**Journal of Diabetes Science and Technology**
Volume 6, Issue 6, November 2012


SYMPOSIUM

# Simultaneous Noninvasive Clinical Measurement of Lens Autofluorescence and Rayleigh Scattering Using a Fluorescence Biomicroscope

John Burd, Ph.D.,[1] Stephen Lum, O.D.,[2] Frederick Cahn, Ph.D.,[1] and Keith Ignotz, B.A., M.B.A.[1]

## Abstract

***Background:***

Lens autofluorescence increases with the age of the subject, and the fluorophores responsible are associated with cataract, retinopathy, and other complications of diabetes. We built a scanning confocal lens fluorescence biomicroscope suitable for routine clinical measurement of lens autofluorescence and light scattering and report data from 127 healthy subjects.

***Method:***

The fluorescence biomicroscope focuses a beam of light from a blue light-emitting diode on the lens and measures fluorescent green light and blue scattered light using a sensitive silicon photomultiplier. The system includes a target fixation light and a video camera for alignment and automatic pupil tracking. Under software control, a volume of measurement is scanned from behind the posterior lens capsule, through the lens to the aqueous humor, and then back again. Software computes the average ratio of lens autofluorescence to scattered light in the central portion of the lens. Self-reported healthy nondiabetic subjects were examined by an optometrist; if their eyes were healthy and without significant cataract, they were entered into the study.

***Results:***

Valid lens autofluorescence data were collected from 127 subjects between 21 and 70 years of age. A linear model for lens autofluorescence intensity with age was highly statistically significant, and the improvement in fit for higher-order polynomial models was not statistically significant. The ratio of lens autofluorescence to light scatter was also calculated; regression analysis showed significant curvature for the relationship of the fluorescence ratio to age, so a nonlinear model was used to estimate the mean ratio of autofluorescence to scatter and its prediction intervals as a function of age.

*continued* →

**Author Affiliations:** [1]Freedom Meditech, San Diego, California; and [2]Luxottica, Milano, Italy

**Abbreviations:** (AGE) advanced glycation end product, (IR) infrared, (DFA) 1-deoxyfructosyl glycation adduct, (LED) light-emitting diode, (UV) ultraviolet

**Keywords:** advanced glycation end products, biomicroscope, diabetes mellitus, fluorescence ratio, lens autofluorescence

**Corresponding Author:** Frederick Cahn, Ph.D., BioMedical Strategies, 2450 Azure Coast Dr., La Jolla, CA 92037; email address *fcahn@biomedicalstrategies.com*

**Abstract cont.**

***Conclusions:***

Our observation of a strongly significant linear regression of fluorescence intensity with age of the subjects agrees with the results from previous studies, as does a nonlinear model for the fluorescence ratio. The fluorescence biomicroscope enables the clinician to identify patients with fluorescence ratio significantly higher than expected for their age.

*J Diabetes Sci Technol 2012;6(6):1251-1259*

## Introduction

The study of the autofluorescence of the human crystalline lens has a long history, beginning with reports from the 19th century.[1,2] Early clinical studies established that lens autofluorescence could be measured noninvasively and that fluorescence intensity increases with the age of the subject.[3,4]

That a noninvasive measurement of lens autofluorescence may have clinical utility is suggested by its physiological basis, which is the accumulation of "advanced glycation end products" (AGEs), a heterogeneous family of yellow–brown and fluorescent proteins that have been modified by glycation (the nonenzymatic reaction of reducing sugars with the free amino groups of proteins and subsequent irreversible reactions).[5] Although most proteins in living systems turn over with sufficient rapidity to avoid significant accumulation of AGEs, some, such as lens crystallins, nerve myelin, and skin collagen, are long lived, and AGEs accumulate in these proteins over a person's lifetime.[5,6] The accumulation of AGEs is believed to contribute to the gradual decline in tissue and organ function that is observed within the aging body.[7] Advanced glycation end products have been implicated in diseases of aging, including cardiovascular diseases.[8–13]

Because glycation is initiated by free reducing sugars, diabetes subjects have an accelerated accumulation of AGEs.[14–22] Thus lens autofluorescence, as a noninvasive measure of AGE accumulation, can be regarded as a measure of cumulative tissue damage due to elevated sugar in plasma and interstitial fluids.

