E

Even while attempting to maintain steady fixation on a point target, humans make regular small, fixational eye movements. These fixational eye movements are typically made without knowledge and include drift, tremor, and microsaccades. Microsaccades are conjugate eye movements, typically of around 10 to 20 deg/s velocity and 25-millisecond duration. Our understanding of the function of microsaccades is incomplete; they appear to play a role in maintaining fixation on a target while also negating perceptual fading due to neural adaptation during periods of steady fixation under laboratory conditions (but see). The functions of fixational eye movements have been more extensively reviewed elsewhere.

The rate at which microsaccades are made is modulated by visual input. For example, brief presentation of a visible texture elicits a pattern of change in the microsaccade rate. This pattern has been termed the “rate signature” and typically follows a sequence of post-stimulus inhibition, followed by a “rebound” increase in rate and a return to the baseline rate over a total period of approximately 500 milliseconds. Microsaccade rate signatures are elicited by any stimulus transient—even those that are not relevant to the task under investigation—and by motor preparation. The latency and magnitude of both the inhibition and rebound phase are modulated by stimulus attributes such as contrast and the expected frequency of occurrence. As microsaccades are generated in the superior colliculus, it is likely that this is the locus for the rate signature, although the exact mechanisms remain unclear. Recent evidence suggests that the rate signature can be modulated by cortical areas and that the frontal eye fields may play a key role in the rebound phase.

Our group has previously shown in a small cohort of healthy observers that sensitivity to visual stimuli can be predicted from properties of the rate signature including the magnitude of the inhibition and rebound phases and the amplitude of the resulting fixational saccade. Further, we have demonstrated the utility of a machine learning approach that can classify stimuli as seen or unseen, depending on the subsequently observed rate signature. This method produced accurate and precise estimates of contrast detection thresholds in a small group of participants with normal vision; however, it has several limitations related to the downsampling of recorded information across subjects, trials, and time that was necessary due to the sparse distribution of microsaccade events in the data. Downsampling of information across subjects relies on the assumption that rate signatures are similar in form across populations with a broad range of contrast sensitivities. Contrast sensitivity can be robustly estimated from rate signatures by probabilistic inference, but more stimulus presentations are currently required to achieve similarly precise estimates to behavioral techniques.
subjects. While this assumption held approximately true for the small population previously tested, it may not hold across wider clinical populations with much greater variation in age and visual and motor function. Downsampling across trials renders the method inflexible to variations in numbers of trials collected, limiting the use of the method outside the laboratory, where competing priorities may influence the amount of time available to collect data. Finally, downsampling across time results in loss of potentially useful information for estimating contrast sensitivity from stimulus-induced changes in microsaccade rate.

The purpose of this study was to develop and test a tractable, practical method for estimating contrast sensitivity from microsaccade rate. Our intention was to develop a method that could be incorporated into conventional frameworks for psychophysical vision testing as well as produce a threshold estimate for a new observer with a microsaccade rate signature of unknown shape. We aimed for the method to be robust to a wide range of contrast sensitivities and to potential differences in the profile of the microsaccade rate signature; therefore, we measured rate signatures in a deliberately broad clinical population. Finally, we also aimed to estimate the time cost of using this approach compared to conventional behavioral measurements.

**METHODS**

The study adhered to the tenets of the Declaration of Helsinki with the exception that it was not formally preregistered in accordance with the 2014 amendment. The study received approval from the institutional ethics review board of the School of Psychology, University of Nottingham. All participants gave written, informed consent to participate and were free to withdraw at any time.

**Participants**

To measure a broad spectrum of contrast sensitivities due to optical or neural deficits, we recruited participants from three groups: older adults with cataract, adults with amblyopia, and younger adults (≤ 40) with normal or corrected-to-normal vision and no known ocular pathology. Our intention was not to compare factors among these groups but rather to recruit a diverse population on which to test the robustness of our methods. Therefore, we did not employ strict inclusion/exclusion criteria to each group. Participants were recruited from the staff and student population of the University of Nottingham and by advertisement in local media.

Volunteers with self-reported amblyopia or cataract first underwent a screening eye examination in which their condition was verified by means of self-reported history, objective and subjective refraction, visual acuity, slit-lamp biomicroscopy, and indirect ophthalmoscopy. Lens opacity was quantified using the LOCS III grading system. Those with or suspected of having amblyopia also undertook a variety of binocular vision assessments to further characterize their condition, including TNO stereopsis testing, cover testing, and Bagolini striated lens testing to evaluate suppression or the presence of anomalous retinal correspondence. Amblyopia was classified as a difference in visual acuity of at least 0.20 logMAR between the two eyes with no apparent ocular pathology. Pelli-Robson contrast sensitivity was also measured for all participants.

