

# Active Learning to Characterize the Full Contrast Sensitivity Function in Cataracts

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**Background:** To characterize contrast sensitivity function (CSF) in cataractous and pseudophakic eyes compared to healthy control eyes using a novel quantitative CSF test with active learning algorithms.

**Methods:** This is a prospective observational study at an academic medical center. CSF was measured in eyes with visually significant cataract, at least 2+ nuclear sclerosis (NS) and visual acuity (VA)  $\geq 20/50$ , in pseudophakic eyes and in healthy controls with no more than 1+ NS and no visual complaints, using the Manifold Contrast Vision Meter. Outcomes included Area under the Log CSF (AULCSF) and CS thresholds at 1, 1.5, 3, 6, 12, and 18 cycles per degree (cpd). A subgroup analysis was performed on cataract eyes with VA  $\geq 20/25$ .

**Results:** A total of 167 eyes were included, 58 eyes in the cataract group, 77 controls, and 32 pseudophakic eyes with respective median AULCSF of 1.053 (0.352) vs 1.228 (0.318) vs 1.256 (0.360). In our multivariate regression model, cataract was associated with significantly reduced AULCSF ( $P=0.04$ ,  $\beta=-0.11$ ) and contrast threshold at 6 cpd ( $P=0.01$ ,  $\beta=-0.16$ ) compared to controls. Contrast threshold at 6 cpd was significantly reduced even in the subgroup of cataractous eyes with VA  $\geq 20/25$  ( $P=0.02$ ,  $\beta=-0.16$ ).

**Conclusion:** The novel qCSF test detected disproportionate significant contrast deficits at 6 cpd in cataract eyes; this remained significant even in the cataractous eyes with VA  $\geq 20/25$ . CSF testing may enhance cataract evaluation and surgical decision-making, particularly in patients with subjective visual complaints despite good VA.

**Keywords:** cataract, contrast sensitivity function, Contrast sensitivity, qCSF method

## Introduction

Cataract surgery is one of the most commonly performed surgical procedures in all of medicine with an estimated 3.5 million cataract surgeries being performed in the United States every year and more than 23 million worldwide.<sup>1</sup> One of the particularities of cataract surgery is that the decision on when to operate is usually based on subjective visual impairment rather than objective, clinically measured visual function metrics. Usually, when a patient feels that their cataract interferes significantly with their everyday vision-related activities, the surgeon will proceed to perform cataract surgery. Symptoms of cataract include worsening vision, muted color intensity, increased glare, halos, image ghosting and poor night vision and have been shown to be strongly correlated with vision-related quality of life.<sup>2</sup>

Despite visual acuity (VA) being the traditionally used functional metric to objectively evaluate visual function in the clinical setting, various vision-related quality of life scales prove that functional vision in cataract patients cannot be adequately approximated by evaluating VA alone.<sup>3–8</sup> VA seems to correlate poorly with patients' perception of visual disability due to their cataracts.<sup>9,10</sup> It is not uncommon that cataract patients with very good visual acuity report subjective visual complaints.<sup>3,11–14</sup> These patients are actually as likely to show comparable improvements in symptoms and visual function following surgery as those with decreased visual acuity.<sup>15–17</sup> This underscores the need for functional metrics that correlate with patient reported outcomes (PRO) better than VA.

Contrast sensitivity (CS) quantifies the amount of lightness or darkness required to see an object compared to its background. The lower the contrast needed to detect an object (contrast threshold), the higher the contrast sensitivity.<sup>18</sup> Cataracts increase the intraocular light scattering and thereby reduce the retinal image contrast.<sup>19</sup> Compared to VA, contrast sensitivity seems to correlate better with subjectively perceived visual impairment<sup>20</sup> and vision-related everyday activities, including mobility,<sup>21</sup> target and face identification,<sup>22</sup> driving,<sup>23</sup> walking,<sup>24</sup> and reading,<sup>25</sup> inserting a key into a lock or a plug into a socket.<sup>20</sup> Further, contrast sensitivity has been shown to be impaired earlier in the course of ocular pathologies when VA is still unaffected,<sup>26,27</sup> the latter often under-estimating the onset and/or severity of visual impairment.<sup>28</sup> Despite its promising role in visual function assessment in cataract, imperfections in the currently available contrast tests have prevented the adoption of contrast sensitivity evaluation in routine cataract consults. Conventional laboratory contrast testing is too time-consuming for clinical purposes.<sup>29</sup> The traditionally used Pelli-Robson chart only evaluates contrast in one spatial frequency with typically large letters. Pre-printed letter charts that evaluate contrast across various spatial frequencies have shown ceiling/floor effects and poor test-retest repeatability.<sup>30–32</sup>

