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Abstract. The time-resolved autofluorescence of the eye is used for the detection of metabolic alteration in diabetic patients who have no signs of diabetic retinopathy. One eye from 37 phakic and 11 pseudophakic patients with type 2 diabetes, and one eye from 25 phakic and 23 pseudophakic healthy subjects were included in the study. After a three-exponential fit of the decay of autofluorescence, histograms of lifetimes $\tau_i$, amplitudes $a_i$, and relative contributions $Q_i$ were statistically compared between corresponding groups in two spectral channels (490 < ch1 < 560 nm, 560 < ch2 < 700 nm). The change in single fluorophores was estimated by applying the Holm–Bonferroni method and by calculating differences in the sum histograms of lifetimes. Median and mean of the histograms of $\tau_2$, $\tau_3$, and $\alpha_3$ in ch1 show the greatest differences between phakic diabetic patients and age-matched controls ($p < 0.000004$). The lack of pixels with a $\tau_2$ of $\sim$360 ps, the increased number of pixels with $\tau_2 > 450$ ps, and the shift of $\tau_3$ from $\sim$3000 to 3700 ps in ch1 of diabetic patients when compared with healthy subjects indicate an increased production of free flavin adenine dinucleotide, accumulation of advanced glycation end products (AGE), and, probably, a change from free to protein-bound reduced nicotinamide adenine dinucleotide at the fundus. AGE also accumulated in the crystalline lens.

Keywords: fluorescence lifetime imaging ophthalmoscopy; diabetes; metabolism; flavin adenine dinucleotide; reduced nicotinamide adenine dinucleotide; advanced glycation end products.

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1 Introduction

Detection of the earliest signs of diabetic retinopathy (DR) increases the probability of reducing further pathological developments, provided that an appropriate therapy will be administered.1,2

Apoptosis of vascular and neural cells has been reported for the early onset of DR.3 Furthermore, in diabetes, neuronal degeneration due to apoptosis in the inner retina has been found to be 10-fold higher than that in vascular cells.4,5

Barber et al.3 found that apoptosis of neural cells is an earlier marker than is damage of vascular cells. No change in concentration of reduced nicotinamide adenine dinucleotide (NADH) was found between excited retina of diabetic rats and controls.6 Further studies were performed on excited retinas of rats under euglycemic and hyperglycemic conditions as well as in vivo on diabetic rats.7 In these studies, no elevated cytosolic NADH/NAD ratio was found under hypoglycemic conditions nor in diabetes, both in vitro and in vivo. But they found an increased polyol synthesis and metabolites upstream of glucose like the sorbitol pathway, which decreases NADPH.

Increased oxidation of sorbitol results in the generation of cytosolic NADH, which in early diabetes leads to metabolic imbalance as well as neural and vascular damage.8

In diabetic rats that were provided with a low dose of insulin, the thicknesses of the inner nuclear and plexiform layers were significantly reduced after 7.5 months. The density of ganglion cells was also reduced. These neurodegenerative changes were observed in the absence of morphologic vascular changes.9

Spectral domain optical coherence tomography showed that the total thickness of the retinal layer, particularly the retinal nerve fiber layer, was significantly thinner in diabetic Otsuka Long-Evans Tokushima fatty rats after 28 weeks than in non-diabetic Long-Evans Tokushima Otsuka rats. Additionally, a decreased number of ganglion cells and an increased frequency of apoptosis have been found by histological investigations.10

Isolated retinal capillary cells showed typical signs of mitochondrial dysfunction, including an increased release of cytochrome c into the cytosol and an accumulation of the proapoptotic protein Bax in mitochondria cells when incubated in 20 mM glucose. However, these changes were inhibited in the presence of superoxide dismutase.11

In diabetic patients, first metabolic alterations were found by Field et al.12 They excited the fundus using flashes of 467 nm light in a 3-deg field and detected the autofluorescence at 535 nm.
Increased fluorescence intensity and a wider distribution of intensity of all pixels in the excited field were found in diabetic patients in relation to controls. These results were interpreted as the result of an impaired electron transport by energy-generating enzymes in the respiratory chain. Because of the specific excitation and emission wavelengths, an increased contribution of flavin adenine dinucleotide (FAD) was assumed.

The problem of in vivo fluorescence measurement is that the crystalline lens and the fundus layers are simultaneously excited. An exact interpretation of the spectrally resolved fundus fluorescence is possible only in eyes with artificial intraocular lenses (IOL) or when the fluorescence of the crystalline lens is separately detected and subtracted from the fluorescence of the whole eye.13,14

In clinical practice, the classification of DR is based on vascular changes according to the Early Treatment Diabetic Retinopathy Study Research Group.15

Using the highly accurate spectral domain optical coherence tomography, it was demonstrated that the thicknesses of the retinal nerve fiber layer, the ganglion cell layer, and the inner plexiform layer were reduced in patients with minimal signs of DR in the pericentral macular area compared with those in the controls. Moreover, in the peripheral macular area, the retinal nerve fiber layer and the inner plexiform layer were found to be thinner than in the controls. The DR status was the only significant explanatory variable for this reduced thickness.16

Measurements of the time-resolved fluorescence of endogenous fluorophores have the potential to detect metabolic alterations in the human fundus.17 The redox pairs of oxidized and reduced NAD-NADH and FAD-FADH2 act as electron carriers in the basic processes of energy metabolism (citrate acid cycle, respiratory chain). Additional fluorophores, such as lipofuscin components, advanced glycation end products (AGE), collagen, elastin, products of the visual cycle (retinol), or components of heme synthesis (protoporphyrin IX), contribute to the autofluorescence of the fundus. In principle, these fluorophores can be discriminated by measuring specific excitation and emission spectra. Due to the transmission of the ocular media, however, excitation of endogenous fluorophores is only possible for wavelengths longer than 400 nm. Because the excitation maxima of most of these fluorophores are at wavelengths shorter than this value, any discrimination based on excitation spectra is difficult. The decay of fluorescence intensity (lifetime) after pulse excitation permits the differentiation of fluorophores with overlapping emission spectra. The free or protein-bound status of a fluorophore can also be distinguished if the emission spectrum is equal in both cases.