One eye of each participant was selected for testing. For participants with amblyopia, the amblyopic eye was tested. For participants with cataract, we chose the eye with poorer visual acuity as long as the vision was still good enough to perform the experiment. For young participants with normal vision, we chose one eye at random.

**Psychophysical and Eye Tracking Procedure**

Data were collected from each participant over 4 to 5 sessions, each a duration of approximately 1 to 1.5 hours. Appropriate refractive correction for the screen distance of 1.5 meters was provided to all participants as wide aperture lenses mounted in a trial frame. The screen distance was maintained by use of a chin and forehead rest. The untested eye was occluded by material that permitted transmission of infrared but not visible light (Optolite Infrared Acrylic; Instrument Plastics, Maidenhead, UK) to permit binocular infrared eye tracking.

During the first session, we initially measured contrast sensitivity functions to Gabor stimuli (random-phase sinusoidal gratings within Gaussian spatial envelope with standard deviation 2.3°) of a range of spatial frequencies (0.5–12 cycles/deg). Gabors were presented for one frame at 85 Hz refresh rate on a gamma corrected CRT display (NEC MultiSync FP2141SB, 1024 × 768 spatial resolution, mean luminance 59 cd/m²; NEC Display Solutions, Tokyo, Japan) via a graphics card that enabled 14 bit luminance resolution (Bits#; Cambridge Research Systems, Kent, UK), using custom software written in PsychoPy2.27 (version 1.83.01). On each trial, the Gabor was randomly oriented at either 45° or 135°, and the observer indicated the orientation by key press (3 or 1 on a numeric key pad, respectively), making their best guess if unsure (two alternative forced choice). Gabor contrast was adjusted on each trial by a 3-down-1-up staircase, changing contrast in logarithmic steps and terminating after 60 trials. The mean of the last 8 reversals was taken as the contrast detection threshold for the particular spatial frequency. Contrast was defined in Michelson units as:

\[
C = \frac{L_{\text{max}} - L_{\text{min}}}{L_{\text{max}} + L_{\text{min}}}
\]

where \(L_{\text{max}}\) and \(L_{\text{min}}\) represent the maximum and minimum luminance of the stimulus, respectively.

From the measured contrast sensitivity function, we then chose the highest spatial frequency that the individual participant could reliably detect at 75% contrast for further testing in the main procedure. During this procedure, the participants’ eye movements were recorded binocularly at 500 Hz using a calibrated infrared eye tracking device (Eyelink 1000; SR Research Ltd, Ottawa, ON, Canada). Participants were instructed to fixate a central white dot (0.1° diameter) and avoid blinking where possible, while Gabor stimuli randomly oriented at either 45° or 135° appeared for one frame at jittered intervals between 1000 and 1400 milliseconds. The contrast of each Gabor was presented according to a method of constant stimuli procedure with 7 levels. Five levels were spaced around the previously measured detection threshold in 0.1 or 0.2 log unit steps depending on the participant; one level was of zero contrast, and the remaining level was of a high contrast (>50%) where task performance was expected to be maximal. Participants were instructed not to respond to these stimuli but to simply continue fixating on the central dot (passive trials). Interleaved at random intervals among the passive presentations were response trials in which the same stimulus was accompanied by an audible tone. Participants indicated the orientation of the Gabor in each response trial by key press (3 or 1 on a numeric keypad for 45° or 135°, respectively), as in the initial phase. Breaks were taken after every three trials to allow participants to blink freely before continuing. This was done to reduce tear film breakup and maintain participants’ comfort. Longer rests were taken after approximately 5 to 8
minutes. Each session typically comprised 10 blocks, yielding a total of 400 passive trials and 80 response trials per contrast level.