The clinical application of lens autofluorescence has been limited by the unavailability of an instrument suitable for routine clinical use. We have developed, and are seeking U.S. marketing clearance for, a scanning confocal lens fluorescence biomicroscope with blue light-emitting diode (LED) excitation that rapidly measures green fluorescence within a volume of measurement that is scanned through the lens. Novel features include the ability to simultaneously measure blue Rayleigh scattered light and an infrared (IR) video system that detects and automatically tracks the position of the pupil so that measurements are fully automated. It is not necessary to dilate the pupil. By computing the ratio of fluorescence to scatter, most potential sources of measurement variation are eliminated, and an easily interpreted index is reported to the clinician.

To establish a baseline of normal eyes, we have used this instrument to measure the fluorescence intensity and fluorescence ratio of 127 self-reported healthy subjects from 21 to 70 years of age with healthy eyes, as verified by optometric examination.

## Methodology

### *Biomicroscope*

The ClearPath DS-120® lens fluorescence biomicroscope optical system (Freedom Meditech, San Diego, CA) consists of a blue (465 nm) LED excitation light with a 430–470 nm band-pass filter, source and collection focusing optics, motor-driven filter wheel with 25% neutral density (scatter), and long-pass (fluorescent emission 500–1650 nm) filters and a silicon photomultiplier light detector (**Figure 1**). In addition, there is a red blinking LED target fixation light positioned within red blinking concentric rings to aid the patient in self-alignment, three IR LED lights to illuminate the eye, and a video camera. A fluorescence reference target is positioned in the optical path during the self-test procedure at startup. Stepper motors control the optics window horizontal motion, chin rest motion,

filter wheel rotation, and optical focus point x, y, z positioning.

### *Startup Procedure*

The system performs a self-test when the fluorescence biomicroscope software starts up. The self-test procedure verifies computer communication with the optical system; resets and zeros the x, y, z positions of the focal point with respect to its switch limits; and starts the filter wheel. The self-test also verifies that the device is operating within normal limits by measuring the fluorescence reference target.

### *Operation*

Only the patient's chin and forehead contact the ClearPath DS-120 lens fluorescence biomicroscope. The patient is positioned with the forehead centered on the headrest. The eye is illuminated by three near-IR 880 nm LED lights and is observed by an IR-sensitive charge-coupled device video camera. An image of the eye is displayed on the computer screen to assist the operator in the alignment of the patient.

The headrest is adjusted manually to bring the corneal plane of the test eye close to the optics window so that the eye is in focus. The patient is instructed to look at the red blinking LED fixation light that is surrounded by red blinking concentric rings. The operator adjusts the optics window and chin rest by clicking arrow icons on the computer screen.

The software includes an automatic tracking program for positioning the pupil. The system automatically aligns its optical axis before a measurement is taken.

The patient is instructed to close and open the eye (to wet the cornea with a tear film to reduce blinking) and the operator clicks on the start icon to begin the scan.

The blue LED light source is focused to achieve a converging excitation beam of blue light that is initially positioned just behind the posterior lens capsule. The collection optics are confocally aligned within a 1 mm diameter and 3 mm long volume of measurement that is scanned through the lens in 0.31 mm steps (**Figure 2**). In the eye, this blue light is scattered by elastic (Rayleigh scattering) and inelastic (fluorescent) interactions with lens proteins.

A filter rejects red and IR light from the positioning lights. A rotating filter wheel chops the beam into blue/green (primarily Rayleigh scattered) and green (fluorescent)