The quantitative contrast sensitivity function method (qCSF) leverages active-learning algorithms to test contrast sensitivity across multiple spatial frequencies, in a clinically feasible manner (2–5 minutes per eye)<sup>33,34</sup> and with both high test-retest reliability and sensitivity in detecting subtle changes in visual function.<sup>35</sup> It has already been applied in retinal disorders,<sup>36–40</sup> amblyopia,<sup>41</sup> glaucoma<sup>42</sup> and aging.<sup>43</sup>

Our group has previously employed the qCSF method and reported significant contrast sensitivity deficits in patients with macular disease and VA  $\geq 20/30$ , that persisted even in eyes with VA  $\geq 20/20^{-1}$ .<sup>44</sup> We herein present an initial prospective observational study employing the qCSF method to characterize contrast sensitivity function in cataractous and pseudophakic eyes compared to non-cataractous healthy controls.

## Methods

This is a prospective, cross-sectional study approved by the Massachusetts Eye and Ear Institutional Review Board (2019P001311) and conducted in accordance with the Declaration of Helsinki and the Health Insurance Portability and Accountability Act. Written informed consent was obtained from all study participants, including consent for publication. Cataract and control subjects were recruited from the Comprehensive Service and Retina Service at the Massachusetts Eye and Ear campus at Longwood. Enrolment took place between December 2016 and February 2019.

Eligible cataract subjects had at least one eye with symptomatic cataract, with at least 2+ nuclear sclerosis (NS) with or without additional cataract subtypes. NO1NC1 of the Lens Opacities Classification System (LOCS) III was considered as 1+NS, while NO2NC1, NO1NC2 or NO2NC2 as 2+NS.<sup>45</sup> Exclusion criteria included VA worse than 20/50, previous surgery, or any documentation of visually significant ocular pathology such as retinal, corneal or optic nerve disease. Control eyes were free of any visual significant ocular pathology. Eyes with a maximum of 1+ cataract of any subtype (no more than NO1NC1, no more than C1 and no more than P1) were included as controls so long as the subject did not complain of any visual symptoms; eyes with intraocular lenses without posterior capsular opacification were included in the pseudophakic group.

## Clinical Assessment

All subjects underwent routine clinical examination, as appropriate for their scheduled clinic visit. This included documenting ocular medical history, intraocular pressure measured by applanation tonometry (Tono-Pen® XL - Reichert Technologies, Depew, New York, USA) Snellen's visual acuity, and slit lamp examination. Fundus examination and refraction were completed in accordance with the patient's clinic visit. Pupillary dilation, if needed, was deferred until after visual testing was completed, so as to not interfere with contrast sensitivity testing at distance. Grading of the cataract was performed as part of the regular exam following dilation.

## Contrast Sensitivity Testing Protocol

Patients were tested for qCSF during their regularly scheduled clinical visits prior to dilation in a standardized environment by a trained research assistant. The study eye or eyes were tested individually, with the fellow eye covered. Subjects used their habitual correction if applicable. If subjects did not have their habitual correction, trial

