Vitreous Fluorophotometer Data Analysis by Deconvolution

R. Theodore Smith, Charles J. Koester, and Charles J. Campbell

The measurement process in fluorophotometry inherently involves a loss of information due to the finite sampling volume of the instrument. Mathematically, the effect is expressed as a convolution of the actual fluorescein distribution with the spread function of the instrument. Scattering by the ocular media can increase the spread function over that due to the instrument alone. A method is proposed for deconvolution of vitreous fluorophotometry data. Simulation studies and analyses of patient data demonstrate recovery of information with this method, including enhancement of retinal peaks and resolution of detail in the posterior vitreous which was not apparent from the original scans. Invest Ophthalmol Vis Sci 27:406-414, 1986

An ideal fluorophotometric instrument would measure the concentration of the fluorochrome in an infinitesimally small volume, and thus provide a precise reading of concentration at each point where a measurement is taken. The reality of the instrumentation is that the measurement volume is the intersection of finite illumination and measurement beams (Fig. 1). Fluorescence anywhere in this diamond-shaped volume is accepted by the detector and contributes to the signal attributed to the central point. The result is a blurring of detail. If the fluorescence is in an infinitesimally thin layer, the resulting signal is the instrument spread function. When there is a distribution of fluorescence, the resulting signal is described mathematically as a convolution between the actual distribution and the spread function.

The purpose of this paper is to demonstrate briefly the effects of convolution on acquired data and then to present a method of deconvolution. The goal of deconvolution is to reverse the effect of convolution and provide a more accurate fluorophotometry tracing with enhanced resolution of detail. Simulation studies using this method are presented in which the experimentally determined instrument spread function is employed to demonstrate resolution of model distributions. Patient studies using this method are presented with emphasis on the basic question of separating retinal and vitreous fluorescence.

This study is restricted to the posterior vitreous, where rapidly varying concentrations near the retina provide the most difficulty. This problem has been addressed using different methods by R. C. Zeimer, J. G. Cunha-Vaz, et al. In their terminology, the spread function or “tailing” refers only to the chorioretinal fluorescence per se as it propagates into the posterior vitreous and obscures fluorophotometric readings there. Their approach is to treat this tailing as a local effect at the retina and subtract it from the complete scan.

The potential importance of resolving fluorophotometry data close to the retina can be seen in the recent work of Bursell, Delori, et al. These authors conclude in a carefully controlled study that normals and diabetics without retinopathy cannot be distinguished on the basis of fluorophotometry data obtained 3 mm from the retina. Although they observe that differences between normals and diabetics are greater closer than 3 mm to the retina, the contribution of retinal fluorescence to this rapidly changing profile makes data obtained for this region, with present methods, insufficiently reliable to distinguish these two groups. A major goal of deconvolution is to push the limit of fluorophotometric resolution closer to the retina itself.

Materials and Methods

Effect of Convolution

A schematic of the measurement diamond is shown in Figure 2, with the volumes of the five subdivisions \( S_i \) and containing unknown fluorescein concentrations \( X_i \). When the instrument is focused on the central point, the total signal \( V_j \) is the sum of the signals \( S_iX_i \), etc received from each of the individual volumes, ie,
Fig. 1. Instrument Spread Function: The diamond-shaped region of intersection between the measurement and illumination beams is the volume actually measured by the instrument and assigned to the central point.

\[ V = S_1X_1 + S_2X_2 + S_3X_3 + S_4X_4 + S_5X_5 \]  
(1)  
A similar equation holds for every point at which measurement takes place, which may be summarized by the convolution equation:

\[ V = S \ast X \]  
(2)