**Identification of Microsaccades and Construction of Rate Signatures**

Processing of raw eye tracking data and identification of microsaccades was performed in MATLAB (R2014b; The Mathworks, Natick, MA, USA). Raw eye tracking data was converted to degrees of visual angle using data from a 9-point calibration performed at the beginning of each block. Data collected during blinks (when pupil size was recorded as 0) were removed along with a buffer of 100 milliseconds on either side. Microsaccades were detected using a velocity-threshold algorithm\(^{12,18}\) with a threshold of 6 times the standard deviation of the median velocity. Detected events with an amplitude of <3 or >60 arcmin or a duration of <6 milliseconds were removed from further analysis. To avoid classifying overshoots as separate microsaccades, any detected events with an interval of <50 milliseconds were combined. To improve the robustness of microsaccade classification, microsaccades were required to overlap in time across both eyes. Microsaccade rates were computed in 2-millisecond bins over an epoch extending from 500 milliseconds before to 1000 milliseconds after stimulus onset. For display and further analysis, computed rates were then binned into 50-millisecond bins over the same epoch.

**Estimation of Contrast Sensitivity From Microsaccades**

Contrast sensitivity for individual participants was estimated from the observed rate signatures by a probabilistic inference method. First, we computed a “maxrate” signature across all presentations of the highest contrast (cmax) stimulus and estimated the corresponding probability of observing a microsaccade in each individual 50-millisecond time bin following stimulus onset (Equation 2, Fig. 1B):

\[
p(\text{maxrate}_t) = 0.05 R_{\text{contrast-cmax}}
\]

where \(R_{\text{contrast-cmax}}\) is the microsaccade rate in Hz at time \(t\) for the maximum contrast stimulus.

Second, we obtained an estimate of the individual participant’s “baserate,” their baseline rate of microsaccades in the absence of any stimulus presentation. Here, we could have taken an analogous approach to the maxrate signature, using data from the 0% condition instead (i.e., red line in Fig. 1A). However, in this case the concept of stimulus onset is notional and time-locked variations simply reflect noisy fluctuations in our measurement of the underlying rate. Therefore, to gain a more robust estimate of baserate, we instead calculated the mean microsaccade rate across all stimulus conditions prior to stimulus onset \((R_{t<0}, \text{where } t = 0 \text{ represents the time of stimulus onset})\) and assumed this to be stable across time. The baserate was then used to infer the probability of observing a microsaccade in each 50-millisecond time bin in the absence of a visible stimulus (Equation 3, Fig. 1B).

\[
p(\text{baserate}_t) = 0.05 R_{<0}
\]

The baserate and maxrate probabilities then acted as two possible generating functions for the microsaccades profiles observed in the full set of contrast conditions. For example, Figure 1A shows the total number of microsaccades produced in each time bin for a subject presented 400 times with a stimulus of each contrast shown. For each individual 50-millisecond time bin, we calculated the probability of the observed data, given the expected probabilities of both baserate and maxrate functions in the same time bin using the binomial distribution:

\[
p(\text{ms}_{\text{no stimulus}}) = \binom{n}{m_{\text{st}}} p(\text{baserate})^m (1 - p(\text{baserate}))^{n-m_{\text{st}}}
\]

(4)

\[
p(\text{ms}_{\text{stimulus}}) = \binom{n}{m_{\text{st}}} p(\text{maxrate})^m (1 - p(\text{maxrate}))^{n-m_{\text{st}}}
\]

(5)

where \(n\) is the number of presentations of the stimulus and \(m_{\text{st}}\) is the total number of observed microsaccades in time bin \(t\) across all trials. The log likelihood of each generating function was then calculated by log transforming these values and summing across all post-stimulus onset time bins (Fig. 1C):

\[
\ln(L_{\text{no stimulus}}) = \sum \ln(p(\text{ms}_{\text{no stimulus}}))
\]

(6)

\[
\ln(L_{\text{stimulus}}) = \sum \ln(p(\text{ms}_{\text{stimulus}}))
\]

(7)

Log likelihood ratios were obtained by subtracting \(\ln(L_{\text{no stimulus}})\) from \(\ln(L_{\text{stimulus}})\). Finally, these values were scaled by first subtracting the value for the zero contrast stimulus from all ratios, then dividing by the ratio for the highest contrast condition (Fig. 1D). In this way, the scaled ratio for the zero-contrast stimulus is fixed at 0 and for the highest-contrast stimulus is fixed at 1. The scaled log-likelihood ratios across all presented contrasts were then fitted by local linear regression using the loess() function in R\(^2\) with span parameter set to 0.75. The contrast at which the fitted function passed through 0.5 was taken as an estimate of contrast sensitivity (see Fig. 1D). In some cases, particularly when low numbers of presentations are made, the fitted function passes through 0.5 twice, in which case we take the higher contrast crossing point as the estimate of contrast sensitivity as this estimate was more stable across different numbers of trials. On rare occasions, again typically with few presentations, the function does not pass through 0.5 at all, in which case we take the contrast at which the function passes closest to 0.5 as the estimate of contrast sensitivity.