frames with their most recent refraction were used. The Manifold Platform (Adaptive Sensory Technology, San Diego, CA) was employed, following a methodology previously described by our group.<sup>39</sup> This novel computational device uses an adaptive computerized Bayesian algorithm to select personalized spatially-filtered optotypes that modulate in both contrast and spatial frequency. Specifically, three filtered Sloan letters of the same spatial frequency are simultaneously displayed in a horizontal line on the testing screen with decreasing contrast. The contrast of the rightmost letter is chosen by the quantitative CSF method and is usually near threshold contrast, with the middle and left letters displayed at two and four times the contrast of the right letter, respectively.<sup>33</sup> As these spatially-filtered optotypes are presented to the patient, the Bayesian algorithm utilized continuously updates and queries the probability of CSF parameters in order to maximize information gain.<sup>33</sup> The patient verbally reports the letters to the experimenter who operates the test with a handheld tablet. The experimenter records either “correct”, “incorrect” or “no response.” The active learning system uses the collected patient data at one spatial frequency to improve sensitivity estimates across all frequencies. This allows for CS testing over a wide range of contrast (128 possible contrasts, 0.002% to 100%) and spatial frequency (19 optotype sizes, approximately 1 to 27 cycles per degree). This also allows relatively quick testing times (2–5 minutes per eye) and have been shown to have robust test-retest reliability.<sup>34,35</sup> The test then estimates a CSF curve based off the 25 CSF trials, generating the area under the log contrast sensitivity function curve (AULCSF), integrated from 1 to 18 cycles per degree. The AULCSF is the broad metric of overall CSF.

## Study Outcome Measures

Main outcomes measured in this study were outputs of the qCSF method, including AULCSF, contrast acuity (CA), and CS thresholds at six spatial frequencies [1, 1.5, 3, 6, 12, 18 cycles per degree (cpd)]. AULCSF represents a global measure of contrast sensitivity function. Contrast acuity measures the smallest optotype visible with the highest level of contrast (ie the spatial frequency where contrast threshold is 100%, illustrated by the intersection of the contrast sensitivity function curve with the x-axis). Contrast sensitivity thresholds quantify the lowest contrast level that can be seen at each spatial frequency. Baseline demographics were collected for all patients. Visual acuities were recorded and converted from Snellen’s VA to logMAR.

## Statistical Analysis

Analysis was carried out using R version 3.4.1. The distribution of continuous numerical data was checked both graphically (histogram and boxplots) and by Shapiro–Wilk Normality Test. Non-normally distributed data was reported as median with Inter-Quartile Range (IGR) and assessed for statistical significance by non-parametric tests like Mann Whitney *U*-Test. Categorical variables were assessed by Pearson’s Chi-squared test with Yates’ continuity correction when applicable. The statistical significance level was set as to two sided *p*-values <0.05. Mixed effects multiple linear regression model fit by REML (Restricted maximum likelihood) was used to account for the inclusion of both eyes of the same patient. These multilevel mixed models were used to assess the following Contrast Sensitivity Function outcomes like AULCSF, CA, and contrast thresholds at 1, 1.5, 3, 6, 12, 18 cpd between the different groups. Predictor variables were selected based on their unadjusted log-rank statistical significance ( $p \leq 0.250$ ) on the univariate analyses for all the potential confounders (eg age, gender, lens status, etc.) separately, for the initial multivariate model. A backward stepwise elimination procedure, based on the Akaike Information Criteria and statistical significance ( $p \leq 0.05$ ), was then used to achieve the multivariate models presented. Finally, using the approach described above, we evaluated the qCSF outcomes among various groups in our cohort.

## Results

A total of 167 eyes were included, 58 eyes of 45 subjects with cataract(s) disease (19 males and 26 females), 77 eyes of 72 healthy controls (40 males and 32 females) and 32 pseudophakic eyes of 27 patients (12 males and 15 females). The median (IQR) age was 69.00 (11.00) years in the cataract group, 54.00 (19.50) years in the control group ( $P < 0.001$ ) and 70.00 (8.00) years in the pseudophakic group ( $P < 0.001$ ). Median (IQR) logMAR visual acuity was 0.097 (0.12) in the cataract group (20/25 Snellen equivalent) versus 0.00 (0.08) in the control group (20/20 Snellen equivalent) ( $P < 0.001$ ),