The excitation and emission spectra and the lifetimes of the expected fundus fluorophores and of the anatomical structures of porcine eyes have been reported.18

The purpose of this study was to determine whether metabolic changes in the time-resolved autofluorescence of the fundus are detectable in diabetic patients who have no signs of DR.

2 Participants and Methods

2.1 Participants

The patients were recruited from the outpatient Clinic of Internal Medicine III of the Jena University Hospital. Inclusion criteria were a diagnosis of type 2 diabetes, no signs of DR, and no or minimal cataracts. No subject was suffering from posterior capsule opacification. The DR stage was blindly evaluated from color fundus images by a retina specialist. The subjects in the control group were volunteers or patients from the Eye Clinic of the Jena University Hospital and did not have diabetes mellitus. They had no ocular disease and had clear media or a mild cataract (nuclear or cortical stadium 1).19 Subjects suffering from systemic diseases that could influence ocular metabolism were not included. Because the fluorescence decay depends on age,20,21 the only subjects who were included in the age-matched comparison between diabetic patients who have no signs of DR and healthy subjects were older than 40 years. The investigation was performed predominantly on subjects with a crystalline lens. For comparison, small groups of pseudophakic (IOL) diabetic patients and healthy subjects were investigated.

All subjects provided written consent to participate in the study. All research procedures were performed according to the Declaration of Helsinki. Approval for the study was obtained from the ethics committee of the Jena University Hospital.

HbA1c was measured using high-performance liquid chromatography (official normal range 4.4% to 5.9%; mean 5.2; TOSOH-Glykoäminoglobin-Analyser-HLC-723-GHbV; Tosoh, Tokyo, Japan). The mean normal range measured in 1079 non-diabetic patients in 2009 was 5.65% ± 0.38%.22 Therefore, we adjusted the HbA1c according to the mean normal value of healthy people to the value of 5.05% (32 mmol/mol) that was found in the Diabetes Control and Complication Trial Research Group (DCCT).23 Blood pressure was measured immediately before the autofluorescence measurement. Table 1 shows the data of the subjects who were included in the study. Values are given as the mean ± standard deviation.

The albumin/creatinine ratio in the urine indicates that some diabetic patients were suffering from microalbuminuria, and some were partly already suffering from macroalbuminuria. Microalbuminuria is assumed for albumin/creatinine ratios of 30 to 300 mg/g, and macroalbuminuria is present for ratios >300 mg/g.

2.2 Measuring System

The time-resolved autofluorescence of the fundus was measured using a modified confocal scanning laser ophthalmoscope (HRA II, Heidelberg Engineering GmbH, Heidelberg, Germany). The optomechanical unit of an HRA II was modified at the Eye Clinic of the University of Jena, Germany, for fundus excitation with a pulsed laser (BDL–440-SMC, Becker&Hickl GmbH, Berlin, Germany), which emitted 75-ps pulses at 448 nm at an 80 MHz repetition rate.18 The fluorescence was detected in two spectral channels (ch1: 490 to 560 nm, ch2: 560 to 700 nm) using multi-channel plate photomultipliers (Ham-R 3809U-50, Hamamatsu, Herrsching, Germany). The fluorescence decay was detected using time-correlated single photon counting (TCSPC) by an SPC 150 board (Becker&Hickl GmbH). An HRT 41 router (Becker&Hickl GmbH) separated the photons from the two spectral channels. Fluorescence decay images were recorded from a 30-deg field with a resolution of ~40 × 40 μm². The excitation power was 100 μW in the cornea plane. Simultaneously with the excitation laser, the fundus was illuminated with an infrared laser (820 nm), which provided contrast-rich fundus images for automatic image registration in both spectral channels. This device was the forerunner model of the demonstrator developed by Heidelberg Engineering.21
### Table 1 Characterization of the subjects included in the study.

| Diabetes type 2 | Healthy subjects |
|----------------|------------------|
|                | Crystalline lens | IOL  | Crystalline lens | IOL  |
| Probands       | 37               | 11   | 25               | 23   |
| Age (years)    | 60.5 ± 15.3      | 61.1 ± 14.7 | 61.9 ± 17.3  | 65.3 ± 14.7 |
| Gender         | 22 M, 15 F       | 8 M, 3 F | 14 M, 11 F     | 10 M, 13 F |
| BMI (kg/m²)    | 29.8 ± 6.1       | 30 ± 6 | 26.6 ± 4.2      | 26.7 ± 4 |
| Systolic blood pressure (mmHg) | 138.9 ± 15.4   | 136.4 ± 16.5 | 134.8 ± 12.2 | 135.1 ± 12.4 |
| Diastolic blood pressure (mmHg) | 82.3 ± 10.8    | 81.5 ± 10  | 78.9 ± 8.8     | 78.1 ± 9  |
| Antihypertonic drugs (%) | 81               | 100   | 28               | 39   |
| Statins (%)    | 43               | 70    | 8                | 13   |
| Time since diagnosis of diabetes (years) | 14.7 ± 9.8     | 16.4 ± 10.7 | 16.4 ± 10.7  | 16.4 ± 10.7 |
| Oral antihyperglycemic agents (%) | 30               | 20    | 24               | 20   |
| Treatment with insulin | 57               | 60    | 24               | 20   |
| Diet (%)       | 13               | 20    | 8                | 13   |
| HbA1C DCCT adjusted (mmol/mol; %) | 49.9 ± 13.9; 7.1 ± 1.4 | 46.3 ± 7.6; 6.8 ± 0.7 |
| Neuropathy (%)  | 24               | 20    | 24               | 20   |
| Albumin/creatinine in urine (mg/g) | 76 ± 233        | 154 ± 198 | 76 ± 233       | 154 ± 198 |
| Albumin/creatinine in urine >20 mg/g (% patients) | 27               | 30    | 27               | 30   |

Note: IOL, intraocular artificial lens.

