Modeling of the rhodopsin bleaching with variational analysis of retinal images

J. Dobrosotskaya\textsuperscript{a}, M. Ehler\textsuperscript{a,b}, E. J. King\textsuperscript{a,b}, R. F. Bonner\textsuperscript{b}, W. Czaja\textsuperscript{a}

\textsuperscript{a} University of Maryland, Department of Mathematics, Norbert Wiener Center, College Park MD 20742
\textsuperscript{b} National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Section on Medical Biophysics, 9 Memorial Drive, Bethesda, MD 20892

ABSTRACT

This paper discusses a variational method of processing the scanning laser ophthalmoscope (cSLO) image sequences in the context of extracting the local rhodopsin density and modeling the bleaching kinetics. This work supports the characterization and detection of early pathological changes in clinical retinal data. Our goals include providing automated tools for tracing early pathological changes over time, in particular rhodopsin density variations and local lesion progression.

Aside from helping to distinguish between healthy and possibly pathological regions, information about the bleaching parameters allows to separate and classify certain elements in the retinal image and may be utilized to refine the output of the edge-detection based method of the microvessel detection.

Our computational approach is a variational technique that approximates measured cSLO image sets optimally within the range of the bleaching model. The characterizing parameters of the approximating curves are computed locally and their spatial changes reflect variations in bleaching kinetics and hence changes in the local rhodopsin density. We prove the consistency of the numerical results by showing that minimization of three different energy functionals measuring the deviation of the approximating curve from the original, leads to almost identical results despite the differences in the variational settings. We also show that the technique is naturally robust to noise.

The curve fitting in the temporal direction of the image stack can be also viewed as a denoising/enhancement routine. The advantages of the temporal correction include a better fit of the image intensity function to the model and the avoidance of local averaging that would impair the spatial resolution.

1. INTRODUCTION AND DESCRIPTION OF PURPOSE

This paper has a very close connection to another submission to SPIE Medical Imaging 2011 by M. Ehler et al.\textsuperscript{4} Both papers address the problem of processing the scanning laser ophthalmoscope (cSLO) image sequences to measure the local rhodopsin density and modeling the bleaching kinetics, with a further goal of creating automated tools for tracing early pathological changes over time, in particular rhodopsin density variations and local lesion progression.\textsuperscript{1,7} However, it concentrates on the computational aspects of the techniques and verification of the numerical results. Details about the clinical significance of our processing method can be found in.\textsuperscript{4}

1.1 Images used in the computations: Instrument and Protocol

The images we used for numerical processing were obtained via the cSLO camera (Heidelberg Retinal Angiograph 4.0, or HRA) which is a confocal system that illuminates only a small spot in the retina at once and then scans the retina line by line. The moving laser beam spot minimizes contributions of scattered fluorescence emission and greatly reduces the signal background. It delivers an intense 488nm laser beam (≈ 200\textmu W/100\mu m\textsuperscript{2}) to the surface of the retina with rapid scanning over an area of ≈ (8mm\textsuperscript{2}).

Sequential cSLO images get brighter with time if the imaging starts with a nearly dark-adapted subject. We follow a simple protocol in which the subject wears vermilion sunglasses (rod-protecting) while waiting and the photographer performs focus adjustment using the infrared reflection imaging (nonbleaching) in the cSLO. A long 488nm excited auto fluorescence movie (≈ 8 frames per sec) is recorded from the start with blinks every 10
sec which refresh the tear film layer on the cornea. The region is exposed to a high enough average photon flux to bleach almost all of the rod rhodopsin after \( \approx 25 \) sec.

This paper discusses a variational method of processing the cSLO image sequences in the context of extracting the local rhodopsin density and modeling the bleaching kinetics. Our computational approach involves approximating measured cSLO image sets optimally within the range of the bleaching model described below.