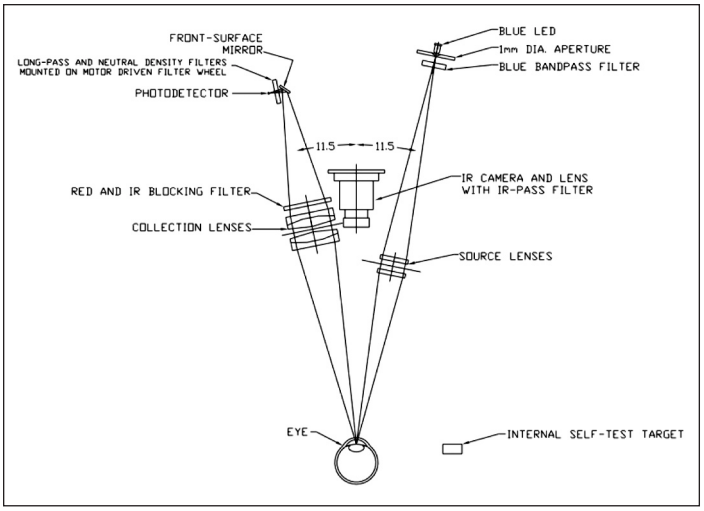


**Figure 1.** Optical system. Dia, diameter.

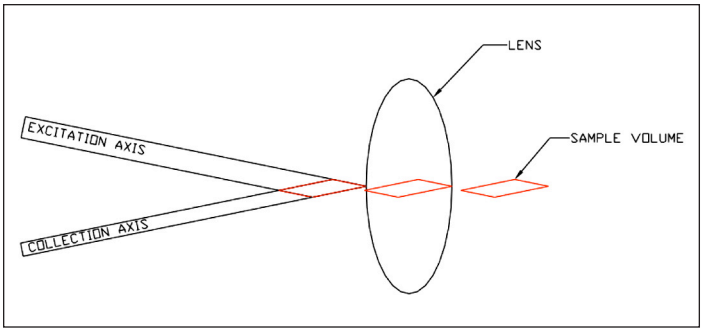


**Figure 2.** Diagram of volume of measurement.

segments. The scattered and fluorescent light is focused on a highly sensitive silicon photomultiplier, and the signals are sent to the analog-to-digital converter on the optics control board and then to the computer.

Under software control, the volume of measurement at the focal point of the light source and detector is scanned from just behind the posterior lens capsule, through the lens, through the anterior lens capsule to the aqueous humor, and then back again. Computer software records both scattered and fluorescent light during the forward and reverse scan and constructs a graph of each that is displayed on the computer monitor. Software detects the front and back surfaces of the lens capsule on the graph, estimates the apparent thickness of the lens, and computes the average of the ratio of lens autofluorescence to scattered light in the central portion of the lens. The software checks that the apparent lens thickness is within a physiological range. Software also detects anomalies in the scan, such as eye blinks, which could cause an inaccurate measurement, or excessive difference between the two scans.

For valid scans, the fluorescence ratio is reported; otherwise, for anomalous scans (too much difference in forward and reverse scans, anterior and posterior edges outside of physiological range, pupil too small, signal out of linear range, or fluorescence ratio outside of physiological range), an error code is reported on the computer monitor, and the fluorescence ratio is not reported. In the case of an error code, the clinician can rescan the eye.

If the scan is valid, software produces a report that is displayed on the screen and that can be printed for the patient and/or for the patient's file. The scan data are also automatically saved on the computer's hard disk.

### *Human Participant Selection Criteria*

Each participant signed an informed consent document, filled out a health questionnaire, and underwent an eye examination (including a slit lamp examination) by an optometrist before being tested with the study device. The following criteria were used to select patients:

*Inclusion Criteria*

- Subject must be aged between 21 and 70 years,
- Subject must be able to read or understand English and give informed consent,
- Subject must complete the health questionnaire, and
- Subject must have healthy eyes, as determined by the optometrist.

*Exclusion Criteria*

- Subject is not deemed healthy based on the comprehensive eye exam;
- Subject has type 1 or type 2 diabetes, as reported on the health questionnaire;
- Subject has had the crystalline lens removed from the test eye or has had the crystalline lens removed and replaced with an intraocular lens implant in the test eye;
- Subject has had a fluorescence angiogram within the past 6 months;
- Subject has undergone a treatment using photodynamic drugs within the past year;
- Subject has been clinically diagnosed with cataracts in the test eye, with any sign of opacification;
- Subject has ocular surface (dry eye) disease;
- Subject is unable to cooperate with or understand clinical instructions; or
- Subject is unable to complete test sequence.

Prior to performing the study, each operator went through a familiarization period. Familiarization included reading and understanding the procedure for the instrument and successfully performing a scan.

### *Fluorescence Intensity Measurement*

Subjects were scanned up to a maximum of eight scans; however, testing was terminated after five successful scans on the subject. A successful scan is one for which the instrument reports a fluorescence ratio instead of an error code. Of the 128 recruited into the study, 1 subject had no successful scans because of droopy eyelids and excessive blinking. There were a total of 628 successful scans among the 127 subjects that had at least one successful scan (mean, 4.9 successful scans/subject).

### *Data Collection*

In addition to reporting the fluorescence-intensity-to-scatter ratio to the clinician on the screen (as previously described), the fluorescence biomicroscope records fluorescence intensity and other scan details in a file on the computer's hard disk. The fluorescence intensity data shown in **Figure 3** was obtained from this file.