**Behavioral Estimation of Contrast Sensitivity**

For each participant, proportion of correct responses from the interleaved response trials was fit by maximum likelihood estimation with a modified cumulative Gaussian function of the form

\[
\psi(x) = 0.5 + (0.5 - fn) \times G(x, \mu, s)
\]

(8)

where \(x\) represents contrast in %, \(fn\) represents the lapse rate defining the upper asymptote, and \(G(x, \mu, s)\) represents the cumulative Gaussian function with mean \(\mu\) and spread (SD) \(s\). The upper asymptote was allowed to vary as a free parameter, while the lower asymptote was fixed at 50%, as per the recommendations of Wichmann and Hill.\(^{30}\) Contrast detection threshold was taken as the 75% correct point on the fitted psychometric functions. This method of measuring the behavioral contrast sensitivity using full psychometric functions from a method of constant stimuli can be considered a laboratory “gold standard” approach against which to compare the ocularmetric estimates. Due to the time taken to measure sensitivity with this method, most current clinical vision tests use highly abbreviated procedures that sacrifice precision for brevity.
FIGURE 1. Schematic representation of the method for estimating contrast sensitivity from microsaccade data. (A) The observed number of microsaccades in each post-stimulus onset time bin for one participant (400 trials per contrast level). (B) The baserate (red line) is calculated as the mean probability of a microsaccade occurring in a time bin (50 ms) prior to stimulus onset (darker shaded gray lines, Equation 3). The maxrate (blue line) is the probability of a microsaccade occurring in each post-stimulus onset time bin when the stimulus is of the highest contrast (Equation 2). Other contrasts are shown in gray. (C) The likelihood of observing the measured number of microsaccades in each post-stimulus onset time bin...
(shown in A) given the baserate (red line, Equation 6) and maxrate (blue line, Equation 7) as generating functions is calculated for each contrast. (D) Loglikelihood ratios between the maxrate and baserate are calculated and scaled such that the value for the zero-contrast stimulus is fixed at 0 and the highest contrast stimulus is fixed at 1. Local regression (loess) is then used to fit a function relating these scaled loglikelihood ratios to stimulus contrast. The contrast at which this function reaches 0.5 is taken as the estimate of contrast sensitivity (arrow). Colors of individual points in panels C and D relate to the rate signatures for different stimulus contrasts in panel A. Dashed vertical lines in panels A and B represent the time of stimulus onset. See text for further details of the method.

Assessment of Efficacy

Estimates of contrast sensitivity from microsaccades were compared to the reference standard of those obtained behaviorally from the interleaved response trials. Overall accuracy and precision was calculated as the mean difference and standard deviation difference from the behavioral estimate (including all trials) respectively across all participants.

For both methods, estimates of accuracy and precision were made with varied numbers of presentations per contrast level up to the total number collected (80 for the behavioral method, 400 for the microsaccade method); presentations were randomly sampled with replacement from the dataset, and this was repeated 200 times to derive bootstrap 95% confidence intervals on the estimates.

To assess the potential utility of the microsaccade method under conditions that produce approximately similar measurement precision to behavioral methods in common use, we considered relationships between contrast sensitivity estimates from microsaccade and behavioral responses with unequal presentation numbers. Specifically, relationships between contrast sensitivity estimates from microsaccades with 400 presentations per contrast level and behavioral responses with 20 presentations per contrast level were measured by Spearman correlation and Bland-Altman analysis. Relationships between both microsaccade and behaviorally measured contrast detection thresholds and Pelli-Robson contrast sensitivity were also investigated by Spearman correlation. All analyses were conducted in R, version 3.2.0.29

RESULTS

A total of 40 people took part in this study (20 with normal vision, 10 with reduced vision due to amblyopia, and 10 with reduced vision due to cataract). Two participants’ data were excluded due to behavioral data not spanning 75% correct (1 with normal vision, 1 with cataract). All included participants (n = 38, 19 with normal vision, 10 with reduced vision due to amblyopia, 9 with reduced vision due to cataract) produced behavioral data that could acceptably fit with the modified cumulative Gaussian function (Equation 8). No inclusion constraints were placed on the eye tracking data. All participants also completed a total of 2800 passive trials with successful eye tracking (blinks removed, 400 presentations per contrast level). All young, visually healthy participants (n = 19, median age 25 years, range 20–40) completed the task with stimulus spatial frequency set at 12 cycles/deg. Clinical characteristics and tested spatial frequencies of all participants with cataract or amblyopia are given in the Table.