Here \( V \) is the vector of measured values and \( X \) the vector of fluorescein concentrations. Simply stated, convolution means that when we are focused at a particular position within the eye, the signal received will be not the fluorescence at that precise point, but a weighted average of that value with other nearby values. When the values of fluorescein concentration are changing rapidly as in the vicinity of the retina, the effect may be substantial. In Figure 3 the solid line represents an ideal assumed fluorescein distribution, which corresponds to the unknown concentrations \( X \) in the equations above. (This model distribution is model II in simulations to follow.) The salient features are a sharp retinal peak, a plateau representing pooled fluorescein adjacent to the retina in a pocket of liquid vitreous, and exponential decay thereafter. Using the experimentally determined instrument spread function (see below), the convolution is performed to obtain \( V \) (Fig. 3, dashed line). \( V \) represents the simulated output scan from the instrument. Note: (1) lowering of the retinal peak, (2) broadening of the retinal peak, (3) loss of detail in the posterior vitreous. These are the effects we aim to reverse with deconvolution.

Deconvolution Methods

In broad terms, deconvolution means the solution of the convolution equation

\[ S \ast X = V \]  
(3)

for an unknown distribution \( X \) when the spread function \( S \) and measured values \( V \) are known. More precisely, if the spread function is the same at every point where measurement takes place, then the measured values \( V \) are given by the convolution integral:

\[ V(x) = \int S(x - y)X(y)dy \]  
(4)

(or appropriately digitized matrix form) from which one hopes to extract the distribution \( X \).

Fig. 2. Instrument Spread Function (Detail): The measurement diamond is subdivided into five compartments of 0.5 mm spacing. The volumes of the individual compartments are \( S_1, S_2, \ldots \) and fluorescein concentrations are \( X_1, X_2, \ldots \). The amounts of fluorescein in the compartments are thus \( S_1X_1, S_2X_2, \ldots \) and so forth.

Fig. 3. Effect of Convolution (Simulation): The measured values show what one would actually obtain by scanning the given model distribution (Model II) with the Fluorotron. This is convolution. Note lowering of the peak and loss of detail.
Fig. 4. Effect of Noise: The deconvolved scan is the exact solution before smoothing. Note the oscillations produced by noise in the patient data.

It must be stated at the outset, however, that for real data an exact solution $X$ is not necessarily the desired goal. The reason is that noise in the data $V$ and errors in determining $S$ for physical systems are generally amplified in the exact deconvolution process. Hence one seeks a reasonable but approximate solution $X$ such that the standard deviation

$$\sigma = \left( \frac{\|S \cdot X - V\|^2}{n} \right)^{1/2}$$

is acceptably small. Here we use the notation for the norm of a vector

$$\|V\|^2 = \sum_{i=1}^{n} V_i^2$$

There is a vast literature on this subject, most of it based on iterative matrix methods. For example, an excellent exposition of the Jansson algorithm as applied to spectroscopy data is found in Blass and Halsey. The Fourier transform approach is a different approach in which equation 3 is transformed to simple multiplication.

$$\hat{S}X = \hat{V}$$

where $\hat{S}$ represents the Fourier transform of $S$, and so on. This is theoretically appealing but is confounded by experimental error in practice. Another method, based on signal theory, is suggested, whereby one assumes that both signal and noise are Gaussian processes. Unfortunately, sharp retinal peaks as arise in fluorophotometry data are not easily fit into this scheme and we found resolution wanting.

The approach here was to develop an iterative matrix method based on a model appropriate to the class of signals being analyzed in the posterior vitreous, rather than rely on a method developed for, eg, spectroscopy data. The model consists of two assumptions about fluorescein concentration in this region. (1) There is a retinal peak; (2) Anterior to the retina the concentration decreases monotonically, or at most can increase within a preset tolerance over a unit interval. The tolerance in condition 2 is an option which can be varied depending on circumstances. It is usually set to a few percent of peak height, but can be relaxed if necessary. Boundary conditions are imposed also to insure a well-determined system, namely, that the concentration behind the retina is ultimately zero, and that as the posterior vitreous concentration reaches mid-vitreous, it attains a value that is constant over a distance equal to half the width of the spread function. The latter condition is applied at the most anterior point under study, generally 6–8 mm from the retina, where fluorescence changes very slowly.