1.2 Rhodopsin Photo-bleaching Model

The kinetics of rhodopsin bleaching\(^2,6,8\) can be described by

\[
B(t) = \frac{I}{I + I_{rh}} \left(1 - e^{-\left(1 + \frac{I}{I_{rh}} \right) \frac{t}{\tau_{rh}}} \right)
\]

where \( I \) is the retinal illuminance, \( \tau_{rh} \) is the regenerating time constant of the visual cycle (\( \approx 7 \) min), \( I_{rh} \) is a bleaching constant that corresponds to the reciprocal of the photo sensitivity, and \( I_{rh} = \frac{L_{rh}}{\tau_{rh}} \). We can specify \( I_{rh} \) from literature values. The bleaching \( B \) describes the loss of absorption at 488nm and increases in the amount of 488nm light reaching the RPE lipofuscin. We denote the steady state by \( B(\infty) \), which is essentially reached at \( t \approx 25 \) sec.

We start with a dark-adapted retina and record an HRA movie until a steady-state bleaching.

The HRA measurements show \( I \gg I_{rh} \), and we evaluate \( B \) only for \( t \ll \tau_{rh} \). Therefore, the above model gets reduced to \( B(t) = 1 - e^{-\frac{D(t)}{\tau_{rh}}} \). The attenuation is related to the amount of rhodopsin absorption \( e^{-\left(1-B(t)\right)c \left(x,y \right)d \left(x,y \right)} \) where \( \epsilon \) is the molar extinction coefficient for rhodopsin at 488nm, \( c \left(x,y \right) \) is the local rhodopsin concentration and \( d \left(x,y \right) \) is the local height of the rod outer segments. The product \( c \left(x,y \right)d \left(x,y \right) \) is the desired local density of rhodopsin and \( \epsilon \) the factor that determines the associated absorption of the exciting light before it reaches the RPE.

The local autofluorescence intensity is

\[
I_{af} \left(x,y,t \right) = I_{af} \left(x,y,\infty \right)e^{-\left(1-B(t)\right)c \left(x,y \right)d \left(x,y \right)}
\]

where \( I_{af} \left(x,y,\infty \right) \) is the steady state corresponding to the completely bleached rhodopsin: \( B(\infty) = 0 \). The rhodopsin density can be determined from the fraction between early unbleached images and later steady state images according to

\[
c \left(x,y \right)d \left(x,y \right) = -\frac{1}{\epsilon} \ln \left( \frac{I_{af} \left(x,y,0 \right)}{I_{af} \left(x,y,\infty \right)} \right).
\]

So, we would like to find \( \alpha, \beta, \gamma \) depending on the pixel position (coordinates) \( x \) and \( y \) such that

\[
I_{af} \left(x,y,t \right) = \alpha \left(x,y \right)e^{-\gamma \left(x,y \right)e^{\beta \left(x,y \right)t}}, \text{ for } t \geq T_0,
\]

where \( \alpha \left(x,y \right) = I_{af} \left(x,y,\infty \right), \beta = -\frac{L_{rh}}{\tau_{rh}}, \) and \( \gamma = \epsilon c \left(x,y \right)d \left(x,y \right) \). The major challenge arising in this problem is investigating the precise kinetics of the rhodopsin bleaching in the presence of noise, possible uneven background illumination, as well as the distortions introduced by the micro eye movements while the movie is recorded and modifications introduced to the image stack in the process of the mutual registration.

2. VARIATIONAL METHOD OF CURVE-FITTING WITHIN THE CONTEXT OF MODELING THE RHODOPSIN BLEACHING

The model. With the goal of fitting the intensity changes over a temporal sequence of cSLO images to the double-exponential bleaching formula introduced by Lamb et al.,\(^8\) we design a variational method that computes a set of the bleaching model parameters that provide a closest fit to the given data.

To compensate for the presence of noise and possible image rescaling we introduce additional parameters into the expected form of the target function. Overall, for the computational convenience, we will be looking for four variable parameters that define a bleaching curve at each pixel position \( \left(x,y \right) \): \( I \left(x,y \right) = d \left(x,y \right) + a \left(x,y \right) \).
\[ e^{-c(x,y)e^{-b(x,y)t}t} \], where \( t \) denotes time after the light exposure of a dark-adapted subject. Here parameter \( a \) may be viewed as a possibly rescaled value of \( \alpha \), \( b \) and \( c \) retain the roles of \( \beta \) and \( \gamma \) respectively, and parameter \( d \) is introduced to compensate for the presence of noise.

