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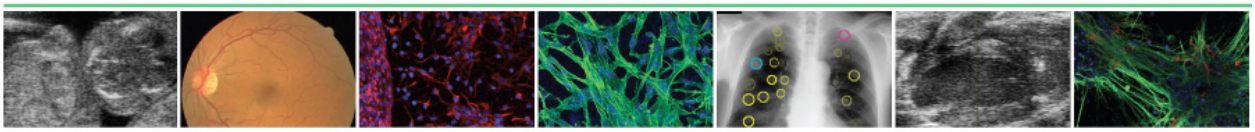

Submitting Abstract: *Poster*

Differential equation model for fading in fluorescence microscopy images

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Introduction

Fluorescence is the basis of several microscope image modalities extensively used in biological and medical research, e.g., such as Laser Scanning *Fluorescence confocal microscopy* (LSFCM) and spinning disk [1].

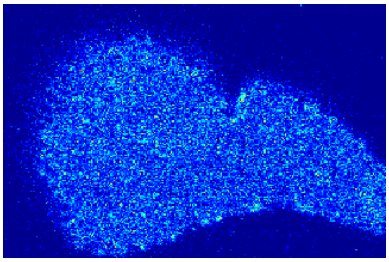

Figure 1 – LSCF Image

When the number of fluorescent molecules is small, high intensity incident radiation is employed to excite the fluorosphores and high amplification gains are needed to make visible the small amount of radiation emitted by them. By this, these images present low *signal to noise ratio* (SNR), are corrupted by a type of multiplicative noise with Poisson distribution, as displayed in Figure 1, and are affected by time intensity decay due to the so called *photoblinking* and *photobleaching* (PBPB) effects. The noise and the PBPB effects together make long time biological observations very difficult.

Several functions laws are presented in the literature to compensate these fading effects [2] and among them the single and double decaying exponentials are the most used. However, a simple and tractable theoretical model based on the physics of the observation process to support these empirical laws is not available.

Here, a theoretical model based on the underlying quantum mechanics physics theory of the observation process associated with this type of images is presented and the common empirical weighted sum of two decaying exponentials (DExp) is derived.

Results with synthetic and real data are presented to assess the robustness of the method and estimate the parameters of the DExp function.

Materials and Methods

From a fluorescence point of view, tagging molecules can be in three main states (see Figure 2), i) *ON-state*, where they are able to fluoresce and be observed, ii) *OFF-state*, where they

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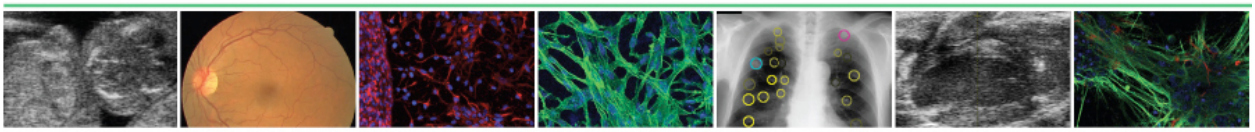


are temporarily not able to fluoresce and therefore are not visible and finally at the iii) *BLEACHED-state* where they become permanently *OFF*.

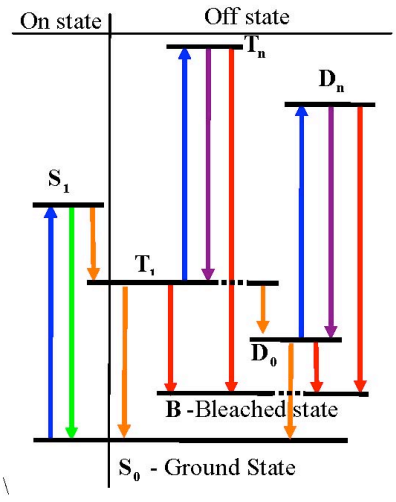


Figure 2 - Photoblinking and photobleaching electronic state transition diagram.

The molecules stay at each *OFF-state* and *ON-state*, called *active* states, according to a power law distribution [Dahan2003] and they can commute between these two states; nevertheless they are not able to recover from the *BLEACHED-state* state. In addition, the average intensity of an image at a given time instant of the experiment is assumed to be proportional to the number of fluorescent molecules at the *ON-state*.