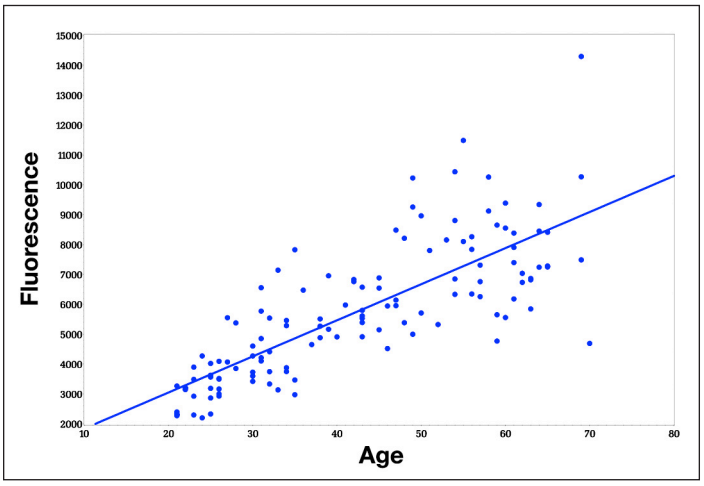


**Figure 3.** Mean fluorescence (arbitrary units) from healthy eyes of 127 healthy subjects versus age (years) from clinical study with regression line (fluorescence = 368 + 138 × age). $R^2 = 0.72$.

Demographic data were analyzed with the Frequencies procedure for SPSS version 19 software for Mac OSX (version 10.6) for descriptive data and the Means procedure for means, medians, and ranges. Fluorescence ratios were analyzed with SAS 9.1 for Windows with procedure PROC GLMMIX.

## Results

### *Demographics*

The study enrolled 128 men and women. One subject, a 41-year-old Asian male, had no valid scans and was removed from the data set. Thus there were 127 valid subjects in the data set.

Demographics of the patient population are shown in **Tables 1–4**. A small majority of the subjects were women. Ages ranged from 21 to 70 years, with a median age of 42 years.

### *Subject Health Characteristics*

Subjects who were enrolled were self-reported as healthy. Health parameters that subjects reported included cholesterol, hypertension, kidney disease, diabetic first-degree relative, and smoking (**Table 5**).

### *Regression of Fluorescence Intensity versus Age*

We performed a linear regression of mean subject fluorescence intensity versus age in years. Since Levene's test showed a highly significant heterogeneity of the variances of the deviations from the mean of replicate observations for each subject, the subject means were weighted using the inverse of the subject-specific variance. The linear model was highly significant, with $p << .001$ (**Table 6**). The improvement in fit for higher-order polynomial models was not statistically significant. **Figure 3** shows the subject mean fluorescence intensities and the linear regression model versus age.

### *Model of Fluorescence Ratio versus Subject Age*

The instrument achieves additional reliability by use of a ratio method that removes many of the potential sources of variation that apply to the measure of absolute fluorescence intensity. Thus, in addition to measuring green fluorescence, the instrument also measures blue scattered light. We used the scattering measurement to calculate the ratio of fluorescence intensity to scatter in order to correct the fluorescence for attenuation effects.

Replicate measurements of individual subjects showed a 5.5% coefficient of variability. Polynomial regression of

**Table 1.**
**Gender**

| Gender | Frequency | Percentage |
|---|---|---|
| Female | 71 | 55.9 |
| Male | 56 | 44.1 |
| Total | 127 | 100.0 |

**Table 2.**
**Age**

| Age range | Frequency | Percentage |
|---|---|---|
| 20–29 | 30 | 23.6 |
| 30–39 | 30 | 23.6 |
| 40–49 | 23 | 18.1 |
| 50–59 | 22 | 17.3 |
| 60–69 | 21 | 16.5 |
| 70 | 1 | 0.8 |
| Total | 127 | 100.0 |
| Mean age | 42.4 | |
| Median age | 42.0 | |

**Table 3.**
**Ethnicity (Reported by Subject)**

| | Frequency | Percentage |
|---|---|---|
| Asian/Pacific Islander | 19 | 15.0 |
| Black | 4 | 3.1 |
| Caucasian | 86 | 67.7 |
| Hispanic | 10 | 7.9 |
| Multi-race or other | 8 | 6.3 |
| Total | 127 | 100.0 |

**Table 4.**
**Height and Weight Characteristics**

| Gender | Statistic | Height (inches) | Weight (pounds) | Body mass index |
|---|---|---|---|---|
| Female | Mean | 63 | 133 | 22.7 |
| | Median | 64 | 130 | 21.6 |
| | Minimum | 49 | 95 | 18.3 |
| | Maximum | 72 | 200 | 33.8 |
| Male | Mean | 69 | 184 | 25.9 |
| | Median | 69 | 176 | 25.8 |
| | Minimum | 61 | 127 | 19.5 |
| | Maximum | 77 | 320 | 42.2 |