**Energy minimization: gradient descent.** We apply the gradient descent algorithm to minimize the following energy defined as a function of four parameters \( a, b, c, d \) and the given temporal data \( f \):

\[
E_f(a, b, c, d) = \int_{T_0}^{T} \left( d + a \cdot e^{-c e^{-b t}e^{-b t}} - f \right)^2 dt,
\]

where \([T_0, T]\) is a time interval of adaptation to light. Here we choose to minimize the square of the \( L_2 \) norm (analogous to the mean square value) of the difference between the model of the temporal intensity change and the given data \( f \) due to the convenience of applying the gradient (steepest) descent technique to a quadratic functional.

In the context of the above energy minimization, the gradient descent method finds the values of \( a, b, c \) and \( d \) as the steady state solutions of the following system of ODE:

\[
\begin{align*}
    a &= -\frac{\int_{T_0}^{T}(d + a \cdot e^{-c e^{-b t}e^{-b t}} - f)e^{-c e^{-b t}e^{-b t}} dt}{\int_{T_0}^{T}e^{-c e^{-b t}e^{-b t}} dt}, \\
    b &= -a \cdot c \cdot \frac{\int_{T_0}^{T}(d + ae^{-c e^{-b t}e^{-b t}} - f)e^{-c e^{-b t}e^{-b t}}e^{-b t}dt}{\int_{T_0}^{T}e^{-c e^{-b t}e^{-b t}} dt}, \\
    c &= a \cdot \frac{\int_{T_0}^{T}(d + ae^{-c e^{-b t}e^{-b t}} - f)e^{-c e^{-b t}e^{-b t}}e^{-b t}dt}{\int_{T_0}^{T}e^{-c e^{-b t}e^{-b t}} dt}, \\
    d &= -\frac{\int_{T_0}^{T}e^{-c e^{-b t}e^{-b t}} dt}{\int_{T_0}^{T}e^{-c e^{-b t}e^{-b t}} dt}.
\end{align*}
\]

(SGD)

Solving this system to steady state allows to find a local minimum of the energy functional \( E_f \) (given a fixed vector of observations \( f \)). In the discretized form, the integration over the time interval \([0, T]\) is naturally replaced with the summation over the image stack. Due to the highly-nonlinear nature of the model, the system of four mutually dependent nonlinear differential equations is solved using an explicit finite difference scheme with the time stepping chosen individually for each parameter (and proportional to its order of magnitude). The evolution stops when the difference between two consecutive values of each parameter is less than .001\% of its magnitude. A typical example of the curve-fitting output for one chosen pixel from the stack of 268 cSLO images is shown in Fig.1(a).

![Figure 1](image)

**Variational properties of the model.** The problem of minimizing this functional and solving the gradient descent equation system has a unique minimizer/solution, provided the parameters \( a, b, c, d \) are restricted to a bounded range around their average values. This can be proven directly by computing the second variation of the above energy we are minimizing (under the condition that the first variation equals zero) and finding the
sufficient conditions under which it is positively definite. According to the theoretical model, the parameters do lie within those bounded ranges, hence the restrictions guaranteeing the uniqueness of the minimizer seem very natural. However, in the presence of noise and other deviations from the expected rhodopsin bleaching behavior (near the center of the fovea, optic disc or the major blood vessels), it is possible to encounter a temporal vector of the intensity values that allows multiple local minimizers, some of which seem to be outliers (for instance, the fitted curve for an averaged intensity vector over a region close to the center of the fovea in Fig. 4).