### 2.3 Fitting of the Fluorescence Decay

The software SPCImage3.6 (Becker&Hickl GmbH) was used to evaluate the time-resolved autofluorescence images. To obtain a sufficient number of photons, a binning factor B = 2 was used, which increased the number of photons at a single pixel by a factor of 25. The fluorescence decay was approximated by the three-exponential model function given in Eq. (1):

\[
I(t) = \text{IRF} \times \sum_{i=1}^{3} a_i \cdot e^{-t/\tau_i} + b,
\]

where \(I(t)\) is the number of photons at time \(t\), \(\text{IRF}\) is the instrument response function, \(a_i\) is the amplitude or pre-exponential factor, \(\tau_i\) is the lifetime of exponent \(i\), and \(b\) is the background.

To avoid the influence of the stepped slope of fluorescence intensity which is caused by the fluorescence of the crystalline lens, a tailfit was employed.

The criterion for an optimal approximation was the minimization of \(\chi^2\) [Eq. (2)]:

\[
\chi^2 = \frac{1}{n-q} \sum_{j=1}^{n} \frac{[N(t_j) - N_e(t_j)]^2}{N(t_j)},
\]

where \(n\) represents the time channels (1024 applied here), \(q\) represents the free parameters \((a_i, \tau_i, \text{and} \, b)\), \(N(t_j)\) represents the detected photons in time channel \(j\), and \(N_e(t_j)\) represents the photons from the convolution of the model function with the IRF.

Assuming that the detection of photons is a Poisson process, the limiting value in Eq. (2) is 1.

In addition to all of the individual amplitudes and lifetimes, the mean lifetime \(\tau_m\) and the relative contribution \(Q_i\) parameters are important for evaluating fluorescence lifetime imaging ophthalmoscopy (FLIO) measurements. Here, the mean lifetime is defined as

\[
\tau_m = \frac{\sum_{i=1}^{p} a_i \cdot \tau_i}{\sum_{i=1}^{p} a_i}.
\]

The relative contribution \(Q_i\) of the component \(i\) corresponds to its respective area under the decay curve.

\[
Q_i = \frac{a_i \cdot \tau_i}{\sum_{i=1}^{p} a_i \cdot \tau_i}.
\]

Pixel-by-pixel fitting of the measured fluorescence decay to a three-exponential model [Eq. (1)] results in images of the lifetimes \(\tau_1, \tau_2, \text{and} \, \tau_3\), and the amplitudes \(a_1, a_2, \text{and} \, a_3\) for the fundus of each subject in both channels. Images of \(\tau_m\) and of \(Q_i\) can be calculated from the lifetimes and amplitudes.

Because the system did not contain an internal fixation target, the 30-deg field was not exactly at the same position at the fundus for all subjects. Thus, the comparison of the FLIO parameter of the complete images was not appropriate. Therefore, after approximation of the fluorescence decay of each pixel of the whole image, a region of interest (RoI) of 71 × 101 pixels, which was located in the superior temporal quadrant that...
included the macula, was selected for further calculations in all measurements. This region was contained in all images.

2.4 Evaluation of Fitting Results

In our study, we calculated the fitting results for each pixel within the RoI (71 × 101 pixels) and determined how frequently each value was determined for each lifetime \( \tau \), amplitudes \( \alpha \), and \( Q \). In this way, one histogram of each \( \tau \), \( \alpha \), and \( Q \) was calculated in both spectral channels for each subject. The histograms of phakic diabetic patients and healthy subjects were compared in two steps.

First, the derived quantities’ mode, mean, and median from these histograms were compared for each individual between the two groups. The statistical comparison was performed using SPSS 21 (IBM Deutschland GmbH, Ehningen, Germany). The two-tailed \( t \) test was used if the error probability in the Kolmogorov-Smirnov test was >0.2 (normal distribution). Equality of variances was assumed if the error probability in the Levene-test was also >0.2. The Mann-Whitney \( U \) test was used if the parameters were not normally distributed. Significant differences in the fitting parameters were assumed for \( p < 0.05 \). The area under curve of the receiver operating characteristics (ROC) was calculated. The ideal value is 1, while a value of 0.5 indicates that the separation between groups is only stochastic. Sensitivity and specificity were given for ROC > 0.72. These results are global because each lifetime \( \tau \), and amplitude \( \alpha \), results from the sum of the contributions from several fluorophores.

In a second step, changes in the single fluorophores were estimated. For this process, the Holm–Bonferroni method\(^\text{24}\) was applied using the software program FLIMX, which was developed at the University of Ilmenau.\(^\text{25,26}\)

Here, the distribution of each fit parameter was divided into \( n \) intervals. The lifetimes in such intervals are, to a certain degree, determined by specific fluorophores. Differences between the fitting parameters in single intervals are considered significant if the error probability \( p \) is lower than the significance level \( \alpha \) divided by the number \( n \) of intervals. The Wilcoxon test was performed for statistical comparison of the fit parameters of diabetic patients and healthy subjects in each interval.

The crystalline lens has a long fluorescence lifetime which influences the FLIO parameters, especially in channel 1. In contrast, an artificial IOL exhibits no fluorescence.

For that reason, the range of fit parameters was different for subjects with crystalline lens and for pseudophakic subjects. The size of the intervals was iteratively determined until the sum of the sensitivity and of specificity as well as ROC was maximal. The ranges and the size of the intervals for the fitting parameters are given in Table 2 for phakic subjects and in Table 3 for pseudophakic subjects.