**Table 5.**
**Self-Reported Health**

| | Frequency | Percentage |
|---|---|---|
| High cholesterol | 17 | 13.4 |
| Hypertension | 9 | 7.1 |
| Kidney disease | 0 | 0.0 |
| Diabetic first-degree relative | 33 | 26.0 |
| Smoking | 42 | 33.1 |
| Total | 127 | 100.0 |

the fluorescence ratio against age showed significance for quadratic term; inclusion of gender, ethnicity, and self-reported health variables did not significantly improve the fit of the model to the data. Because the quadratic function shows a nonphysiological negative slope at high age, we used a nonlinear model to compute the mean predicted ratio and 80% and 95% prediction intervals (**Figure 4**):

$$y = \kappa/(1\_+ \exp(-\beta^*(x-\mu)),$$

where $y$ is the fluorescence ratio, $x$ is the subject age, $\kappa$ is the level of asymptote, $\beta$ is the rate of rise, and $\mu$ is the location of the inflection point. The parameters $\kappa$, $\beta$, and $\mu$ are estimated using nonlinear least squares regression, using only a single measurement for each subject in order to obtain the prediction interval for a single observation. The model was highly significant ($p < .0001$), with estimates of $\kappa = 0.22$, $\beta = 0.15$, and $\mu = 20.4$.

## Discussion

Our observation of a strongly significant linear regression of fluorescence intensity with age of the subjects agrees with the results with previous studies (except for differences of scale).[23–25] Similar relationships have been reported for skin autofluorescence.[26–28]


**Figure 4.** Observed fluorescence ratios from healthy eyes of 127 healthy subjects from clinical study with regression line (green) and upper one-sided 80% and 95% prediction intervals.

### *Significance of Lens Autofluorescence*

Kurzel and coauthors[29] identified fluorophores with five emission maxima in the ultraviolet (UV) and visible fluorescence spectra in the intact human lens. The UV bands include the amino acid tryptophan, a natural fluorophore in proteins, as well as other fluorophores that are responsible for lens autofluorescence that increases with age. A blue/green (450 nm excitation/550 nm emission) fluorophore, corresponding to the excitation and emission bands used in this study, is substantially increased in brunescent lenses.[29]

The fluorophores responsible for age-dependent lens fluorescence have been chemically identified as AGEs that are formed by a process of glycation.[6,30–32] Abiko and coauthors[33] measured lens autofluorescence in Chinese hamsters *in vivo*, removed the eyes, and analytically measured AGEs in lens extracts; lens autofluorescence was correlated with AGEs as measured by enzyme-linked immunosorbent assay. Other studies that identify AGEs

**Table 6.**
**Analysis of Variance for Linear Regression of Fluorescence Intensity (Arbitrary Units) versus Age (Years) Shown in Figure 3**

| Model | | Sum of squares | df | Mean square | F | Significance |
|---|---|---|---|---|---|---|
| 1 | Regression | 141,872 | 1 | 141,872 | 325 | 0.000 |
| | Residual | 54,457 | 125 | 435 | | |
| | Total | 196,329 | 126 | | | |

as the fluorophores responsible for lens autofluorescence include Kamei and Kato,[32] who correlated the analytically determined 1-deoxyfructosyl glycation adduct (DFA) with the fluorescence of normal and colored human lenses of different ages. Both DFA and lens autofluorescence were shown to be reliable indicators of the extent of glycation, and both increased significantly with chronological age and lens brunescence. Das and coauthors[34] used immunochemical assays to quantitate AGEs, which were shown to be correlated with lens fluorescence.

