Suppression of Lens Fluorescence in FLIO Images of Cataract Patients

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Abstract: Abnormally long lifetimes in FLIO data almost always result from contamination of the fundus fluorescence by fluorescence of the crystalline lens. Here, we show how the problem can be identified and the correct fundus lifetimes be obtained.

Keywords: FLIM, FLIO, Lens Fluorescence, Cataract

Example of Long-Lifetime Data

An example of a FLIO image with unexpectedly long lifetime is shown in Fig. 1. The image shows the amplitude-weighted lifetime, tm, of a triple-exponential decay model. The image shows a uniformly blue area, without any indication of an image. To inexperienced users it may look like a malfunction of the instrument or the data analysis software.

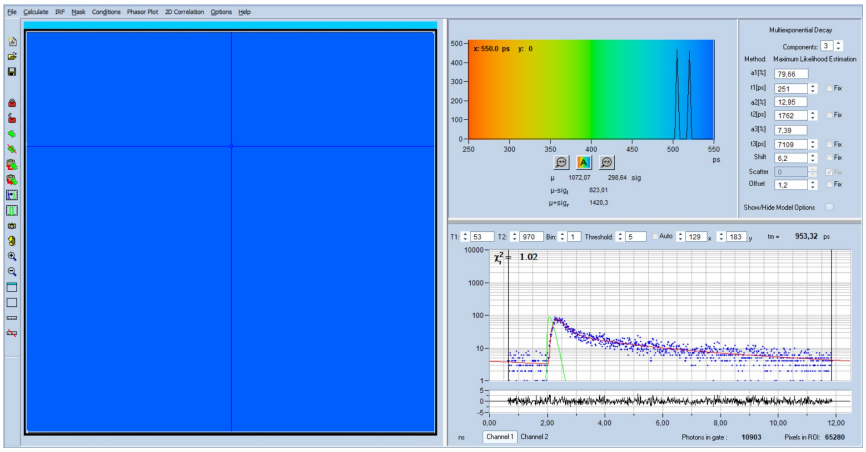

Fig. 1: FLIO data with long tm

The explanation of the unusual image is easy: The lifetimes of all pixels are out of the lifetime-display range, in this case 250 ps to 550 ps. Moreover, the intensity settings, 'brightness' and 'contrast', both have been set to 100%, see Fig. 2, left.

Fig. 2: Brightness and Intensity settings for the FLIO image

100% settings are commonly used in FLIO images. They are, however, not a good solution because they suppress any intensity contrast in the image. With the lifetime out of range, you cannot tell whether or not an image has been recorded at all. We therefore discourage to use 100% intensity and contrast. With both values set to 70% the image becomes as shown in Fig. 3. The figure clearly

shows that an image was recorded. Obviously, the problem is that the lifetimes are off range. An image within a lifetime range from 250 ps to 2000 ps is shown in Fig. 4. It looks like a normal FLIO image - with the exception that the lifetimes are too long.

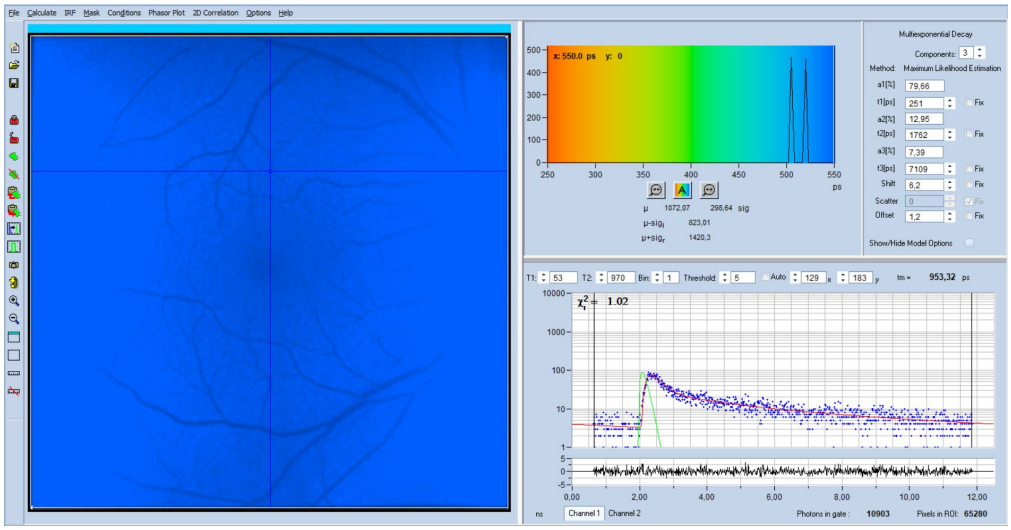

Fig. 3: Same data as in Fig. 1, but with 70% contrast and 70% brightness.