Figure 2 shows four example rate signatures when stimulus contrast was maximal. Heterogeneity in shape of rate signatures between participants was a feature of the data, and we found no pronounced relationship between shape features and group (normal, cataract, or amblyopia). While the “classical” rate signature shape was observed in some participants (e.g., Fig. 2A); other participants had clear differences such as a lack of pronounced inhibition (Fig. 2B), lack of rebound (Fig. 2C), latency differences in inhibition or rebound phase (Fig. 2D), or some combination of these.

Figure 3 shows the association between thresholds measured by the two methods when the behavioral method uses 20 presentations per contrast level and the microsaccade method uses 400 presentations per contrast level. This ratio in presentations per contrast level of 1:20 was chosen to produce approximately similar precision between the two methods (see later). Spearman’s rank correlation between thresholds from the two methods was 0.74 (P < 0.001). Median absolute difference in contrast sensitivity estimates between the two methods (microsaccade or behavioral) was 7.6%. Bland-Altman 95% limits of agreement were −20.7% to 25.0%. On average, microsaccade-based estimates of contrast sensitivity were 1.2% higher than behavioral estimates.

Figure 4 shows the accuracy and precision of contrast detection thresholds across all participants estimated from microsaccade rates when the behaviorally measured thresholds are used as the reference standard and the number of presentations per contrast level is varied. Figure 4 also shows similar data for the behavioral thresholds, derived by randomly subsampling the data. Precision—quantified as standard deviation of threshold errors (difference from reference standard)—improved with increasing number of presentations for both methods (Fig. 4B).

Our data suggest that a ratio in number of presentations of more than 20:1 between the two modalities is required to equate precision in output thresholds using the current approach. Allowing for 1500 milliseconds per presentation in both modalities (sufficient time for a behavioral response to be made or the microsaccade rate to return to baseline), measuring contrast sensitivity to a stimulus with equivalent precision would take approximately 70 minutes using microsaccades, compared to around 3.5 minutes behaviorally using 7 contrast levels, 20 presentations per level.

Contrast sensitivity measured using the Pelli-Robson letter chart was highly quantized and did not correlate with the laboratory-based behavioral estimates or microsaccade-based estimates of contrast sensitivity (Fig. 5; microsaccade Spearman’s rho 0.12, P = 0.46, behavior Spearman’s rho 0.06, P = 0.74).

DISCUSSION

Microsaccade rate signatures elicited by visual stimuli are modulated by properties of the stimulus such as contrast. Previous studies have demonstrated the potential utility of involuntary microsaccades as an objective marker of
visual sensitivity, although these studies were limited to young, visually normal participants with a narrow range of contrast thresholds.\textsuperscript{13,18} It follows that there may be a future use of such ocularmetric measures in objectively estimating visual sensitivity in clinical populations.

In the present study, we deliberately recruited participants with cataract and amblyopia, as well as young visually healthy participants, to test our methods on a clinically diverse population with varied ages and visual functions. We found considerable heterogeneity in microsaccade rate signatures across our study population, including in the timing of the individual phases, and indeed whether each distinct phase occurs at all (Fig. 2). These between-individual differences in microsaccade signature bore no apparent relationship in our sample with contrast sensitivity, clinical condition (normal, amblyopia, or cataract), or age.

Given the apparent heterogeneity of rate signature shapes in the wider population observed herein, it is important that methods that estimate aspects of visual or nonvisual function from microsaccade rates are robust to differences in rate signature. In this study, we have demonstrated a tractable, probabilistic inference-based approach to estimating contrast sensitivity from the microsaccade rate signature. The method accommodates the heterogeneity of signatures we report and so works for any individual with a measurable microsaccade rate signature that exhibits a graded response to contrast, as was the case for all participants in this study.