These mild assumptions are broad enough to include nearly any reasonable distribution of fluorescein, yet seem to be adequate to dampen any nonphysiologic oscillations that arise from data noise or errors in the spread function.

The "least squares" solution is then a function $X$ for which

$$\|S \cdot X - V\|^2$$

is minimum and which satisfies 1 and 2. $X$ is determined iteratively as follows:

i) $S \cdot X_0 = V$ is solved exactly for $X_0$ by Gaussian elimination

ii) $X_0$ is inspected for the location of the retinal peak

iii) Relative minima in $X_0$ (ie, points not satisfying (2) are identified and eliminated as follows. If $X_j = (x_1, \cdots, x_{j-1}, x_j, x_{j+1}, \cdots, x_n)$ has a minimum $x_j$, then $x_{j-1}, x_j, x_{j+1}$ are replaced by $x_{j-1}, x_j, x_{j+1}$ such that

a) $X_1 = (x_1, \cdots, x_{j-1}, x_j, x_{j+1}, \cdots, x_n)$ satisfies the model and

b) $\|S \cdot X_1 - V\|^2$ is a minimum

The determination of $(x_{j-1}, x_j, x_{j+1})$ is easily accomplished in the space $x_{j-1} > x_j > x_{j+1}$ since it must lie on a boundary component

$$x_{j-1} > x_j > x_{j+1}$$

Thus the problem of minimizing $\|S \cdot X_1 - V\|^2$ is a straightforward 2-dimensional least squares calculation.

If $X_1$ has a minimum the process is repeated to give $X_2, X_3$, etc until a solution $X$ is obtained which satisfies (2) and for which $\|S \cdot X - V\|^2$ is least. Of course, one could argue that this iterative approach does not yield a global minimum among all possibilities satisfying (2). However, investigations with a program that optimizes
on all points simultaneously have demonstrated essentially equivalent results, while simply requiring much lengthened computations. The smoothing effect of the least squares method may be seen by comparing Figures 4 and 5. In Figure 4 deconvolution has been performed using Gaussian elimination alone (the exact solution). Note the oscillations in the solution produced by noise in the input data. In Figure 5 the least squares method has been applied to produce a new solution which now decreases in a physiologic manner after the retinal peak and has a small inherent standard deviation from exactness.

**Determination of Instrument Spread Function**

The instrument used in all studies was the Coherent Fluorotron Master (Coherent Medical Division; Palo Alto, CA), whose properties are described in detail. A model eye was constructed that reproduced the basic optics of the eye, with provision for filling a chamber of variable thickness with a fluorescein concentration of known concentration. A solution of 1000 ng/ml was used in a chamber with a thickness of 0.1 mm. The model eye was scanned three times with the Fluorotron and the tracings analyzed. A perfect instrument would record a square function 100 μm wide. Instead, each tracing was a triangular function, representing the instrument spread function. The width at half height of the resulting signal, ie, of the instrument spread function, is 0.7 mm. This number is thus a necessary lower bound on the width of any patient spread function and is nearly attained in some young patients.

The five point spread function then used for deconvolution was obtained by normalizing the above data at ½-mm intervals. The normalized instrument spread function is thus:

\[ S = (0.02, 0.18, 0.60, 0.18, 0.02) \]

**Patient Scan Selection and Deconvolution**

Ten patient scans were selected retrospectively for study; they had well-defined retinal peaks on the pre-injection and/or bolus scans, as measured by the Coherent Fluorotron. Prior to fluorescein angiography and vitreous fluorophotometry an intravenous bolus of 500 mg Na fluorescein 10% had been administered. Data were taken at ½-mm intervals to localize peaks as precisely as possible on the pre-injection, 5-min bolus and 1-hr measurement scans.

