table 1 gives a summary of the parameters describing both groups of lens images divided on the basis of their nuclear densities.

DISCUSSION

Scheimpflug photography is a well-recognized method for obtaining clear sagittal views of the crystalline lens in vivo. The NEI Scheimpflug Cataract Imaging System was developed to correct deficiencies in, as well as improve on, the older Scheimpflug systems and to allow easy, accurate, and reproducible imaging and analysis of nuclear cataracts. Briefly, the present system uses a quick, user-friendly, automated image analysis system and allows for comparability of density measurements (in optical density units) even with different lamp voltages. Multilinear densitometry is performed along a 0.41 mm high ROI along the axial center because any lens change along the visual axis of the eye could directly affect vision. Although this selected region samples only a small section of the entire lens nucleus, the density measurements obtained are considered representative of the whole nucleus because nuclear opaci-
FIGURE 5. The 99% range for the difference \((X_2 - X_1)\) between two measurements for the lower density group (nuclear densities < 0.30 odu): ±0.023 odu.

The concepts of a threshold of change \((T)\)\(^{18}\) and a tolerance interval\(^{19}\) were meant to define a range within which differences between replicate measurements were assumed to be due to measurement error. This threshold value was applied by Chylack and coworkers in a clinical setting for measuring longitudinal growth of nuclear cataracts.\(^{20}\) Subsequent nuclear density measurements on the same lens that exceeded the threshold range were considered significant changes, outside measurement error. However, there was no mention of whether the threshold range was uniform throughout the entire interval of densities.

The present study, on the other hand, shows a fixed, narrow 99% range (±0.023 odu) for lenses with nuclear densities < 0.30 odu (clear lenses to lenses with moderately severe nuclear opacities). These limits may be applied to longitudinal studies of nuclear cata-

### TABLE 1. Parameters Describing Two Groups of Lens Images Divided on Basis of Nuclear Density

<table>
<thead>
<tr>
<th>Nuclear Density* (optical density units)</th>
<th>&lt;0.30</th>
<th>≥0.30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of images</td>
<td>125</td>
<td>18</td>
</tr>
<tr>
<td>Mean density</td>
<td>0.094</td>
<td>0.48</td>
</tr>
<tr>
<td>SD</td>
<td>0.009†</td>
<td>0.056‡</td>
</tr>
<tr>
<td>99% range</td>
<td>±0.023</td>
<td>±0.14X₁</td>
</tr>
</tbody>
</table>

* The average of two repeat measurements \(X_1\) and \(X_2\) on the same lens.
† Standard deviation of the differences of two repeat measurements.
‡ Standard deviation of the ratio of two repeat measurements.
racts—comparing the nuclear density from one visit against the density obtained on a succeeding visit. For any future pair of measurements \((X_1, X_2)\), either the range of \(\pm 0.023\) or the tube plot in Figure 5 may be used. If \(|X_2 - X_1| \leq 0.023\) (the point \([X_1, X_2]\) falls inside the tube), the difference is ascribed to measurement error. If \(|X_2 - X_1| > 0.023\) (the point \([X_1, X_2]\) falls outside the tube), we conclude that there is a change beyond measurement error. For clinical trials wherein persons with low density opacities are recruited, the 99% range for the low density group may be applied.

Similarly, for any future measurements \((X_1, X_2)\) with density \(X_1 \geq 0.30\) odu, if \(|X_2/X_1 - 1| > 0.14\) (the point \([X_1, X_2]\) falls outside the funnel), we conclude that there is a change beyond measurement error. Because of the smaller number of sampled lenses with nuclear densities \(\geq 0.30\) odu, and the greater variability observed in the differences between repeat measurements in these same lenses, the 99% range is, as expected, wider. These limits are still of value when used for natural history studies.

However, even though a wider 99% range was noted for the higher density group of images, we are most interested in the very early stages of cataract when the reproducibility of this system is excellent and when these lenses are being objectively followed longitudinally, as in clinical trials of anti-cataract drugs.

In summary, we have shown the excellent reproducibility of the NEI Scheimpflug Cataract Imaging System for nuclear cataracts and its potential usefulness in future studies on nuclear cataracts, especially for natural history studies and clinical trials of anti-cat-

Key Words

NEI Scheimpflug Cataract Imaging System, nuclear cataracts, 99% range, densitometry, LOCS II

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References


APPENDIX

Because the standard deviation of the difference between two repeat measurements increased with the
magnitude of measurements for densities above 0.30 odu, we need a model that allows evaluation of the 99% range separately for the lower and higher density intervals. The following model was suggested:

Denote by \( A \) the actual density. It is reasonable to assume that a measurement \( X \) can be written in the form: \( X = A + A \xi + \eta \) (*), with the total error consisting of two parts: a multiplicative error \( A \xi \) and the additive error \( \eta \). The multiplicative error \( A \xi \) increases with the magnitude of actual density \( A \), with \( \xi \sim N(0, \sigma^2) \) being a mean zero normal random variable with the variance \( \sigma^2 \). The additive error \( \eta \sim N(0, \sigma^2) \) is a mean zero normal random variable with the variance \( \sigma^2 \). The additive error \( \eta \) does not depend on the actual magnitude of the density. Measurement errors \( A \xi \) and \( \eta \) are assumed to be independent for different measurements.