### Table 2
Range of fitting parameters for phakic subjects.

| Parameter | Interval size | Range ch1 | Range ch2 |
|-----------|---------------|-----------|-----------|
| \( \alpha_1 \) in % | 2 | 74 to 92 | 60 to 88 |
| \( \alpha_2 \) in % | 1 | 8 to 19 | 14 to 34 |
| \( \alpha_3 \) in % | 0.5 | 1 to 6.5 | 1.5 to 5 |
| \( \tau_1 \) in ps | 5 | 35 to 105 | 45 to 105 |
| \( \tau_2 \) in ps | 10 | 330 to 690 | 300 to 590 |
| \( \tau_3 \) in ps | 50 | 2500 to 4600 | 1800 to 3500 |
| \( \tau_m \) in ps | 10 | 120 to 370 | 150 to 360 |
| \( Q_1 \) in % | 2 | 12 to 36 | 9 to 35 |
| \( Q_2 \) in % | 1 | 13 to 37 | 30 to 47 |
| \( Q_3 \) in % | 2 | 26 to 74 | 20 to 50 |

### Table 3
Range of fitting parameters for pseudophakic subjects.

| Parameter | Interval size | Range ch1 | Range ch2 |
|-----------|---------------|-----------|-----------|
| \( \alpha_1 \) in % | 2 | 72 to 90 | 60 to 80 |
| \( \alpha_2 \) in % | 1 | 10 to 24 | 18 to 29 |
| \( \alpha_3 \) in % | 0.5 | 0.5 to 5 | 2 to 6 |
| \( \tau_1 \) in ps | 5 | 45 to 85 | 45 to 85 |
| \( \tau_2 \) in ps | 10 | 330 to 460 | 330 to 460 |
| \( \tau_3 \) in ps | 50 | 2000 to 3000 | 1800 to 2300 |
| \( \tau_m \) in ps | 10 | 100 to 250 | 160 to 270 |
| \( Q_1 \) in % | 2 | 19 to 41 | 12 to 34 |
| \( Q_2 \) in % | 1 | 28 to 45 | 38 to 49 |
| \( Q_3 \) in % | 2 | 22 to 46 | 22 to 46 |

3 Results

The decay of the fluorescence intensity was fitted by the three-exponential model function of Eq. (1) for each pixel in both spectral channels. As demonstrated in Fig. 1, the model function sufficiently approximates the measured decay.

After the fitting, images of the lifetimes, amplitudes, and relative contributions were calculated for both spectral channels. Figure 2 shows these images in the short-wavelength channel for a healthy subject and for a patient with diabetes but no signs of DR. The bluer color in the images of the patient with diabetes indicates a prolongation of the lifetimes.

![Fig. 1 Tailfit of a paramacular pixel by a three-exponential model function. The fit parameters are \( \alpha_1 = 82.3\% \), \( \alpha_2 = 11.3\% \), \( \alpha_3 = 6.4\% \), \( \tau_1 = 105\) ps, \( \tau_2 = 639.8\) ps, \( \tau_3 = 4294.4\) ps, and \( \chi^2 = 1.14 \).](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/061106-4/June-2015-Vol-20(6)/Schweitzer-et-al.-Fluorescence-lifetime-imaging-ophthalmoscopy-in-type-2-diabetic-patients)
Fig. 2 Paired comparison of the amplitudes $\alpha_i$ (upper two rows of images), lifetimes $\tau_i$ (middle two rows of image), and relative contributions $Q_i$ (lower two rows) of a diabetic patient and a healthy subject in the short-wavelength channel. Images of the diabetic patients are in the upper part, and images of the healthy subject are in the lower part of each double row of images. The regions of interest are highlighted (white box).
The size and the position of the ROIs selected for quantitative comparison of the histograms of FLIO parameters are also shown in Fig. 2.

Generally, each histogram shows how often the same value of each parameter (lifetime, amplitude, and relative contribution) was determined in the analyzed field of a subject.

Figure 3, as an example, depicts the histogram of the lifetime \( \tau_2 \) of a diabetic patient in ch1 and ch2. The differences in the distribution of the lifetime \( \tau_2 \) in the two spectral channels indicate that the fluorescence results from different substances. Furthermore, this difference was detected equally in both the diabetic patients and the healthy subjects. From these distributions, statistical parameters were extracted for each subject.

The results of the statistical comparison are given for subjects with a crystalline lens in Table 4 for channel 1 (490 to 560 nm) and in Table 5 for channel 2 (560 to 700 nm).

In the short-wavelength channel, the best discrimination between healthy subjects and diabetic patients who have no signs of DR was achieved for the median and mean parameters in the distributions of \( \alpha_1, \tau_2, \tau_1, \tau_{\text{fit}}, Q_1, Q_2, \) and \( Q_3 \). The statistical values of skewness and kurtosis mostly did not reach significance. The highest ROC value (0.875) with 83.8% sensitivity and 76% specificity was found for the median of \( \tau_2 \) in ch1 with an error probability \( p = 0.000001 \). Good discrimination was also achieved for the medians of \( \alpha_1 \) and \( \tau_2 \) in the short-wavelength channel with error probabilities of \( p = 0.000005 \).

No significant difference between the two groups was obtained for the amplitude \( \alpha_1 \). In contrast, the difference in the lifetime \( \tau_2 \) between the two groups is highly significant, supporting the assumption that the composition of the fluorescent substances changes.

The best discrimination was obtained in the long-wavelength channel (560 to 700 nm) using the median and mean of the distributions of \( \alpha_1, \tau_2, \tau_1, Q_1, Q_2, \) and \( Q_3 \) in the selected ROIs at the fundus (Table 5).

The statistical comparison was also performed for pseudophakic patients and controls (Table 6). Only 10 subjects were included in the diabetic group; thus, the value of the statistical comparison is limited. The median and mean were only significantly different for \( \tau_2 \) in the short-wavelength channel, but on a much lower level than in phakic eyes. Surprisingly, the best discrimination was possible for the kurtosis of the amplitude \( \alpha_1 \). In the long-wavelength channel, the differences for the median and mean reached significance for \( \tau_1 \) and \( \tau_2 \).

Figure 4 shows median boxplots for all FLIO parameters in ch1 and ch2 for phakic subjects. For pseudophakic subjects, only significantly different parameters are given.

To obtain more information about the substance-specific changes in the eyes of diabetic patients as an early sign of metabolic alterations before the signs of DR are visible, the Holm–Bonferroni test was applied. Many fluorophores are excited in the eye. Thus, each lifetime \( \tau_i \) in a three-exponential approximation includes lifetimes from different fluorophores. Thus, the histograms that are created from the fitting parameters for all of the RoI pixels reflect the contributions of several fluorophores.