In order to reduce the noise (including local distortions due to the eye movements and the mutual image registration), we apply the fitting routine to the image stack obtained by averaging each pixel over its surrounding 8×8 pixels. The fitting curves then enable us to detect local rhodopsin changes up to a resolution of about 100µm. The output of such simulation is shown in Fig. 2. Running the gradient descent minimization of the above model with no additional restrictions at each pixel location (x, y) produces four values: a(x, y), b(x, y), c(x, y), d(x, y), which one can view as the four recovered parameter matrices. An example of such output is shown in Fig. 3. One can naturally expect the fitting parameters for pixels near the major blood vessels or the optic disc to be different from parameters computed for the rest of the pixel set. However, the results show that the optimal curve behavior near the fovea is also significantly deviating from the neighboring areas. To improve the algorithm performance, we used the results of the first fitting simulation to construct a better numerical model.

According to the theoretical model, parameter b(x, y) (or β(x, y)) can be considered constant throughout the image. Since our computations showed only minor variations of b(x, y) away from the blood vessels, fovea and optic disc, we adjusted the mathematical model by fixing the value of b and minimizing the same energy with respect to the other three parameters. In terms of the gradient descent it simply meant excluding the second differential equation from the system. To determine the true value of b, we computed an average of the intensity curves over a large area away from the fovea and the optic disc (an annulus-like area shown in Fig. 4(a)) and ran the fitting routine (Fig. 1(b)). The adjusted method produced much smoother parameter distributions that are shown in Fig. 3.

Processing non-averaged images is harder due to a higher noise level at each pixel (as shown in Fig.1(a)), so to investigate the behavior of the resulting curves we consider the intensity vectors obtained as local averages. Just as the theoretical model predicts, the fitted bleaching curves away from the major blood vessel, optic disc and fovea are relatively close, while the curves corresponding to the above listed areas are the outliers - Fig. 4.

**Estimating the deviation of the original data from the fitted double-exponential curves**

Computing the approximating double-exponential curve at each pixel of the image stack produces another 3D array of values (indexed by the 2D location and time) which can be viewed as an image stack. Taking the difference between the original intensity value and the value on the fitted curve produces yet another image stack of the differences between the original and the "fitted" image at each time moment. Computing an average of the differences over the entire time sequence produces a matrix with the maximum value of .42 while the average intensity value in the stack equals 56. Moreover, looking at the particular images in the difference stack, we observe that the maximum values are attained near the major blood vessels (see Fig. 3(d)), where the fitting...
Figure 3. Values of the parameters $a, c$ and $d$ determined via the gradient descent minimization with a fixed value of $b = 0.0368$: (a) values of $a$ between 43 and 108, (b) values of $c$ between 0.1 and 0.5, (c) values of $d$ between -18 and -12, (d) difference between the actual image from the stack (the 10th) and the corresponding image in the “fitted” stack.

(model is expected to be less consistent. In this manner, the deviations of each fitted curve from the original (which appears to be non-monotone even over several consecutive time moments, and hence can be treated as a noisy signal) can be attributed to the micromovements of the eye. Our model was specifically designed to incorporate all possible deviations and noise into the additive and multiplicative parameters of minimization, so it can automatically take care of the multiplicative and additive noise present in the image intensity data.

3. VERIFICATION OF THE RESULTS

We used two different approaches to verify the consistency of our numerical output: comparing the outputs of our routine using the initial and denoised inputs, as well as minimizing the discrepancy from the model with respect to different norms (i.e. minimizing different energies based on the same theoretical model).

3.1 Denoising consistency test

Let us consider the cSLO image intensity curves corresponding to each pixel in the “time-direction”, i.e. the temporal vectors of intensity values at each fixed pixel position. As mentioned before, due to microscopic eye movements as well as other factors introducing noise and distortion (for instance, mutual registration of the image sequence) the above vectors look unlike smooth functions that could be suggested by the context of the model. This opens the question of using temporal denoising, which has an advantage of introducing no additional local smoothing, both to enhance the image stack quality and to test the consistency of curve fitting before and after denoising. However, the variational fitting technique is robust to noise (see Fig. 5) and can itself be used for the image stack denoising/enhancement.)
Indeed, the same variational curve-fitting applied to the vector of observations \( f \) preprocessed via adaptive translation-invariant thresholding (which was successfully used for 2D signals with jumps\(^3\)), i.e. using a technique of a different class unrelated to curve fitting, produced a curve almost identical to the original fitting result (see Fig. 5) with minor (<1.25%) variations in the computed parameters \( a \) and \( c \). The values of the additive parameter \( d \) differ by about 6%, however, this could be expected since this parameter was intended to incorporate the additive noise.