The measurement scan was then entered at ½-mm intervals from the retinal peak as the measured data input to the deconvolution process. Deconvolution was performed on this data using the least squares protocol and the instrument spread function given above. A standard deviation for each scan

\[ \sigma = (\|S \cdot X - V\|^2/n)^{1/2} \]  

was calculated which reflects the difference between the measured data and the convolution of the solution with the spread function. An average concentration of fluorescein in the posterior vitreous (the 6 mm adjacent to the retina) was also calculated. Posterior vitreous average concentrations were also determined using the Optimized Protocol package supplied by Coherent for comparison.

**Deconvolution of Simulated Scans**

Two model distributions of fluorescein in the posterior vitreous were chosen on which to test deconvolution. Model I consists of a retinal peak followed by an exponential decay. Since the proposed resolution is in ½-mm steps, the model does not show finer detail such as a square function for the retina itself. Such resolution would require smaller steps, on the order of 0.1 mm. Hence the retinal peak is represented by a single point, and connecting this point to the next closest data point results in a triangular shape for the retinal peak model. The model is convolved with the instrument spread function to simulate measured data (see Fig. 6).

Model II interposes a plateau in the fluorescein concentration immediately adjacent to the retinal peak (previously depicted in Fig. 3). This model was suggested by the fact that, in cases of posterior vitreous detachment with liquid vitreous in front of the retina, bulk flow rather than diffusion would be operative with nearly constant resulting concentrations over that region. This possibility is also suggested by Zeimer et al. in their determination of diffusion coefficients from fluorophotometry data.
Fig. 6. Model Fluorescein Distribution I: A retinal peak is followed by exponential decay. The simulated measured values, obtained by convolution, show the effect of the measurement process. They are the input for the deconvolution process.

The simulations consist of solving the equations

\[ S_0 \cdot X = V_0 \]  

(9)

for X, where \( S_0 \) is the instrument spread function and \( V_0 \) is the simulated measured data obtained by convolving \( S_0 \) with one of the model distributions. To determine the ability of the deconvolution protocol to resolve these model peaks under experimental conditions, two sources of error are introduced: (A) Noise in the test data \( V_0 \); (B) Error in spread function \( S_0 \).

**Case A: Random Noise in Test Data**

If \( V_0 \) is the simulated measured data (obtained by convolution from either Model I or Model II), replace \( V_0 \) by

\[ V = V_0 + n \]

where \( n \) is a vector of random noise of magnitude consistent with Fluorotron capabilities (±1.5 ng/ml).

Deconvolution is then performed using the equation:

\[ S_0 \cdot X = V \]

and the result X is compared to Model I or Model II as the case may be.

**Case B: Error in Spread Function**

The most basic error in spread function input would be to choose a spread function which is either too wide or too narrow. More precisely, for a given spread function \( S \) let \( w \) denote the width at half height. Thus \( w \) may serve as a measure of the overall breadth of the spread function. For the instrument spread function \( S_0 \), \( w_0 = .7 \) mm. To compare a given spread function \( S \) to \( S_0 \) define the spread factor

\[ \psi = w/w_0 \]  

(10)

as a measure of a broadening or narrowing error in the spread function.

For these simulations symmetrical spread functions \( S \) were constructed which had spread factors 0.9 and 1.2 respectively when compared to \( S_0 \). Random noise of amplitude ±1.5 ng/ml was also added to the simulated measured data just as in Case A. These simulations thus consist of solving the equations

\[ S \cdot X = V \]

where \( S \) is a spread function with spread factor 0.9 or 1.2 compared to the correct spread function \( S_0 \), and \( V = V_0 + n \). These equations are solved using the least squares deconvolution protocol for X, and X is again compared to Model I or Model II, depending on which model was used to generate \( V_0 \). (Note, of course, that \( V_0 \) is always generated from convolution with \( S_0 \).)

**Results**

**Simulation Studies**

In each figure, three tracings are depicted. The “Test Data” corresponds to the vector \( V \) obtained by convolving a model scan with \( S \). The “Deconvolved Scan” is the solution X found by the least squares protocol. The original model scan is also graphed for comparison with \( X \). The scale in each figure is the same so comparison can be made between figures.