Here, the following *continuous time set of differential equations* is proposed to describe the number of molecules at the *ON-state* and *OFF-state* along the time, according the Jablonsky diagram displayed in Figure 2.

$$n(t) = n_{ON}(t) + n_{OFF}(t) \quad (1)$$

$$\frac{dn_{ON}}{dt}(t) = \beta_{OFF} n_{OFF}(t) - \beta_{ON} n_{ON}(t) \quad (2)$$

$$\frac{dn}{dt}(t) = -\xi n_{OFF}(t) \quad (3)$$

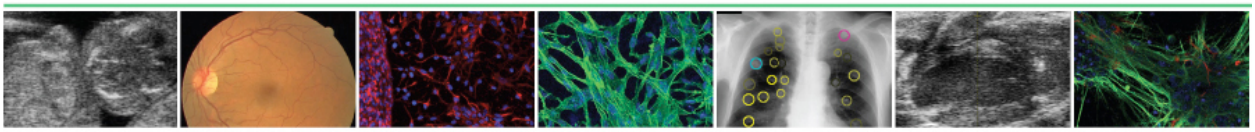
where n(t) is the total number of active molecules at instant t and $n_{ON}(t)$ and $n_{OFF}(t)$ are the number of active molecules at the *ON-state* and *OFF-state* respectively, at the same instant. $\xi=I+\tau$ is the decay rate of the active molecules associated with the transitions to the permanent *BLEACHED-state*, where I is proportional to the amount of incident radiation and τ is associated with other factors not related with illumination.
This means that even when no radiation illuminates the specimen, I=0, the number of active molecules decreases. However, since the main factor for the intensity decay is the incident radiation it is expected that $I>>\tau$.

Equation (2) models the photoblinking effect where it is assumed that the variation of the number of molecules at the *ON-state* is proportional, with constant β_{OFF}, to the number of molecules at the *OFF-state* and negatively proportional, with constant β_{ON}, to the number of molecules at the *ON-state*.

The magnitudes of β_{ON} and β_{OFF} are related with the *statistical aging* effect [3] that leads to an increasing number of active molecules at the *OFF-state* and an identical decreasing number at the *ON-state*. Therefore, the transition rate from the *OFF-state* to the *ON-state* is smaller than the inverse transition, which means that $\beta_{ON} > \beta_{OFF}$.

Results

Figure 3 shows the evolution of the average intensity per image, where time is in seconds, for two real data sequences, (A) acquired without using any LSFCM technique and (B) using the *fluorescence loss in photobleaching* (FLIP) technique. As can be observed in this figure, the fits of the data using two-exponentials models (red and orange curves) yield better results than the one-exponential models (blue and cyan curves), which is confirmed by the corresponding values of the root mean square error (RMSE) displayed in the plot legends.



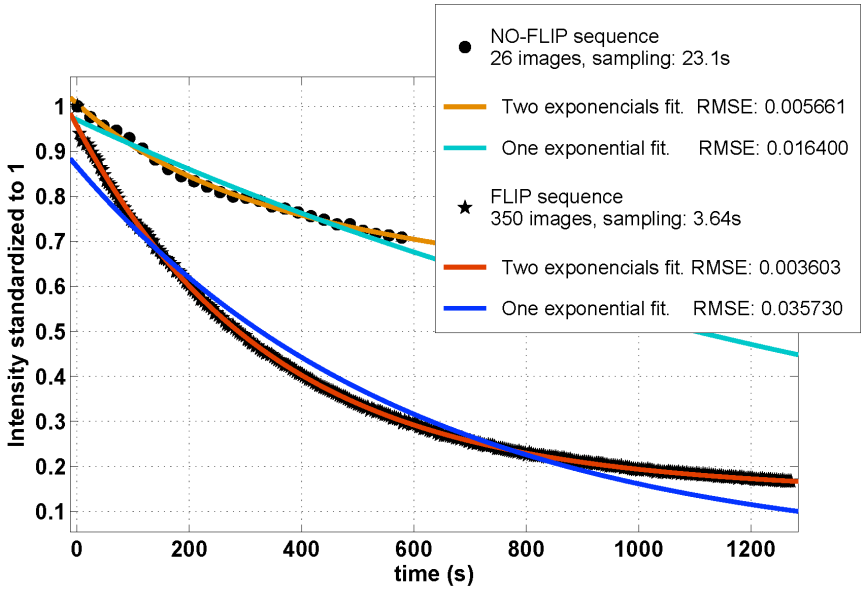


Figure 3 - Standardized average intensity per image as a function of the experiment time, for two LSFCM real data sequences, one without using FLIP nor any other technique (dark circles in A) and the other using the FLIP technique (dark stars in B). Red and orange curves stand for the fits of the data with two-exponentials models. Blue and

Discussion and Conclusions

In this work a differential equation based mathematical model that takes into account the underlying quantum physics of the fluorescence process, common to all microscopy imaging modalities, is presented and a closed form solution is provided. This solution correspond to the well known empirical decreasing double exponential law often described in the literature that is obtained from experimental data. Here, however, we use a theoretical reasoning to derive the same law where the parameters of the model have a physical meaning.

Experiments with synthetic and real data are used to assess the results and the values of the parameters are critically analyzed by taking into account their physical meaning.

This model is incorporated in Bayesian denoising algorithm to compensate PBPB fading effect in long biological experiments where the last images of the sequence are usually useless. By using the model and temporal correlation between images it is possible to recover relevant information even from these images that are almost faded.

References

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