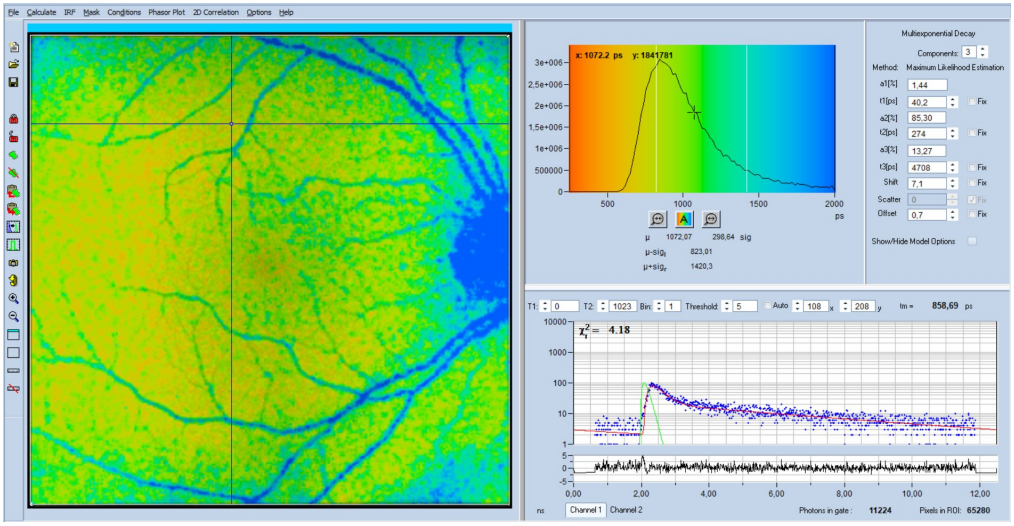

Fig. 4: Same data as in Fig. 3, but with lifetime range 250 ps to 2000 ps

Where is the Long Lifetime Coming from?

A look at the decay data in Fig. 1 to Fig. 4, upper right shows that there is a slow decay component, with a very long lifetime, t3 = 4.7 ns, and a large amplitude, a3 = 13%. The other decay components are, more or less, in the normal range. It is clear that the long average lifetime, tm, is caused by the presence of this slow component.

A clue of where the slow component comes from is obtained from a close inspection of the decay data, see Fig. 5.

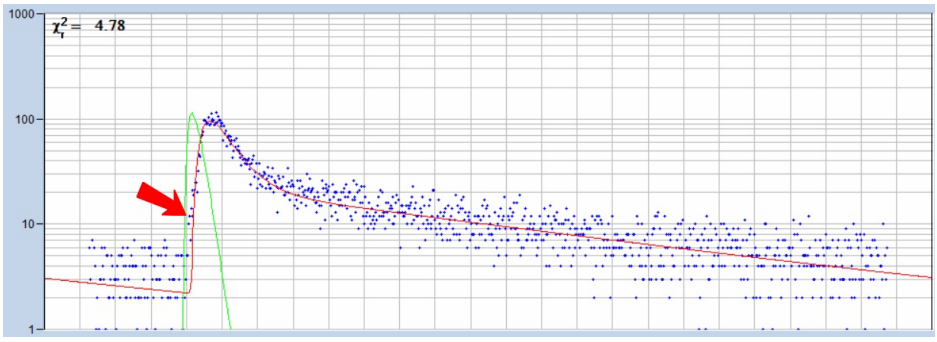

Fig. 5: Decay curve in selected spot of the image, fit by triple-exponential standard model

Not only that the rising edge of the data has an unusual shape, the triple-exponential model is also unable to fit the rising edge of the data correctly. Obviously, there are photons which arrive earlier than the conventional triple-exponential model can account for. The only anatomic structures the early photons can come from are the cornea and the crystalline lens.