**Table 1** Baseline Characteristics

	Cataract	Control	Pseudophakic	p-value
Total Patients N (%)	45 (31.25)	72 (50.00)	27 (18.75)	
Sex, Female: N (%)	26 (57.78)	32 (44.44)	15 (55.56)	0.32
Age: Median (IQR)	69.00 (11.00)	54.00 (19.50)	70.00 (8.00)	<0.001
Total Eyes N (%)	58 (34.73)	77 (46.11)	32 (19.16)	
Eye, OD: N (%)	31 (53.45)	45 (58.44)	17 (53.12)	0.80
logMAR: Median (IQR)	0.097 (0.12)	0.000 (0.06)	0.010 (0.08)	

**Abbreviations:** IQR, inter-quartile range; logMAR, logarithm of the minimum angle of resolution.

versus 0.010 (0.08) in the pseudophakic group (20/20 Snellen equivalent)(P < 0.001). Baseline characteristics are summarized in Table 1.

### CSF Outcome Measures in Cataract Eyes versus Controls versus Pseudophakic Eyes

AULCSF, CA and the contrast sensitivity thresholds at each specific spatial frequency for the cataract, control and pseudophakic group are shown in Table 2. (Table 2) AULCSF in the cataract group was 1.053 (0.352) versus 1.228 (0.318) in the control group versus 1.256 (0.360) in the pseudophakic group. When controlling for age in our multivariate regression model, the presence of cataract was associated with significantly reduced AULCSF compared to controls (P= 0.04,  $\beta$ = -0.11) while pseudophakia was not associated with significantly different AULCSF compared to controls (P=0.27,  $\beta$ = 0.07).

CA in the cataract group was 1.201 (0.170) versus 1.308 (0.216) in the control group versus 1.268 (0.178) in the pseudophakic group. When controlling for and age in our multivariate regression model, the presence of cataract was not associated with significantly reduced CA compared to controls (P= 0.12,  $\beta$ = -0.05) and pseudophakia was not associated with significantly different CA compared to controls (P=0.13,  $\beta$ = 0.05).

CSF threshold at 1 cpd in the cataract group was 1.339 (0.185) versus 1.387 (0.208) in the control group versus 1.409 (0.268) in the pseudophakic group. When controlling for age and sex in our multivariate regression model, the presence of cataract was not associated with significantly reduced CSF threshold at 1 cpd compared to controls (P= 0.80,  $\beta$ = 0.01) and pseudophakia was not associated with significantly different AULCSF compared to controls (P= 0.11,  $\beta$ = 0.06).

CSF threshold at 1.5 cpd in the cataract group was 1.365 (0.199) versus 1.448 (0.230) in the control group versus 1.450 (0.242) in the pseudophakic group. When controlling for age and sex in our multivariate regression model, the presence of cataract was not associated with significantly reduced CSF thresholds at 1.5 cpd compared to controls (P= 0.79,  $\beta$ = -0.01) and pseudophakia was not associated with significantly different AULCSF compared to controls (P= 0.09,  $\beta$ = 0.07).

CSF threshold at 3 cpd in the cataract group was 1.347 (0.225) versus 1.460 (0.275) in the control group versus 1.447 (0.237) in the pseudophakic group. When controlling for age in our multivariate regression model, the presence of

**Table 2** Contrast Sensitivity Outcome Metrics for the Cataract, Control and Pseudophakic Group

	Cataract (N = 58)	Control (N = 77)	Pseudophakic (N = 32)
AULCSF	1.05 (0.35)*	1.22 (0.32)	1.26 (0.36)
CA	1.20 (0.17)	1.31 (0.22)	1.27 (0.18)
1 cpd	1.34 (0.19)	1.39 (0.21)	1.41 (0.27)
1.5 cpd	1.37 (0.20)	1.45 (0.23)	1.45 (0.24)
3 cpd	1.35 (0.23)	1.46 (0.28)	1.48 (0.24)
6 cpd	1.05 (0.46)*	1.25 (0.24)	1.31 (0.40)
12 cpd	0.39 (0.56)	0.69 (0.49)	0.64 (0.47)
18 cpd	0.00 (0.05)	0.18 (0.48)	0.04 (0.37)