Section 4.1 continues the discussion about applying the above technique to denoising of an image stack.

### 3.2 Comparison with other variational models: minimizing the \( H_1 \) and \( L_1 \) norms.

We compare out initial model with two additional variational models in order to show that the results are consistent despite the differences in the nature of the minimized functionals, thus verifying the accuracy of the numerical output.

**The \( H^1 \) model.** Since the curve we expect to recover is smooth, we can try to minimize the distance between the actual signal and the fitting curve in a higher order norm. The \( H^1 \) norm includes an additional term measuring the mean-square distance between the expected derivative of the curve according to the model and the numerically obtained approximation to the derivative of the given data:

\[
E_2(u) = E_2(a, b, c, d) = \int_{T_0}^{T_1} |\nabla(u - f)|^2 dt + \lambda \int_{T_0}^{T_1} |u - f|^2 dt
\]

where \( u(t) \) is the function expressing the expected behavior with respect to the model \( u(t) = d + ae^{-ce^{-bt}} \).

Solving this minimization problem via the gradient descent method leads us to solving the following system of ordinary differential equations:

\[
\begin{align*}
    a_t &= - \int_{T_0}^{T_1} (d + ae^{-ce^{-bt}} - f) e^{-ce^{-bt}} dt + \lambda \int_{T_0}^{T_1} (e^{-ce^{-bt}} - e^{-bt}) abce^{-ce^{-bt}} dt, \\
    b_t &= -a \cdot c \cdot \int_{T_0}^{T_1} (d + ae^{-ce^{-bt}} - f) e^{-ce^{-bt}} dt + \lambda \int_{T_0}^{T_1} (e^{-ce^{-bt}} - e^{-bt}) abce^{-ce^{-bt}} dt, \\
    c_t &= a \cdot \int_{T_0}^{T_1} (d + ae^{-ce^{-bt}} - f) e^{-ce^{-bt}} dt - \lambda bc \int_{T_0}^{T_1} (e^{-ce^{-bt}} - e^{-bt}) abce^{-ce^{-bt}} dt, \\
    d_t &= - \int_{T_0}^{T_1} e^{-ce^{-bt}} dt.
\end{align*}
\]
The $H_1$ model involves evaluation of the numerical derivatives, thus making the value of the intensity-shift parameter $d$ less influencing.

The $L^1$ model. We can also try to minimize the distance between the actual data vector and the fitting curve with respect to the $L^1$ norm (i.e. the sum of the absolute values of their differences at each time step). In this case we are interested in minimizing the following energy functional:

$$E_3(u) = E_3(a, b, c, d) = \|u - f\|_{L^1} = \int_{T_0}^{T_1} |u - f| dt.$$  

Solving this minimization problem via the gradient descent method leads us to the following system of ordinary differential equations:

$$\begin{align*}
    a_t &= \int_{T_0}^{T} \text{sign}(d + a \cdot e^{-c \cdot e^{-b \cdot t}} - f) e^{-c \cdot e^{-b \cdot t}} dt \\
    b_t &= a \cdot c \cdot \int_{T_0}^{T} \text{sign}(d + a \cdot e^{-c \cdot e^{-b \cdot t}} - f) \cdot e^{-c \cdot e^{-b \cdot t}} \cdot e^{-b \cdot t} \cdot t) dt \\
    c_t &= \int_{T_0}^{T} \text{sign}(d + a \cdot e^{-c \cdot e^{-b \cdot t}} - f) \cdot e^{-c \cdot e^{-b \cdot t}} \cdot b \cdot t) dt \\
    d_t &= \int_{T_0}^{T} \text{sign}(d + a \cdot e^{-c \cdot e^{-b \cdot t}} - f) + mu \cdot (d - d) dt 
\end{align*}$$