Therefore, similar to each approximated \( \tau_i \), the contribution of individual fluorophores with slightly different lifetimes can be estimated if the range of \( \tau_i \) is divided into intervals. In each interval, the difference in each fitting parameter was tested between the distributions corresponding to healthy subjects and diabetic patients. The discriminations between the groups are independent of the intervals; thus, the results of intervals with high sensitivity and high specificity can be combined to obtain the best separation. Table 7 summarizes the results of the Holm–Bonferroni test for phakic diabetic patients and healthy subjects.

The best discrimination between healthy subjects and diabetic patients who have no signs of DR was obtained for the lifetime \( \tau_2 \) in ch1 (490 to 560 nm) in the interval of 400 to 410 ps (ROC = 86%, sensitivity = 83.8%, specificity = 84%), \( \tau_3 \) for 3050 ps in the interval 2800–3100 ps (ROC = 84%, sensitivity = 75.7%, specificity = 86%), and \( Q_2 \) for 29% (ROC = 85%, sensitivity = 89.2%, specificity = 72%).

In the long-wavelength channel, the best separations were found for \( \tau_1 \) at 2100 ps (ROC = 85%, sensitivity = 89.2%, specificity = 80%) and for \( Q_2 \) at 43% (ROC = 87%, sensitivity = 75.7%, specificity = 96%).

In the comparison between the controls and the diabetic patients with an artificial IOL, significance in the Holm–Bonferroni test was only reached for \( \tau_1 \) at 65 ps (ROC = 81%, sensitivity = 80%, specificity = 69.6%) in ch1 (Table 8). The intervals of the fitting parameters are given for the parameters whose ROC values are at least 0.7. We speculate that the low number of diabetic patients with an artificial IOL led to the lower number of significant parameters compared with the results in Table 7. On the other hand, these results point at the considerable influence of changes in the fluorescence of diabetic lenses.

Graphical analysis of the histograms is a further step in the interpretation of metabolic alterations at the fundus of diabetic patients. The sum and difference histograms of \( \tau_2 \) in ch1 for diabetic patients and for healthy subjects with a crystalline lens are given in Fig. 5.

We observe a shift in the sum histograms for \( \tau_2 \) to longer fluorescence decay times in the fundus of diabetic patients who show no signs of DR compared with those for the controls. The curve in the difference histogram of \( \tau_2 \) in Fig. 5 clearly shows that in the RoI of diabetic patients, the number of pixels with a decay time of \( \sim 380 \) ps is reduced, whereas more pixels with fluorescence decay times longer than \( \sim 450 \) ps are present compared with those in the controls.

As demonstrated in Fig. 6, there is also a shift in the sum histograms of \( \tau_3 \) to longer fluorescence decay times in the fundus of diabetic patients who show no signs of DR when compared with the controls.
Table 4  Statistical comparison based on the fit parameters of the fundus fluorescence in channel 1 (490 to 560 nm) for the phakic eyes of healthy subjects and diabetic patients who have no signs of diabetic retinopathy (DR).

| Fit parameter | Test  | Modal | Median | Mean  | SD    | Skewness | Kurtosis |
|---------------|-------|-------|--------|-------|-------|----------|----------|
| Channel 450 to 560 nm | \(\alpha_1\) | \(t\) test | 0.008 | 0.011 | ns | ns | 0.003 |
|                 | Mann-Whitney | | 0.013 | | | | |
| \(\alpha_3\) | \(t\) test | 1E-06 | 1E-06 | | | | |
|                | Mann-Whitney | | 8E-05 | 2E-05 | ns | ns | |
|                | ROC | 0.829 | 0.842 | 0.845 | 0.827 | ns | ns |
|                | Sensitivity | 0.892 | 0.81 | 0.8 | 0.76 | | |
|                | Specificity | 0.72 | 0.72 | 0.845 | 0.824 | | |
| \(\tau_1\) | \(t\) test | 0.004 | 0.008 | | | | |
|                | Mann-Whitney | | 0.036 | 0.024 | 0.008 | ns | |
| \(\tau_2\) | \(t\) test | 1E-06 | 1E-06 | 1E-06 | 3E-06 | ns | ns |
|                | ROC | 0.862 | 0.875 | 0.875 | 0.854 | | |
|                | Sensitivity | 0.784 | 0.838 | 0.946 | 0.838 | | |
|                | Specificity | 0.8 | 0.76 | 0.72 | 0.72 | | |
| \(\tau_3\) | \(t\) test | 4E-06 | 4E-06 | | ns | ns | |
|                | Mann-Whitney | | 0.001 | 5E-05 | | | |
| \(\tau_m\) | \(t\) test | 3E-06 | 3E-06 | | ns | | |
|                | Mann-Whitney | | 6E-05 | 2E-05 | 0.024 | | |
|                | ROC | 0.803 | 0.837 | 0.837 | 0.826 | | |
|                | Sensitivity | 0.73 | 0.73 | 0.73 | 0.73 | | |
|                | Specificity | 0.76 | 0.76 | 0.76 | 0.72 | | |
| \(Q_1\) | \(t\) test | 1E-06 | 3E-06 | ns | ns | | |
|                | Mann-Whitney | | 6E-05 | | 0.018 | | |
|                | ROC | 0.781 | 0.827 | 0.829 | | | |
|                | Sensitivity | 0.72 | 0.72 | 0.72 | | | |
|                | Specificity | 0.703 | 0.757 | 0.784 | | | |
| \(Q_2\) | \(t\) test | 1E-07 | 1E-07 | ns | ns | ns | |
|                | Mann-Whitney | | 1E-05 | | | | |
|                | ROC | 0.822 | 0.848 | 0.848 | | | |
|                | Sensitivity | 0.72 | 0.76 | 0.76 | | | |
|                | Specificity | 0.757 | 0.838 | 0.811 | | | |
| \(Q_3\) | \(t\) test | ns | 1E-07 | 1E-07 | 0.029 | ns | |
|                | ROC | 0.85 | 0.852 | | | | |
|                | Sensitivity | 0.865 | 0.865 | | | | |
|                | Specificity | 0.72 | 0.72 | | | | |