Advanced glycation end products, measured chemically in lens extracts, are also correlated with age and are elevated in cataractous and brunescent lenses.[21,32,34–43] Thus increased lens autofluorescence can be a biomarker indicative of more extensive underlying damage to the proteins due to glycation.[5,32]

### *Clinical Studies of Lens Autofluorescence*

Consistent with the identification of lens autofluorescence with the accumulation of AGEs due to glycation reactions, noninvasive clinical studies of blue/green lens autofluorescence have shown that, in addition to the increase of lens autofluorescence with age,[25,44] lens autofluorescence is increased in patients with diabetes mellitus.[23,45–49] Furthermore, within the diabetes patient population, lens fluorescence is also correlated with the development of the diabetes-related pathologies of cataract and retinopathy[50–52] and with systemic pathologies.[53]

### *Fluorescence Ratio versus Age*

Yu and coauthors[54] suggested that the ratio of fluorescence intensity to scattering would help compensate fluorescence measurements for attenuation effects; their clinical data indicated that the fluorescence ratio could provide better discrimination between individuals with and without diabetes than fluorescence intensity. Our data showing a nonlinear relationship of fluorescence ratio to age in healthy subjects are consistent with the data presented by Yu and coauthors.[54] Our data are based on only healthy subjects, so we carried out a nonlinear regression to generate predicted fluorescence ratios versus age as well as 80% and 95% prediction intervals. There were 9 subjects above the 95% confidence interval, which is greater than the 6 expected for the 127 patient sample, suggesting the possibility that some of the subjects who self-reported as healthy may have developed diabetes. However, the study is limited in the numbers of subjects in the higher age groups and by the lack of confirmative laboratory data. In the future, we plan to carry out clinical trials on both healthy subjects and subjects who have type 2 diabetes with sufficient power to determine the ability of the fluorescence ratio to screen for risk for undiagnosed diabetes.

## Conclusion

The fluorescence biomicroscope enables the clinician to measure lens autofluorescence in routine eye examinations. Patients with lens autofluorescence significantly higher than expected for their ages may have an accelerated accumulation of AGEs and increased risk for the pathologies associated with diabetes and AGEs.

**Funding:**

This work was funded by Freedom Meditech, San Diego, CA and supported by Eye Exam of California.

**Disclosures:**

Frederick Cahn is a consultant to Freedom Meditech. John Burd and Keith Ignotz are employees of Freedom Meditech.

**References:**

1. Regnauld J. Sur la fluorescence des milieux de l'oeil chez l'homme et quelques mammiferes. L'Institut. 1858;26:410.
2. Setschenow J. Ueber die Fluorescenz der durchsichtigen Augenmedien beim Menschen und einigen anderen Säugethieren. Albrecht Graefes Archiv Ophthalmologie. 1859;5(2):205–10.
3. Vannas M, Wilska A. Eine Methode zur Meesung der Fluorenzenz (lor lehnden menschlichen Augenlinee und eine Untersuchung iiber ifire Abhiingigkeit. Alter Klin Monatabl Augenh. 1935;95:53–64.
4. Klang G. Measurements and studies of the fluorescence of the human lens *in vivo*. Acta Ophthalmol. 1948;31:1–152.
5. Dyer DG, Blackledge JA, Katz BM, Hull CJ, Adkisson HD, Thorpe SR, Lyons TJ, Baynes JW. The Maillard reaction *in vivo*. Z Ernahrungswiss. 1991;30(1):29–45.
6. Monnier VM, Cerami A. Nonenzymatic browning *in vivo*: possible process for aging of long-lived proteins. Science. 1981;211(4481):491–3.
7. Fleming TH, Humpert PM, Nawroth PP, Bierhaus A. Reactive metabolites and AGE/RAGE-mediated cellular dysfunction affect the aging process: a mini-review. Gerontology. 2011;57(5):435–43.
8. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res. 2004;63(4):582–92.
9. Cooper ME. Importance of advanced glycation end products in diabetes-associated cardiovascular and renal disease. Am J Hypertens. 2004;17(12 Pt 2):31S–38S.
10. Mendez JD, Xie J, Aguilar-Hernandez M, Mendez-Valenzuela V. Molecular susceptibility to glycation and its implication in diabetes mellitus and related diseases. Mol Cell Biochem. 2010;344(1-2):185–93.
11. Thorpe SR, Baynes JW. Role of the Maillard reaction in diabetes mellitus and diseases of aging. Drugs Aging. 1996;9(2):69–77.

12. Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands JL, Smit A. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. Cardiovasc Diabetol. 2008;7:29.

13. Monnier VM, Sell DR, Genuth S. Glycation products as markers and predictors of the progression of diabetic complications. Ann N Y Acad Sci. 2005;1043:567–81.

14. Garlick RL, Mazer JS, Chylack LT Jr, Tung WH, Bunn HF. Nonenzymatic glycation of human lens crystallin. Effect of aging and diabetes mellitus. J Clin Invest. 1984;74(5):1742–9.

15. Lutze M, Bresnick GH. Lenses of diabetic patients "yellow" at an accelerated rate similar to older normals. Invest Ophthalmol Vis Sci. 1991;32(1):194.

