# Expanding the applicability of the multi-photon fluorescence recovery after photo-bleaching technique *in vivo* using a new diffusion-convection model


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## Abstract


Multi-photon fluorescence recovery after photobleaching (MP-FRAP) is a well-established microscopy technique used to measure diffusion in biological systems. In this poster, we present the derivation of a new fluorescence recovery model that explicitly accounts for the possibility of convective flows in a system. We test this "diffusion-convection" model through both computer generated data and fitting, and *in vitro* experimentation. We also demonstrate our model *in vivo*. Our results show that the diffusion-convection model significantly improves the capabilities of MP-FRAP by yielding accurate values for the diffusion coefficient, even when significantly large flow speeds are present.


## Introduction

MP-FRAP is performed by using an intense laser flash to irreversibly photobleach a region of interest, then monitoring the region of interest as still-fluorescent molecules from outside the region diffuse inward to replace the bleached molecules.

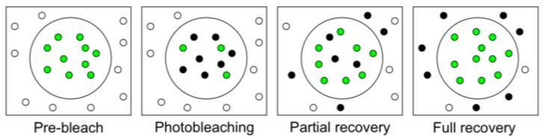


Figure 1: FRAP. Green dots indicate fluorescing molecules, black dots represent photobleached molecules (non-fluorescent), and white dots represent unexcited fluorescent molecules.

The resulting fluorescence recovery curve can be modeled mathematically and the relevant parameters determined from the data by a least-squares fitting algorithm.

$$F(t) = F_o \sum_{n=0}^{\infty} \frac{(-\beta)^n}{n!} \frac{1}{1+n+2nt/\tau_D} \frac{1}{\sqrt{1+n+2nt/R\tau_D}}$$

Equation 1: The diffusion-only MP-FRAP model includes two fitting parameters: the bleach depth parameter, β, and the characteristic diffusive recovery time, $\tau_D$.

One of the goals of our research is to improve MP-FRAP by including the effect of convective flow in the derivation of the fluorescence recovery model. In this new "diffusion-convection" model a third fitting parameter, $\tau_v$, is introduced, which describes the characteristic recovery time due to linear flow.

$$F(t) = F_o \sum_{n=0}^{\infty} \frac{(-\beta)^n}{n!} \frac{\exp\left[\frac{-4n(t/\tau_v)^2}{1+n+2nt/\tau_D}\right]}{(1+n+2nt/\tau_D)(1+n+2nt/R\tau_D)^{1/2}}$$

Equation 2: The diffusion-convection MP-FRAP model. The exponential describes the component of recovery due to convective flow.

## Results

IN SILICO: Computer generated data was used to establish the conditions (input parameters and noise) under which the fitting algorithm could correctly return the input parameters.

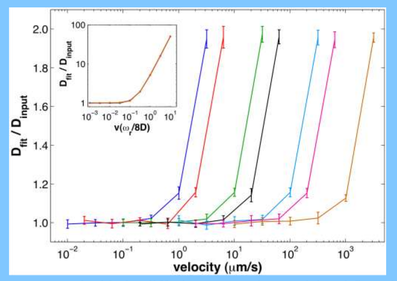


Figure 2: Data generated with the diffusion-convection model and fit to the diffusion-only model. Each colored line represents a different value of input diffusion coefficient. When the horizontal axis is scaled, we find that the diffusion-only model begins yielding erroneous diffusion coefficients at a scaled velocity of $v_s \sim 0.1$.

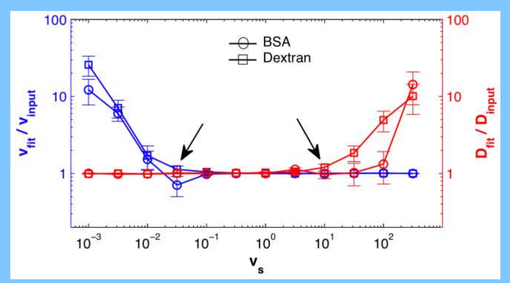


Figure 3: Data generated and fit using the diffusion-convection model. When either diffusion (low $v_s$) or flow (high $v_s$) dominate the recovery, the non-dominant parameter is poorly fit. For a wide range of balanced recoveries (between arrows), both parameters are well fit. The new model extends accurate recovery of the diffusion coefficient to scaled velocities on the order of vs ~ 1.

IN VITRO: Experimental data taken in a system with know diffusion and flow was used to establish the physical validity of the diffusion-convection model and to more accurately establish the conditions under which the two models are relevant.