Correlation between contrast detection thresholds estimated using gold-standard behavioral methods and from microsaccades was strong (Fig. 3). Mean difference between estimates from the two methods was small (1%), indicating that there is very little bias in the estimates from microsaccades relative to behavioral estimates. Though median absolute

### Table. Clinical Characteristics and Tested Spatial Frequencies for Each Participant With Amblyopia or Cataract, Identified by A or C, Respectively, in the First Column

<table>
<thead>
<tr>
<th>Participant</th>
<th>Age, y</th>
<th>Sex</th>
<th>Eye</th>
<th>Visual Acuity, logMAR</th>
<th>LOCS III Lens Classification\textsuperscript{25}</th>
<th>Spatial Frequency, Cycles/Deg</th>
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<tbody>
<tr>
<td>A1</td>
<td>34</td>
<td>F</td>
<td>L</td>
<td>0.20</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>A2</td>
<td>20</td>
<td>F</td>
<td>L</td>
<td>0.46</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>A3</td>
<td>19</td>
<td>F</td>
<td>L</td>
<td>0.20</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>A4</td>
<td>19</td>
<td>F</td>
<td>L</td>
<td>0.34</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>A5</td>
<td>50</td>
<td>M</td>
<td>R</td>
<td>0.62</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>A6</td>
<td>61</td>
<td>F</td>
<td>L</td>
<td>0.26</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>A7</td>
<td>20</td>
<td>F</td>
<td>R</td>
<td>0.62</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>A8</td>
<td>21</td>
<td>M</td>
<td>L</td>
<td>0.50</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>A9</td>
<td>58</td>
<td>M</td>
<td>R</td>
<td>0.34</td>
<td>-</td>
<td>8</td>
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<tr>
<td>A10</td>
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<td>L</td>
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<td>-</td>
<td>1</td>
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<tr>
<td>C1</td>
<td>71</td>
<td>F</td>
<td>L</td>
<td>0.10</td>
<td>NO 3.0, NC 3.0, C 2.0, P 0.0</td>
<td>2</td>
</tr>
<tr>
<td>C2</td>
<td>63</td>
<td>F</td>
<td>L</td>
<td>0.20</td>
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<td>4</td>
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<tr>
<td>C3</td>
<td>75</td>
<td>F</td>
<td>L</td>
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<td>M</td>
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<td>0.14</td>
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<td>4</td>
</tr>
<tr>
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<td>M</td>
<td>L</td>
<td>0.10</td>
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<td>2</td>
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<tr>
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<td>M</td>
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<tr>
<td>C7</td>
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<td>F</td>
<td>R</td>
<td>0.14</td>
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<tr>
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<td>F</td>
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<td>0.12</td>
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<td>2</td>
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<tr>
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<td>0.40</td>
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<td>2</td>
</tr>
</tbody>
</table>

**Figure 2.** Examples of rate signatures from four participants when stimulus contrast was maximal. Note the heterogeneity in shape of the rate signatures. Dashed vertical lines mark the time of stimulus onset. The clinical condition of each participant is indicated (A and C, normal; B, cataract; D, amblyopia), though we did not find any clear relationship between rate signature shape and clinical condition.
differences between the two measures were 7.6% (indicating that for 50% of participants, differences between the two estimates were smaller than this), Bland-Altman analysis showed 95% limits of agreement spanning approximately 20% either side of the mean. This indicates that while the two measurements of contrast sensitivity were closely correlated, in some cases they did not exhibit strong agreement, leading to the wide 95% limits. This limited agreement between the two modalities is likely to be multifactorial; the limited precision of both measures is likely to be one factor, potentially leading to large differences between their estimates in some cases. Another source of discordance may be the differences in nonvisual processing between converting a detected visual stimulus to a change in involuntary microsaccade behavior and making a sensory decision and voluntary motor output as required for a key press.

**Figure 3.** (A) Relationship between contrast detection thresholds estimated behaviorally and from microsaccades. In this instance, behavioral thresholds were estimated from 20 presentations per level, whilst microsaccade-based thresholds were estimated from 400 presentations per level, a ratio of 1:20. The dashed line represents unity. (B) Bland-Altman plot showing difference in thresholds between the two methods against their mean. Dashed line shows mean difference, dotted lines show 95% limits of agreement.