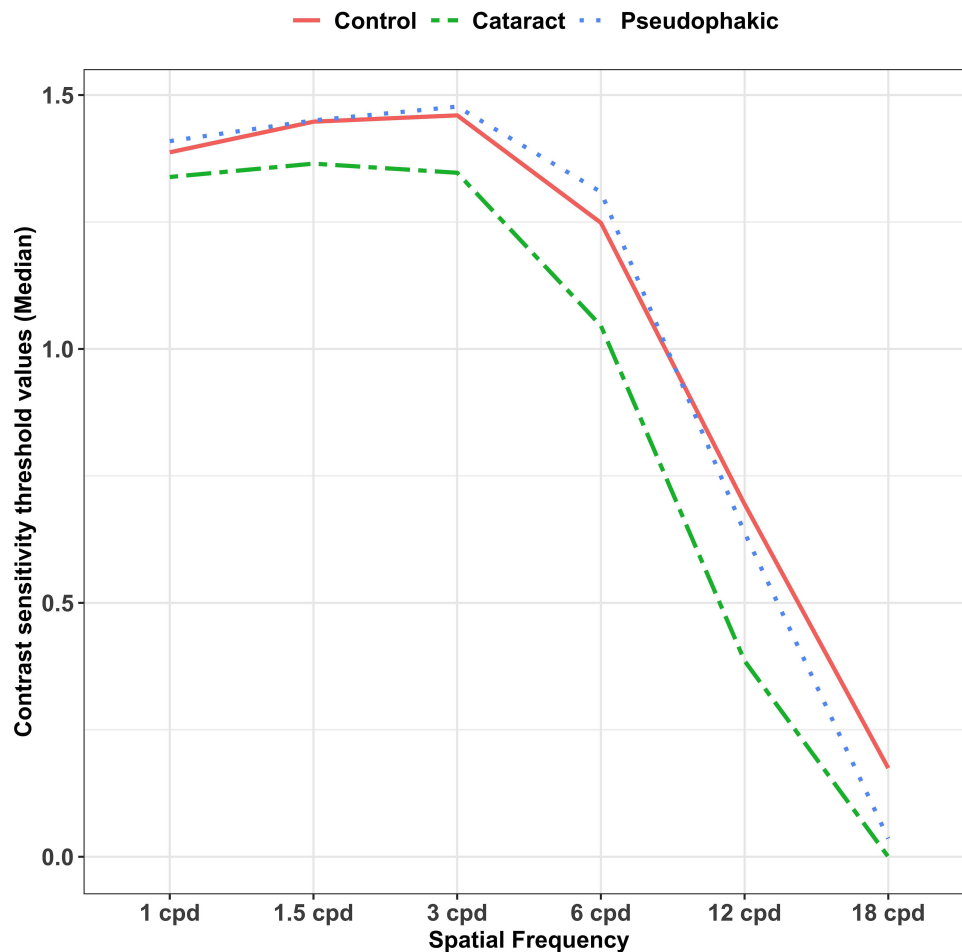
**Notes:** Values for contrast outcomes are presented as median (IQR). \*Signifies significantly different values compared to controls.

**Abbreviations:** AULCSF, area under the log contrast sensitivity function curve; CA, contrast acuity; cpd, cycles per degree.