Note: ns, nonsignificant; ROC, receiver operating characteristic.
Table 5  Statistical comparison based on the fit parameters of the fundus fluorescence in channel 2 (560 to 700 nm) for the phakic eyes of healthy subjects and diabetic patients who have no signs of DR.

| Fit parameter | Test  | Modal  | Median  | Mean  | SD   | Skewness | Kurtosis |
|---------------|-------|--------|---------|-------|------|----------|----------|
| Channel 560 to 700 nm | $\alpha_3$ | $t$ test | 0.004 | 0.004 | ns   | 0.004    | 0.004    |
|                |       | Mann-Whitney | 0.003 | 0.001 |      |          |          |
| $\tau_1$      | $t$ test | 0.022 | 0.038 | 0.018 | ns   | ns       |          |
|                | Mann-Whitney | 0.0005 |        |       |      |          |          |
| ROC           | 0.724 | 0.735 | 0.746 | 0.75  |      |          |          |
| Sensitivity   | 0.81  | 0.838 | 0.784 | 0.757 |      |          |          |
| Specificity   | 0.6   | 0.64  | 0.64  | 0.72  |      |          |          |
| $\tau_2$      | $t$ test | 8E-05 | 9E-05 | 0.001 |      |          |          |
|                | Mann-Whitney | 0.0001 | 0.008 | 0.018 |      |          |          |
| ROC           | 0.783 | 0.798 | 0.791 | 0.776 |      |          |          |
| Sensitivity   | 0.757 | 0.78  | 0.757 | 0.757 |      |          |          |
| Specificity   | 0.72  | 0.64  | 0.73  | 0.76  |      |          |          |
| $\tau_3$      | $t$ test | 1E-06 | 2E-05 | 2E-05 | 2E-05 | ns       | ns       |
|                | ROC   | 0.84  | 0.841 | 0.845 | 0.81  |          |          |
| Sensitivity   | 0.784 | 0.811 | 0.811 | 0.73  |      |          |          |
| Specificity   | 0.76  | 0.76  | 0.76  | 0.68  |      |          |          |
| $\tau_m$      | $t$ test | 0.001 | 0.001 | 0.001 | ns   | ns       |          |
|                | ROC   | 0.746 | 0.762 | 0.759 |      |          |          |
| Sensitivity   | 0.703 | 0.703 | 0.703 |      |      |          |          |
| Specificity   | 0.6   | 0.64  | 0.64  |      |      |          |          |
| $Q_1$         | $t$ test | 0.005 | 0.005 | 3E-05 | ns   | 0.001    |          |
|                | ROC   | 0.815 | 0.777 |      |      |          |          |
| Sensitivity   | 0.76  | 0.68  |      |      |      |          |          |
| Specificity   | 0.703 | 0.785 |      |      |      |          |          |
| $Q_2$         | $t$ test | 1E-07 | 7E-05 | 0.001 | ns   | ns       |          |
|                | ROC   | 0.872 | 0.851 | 0.857 | 0.734 |          |          |
| Sensitivity   | 0.76  | 0.8   | 0.84  | 0.56  |      |          |          |
| Specificity   | 0.919 | 0.757 | 0.73  | 0.676 |      |          |          |
| $Q_3$         | $t$ test | 4E-05 | 6E-05 | 6E-05 | 5E-06 | ns       | ns       |
|                | ROC   | 0.791 | 0.794 | 0.794 | 0.83  |          |          |
| Sensitivity   | 0.73  | 0.703 | 0.838 | 0.757 |      |          |          |
| Specificity   | 0.68  | 0.76  | 0.68  | 0.72  |      |          |          |
Likewise, considering the difference histogram for the decay time $\tau_3$ in Fig. 6, fewer pixels with a lifetime of $\sim 3000$ ps are present in diabetes, and more pixels with longer decay times of $\sim 3900$ ps are present in the selected ROIs.

Longer decay times for $\tau_2$ and $\tau_3$ in the short-wavelength channel as well as higher values of $\alpha_3$ were observed in the diabetic patients versus controls.

| Fit parameter   | Test    | Modal | Median | Mean  | SD    | Skewness | Kurtosis |
|-----------------|---------|-------|--------|-------|-------|----------|----------|
| Channel 490 to 560 nm $\alpha_3$ | Mann-Whitney | ns    | ns     | ns    | ns    | ns       | 0.007    |
|                  | ROC     |       |        |       |       |          | 0.796    |
|                  | Sensitivity |       |        |       |       | 0.739    |          |
|                  | Specificity |       |        |       |       | 0.7      |          |
| $\tau_2$        | $t$ test | ns    | 0.042  | 0.031 | ns    | ns       | ns       |
| $\tau_3$        | $t$ test | 0.031 | ns     | ns    | ns    | 0.047    | ns       |
| $\tau_m$        | $t$ test | 0.035 | ns     | ns    | ns    | ns       |          |
|                 | Mann-Whitney |       |        |       |       | 0.028    |          |
| $Q_3$           | $t$ test | ns    | ns     | ns    | 0.036 | ns       |          |
|                 | Mann-Whitney |       |        |       |       | 0.04     |          |
| Channel 560 to 700 nm $\alpha_3$ | Mann-Whitney | ns    | ns     | ns    | ns    | ns       | 0.042    |
| $\tau_1$        | $t$ test | ns    | 0.017  | 0.014 | ns    | ns       | ns       |
| $\tau_3$        | Mann-Whitney | ns    | 0.038  | 0.028 | ns    | ns       | ns       |
| $Q_2$           | Mann-Whitney | ns    | ns     | ns    | ns    | ns       | 0.02     |

4 Discussion

The detection of early metabolic changes in the fundus is important for the development of a specific treatment for DR. First in vivo spectral measurements of metabolic alterations at the fundus of diabetic patients were published by Field et al.\textsuperscript{12} It is assumed that alterations in the retinal vessel system\textsuperscript{7} or thinning of fundus layers\textsuperscript{16} are most likely consequences of metabolic malfunction.