The whole sample was divided into two groups: \( n \) eyes with the average densities below 0.30 and \( m \) eyes with the average densities 0.30 or higher, and the 99% range was obtained separately for each group.

a) When actual density \( A \) is low, then \( A \xi \) is much smaller than \( \eta \), and \( A \xi \) can be ignored. In the low-density group, model (*) is approximated by a model with an additive error only: \( X = A + \eta \). (1)

If \( X_{i1} = A_i + \eta_{i1} \) denotes the first measurement on the \( i \)th eye, and \( X_{i2} = A_i + \eta_{i2} \) denotes the second measurement on the same eye, then the difference \( D_i \) between two repeat measurements is:

\[
D_i = X_{i2} - X_{i1} = (A_i + \eta_{i2}) - (A_i + \eta_{i1}) = \eta_{i2} - \eta_{i1}.
\]

The random variables \( \eta_{i1} \) and \( \eta_{i2} \) are assumed to be independent of each other and independent for different values of \( i \). The variance of \( D \) is:

\[
\text{var}(D) = 2 \sigma^2.
\]

The 99% range for the difference \( (X_2 - X_1) \) using measurements on the \( n \) eyes in the low-density group is:

\[
\pm 2.58 \text{SD}(D) = \pm 2.58 \sqrt{\frac{\sum (D_i - \bar{D})^2}{n - 1}} \tag{1a}
\]

where \( \bar{D} \) denotes the mean value of the difference \( D_i \) between two repeat measurements for the \( n \) eyes in the low density group. Figure 2 shows that the scatter of the differences \( D_i \) is homogenous for the lower density group, thus justifying the use of \( \text{std}(D) \) in this group.

b) When the actual density \( A \) is high, then \( A \) is much larger than \( \eta \) and \( \eta \) can be ignored. In the high density area, model (*) can be reduced to an approximate model with a multiplicative error only: \( X = A (1 + \xi) \). (2)

Let \( X_{i1} = A_i (1 + \xi_{i1}) \) denote the first measurement on the \( i \)th eye and \( X_{i2} = A_i (1 + \xi_{i2}) \) denote the second measurement on the same eye. The random variables \( \xi_{i1} \) and \( \xi_{i2} \) are assumed to be independent of each other and independent for different values of \( i \). Instead of the difference of the two repeat measurements, we now look at the ratio of two repeat measurements to see how it differs from unity (perfect agreement). For each eye we calculate the value:

\[
G_i = \frac{X_{i2}}{X_{i1}} - 1 = \frac{A_i (1 + \xi_{i2})}{A_i (1 + \xi_{i1})} - 1 = \xi_{i2} - \xi_{i1},
\]

provided \( \xi \) is small. The variance of \( G \) is:

\[
\text{var}(G) \approx 2 \sigma^2.
\]

The 99% range for the difference \( (X_2 - X_1) \) using measurements on the \( m \) eyes in the high-density group is:

\[
\pm 2.58 \text{SD}(G) = \pm 2.58 \sqrt{\frac{\sum (G_i - \bar{G})^2}{m - 1}} \tag{2a}
\]

where \( \bar{G} \) denotes the mean value of \( G \) for the \( m \) eyes in the high-density group.

It is worth noting that using the value \( G_i = (X_{i2}/X_{i1}) - 1 \) in the region of high density is equivalent to using the difference (logarithm) of logarithms of two repeat measurements, provided \( G_i \) is small. To show this, we use that \( G_i = (X_{i2}/X_{i1}) - 1 \) or \( X_{i2}/X_{i1} = 1 + G_i \). From this, \( \log X_{i2} - \log X_{i1} = \log(X_{i2}/X_{i1}) = \log(1 + G_i) \approx G_i \). As was mentioned above, Figure 3 shows that the scatter of the differences (logarithm) is homogenous in the higher density interval (\( > 0.30 \) odu). We have just shown that (logarithm) is homogenous for densities over 0.30 odu, thus justifying the use of \( \text{std}(G) \) in the higher density interval.