**Case A: Effect of Random Noise**

Figure 7 (Model I) and Figure 8 (Model II) show the results of deconvolution when random noise is added to the test data but the spread function is known exactly (spread factor \( \psi = 1 \)). In each case the deconvolved
Fig. 8. Deconvolution with Exact Spread Function (Model II): Here again the solution superimposes almost exactly on the model. The peak and plateau coincide.

Fig. 10. Effect of Narrowed Spread Function (Model II): Here again the solution falls between the test data and the model. There is improvement on the test data, but the plateau is not completely resolved.

Fig. 9. Effect of Narrowed Spread Function (Model I): The solution falls between the test data and the model. Hence there is improvement, but resolution is not complete.

Fig. 11. Effect of Widened Spread Function (Model I): The solution peak is higher than the model peak, and at 1/2 mm there is an overshoot. Input of too wide a spread function does not yield an improvement over the test data.

Case B: Effect of Spread Function

Figure 9 (Model I) and Figure 10 (Model II) show the results of deconvolution when too narrow a spread function is used ($\psi = 0.9$). In each case the deconvolved scan is closer to the model data than the test data. The peak height falls between that of the test data and that of the model. In Model II the preretinal plateau is not resolved completely. Thus in these cases deconvolution is an improvement on the test data, but resolution is not complete. Of course, in the limiting case of deconvolution with a very narrow spread function $\psi \to 0$, the deconvolved scan would simply reproduce the test data, with no gain in resolution.

Figure 11 (Model I) and Figure 12 (Model II) show the results of deconvolution when too wide a spread function is used ($\psi = 1.2$). In each case the peak height is higher than that of the model, and there is a tendency to overshoot immediately past the peak. At any given point the deconvolution may be closer or further from the model as compared with the test data.

In summary, simulations using deconvolution are used to resolve two model distributions. These simulations demonstrate that deconvolution yields an improvement over the test data in the presence of noise in cases where the spread function used is narrower than or equal to the true spread function. When the
spread function is known exactly, deconvolution provides exact resolution of the model distributions. Use of too wide a spread function may yield unreliable results.

**Patient Studies**

In general our results for average concentration were in good agreement with those obtained from the Optimized Protocol package supplied by Coherent and based on the methods in. However, the shape of the distribution in the posterior vitreous supplied some new information. The height of the retinal peak was greater in every case, and there was better separation of posterior vitreous fluorescence from retinal fluorescence in every case. Other specific findings, including the shift of a retinal peak, are noted in the examples below.

**Fig. 12.** Effect of Widened Spread Function (Model II): The solution peak overestimates the model peak and the plateau is replaced by a trough artifact. Use of too wide a spread function can introduce errors in deconvolution.

**Fig. 13.** Active Toxoplastic Chorioretinitis with Severe Vitreous Leakage: Deconvolution shows that the true retinal peak of fluorescence is located .5 mm posterior to the apparent peak, a phenomenon seen when high vitreous fluorescence levels are present.

**Fig. 14.** Partial Branch Retinal Vein Occlusion, Inactive: Note the enhanced resolution at retinal and vitreous fluorescence.

**Fig. 15.** Senile Macular Degeneration with Marked Attenuation of RPE Density: Deconvolution shows significant accentuation of the retinal peak.

Figure 13 represents a case of active toxoplastic chorioretinitis with marked early vitreous leakage. Deconvolution shows that the true retinal peak is .5 mm posterior to the measured apparent peak. This then is an example of a case in which vitreous fluorescence moves the measured peak anteriorly by the convolution phenomenon. (That this could occur was demonstrated by simulations in Koester et al).6

Figure 14 is an example of a patient with partial branch retinal vein occlusion. The deconvolution shows better delineation of the retinal from the vitreous fluorescence than does the raw data.

Figure 15 is an example of a patient with senile macular degeneration with significant window defects on fluorescein angiogram. Deconvolution with the instrument spread function improves the sharpness of the retinal peak.