The new technique of FLIO permits the discrimination of fluorophores according to the decay of fluorescence after pulse excitation and, to a certain degree, according to the emission spectra. Based on spectral and time-resolved in vitro measurements of autofluorescence on isolated structures\textsuperscript{27} of porcine eyes and of fundi in toto\textsuperscript{28} as well as of human fundus structures,\textsuperscript{29} we assume a relation between FLIO parameters and fundus layers. Additionally, cross-sections in FLIO images also show a relation between anatomical structures and lifetimes as well as amplitudes.\textsuperscript{30}

Thus, the amplitude $\alpha_4$ and the decay time $\tau_1$ might correspond to some extent with the retinal pigment epithelium, $\alpha_3$ and $\tau_2$ likely correspond to the neuronal retina, and $\alpha_3$ and $\tau_3$ likely demonstrate the influence of connective tissue and of the lens.

The decay times and the amplitudes in the short-wavelength channel (490 to 560 nm) differ from those in the long-wavelength channel (560 to 700 nm); thus, different substances contribute to the total fluorescence in the two channels.

In particular, the long fluorescence decay of the crystalline lens is present in the short-wavelength channel, where the $\tau_3$ is much longer than the $\tau_1$ in the long-wavelength channel. This contribution is confirmed because the $\tau_3$ in the short-wavelength channel in pseudophakic eyes is much shorter than that in eyes with a crystalline lens.

To investigate such metabolic alterations, diabetic patients who have no signs of DR and age-matched healthy subjects were investigated by FLIO. Although the mean duration of diabetes mellitus was 15 years, the mean HbA1c value was 7.1 $\pm$ 1.4\%, which is in the 6.5 to 7.5\% range recommended by the EASD/ADA\textsuperscript{31} and by the National German Guideline.\textsuperscript{32}

In contrast to optical coherence tomography measurements of the fundus layers and visible changes in the retinal vessel system, FLIO measurements detect functional metabolic changes. Considering amplitudes and lifetimes after a three-exponential fit of fluorescence decay in fields of 71 $\times$ 101 pixels, the best discriminating parameters were the median and mean in histograms of $\tau_2$. Because $\tau_2$ presumably corresponds to the neuronal retina, metabolic alterations in this anatomical structure might be assumed to occur in the fundus of diabetic patients before DR is detectable in color fundus photographs. Early alterations in the vessel system are detectable by fluorescein angiography. As this is an invasive test, it was not applied for ethical reasons in people who have had no visual complaints.

In addition to the statistical evaluation of the distribution of individual FLIO parameters, the sum of the individual histograms of the amplitudes, decay times, and relative contributions was considered for both groups. Similar to each individual histogram, the sum histograms contain the fluorescence of several
fluorophores. Thus, it is difficult to attribute any alterations to changes of only one fluorophore.

Calculations of the difference between the sum histogram of diabetic patients and that of healthy subjects revealed decreased abundance at $\sim 380$ ps for $\tau_2$ and at $\sim 3000$ ps for $\tau_3$; however, more pixels had longer $\tau_2$ decay times of $\sim 480$ ps and a longer $\tau_3$ of $\sim 3800$ ps.

There are several possibilities for interpretation of this change. A change in the composition of elastin (lifetime $\tau_1 = 380$ ps/amplitude $\alpha_1 = 72\%$ and $\tau_2 = 3.59$ ns/amplitude $\alpha_2 = 28\%$) would be thinkable, in principle. But we found no hints for such changes of elastin in diabetes in the literature.

More relevant is the interpretation in connection with the underlying chemical species. The decay time of 380 ps corresponds to free NADH; thus, a lack of free NADH versus protein-bound NADH, which has decay times of several nanoseconds, can be assumed. The increased contribution of protein-bound NADH is indicated by the longer $\tau_2$ decay time and by the increased amplitude $\alpha_3$ in the short-wavelength channel. This reason for the changed lifetime is unlikely because the maximal excitation of NADH is at 340 nm and the emission maximum is at 460 nm. In earlier studies, we excited pure NADH at 446 nm and detected a weak fluorescence with a maximum at 530 nm. Because it was unlikely to excite NADH at such long wavelength, we interpreted this fluorescence as result of pollution. Apparently, the minimal contribution of the NADH fluorescence can be detected by the highly sensitive TCSPC technique.
**Table 7** Results of the Holm–Bonferroni test for diabetic patients who have no signs of DR and for healthy subjects.

| Fit parameter | Significance | ROC | Sensitivity | Specificity | Best separation | Further separation intervals |
|---------------|--------------|-----|-------------|-------------|-----------------|-----------------------------|
| **Channel 490 to 560 nm** | | | | | | |
| $\alpha_1$ | ns | 0.7 | 0.622 | 0.8 | 82 | | |
| $\alpha_3$ | 0.001 | 0.73 | 0.946 | 0.52 | 1.5 | 1.5, 5.5 |
| $\tau_1$ | 0.001 | 0.8 | 0.811 | 0.64 | 70 | | |
| $\tau_2$ | 0.001 | 0.86 | 0.838 | 0.84 | 400 | 400, 410, 480 to 580 |
| $\tau_3$ | 0.001 | 0.84 | 0.757 | 0.84 | 3050 | 2800 to 3100, 4400 |
| $\tau_m$ | 0.01 | 0.77 | 0.838 | 0.64 | 150 | 140 to 160, 270, 330 to 360 |
| $Q_1$ | 0.001 | 0.77 | 0.891 | 0.64 | 34 | 34 to 36 |
| $Q_2$ | 0.001 | 0.85 | 0.892 | 0.72 | 29 | 21, 28 to 31 |
| $Q_3$ | 0.001 | 0.78 | 0.838 | 0.72 | 40 | 38 to 40 |
| **Channel 560 to 700 nm** | | | | | | |
| $\alpha_1$ | ns | 0.65 | 0.649 | 0.68 | 74 | | |
| $\alpha_2$ | ns | 0.63 | 0.757 | 0.64 | 23 | | |
| $\alpha_3$ | 0.05 | 0.71 | 0.568 | 0.84 | 4.5 | 4 to 5 |
| $\tau_1$ | 0.01 | 0.77 | 0.676 | 0.76 | 65 | 65, 80 |
| $\tau_2$ | 0.01 | 0.79 | 0.676 | 0.8 | 450 | 37 to 380, 430 to 460 |
| $\tau_3$ | 0.001 | 0.85 | 0.892 | 0.8 | 2100 | 2050 to 2150, 2650 to 2900 |
| $\tau_m$ | 0.01 | 0.73 | 0.913 | 0.56 | 160 | 160 |
| $Q_1$ | 0.05 | 0.7 | 0.541 | 0.88 | 18 | 18, 20 |
| $Q_2$ | 0.001 | 0.87 | 0.757 | 0.96 | 43 | 35 to 39, 43 to 45 |
| $Q_3$ | 0.01 | 0.76 | 0.757 | 0.76 | 40 | 40 to 44 |