16. Sensi M, Pricci F, Pugliese C, De Rossi MG, Celi FS, Cristina A, Morano S, Andreani D, Di Mario U. Enhanced nonenzymatic glycation of eye lens proteins in experimental diabetes mellitus: an approach for the study of protein alterations as mediators of normal aging phenomena. Arch Gerontol Geriatr. 1992;15 Suppl 1:333–7.

17. Van Boekel MA, Hoenders HJ. Glycation of crystallins in lenses from aging and diabetic individuals. FEBS Lett. 1992;314(1):1–4.

18. Nakayama H, Mitsuhashi T, Kuwajima S, Aoki S, Kuroda Y, Itoh T, Nakagawa S. Immunochemical detection of advanced glycation end products in lens crystallins from streptozocin-induced diabetic rat. Diabetes. 1993;42(2):345–50.

19. Zarina S, Zhao HR, Abraham EC. Advanced glycation end products in human senile and diabetic cataractous lenses. Mol Cell Biochem. 2000;210(1-2):29–34.

20. Gul A, Rahman MA, Salim A, Simjee SU. Advanced glycation end products in senile diabetic and nondiabetic patients with cataract. J Diabetes Complications. 2009;23(5):343–8.

21. Nagaraj RH, Linetsky M, Stitt AW. The pathogenic role of Maillard reaction in the aging eye. Amino Acids. 2012;42(4):1205–20

22. Hashim Z, Zarina S. Advanced glycation end products in diabetic and non-diabetic human subjects suffering from cataract. Age (Dordr). 2011;33(3):377–84.

23. Bleeker JC, van Best JA, Vrij L, van der Velde EA, Oosterhuis JA. Autofluorescence of the lens in diabetic and healthy subjects by fluorophotometry. Invest Ophthalmol Vis Sci. 1986;27(5):791–4.

24. Van Wirdum E, Mota MC, van Best JA, Leite E, Kappelhof JP, Faria de Abreu JR, Paul LC, Freitas Martins J, Oosterhuis JA, Marques A. Lens transmission and autofluorescence in renal disease. Ophthalmic Res. 1988;20(5):317–26.

25. Occhipinti JR, Mosier MA, Burstein NL. Autofluorescence and light transmission in the aging crystalline lens. Ophthalmologica. 1986;192(4):203–9.

26. Lyons TJ, Bailie KE, Dyer DG, Dunn JA, Baynes JW. Decrease in skin collagen glycation with improved glycemic control in patients with insulin-dependent diabetes mellitus. J Clin Invest. 1991;87(6):1910–5.

27. Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, den Hollander NC, Thorpe SR, Baynes JW, Navis G, Gans RO, Smit AJ. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. J Am Soc Nephrol. 2005;16(12):3687–93.

28. Januszewski AS, Sachithanandan N, Karschimkus C, O'Neal DN, Yeung CK, Alkatib N, Jenkins AJ. Non-invasive measures of tissue autofluorescence are increased in type 1 diabetes complications and correlate with a non-invasive measure of vascular dysfunction. Diabet Med. 2012;29(6):726–33.

29. Kurzel RB, Wolbarsht ML, Yamanashi BS. Spectral studies on normal and cataractous intact human lenses. Exp Eye Res. 1973;17(1):65–71.

30. Liang JN, Rossi MT. *In vitro* non-enzymatic glycation and formation of browning products in the bovine lens alpha-crystallin. Exp Eye Res. 1990;50(4):367–71.

31. Dyer DG, Blackledge JA, Thorpe SR, Baynes JW. Formation of pentosidine during nonenzymatic browning of proteins by glucose. Identification of glucose and other carbohydrates as possible precursors of pentosidine *in vivo*. J Biol Chem. 1991;266(18):11654–60.

32. Kamei A, Kato M. Contribution of glycation to human lens coloration. Chem Pharm Bull (Tokyo). 1991;39(5):1272–6.

33. Abiko T, Abiko A, Ishiko S, Takeda M, Horiuchi S, Yoshida A. Relationship between autofluorescence and advanced glycation end products in diabetic lenses. Exp Eye Res. 1999;68(3):361–6.

34. Das BK, Sun TX, Akhtar NJ, Chylack LT Jr, Liang JJ. Fluorescence and immunochemical studies of advanced glycation-related lens pigments. Invest Ophthalmol Vis Sci. 1998;39(11):2058–66.