Another interesting example (Fig. 16) shows a midvitreous “camel hump” in a patient with optic atrophy.
Such phenomena have been reported in retinitis pigmentosa\(^7\) and in vitreous abnormalities.\(^8\) The mid-vitreous hump is accentuated by deconvolution. This is a case in which it was clearly advantageous to relax condition 2 of the model and to permit relative minima.

The patient in Figure 17 also had massive early leakage in the bolus scan due to active uveitis. This was the only eye in which the deconvolution gave an average concentration (24.0 ng/ml) which differed markedly from that obtained using the Coherent Optimized Protocol (−2.5 ng/ml). The latter negative value was caused by the early leakage, which the Optimized Protocol subtracted from the measurement scan.

Figure 18 demonstrates the ability of the least squares deconvolution protocol to smooth out a different sort of artifact, a blink. The artifact is much larger than usual background noise, and would be accentuated by deconvolution if least squares smoothing were not employed.

**Discussion**

Simulation studies show that deconvolution can completely resolve detail in fluorophotometry tracings near the retina that has been obscured by the intrinsic spread function of the instrumentation. This precise resolution requires precise knowledge of the spread function.

For the deconvolution of patient scans, the exact spread function of a given eye includes the instrument spread function and other factors and cannot be known precisely. However, deconvolution using the instrument spread function alone reliably provides improved resolution. The principal improvements are (Figs. 13–18): (1) enhancement of retinal peaks, (2) better separation of posterior vitreous from retinal fluorescence, (3) correct location of retinal peaks (Fig. 13). Accurate separation of retinal and vitreous fluorescence is most important since quantification of the breakdown of the retina/vitreous barrier depends on it (cf Bursell et al).\(^2\) The gradient for flow from retina to vitreous is also essential to understanding the kinetics of the distribution of fluorescein,\(^5\) and more accurate retinal peak heights as well as nearby vitreous values could aid these studies.

The spread function S near the retina for a given eye is a complex aggregate of the contributions from (at least) the following: (1) instrument spread function, (2) scattered light within the lens, (3) scattered light in retina or vitreous, (4) refractive error.

It is clear therefore that S must be wider than S\(_o\) (the instrument spread function) in every case. Hence, use of the instrument spread function for deconvolution guarantees that the true spread function is at worst underestimated. This investigation has thus used the

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**Fig. 16.** Optic atrophy and Mid-Vitreous “camel hump”: Note the increased resolution and shift of this phenomenon by deconvolution.

**Fig. 17.** Active Inflammation and Massive Early Leakage: Deconvolution enhances the separation of retinal from vitreous fluorescence in this extreme case. Artifact is easily introduced by other methods of analysis.

**Fig. 18.** Blink Artifact: Note how the least squares solution smooths out the blink at 3.5 mm. Without least squares smoothing the artifact would instead be accentuated (compare Figs. 4 & 5).
instrument spread function for all patient studies, since the simulations show that deconvolution with an underestimated spread function reliably yields an improvement over the measured data even if resolution is not complete.

Accurate estimates of the spread functions of patient eyes could improve resolution further. For example, the retinal peak of the 5-min bolus scan provides one in vivo estimate, treating the retina as a thin test film distribution. However, the assumption that the bolus peak derived from a thin film only would not be valid in the case of eg (1) significant reduction in RPE density, resulting in a large choroidal contribution (cf\(^5\)) or (2) early vitreous leakage at 5 min. Hence use of the bolus peak might lead to an over-estimate of spread function width, with a less reliable deconvolution as shown by the simulation studies. Further study is needed to see if in vivo spread functions can be determined with sufficient accuracy for use in deconvolution.

In summary, deconvolution methods are a promising adjunct to the analysis of fluorophotometry data. Posterior vitreous average concentrations are readily obtained which are comparable to those derived from present modes of analysis. Detail of fluorescein concentrations (and resolution of retinal and vitreous fluorescence) is obtained which is not available from other methods.

**Key words:** vitreous fluorophotometry, convolution, deconvolution, spread function, least squares

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### References