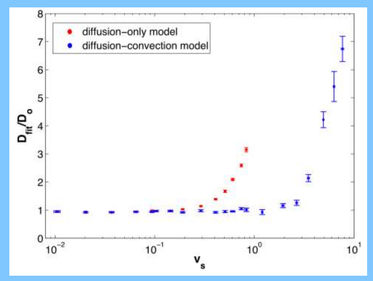


Figure 4: Experimental data taken in the presence of flow and fit to both the diffusion-only and diffusion-convection models. The results show that the diffusion-convection model correctly determines the diffusion coefficient in the presence of flows an order of magnitude greater than those at which the diffusion-only model breaks down.

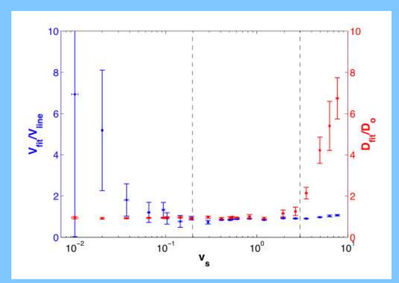


Figure 5: Experimental data taken in the presence of flow and fit to the diffusion-convection model. The dotted lines delineate three distinct regions: 1) diffusion-dominated, in which only the diffusion coefficient is well fit; 2) balanced, in which the diffusion coefficient and flow speed are well-determined; 3) flow-dominated, in which only the flow speed is accurately determined.

IN VIVO: We chose to demonstrate the effectiveness of the diffusion-convection model *in vivo* by measuring diffusion (and convection) within tumor vessels. This model was selected because blood flow through tumor vessels exhibits a wide range of flow speeds with which to fully test the diffusion-convection model *in vivo* in analogy with our *in vitro* experimentation. Moreover, measurement of plasma viscosity (using a simple conversion via the Stokes-Einstein equation) has important implications in the study of several disease states.

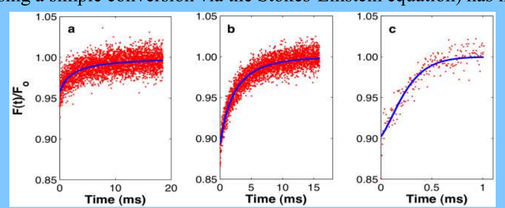


Figure 6: Experimental fluorescence recovery curves of FITC-dextran, flowing in vessels of 4T1 tumors growing in dorsal skinfold chambers. Each curve represents a different fitting regime for the diffusion/convection model: (a) diffusion-dominated, in which only the diffusion coefficient is accurately determined; (b) balanced, in which both the diffusion coefficient and flow speed are well fit; (c) flow-dominated, in which only the flow speed is accurately determined. The values for the diffusion coefficient and flow speed obtained from the fit to each curve (data not shown) bear out the expectations for each regime.

## Methods

MP-FRAP is performed using a tunable mode-locked Ti:sapphire pulsed laser. Laser power is modulated rapidly between monitoring and bleaching intensities by a Pockels Cell. Fluorescence emission is collected by a photomultiplier tube, connected to a multi-channel scaler, which sums photon counts over a large number of bleach-recovery cycles. Fits to the data are generated by the lsqcurvefit function in MATLAB.

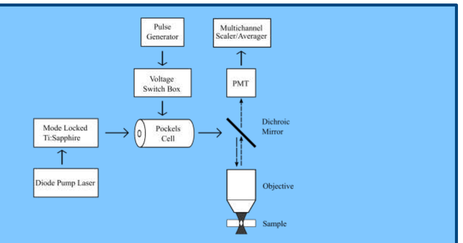


Figure 7: Equipment diagram.

*In silico* data was generated in MATLAB using equations 1 & 2 for the diffusion-only and diffusion-convection models, respectively. Testing of each model was conducted by generating data using the diffusion-convection model and then fitting the data to both models and comparing input and output parameters.

*In vitro* samples were produced by allowing a solution of fluorescein isothiocyanate (FITC), conjugated to either bovine serum albumin (BSA) or $2*10^{-6}$ mW dextran, to flow freely through a narrow channel. Fits to the diffusion coefficient were compared to values taken without flow, while velocity fits were compared to line-scans taken concurrently with the MP-FRAP data.

*In vivo* measurements were made in blood vessels of tumors implanted within dorsal skinfold chambers on male BALB/cBYJ mice. Fits to the diffusion coefficient were compared to *in vitro* values, while velocity fits were compared to line-scans.

## Summary and Conclusion

We derived a new mathematical model to describe fluorescence recovery after photobleaching in the presence of convective flow, as well as diffusion. We also explored the enhanced capabilities of our diffusion-convection model. Our *in silico*, *in vitro*, and *in vivo* data all concur and show that the diffusion-convection model broadens the applicability of the MP-FRAP technique by enabling the accurate recovery of diffusion coefficients in the presence of significant convective flow.

## Acknowledgements


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