35. Pokupec R, Kalauz M, Turk N, Turk Z. Advanced glycation end-products in human diabetic and non-diabetic cataractous lenses. Graefes Arch Clin Exp Ophthalmol. 2003;241(5):378–84.

36. Franke S, Dawczynski J, Strobel J, Niwa T, Stahl P, Stein G. Increased levels of advanced glycation end products in human cataractous lenses. J Cataract Refract Surg. 2003;29(5):998–1004.

37. Ranjan M, Beedu SR. Spectroscopic and biochemical correlations during the course of human lens aging. BMC Ophthalmol. 2006;6:10.

38. Jacobs R, Krohn DL. Variations in fluorescence characteristics of intact human crystalline lens segments as a function of age. J Gerontol. Nov 1976;31(6):641–7.

39. Cheng R, Lin B, Ortwerth BJ. Separation of the yellow chromophores in individual brunescent cataracts. Exp Eye Res. 2003;77(3):313–25.

40. Kamei A. Glycation and insolubility of human lens protein. Chem Pharm Bull (Tokyo). 1992;40(10):2787–91.

41. Yano M, Matsuda S, Bando Y, Shima K. Lens protein glycation and the subsequent degree of opacity in streptozotocin-diabetic rats. Diabetes Res Clin Pract. 1989;7(4):259–62.

42. Oimomi M, Maeda Y, Hata F, Kitamura Y, Matsumoto S, Baba S, Iga T, Yamamoto M. Glycation of cataractous lens in non-diabetic senile subjects and in diabetic patients. Exp Eye Res. 1988;46(3):415–20.

43. Lyons TJ, Silvestri G, Dunn JA, Dyer DG, Baynes JW. Role of glycation in modification of lens crystallins in diabetic and nondiabetic senile cataracts. Diabetes. 1991;40(8):1010–5.

44. Siik S, Airaksinen PJ, Tuulonen A, Alanko HI, Nieminen H. Lens autofluorescence in healthy individuals. Acta Ophthalmol (Copenh). 1991;69(2):187–92.

45. Van Best JA, Vrij L, Oosterhuis JA. Lens transmission of blue-green light in diabetic patients as measured by autofluorophotometry. Invest Ophthalmol Vis Sci. 1985;26(4):532–6.

46. Mosier MA, Occhipinti JR, Burstein NL. Autofluorescence of the crystalline lens in diabetes. Arch Ophthalmol. 1986;104(9):1340–3.

47. Van Wirdum E, van Best J, Bruining GJ, de Beaufort C, Oosterhuis J. Blood-retinal and blood-aqueous barrier permeability, lens autofluorescence and transmission in insulin-dependent diabetic youngsters. Graefes Arch Clin Exp Ophthalmol. 1989;227(1):26–9.

48. Larsen M, Kjer B, Bendtson I, Dalgaard P, Lund-Andersen H. Lens fluorescence in relation to metabolic control of insulin-dependent diabetes mellitus. Arch Ophthalmol. 1989;107(1):59–62.

49. Kessel L, Sander B, Dalgaard P, Larsen M. Lens fluorescence and metabolic control in type 1 diabetic patients: a 14 year follow up study. Br J Ophthalmol. 2004;88(9):1169–72.

50. Sparrow JM, Bron AJ, Brown NA, Neil HA. Autofluorescence of the crystalline lens in early and late onset diabetes. Br J Ophthalmol. 1992;76(1):25–31.

51. Mori F, Ishiko S, Abiko T, Kitaya N, Kato Y, Kanno H, Yoshida A. Changes in corneal and lens autofluorescence and blood glucose levels in diabetics: parameters of blood glucose control. Curr Eye Res. 1997;16(6):534–8.

52. Munch IC, Larsen M, Borch-Johnsen K, Glumer C, Lund-Andersen H, Kessel L. Cumulative glycaemia as measured by lens fluorometry: association with retinopathy in type 2 diabetes. Diabetologia. 2011;54(4):757–61.

53. Larsen M, Kjer B, Bendtson I, Dalgaard P, Lund-Andersen H. Lens fluorescence in relation to nephropathy in insulin-dependent diabetes mellitus. Graefes Arch Clin Exp Ophthalmol. 1992;230(1):6–10.

54. Yu NT, Krantz BS, Eppstein JA, Ignotz KD, Samuels MA, Long JR, Price J. Development of a noninvasive diabetes screening device using the ratio of fluorescence to Rayleigh scattered light. J Biomed Opt. 1996;1(3):280–8.