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A Photobleaching/Photoblinking analytical model for LSFCM imaging

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Laser scanning fluorescence confocal microscope (LSFCM) imaging is an extensively used modality in biological research [1]. However, these images present low signal to noise ratio and a time intensity decay effect due to the so called *photoblinking/photobleaching* (PBPB) phenomenon that corresponds to an intensity fading of a fluorescent probe along the time, as shown in Figure 1. This effect is caused by quantum phenomena associated with the electronic excitation and photochemical reactions among the fluorescent and the surrounding molecules induced by the incident radiation that temporarily or irreversibly destroy their ability to fluoresce. Since illumination is needed to excite and observe the tagging fluorescent proteins in the specimen and all the fluorophores will eventually photobleach upon extended excitation, the acquisition of this type of images becomes a hard task for long exposures.

There are in the literature several proposals [2] to model this fading effects and among them the single and double exponential are the most used. However, simple and tractable theoretical models based on the physics of the observation process to support these empirical laws are not available. In this work, that theoretical model, supported on the underlying physics of the process, is derived to describe the PBPB effect (see Figure 2).

From a fluorescence point of view, tagging molecules can be in three main states [3] (see Figure 3), i) ON-state, where they are able to fluoresce and be observed, ii) OFF-state, where they are temporarily not able to fluoresce and therefore are not visible and finally at the iii) BLEACHED-state where they become permanently OFF. Here, a continuous time differential equation dynamic model is proposed to describe the number of molecules at the ON-state, n_{ON}, along the time. The model is based on the underlying quantum mechanic physics theory of the observation process associated with this type of images and the common empirical weighted sum of two decaying exponentials (DExp), usually used in the literature, is derived from the model. The parameters β_{ON} and β_{Off} are the transitions rate from and to the ON-state respectively and ξ is the decay rate associated with the transitions for the permanent BLEACHED state.

Experiments with synthetic and real data are presented to validate the PBPB model and estimate the physical variables associated with the process. Intensity decay from real data and the corresponding theoretical curve are compared and displayed in Figure 4.

References

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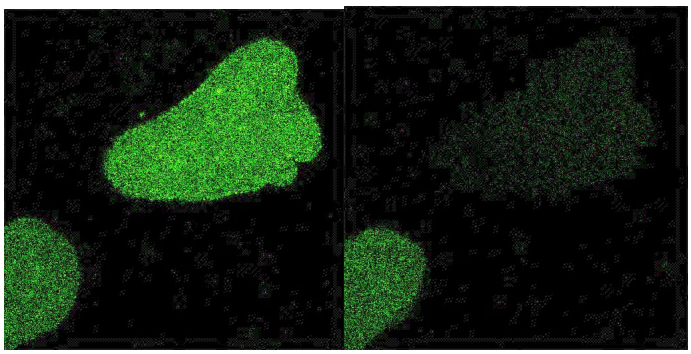

Figure 1. Two images of a real LSFCM sequence with PBPB under *Fluorescence loss in photobleaching* (FLIP) experiments.

$$n(t) = n_{ON}(t) + n_{OFF}(t)$$

$$\frac{dn_{ON}}{dt}(t) = \beta_{OFF} n_{OFF}(t) - \beta_{ON} n_{ON}(t)$$

$$\frac{dn}{dt}(t) = -\beta n_{OFF}(t)$$

$$\frac{d^2 n_{ON}(t)}{dt^2} + (\alpha + \xi)\frac{dn_{ON}(t)}{dt} + \beta_{ON}\xi n_{ON}(t) = 0$$

where $\alpha = \beta_{ON} + \beta_{OFF}$.

Figure 2. Differential equation model describing the PBPB effect. a) (left), set of equations describing the dynamics of the total number of fluophores and of the ones at the ON-state and b) (right), second order differential equation describing the time varying PBPB effect, obtained form a).

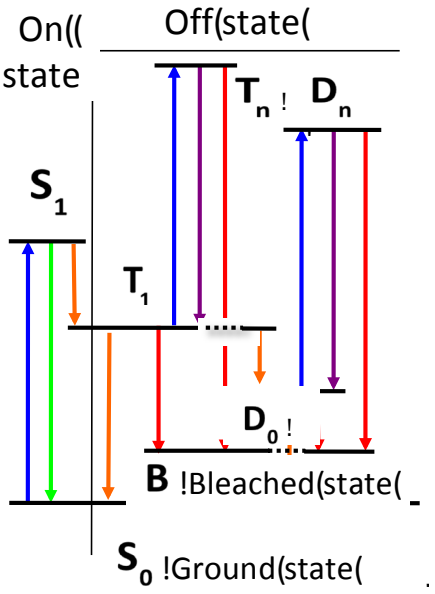

Figure 3 – PBPB electronic state transition Jablonsky diagram.

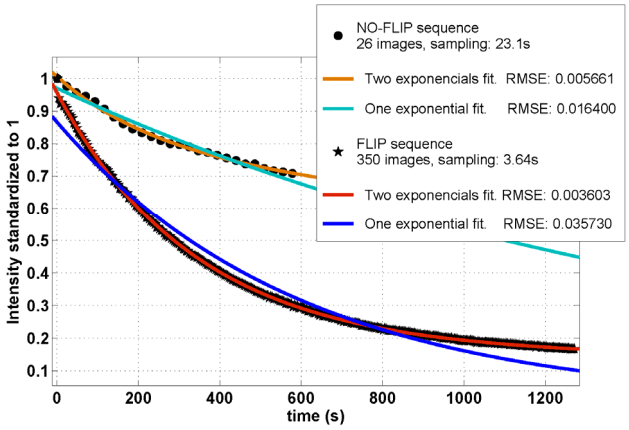

Figure 4 - Standardized average intensity per image as a function of the experiment time, for two LSFCM real data sequences, one without using any fluorescence decay acceleration technique (black circles) and the other using the FLIP technique (black stars). Red and orange curves stand for the fits of the data with two-exponentials models. Blue and cyan curves stand for the fits of the data with one-exponential models. The root mean square error (RMSE) is displayed in the plot legend.

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