The Shifted-Component Model

To account for lens and cornea fluorescence in FLIO data we introduced the 'Shifted-Component' Model in SPCImage [2], see Fig. 6. Every decay component of the model function can be equipped with a shift in time. Negative values mean a shift to shorter times, positive shift a shift to longer times.

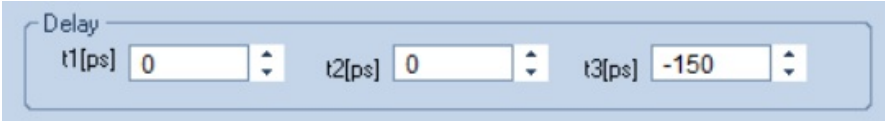

Fig. 6: Parameters of Shifted Component Model

It turns out that a triple-exponential model with a shift of -150 ps in the third component fits the data of adult eyes perfectly, see Fig. 7. The model not only fits the rising edge beautifully, there is also no oscillation in the residuals. A shift in the first or second component does not deliver a similarly good fit. That means, the third decay component indeed comes from the front structures of the eye.

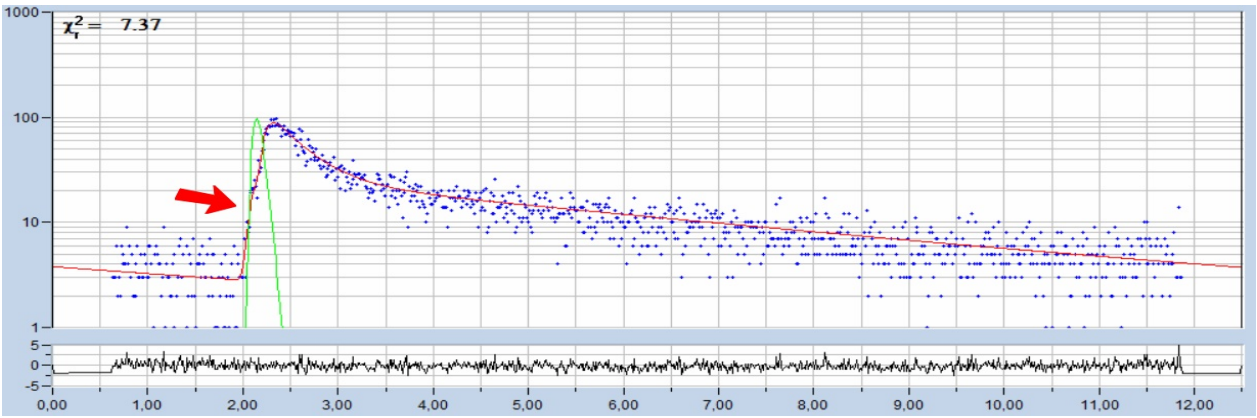

Fig. 7: Fit of decay data with shifted component model, t3 = -150 ps

IRF Considerations

When the automatic IRF optimisation function of SPCImage is used for FLIO it *must* be used with the shifted-component model, see Fig. 8. With the standard double or triple exponential model the procedure compensates the imperfection of the model with a wrong IRF. This IRF is too long, with the result that the short lifetime component, t1, is determined too short. Worse, the obtained IRF depends on the amount of lens fluorescence. This leads to different t1 and t2 values for different amounts of lens fluorescence. For the relationship of IRF modelling and shifted-component model please see [1, 3] and [4].

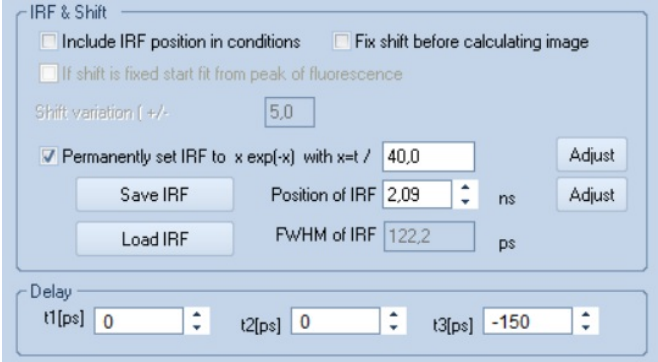

Fig. 8: The IRF optimisation must be performed with the shifted-component model

Analysis with the Shifted-Component Model

Assuming that the third (i.e. the slowest) decay component comes from the lens its lifetime contribution can be rejected from the FLIO data. Analyse the data with the shifted-component model, and, instead of using an average, tm, of all three decay components, use only the components t1 and t2. These are the decay component that come from the fundus. The amplitude-weighted average of t1 and t2 is available in SPCImage. Go into 'Colour' and select 'tm12' for colour coding tm12 in the image, see Fig. 9.