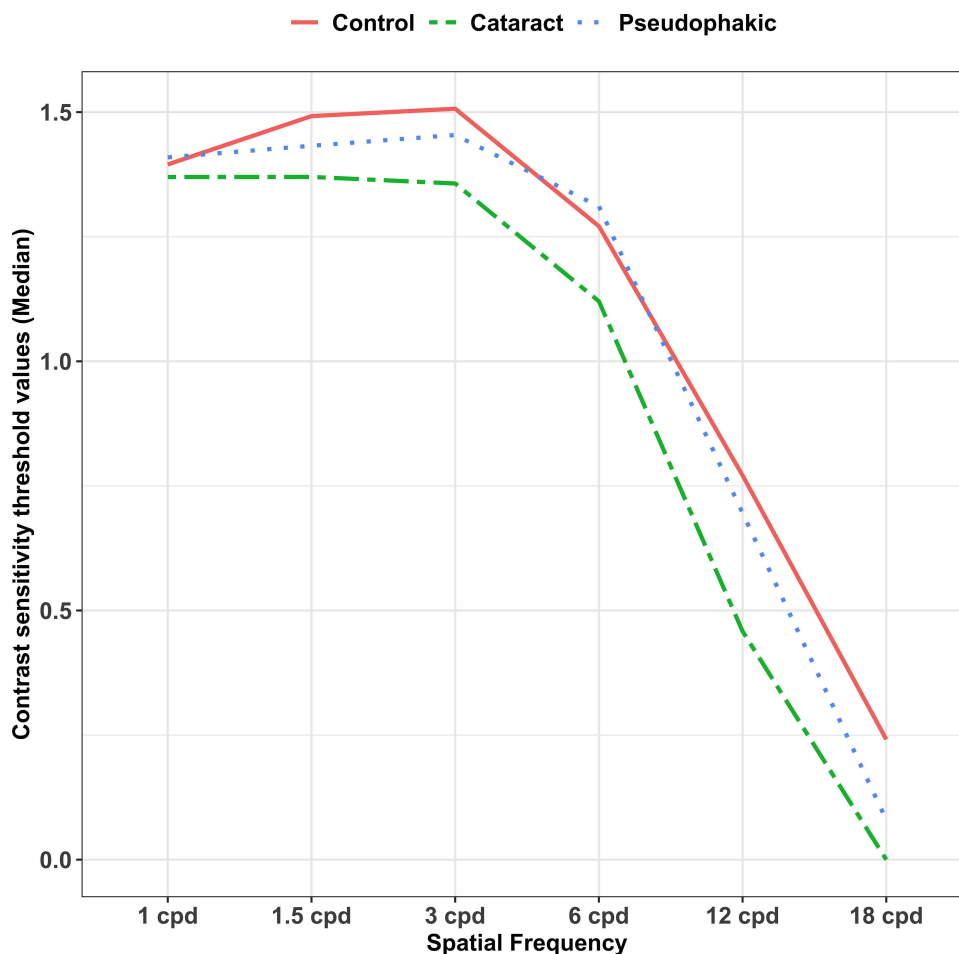
cataract was not associated with significantly reduced CSF thresholds at 3 cpd compared to controls ( $P=0.07$ ,  $\beta=-0.08$ ) and pseudophakia was not associated with significantly different AULCSF compared to controls ( $P=0.32$ ,  $\beta=0.05$ ).

CSF threshold at 6 cpd in the cataract group was 1.046 (0.457) versus 1.248 (0.239) in the control group versus 1.309 (0.406) in the pseudophakic group. When controlling for age in our multivariate regression model, the presence of cataract was associated with significantly reduced CSF thresholds at 6 cpd compared to controls ( $P=0.01$ ,  $\beta=-0.16$ ) while pseudophakia was not associated with significantly different AULCSF compared to controls ( $P=0.48$ ,  $\beta=0.05$ ).

CSF threshold at 12 cpd in the cataract group was 0.386 (0.561) versus 0.694 (0.485) in the control group versus 0.638 (0.469) in the pseudophakic group. When controlling for age in our multivariate regression model, the presence of cataract was not associated with significantly reduced CSF threshold at 12 cpd compared to controls ( $P=0.10$ ,  $\beta=-0.12$ ) and pseudophakia was not associated with significantly different AULCSF compared to controls ( $P=0.20$ ,  $\beta=0.11$ ). CSF thresholds at 18 cpd in the cataract group was 0.000 (0.051) versus 0.175 (0.480) in the control group versus 0.036 (0.368) in the pseudophakic group. When controlling for in our multivariate regression model, the presence of cataract was not associated with significantly reduced CSF thresholds at 18 cpd compared to controls ( $P=0.17$ ,  $\beta=-0.07$ ) and pseudophakia was not associated with significantly different AULCSF compared to controls ( $P=0.40$ ,  $\beta=0.05$ ) Figure 1 represents the AULCSF, CA and reductions in contrast sensitivity thresholds at each spatial frequency in cataract eyes compared to control eyes and pseudophakic eyes (Figure 1).