In both of the above models we use an explicit numerical solver to implement the gradient descent minimization. We perform the comparison of the above models by computing the approximating double-exponential curves for the same intensity vector, in particular the comparison of minimizers is performed on the signal obtained by averaging the intensity values over the annulus within 5-9 degrees (as shown in Fig.1(b)). The resulting approximating curves, when plotted, are almost indistinguishable, so, instead we list the individual parameter values recovered via each model:

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_2$</td>
<td>87.08</td>
<td>0.037</td>
<td>0.33</td>
<td>-14.74</td>
</tr>
<tr>
<td>$H_1$</td>
<td>87.376</td>
<td>0.0362</td>
<td>0.323</td>
<td>-15.032</td>
</tr>
<tr>
<td>$L_1$</td>
<td>87.774</td>
<td>0.0367</td>
<td>0.327</td>
<td>-15.234</td>
</tr>
</tbody>
</table>

4. INDIRECT APPLICATIONS

4.1 Curve fitting as a method of denoising the retinal movies in the temporal direction

In section 3.1 we discussed the resistance of our variational curve fitting technique to noise. Moreover, studying the difference between the original image stack and the stack obtained as a union of the double-exponential approximating curves leads to the hypothesis that the deviations of an image intensity from the curve predicted by the rhodopsin bleaching model can be considered noise, or deviations related to micro-movements of the eye. If we view each intensity curve at a pixel position as a denoised vector, we can view the union of those new values as a denoised image stack - see Fig.6. The convergence to a steady state, implied by the bleaching model, can be visually traced in the modified stack of images (the stack composed of the exponential curves optimally approximating the original ones). Extrapolation in time allows to make a steady state prediction for a given sequence of images.

4.2 The rhodopsin density map as a microvessel detection tool

The state of microvascular structure is a natural characteristic of the retina, it is clinically important to observe the changes in this structure over time. Traditional edge detection techniques can answer the question of localizing the blood vessels only up to a certain extent. It is very difficult for any automated technique to detect the smallest, not clearly visible vessels, without picking up extra details.

Aside from helping to distinguish between healthy and possibly pathological regions, information about the bleaching parameters allows to separate and classify certain elements in the retinal image and may be utilized to refine the micro vessel detection. For these purposes we do not use any local averaging to avoid smoothing the edges and apply the fitting routine to the temporal vectors associated with each separate pixel. Indeed, the matrix composed of the reconstructed values of the parameter $c$ shows clear and refined silhouettes of the blood vessels, some of which are almost completely invisible on a regular image from the stack (Fig.).
Figure 6. (a) A cut from the 20th image of the stack, (b) the respective values of parameter \(c(x, y)\)(rescaled product of the local rhodopsin concentration and the local height of the rod outer segments), (c) the respective cut from the 20th image of the denoised stack.

5. CONCLUSIONS

Summary A stable numerical implementation of the rhodopsin photo-bleaching was developed and tested on the sequences of the cSLO images. The results show consistency with the theoretical model and resistance to noise. Additional applications of this technique such as the microvessel detection and image stack denoising were discussed and tested.

Future work Authors are planning to utilize the CUDA technology and various parallel computing methods in order to improve the computational speed of our processing routine. We are planning to work on the quality of the recovered parameter images (for instance, to eliminate double-contour artifacts near the major blood vessels as in Fig. 6(b)) by researching available registration techniques and choosing those that are most efficient for the class of the HRA retinal images. We are looking forward to consulting with the ophthalmologists and, after extensive testing on healthy subjects, utilizing the methods we developed within the tools for precise analysis of retinal pathologies in some of the patients.

6. ACKNOWLEDGEMENTS

The research was supported in part by NSF through grant CBET 0854233, and by NGA through grant HM 15820810009. The authors also thank Emily Chew, Denise Cunningham and the National Eye Institute, NIH, for valuable input.

REFERENCES