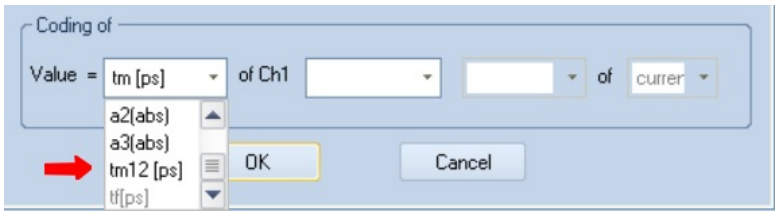

Fig. 9: Selection of the average of t1 and t2 for colour coding

An example for the data shown in Fig. 1 is presented in Fig. 10. The shifted-component model with three decay components and a shift of t3 of -150 ps was used. The colour-coded lifetime is t12, i.e. an amplitude-weighted lifetime of only t1 and t2. As can be seen from the image and the lifetime histogram, the tm12 values are in the normal range of the fundus lifetime.

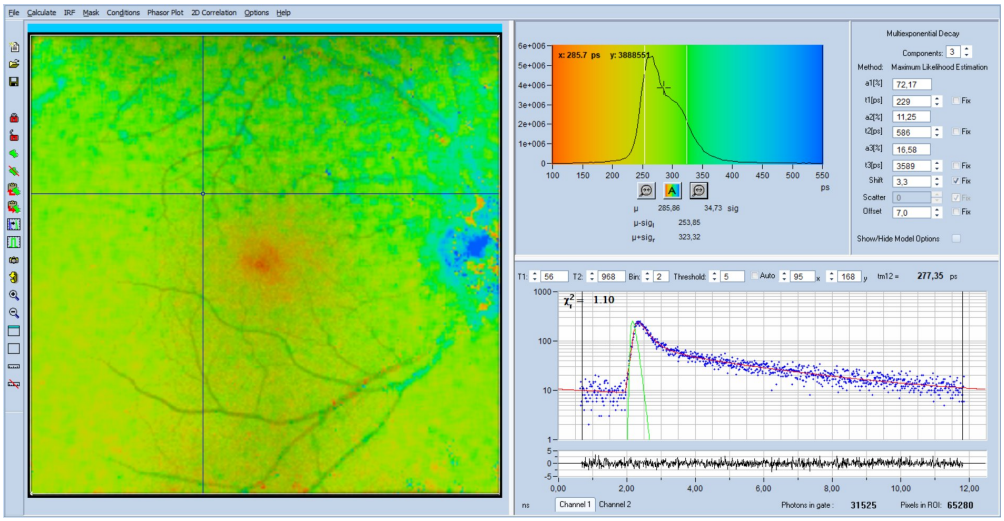

Fig. 10: Analysis of the data shown in Fig. 1 with shifted-component model, colour-coding of tm12

Similar results have been presented in [5]. The authors compare pre-surgery and post-surgery FLIO results from a cataract patient. As in the data presented here, pre-surgery data were heavily contaminated by lens fluorescence. Conventional triple-exponential decay analysis produced tm values which were completely out of range. Nevertheless, the shifted-component model was able to extract correct fundus lifetimes, which were later confirmed by post-surgery measurements. Results shown in [4] demonstrate that even FLIO data cataract-free eyes contain a noticeable amount of lens fluorescence. Shifted-component tm12 fundus lifetimes were about 20% shorter than the conventional tm lifetimes.

Advice for Obtaining Correct FLIO Images

When the lifetimes are unusually long, check the amplitude of the slowest decay component. Values larger than 5% indicate that lens fluorescence is the problem. The source of strong lens fluorescence can be cataract or poor focusing. Therefore, focus correctly on the fundus. Imperfect focusing reduces the detection efficiency of the fundus fluorescence and thus increases the relative amount of lens fluorescence. Use the shifted-component model of SPCImage in combination with the synthetic IRF. Use the average lifetime of the first two components, tm12, to reject lens fluorescence from the data and to obtain pure fundus lifetimes. Record as many photons as possible. Suppression of lens fluorescence requires triple-exponential data analysis and thus a large number of photons.

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