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AN APPROACH TO DETERMINE DIFFUSION COEFFICIENTS IN INHOMOGENEOUS TISSUES WITH FRAP

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INTRODUCTION

The diffusion coefficient (D) determines the diffusion rate of a given molecule through a particular material. For biological tissues, D is an important parameter if one is to study nutrient supply and transport of signaling molecules. Local D’s can be determined with fluorescence recovery after photo bleaching (FRAP) [Carrero, 2003]. However, methods to quantify D from FRAP data typically assume homogeneity, which is not true for many biological tissues, and cannot deal with boundary conditions such as difficult geometrical restrictions. Also, limitations to the intensity distribution of acquired images exist.

Our aim was to develop a method that can account for inhomogeneity and geometrical boundary conditions, while being independent of image size. This abstract provides a validation and shows the relevance for inhomogeneous tissues.

METHOD

Agarose discs were loaded with 66 kDa fluorescein-albumin. After bleaching an area with 9 or 18 µm radius twice, 110 images (1/s) of 56*56 µm were acquired (Zeiss LSM510, 20x obj). Two bleach area sizes were used for validation: D must be independent of bleach area size. Intensity recovery of the bleach area is determined from the images (fig 1).

![Fig 1. Intensity recovery with time (s) in an 18 µm bleach area in agarose, along with the best model fit.](image)

To compute D the measurement is simulated with a 2D FE analysis encompassing a 1 mm diameter area and constant border intensity. The initial intensity distribution in the central 56*56 µm of the model was derived from the first acquired image after bleaching, by transferring the mean intensities in seven rings around the bleach area to the input mesh for the FE simulation. Using an automated iterative procedure, the intensity recovery curve as computed with the FE analysis was fitted on the experimental curve (fig 1). Fitting parameters are D and the initial intensity of the area outside the 35 µm of the image (I_{out}).

RESULTS

Fitted curves highly correlated with measured data (fig 1), D and I_{out} were independent of initial values, and I_{out} was always trustworthy. Variations of D were within 3% for both 9 and 18 µm bleach areas at different spots. Differences between both sizes were within the same range, but depended on the number of rings used to describe the initial intensity distribution.

Applicability is shown by a hypothetical FRAP measurement near the transition between two tissues (e.g. cartilage-bone), with D_{tissue1}=10*D_{tissue2}. Images and intensity recovery of the bleach area are distinct from the situation without such transition (fig 2). Hence, neglecting the transition results in erroneous estimation of D. The present method can account for it and thus correctly determines D_{tissue1} in both cases.

![Fig 2. Left: Hypothetical FRAP images at t=2, 5 and 25 s, with (upper) or without (lower) tissue transition. Right: Normalized intensity recovery with time (s).](image)

CONCLUSION

A new method to obtain D from FRAP experiments is developed, based on a fitting procedure with a 2D diffusion FE model. This method can deal with tissue anisotropy and geometrical restrictions. Combination of FRAP with this method is particularly promising because geometrical data is inherently obtained from the FRAP images themselves.

REFERENCES