**Table 8** Results of the Holm-Bonferroni test for pseudophakic diabetic patients who have no signs of DR and for healthy subjects.

| Fit parameter | Significance | ROC | Sensitivity | Specificity | Separation interval |
|---------------|--------------|-----|-------------|-------------|---------------------|
| **Channel 490 to 560 nm** | | | | | |
| $\alpha_2$ | ns | 0.7 | 0.7 | 0.696 | 15 |
| $\tau_1$ | 0.05 | 0.81 | 0.8 | 0.696 | 65 |
| $\tau_2$ | ns | 0.73 | 0.7 | 0.652 | 410 |
| $\tau_3$ | ns | 0.76 | 0.7 | 0.739 | 2500 |
| $\tau_m$ | ns | 0.72 | 0.6 | 0.739 | 140 |
| $Q_3$ | ns | 0.8 | 0.7 | 0.826 | 26 |
| **Channel 560 to 700 nm** | | | | | |
| $\tau_1$ | ns | 0.71 | 0.9 | 0.565 | 85 |
| $\tau_2$ | ns | 0.72 | 0.8 | 0.652 | 410 |
| $\tau_3$ | ns | 0.79 | 0.9 | 0.652 | 2100 |
| $Q_3$ | ns | 0.72 | 0.8 | 0.652 | 28 |
A shift of NADH from the free to the protein-bound state results from an increased contribution of glycolysis to energy production. A change from free to protein-bound NADH cannot completely be excluded. Considering all these aspects, the detected changes in the time-resolved autofluorescence in type 2 diabetes are the result of several metabolic alterations, most probably the accumulation of AGEs in the lens and the production of free FAD and accumulation of AGEs in the neuronal retina at the fundus. A change from free to protein-bound NADH cannot completely be excluded.

In conclusion, FLIO is a promising new method for investigation of the metabolism of the eye based on fluorescence lifetime of endogenous fluorophores. To get information about the fundus fluorescence alone, the fluorescence decay of the lens should be measured separately and be used in the fit of the fluorescence decay, detected from the whole eye. To specify the interpretation of metabolic alterations, techniques should be developed, permitting FLIO measurements in single fundus layers of the living human eye.

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References

1. Y. Ohkubo et al., “Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin dependent diabetes mellitus: a randomized prospective 5-year study,” Diab. Res. Clin. Pract. 28, 103–117 (1995).
2. UK Prospective Diabetes Study (UKPDS) Group, “Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33),” Lancet 352, 837–853 (1998).
3. A. J. Barber, T. W. Gardner, and S. F. Abcouwer, “The significance of vascular and neural apoptosis to the pathology of diabetic retinopathy,” Invest. Ophthalmol. Vis. Sci. 52, 1156–1162 (2011).
4. A. J. Barber et al., “Neural apoptosis in the retina during experimental and human diabetes: early onset and effect of insulin,” J. Clin. Invest. 102, 783–791 (1998).
5. A. B. El-Remessy et al., “Neuroprotective and blood-retinal barrier-preserving effect of cannabinoid in experimental diabetes,” Am. J. Pathol. 168, 235–244 (2006).
6. R. M. H. Diederan et al., “Reexamining the hyperglycemic pseudohypoxia hypothesis of diabetic oculopathy,” Invest. Ophthalmol. Vis. Sci. 47, 2726–2731 (2006).
7. M. S. Ola et al., “Analysis of glucose metabolism in diabetic rat retinas,” Am. J. Physiol. Endocrinol. Metab. 290, E1057–E1067 (2006).
8. Y. Ido et al., “Early neural and vascular dysfunction in diabetic rats are largely sequelae of increased sorbitol oxidation,” Antioxid. Redox Signal. 12, 39–51 (2010).
9. E. Lieth et al., “Retinal neurodegeneration: early pathology in diabetes,” Clin. Exp. Ophthalmol. 28, 3–8 (2000).
10. J. H. Yang et al., “Retinal neurodegeneration in type II diabetic Otsuka Long-Evans Tokushima fatty rats,” Invest. Ophthalmol. Vis. Sci. 54, 3844–3851 (2013).
11. R. U. Kowluru and S. N. Abbas, “Diabetes-induced mitochondrial dysfunction in the retina,” Invest. Ophthalmol. Vis. Sci. 44, 5327–5334 (2003).
12. M. G. Field et al., “Rapid, noninvasive detection of diabetes-induced retinal metabolic stress,” Arch. Ophthalmol. 126(7), 934–938 (2008).
13. F. C. Delori et al., “In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics,” Invest. Ophthalmol. Vis. Sci. 36(3), 718–729 (1995).
14. D. Schweitzer, “Ophthalmic applications of FLIM,” Chapter 20 in Fluorescence Lifetime Spectroscopy and Imaging, L. Marcu, P. M. W. French, and D. S. Elson, Eds., pp. 423–447, CRC Press, Boca Raton, FL (2014).
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