**Figure 1** Area under the Log contrast sensitivity function curve (AULCSF), contrast acuity (CA) and reductions in contrast sensitivity thresholds at each spatial frequency in cataract eyes compared to control eyes and pseudophakic eyes.



**Figure 2** Area under the Log contrast sensitivity function curve (AULCSF), contrast acuity (CA) and reductions in contrast sensitivity thresholds at each spatial frequency in cataract eyes compared to control eyes and pseudophakic eyes in the subgroup analysis on eyes with very good visual acuity (VA  $\geq 20/25$ ).

### Subgroup Analysis on Eyes with Very Good Visual Acuity (VA $\geq 20/25$ )

In a subgroup analysis including only the eyes with vision equal or better than 20/25, (27 cataract versus 63 control eyes), after controlling for age, contrast threshold at 6 cpd remained statistically significantly reduced in the cataract group when compared to the control group ( $P=0.03$ ,  $\beta=-0.14$ ). Reductions in AULCSF, CA and in the lower (1, 1.5, 3 cpd) and higher (12, 18 cpd) spatial frequencies were also observed, though without reaching statistical significance (Figure 2).

### Discussion

In this initial prospective cross-sectional observational study, we employed the novel qCSF method to characterize contrast sensitivity function in cataract disease and pseudophakic eyes compared to healthy controls. We detected a generalized decrease in the broad metric of CSF, AULCSF, and disproportionate deficits in contrast sensitivity thresholds in cataract eyes with subjective symptomatology versus healthy controls. Importantly, these disproportionate deficits in contrast sensitivity persisted even in cataract eyes with VA as good as 20/25 or better. This suggests that contrast sensitivity measured with the qCSF method may be able to detect more subtle changes in visual function in eyes with cataract than the VA does.

Perhaps most importantly, we detected a disproportionate decrease in contrast thresholds at the spatial frequency of 6 cpd, a spatial frequency that is particularly linked with vision-related everyday life activities. It has been shown that contrast sensitivity at 3 and 6 cpd is associated with everyday activities such as finding a door on a wall or discerning

a bus from a car<sup>31</sup> while contrast sensitivity thresholds specifically at 6 cpd was found to be the best predictor of road sign and object identification.<sup>22</sup> The presence of cataract was not associated with significantly reduced CSF threshold at lower (1, 1.5, 3 cpd) or higher (12, 18 cpd) spatial frequencies. Pseudophakia was not associated with significantly different contrast outcome measures compared to control eyes. Of note, the contrast sensitivity deficits at 6 cpd detected herein in cataractous eyes would have gone unnoticed if contrast sensitivity testing was performed using the traditional Pelli-Robson chart that only tests contrast at the spatial frequency of 0.5–1cpd (when tested at 1 meter distance as the manual suggests). This is clinically relevant, and may lead physicians to overestimate the functional vision of patients during the cataract surgery decision-making process.

Many cataract studies have incorporated various contrast sensitivity tests attempting to evaluate patients' visual function in a more accurate and comprehensive manner.<sup>4–8</sup> Measuring contrast sensitivity thresholds across a wide spectrum of spatial frequencies seems to provide valuable information regarding how visual deficits may affect patients' vision-related quality of life. As the traditional Pelli-Robson test is limited in that respect, other contrast sensitivity tests have been developed and reported in the clinical studies. However, none of these tests have yet been established and incorporated into the routine clinical practice. Currently available tests that evaluate contrast thresholds across various spatial frequencies such as the Vistech or the Functional Acuity Contrast Test (FACT) tests have been repeatedly shown to have ceiling effects, floor effects and poor test-retest repeatability such that they are not used in clinical practice.<sup>30,46,47</sup> With the advent of the qCSF method, active learning algorithms enable the global shape of contrast sensitivity function to be tested in a time-efficient (2–5 minutes per eye) manner, across multiple spatial frequencies; at the same time the qCSF test has been shown to have high sensitivity in detecting subtle changes in visual function and great test-retest repeatability.<sup>34,35</sup>