**Figure 4.** Effect of number of presentations per contrast level on accuracy and precision of threshold estimates across all participants for both behavioral (blue squares) and microsaccade (red dots) approaches. (A) Bias (accuracy) versus standard deviation (SD) of threshold estimates (precision) for different numbers of presentations per contrast level. Dashed circles show lines of equal root mean square error (RMSE). Number of presentations per level increases toward the bottom of the plot for both methods, numbers shown in italics give examples of the number of presentations for their nearest points. (B) The effect of number of presentations per contrast level on precision (standard deviation) of threshold estimates for both methods. Error bars in both plots represent 95% confidence intervals derived from 200 bootstrap repeats.
likely that the number of stimulus presentations contributing (see Fig. 1D). The procedure can then continue to present rate) and $P$ (observed microsaccades | baserate) exceeds 0.5 (see Fig. 1D). The procedure can then continue to present further stimuli in the usual way. In this format of testing, it is likely that the number of stimulus presentations contributing to one “seen/unseen” response will need to be at least 20 to approach the precision of a short behavioral method of

Precision of threshold estimates from microsaccades was worse than that of the behavioral method, and equating precision between the methods required microsaccade rate signatures to be measured across many more stimulus presentations per contrast level than would be made by an equivalently precise behavioral technique. However, the total time taken to measure contrast sensitivity using our method could be drastically reduced by approaches that reduce the amount of time required for each presentation. In the present study, we have observed microsaccade rate signatures following the presentation of single stimuli. Future work could be directed toward understanding how rate signatures triggered by multiple, sequentially presented stimuli combined. If the combined signatures can be separated, it would enable the presentation of stimulus “trains” in which multiple stimuli are presented in rapid sequence, and the microsaccade rate signature to all stimuli combined is then separated into responses to individual stimuli. Such an approach may allow multiple presentations to be made per second, either at the same contrast or at varied contrasts, greatly reducing test duration, but this remains a target for further work.

An advantage of our method is that it may be incorporated into conventional psychophysical frameworks. In this study, we took the simple approach of the method of constant stimuli that can be used with our method without adaptation. Adaptive methods such as staircase procedures or Bayesian techniques may also incorporate our method by repeatedly presenting the same stimulus in order to build up a microsaccade rate signature which can then be classified as “seen” or “not seen” depending on whether the scaled log-likelihood ratio between $P$ (observed microsaccades | max-rate) and $P$ (observed microsaccades | baserate) exceeds 0.5 (see Fig. 1D). The procedure can then continue to present further stimuli in the usual way. In this format of testing, it is likely that the number of stimulus presentations contributing to one “seen/unseen” response will need to be at least 20 to approach the precision of a short behavioral method of

Constant stimuli procedure. It should be noted, however, that current clinical psychophysical procedures, such as automated perimetry, commonly use highly abbreviated thresholding procedures $^{32}$ that result in considerably lower precision than even a short method of constant stimuli procedure, as investigated herein. $^{33,34}$ Fewer stimulus presentations per “response” may be required for the microsaccade method to deliver sufficiently precise threshold estimates for clinical purposes that are currently adequately served by such methods.

Even given the poor precision of the threshold estimates produced by the current approach compared to behavioral methods, there remain advantages over any behavioral method that requires subjects to respond. Such methods may be unable to return an estimate of any kind in clinical populations where responses cannot be readily measured, such as young children and those with cognitive disabilities. In these populations, microsaccade-based measures may be able to elicit clinically useful estimates of visual sensitivity, providing an alternative or complement to electrophysiological tests. Of course, further development of our methods would be needed before they could be tested in such populations. For example, since fixation stability is likely to be poor in such populations, it may be necessary to develop gaze-contingent stimulus display methods that are compatible with measurement of microsaccade rate signatures. Future efforts to improve the clinical utility of microsaccade-based measures of visual sensitivity should also aim to improve precision and therefore reduce the time taken to produce clinically useful measurements. Further development of the algorithms used to detect microsaccades could help in this respect, as could better understanding of the causes of heterogeneity in rate signature shape and how these can be exploited.

In conclusion, the shape of microsaccade rate signatures following presentation of brief, transient stimuli varies considerably among individuals, though the graded response to contrast is ubiquitous across these variations in shape. We have demonstrated a simple, tractable method for estimating contrast sensitivity from microsaccade rate following presentation of visual stimuli. The method appears robust to variation in contrast sensitivity and to heterogeneity in the shape and features of the microsaccade rate signature. It is flexible to different numbers of stimulus presentations and can be incorporated into conventional psychophysical frameworks, albeit currently requiring considerably longer test time than laboratory behavioral methods to achieve similarly precise results. This method, therefore, holds potential for further development toward future clinical use, though the immediate focus of development needs to be on improving the efficiency of data collection to ensure clinical viability.

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