An increasing importance is being placed on patient reported outcomes in cataract surgery, a measure that is guided mainly by the subjective visual impairment reported by the patient rather than deteriorating objectively measured visual function metrics. A visual function outcome that correlates to subjective visual impairment and vision-related everyday life activities better than visual acuity does, will enhance the clinician's understanding of the patient's experience of their vision. This will in turn guide surgical decision making in a patient-centered manner.

Unsurprisingly, cataract surgery improves not only visual function but also overall quality of life, self-assessed health and mental health, cognition, depression, earnings, mobility, self-care, life satisfaction, self-efficacy and self-reported overall happiness.<sup>48–50</sup> Further, patient happiness after cataract surgery is strongly correlated with subjectively perceived improvement in their functional vision.<sup>48</sup> For cataract surgeons, being able to routinely evaluate visual function in a way that is both objective and highly correlated with patients' subjective visual complaints, would render surgical decision making a patient-centered process. Contrast sensitivity has been suggested in the past as a visual function metric that strongly correlates with subjective visual impairment and vision-related quality of life. Now with the time-efficient, sensitive and highly reliable qCSF method, contrast sensitivity has the potential to be incorporated in the routine clinical practice.

There are certain limitations in this study. This is only a moderate sample size cross-sectional study that included patients with at least 2+ nuclear sclerosis that may or may not also have other coexisting cataract subtypes. Therefore our results cannot be generalized to all cataract subtypes nor directed towards specific cataract subtypes. Another limitation of this exploratory study is the lack of standardization of cataract grading among several providers. Ideally future studies should employ the LOCS III system to improve the consistency of the grading.<sup>45</sup> We set out to compare eyes with visually significant cataract to eyes that lacked visually significant ocular pathology. While none of the control subjects had visual symptoms, some did have mild opacification of the lens, no greater than 1+ NS. The fact that we still found statistically significant reductions in contrast sensitivity in our cataract study group compared to a control group with some mild cataract only strengthens our findings. Additionally, while pseudophakia was not associated with decreased CSF compared to cataract or controls, a sub-analysis based on type of intraocular lens (IOL) was not performed. Further studies utilizing qCSF testing to characterize CSF in pseudophakic eyes with different types of IOL would be valuable. A longitudinal follow up study is ongoing aiming to showcase the improvement in contrast sensitivity function after cataract surgery, investigating whether the beneficial effect on cataract surgery is even greater than the one currently measured employing VA as the only visual function metric.

In conclusion, employing the qCSF method, we detected disproportionate deficits in contrast sensitivity thresholds in cataract eyes versus controls, at specific spatial frequencies that are particularly linked with vision-related everyday life

activities; these disproportionate deficits in persisted even in cataract eyes with VA  $\geq 20/25$ , suggesting that contrast sensitivity may be able to detect more subtle changes in visual function of eyes with cataract than the traditional VA testing does. These deficits would have been missed using the traditional Pelli-Robson chart. Contrast sensitivity measured with the qCSF emerges as a promising adjunct visual function endpoint, with the potential to be incorporated in the standard routine cataract evaluation to enhance surgical decision-making for cataract surgery.

## Data Sharing Statement

Data will be available upon reasonable request to Filippos Vingopoulos, filippos\_vingopoulos@meei.harvard.edu.

## Author Contributions

Concept and design: FV, MK, RAV, ACL, ZKL, JBM. Subject identification: RAV, ACL, ZKL, JBM. Data collection: FV, MK, RFS, RK. Data analysis: IG, MK. Data interpretation: FV, MK, IG. Initial manuscript draft: FV, MK. Critical Revision of the manuscript: FV, MK, IG, RFS, RK, RAV, ACL, ZKL, JBM. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

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