

# *In vivo* fluorescence tomography of m-THPC in a colon cancer mice model

Pontus Svenmarker<sup>1</sup>, Johan Axelsson<sup>1</sup>, Haiyan Xie<sup>1</sup>, Niels Bendsoe<sup>2</sup>, Katarina Svanberg<sup>3</sup> Susanna Grafe<sup>4</sup> and Stefan Andersson-Engels<sup>1</sup>

<sup>1</sup> Department of Physics, Lund University, P.O box 118, SE-221 00 Lund, Sweden

<sup>2</sup> Department of Dermatology and Venereology, Lund University Hospital, P.O box 118, SE-221 00 Lund, Sweden

<sup>3</sup> Department of Oncology, Lund University Hospital, P.O box 118, SE-221 00 Lund, Sweden

<sup>4</sup> BioLitec AG, Winzerlaer Strasse 2, 07745 Jena, Germany  
pontus.svenmarker@fysik.lth.se

<http://www.physics.lu.se/biophotonics>

**Abstract:** The pharmacokinetics of a pegylated liposome-encapsulated m-THPC sensitizer is investigated in an female NMRI nu/nu mice model with murine HT29 colon cancer inoculated in both the left and right hind thigh. The aim is to non-invasively *in vivo* quantify the distribution of the m-THPC concentration by fluorescence tomography. In total 25 animal were used. Fluorescence (720nm) was induced by a laser diode (652nm), which was raster scanned across the ventral end of the mouse and captured by a CCD-camera on the dorsal end. All data were correlated with chemical extraction.

## 1 Introduction

Photodynamic therapy (PDT) as a cancer treatment modality has shown promising results both in terms of efficiency and selectivity. Three components are needed for a PDT response: light, oxygen and sensitizer. Among the most potent sensitizer available today is meso-tetra(hydroxyphenyl)chlorin (mTHPC), which forms aggregates in aqueous surroundings, leading to limited transportation within biological tissue, tumor selectivity and PDT efficiency. To improve on the distribution properties of the sensitizer, liposomes and more recently pegylated liposomes have been used as drug delivery vehicles. In this study, the pharmacokinetics of a pegylated liposomeencapsulated m-THPC sensitizer is investigated in an animal model. The aim is to non-invasively *in vivo* quantify the distribution of the m-THPC concentration by optical fluorescence tomography.

## 2 Teory

### 2.1 Steady-state optical tomography

Light propagation in turbid media can be described by the diffusion equation. For steady-state illumination, it is expressed as

$$-\nabla \cdot D(\mathbf{r})\nabla U(\mathbf{r}) + \mu_a(\mathbf{r})U(\mathbf{r}) = q(\mathbf{r}) \quad (1)$$

where  $\mu_a$  is the absorption coefficient,  $D = 1/3(\mu_a + \mu'_s)$  is the diffusion coefficient,  $\mu'_s$  the reduced scattering coefficient,  $q$  the source and  $U$  the photon density at position  $\mathbf{r}$ . The boundary data  $y$  consists of intensity measurements for different projection through the media. It can be used for reconstructing an interior image of  $\mu_a$ , when assuming a known  $\mu'_s$ . The image is found from minimizing the objective function  $\Omega$ .

$$\Omega = \min_{\mu_a} \{ \|y - F(\mu_a)\|^2 + \lambda \|\mu_a - \mu_{a0}\|^2 \} \quad (2)$$

Here  $F$  is the forward mapping of the inertial parameters to the boundary measurements. Tikhonov regularisation, with hyper parameter  $\lambda$ , is also applied to help find a solution for the otherwise ill-posed problem. By taylor expanding Eq. (2) and setting the derivative with respect to  $\mu_a$  to zero, an update equation can be derived

$$(J^T J + \lambda L^T L) \delta \mu_a = J^T (y - F(\mu_a)) - \lambda L^T L \delta \mu_a \quad (3)$$

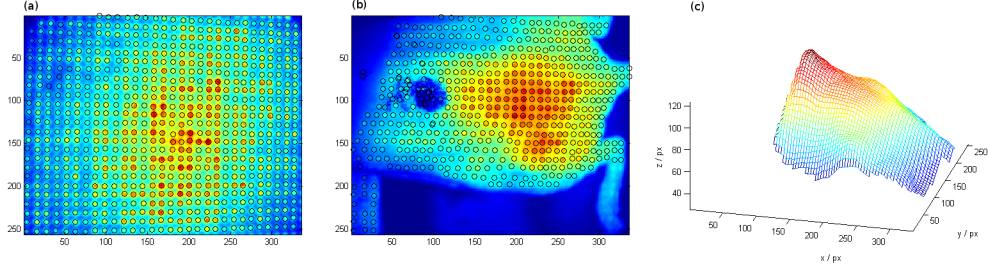


Fig. 1. Surface shape rendering of a mouse. (a) Flat surface, (b) Dot offset caused by the mouse and (c) the mouse surface.

where  $J$  is the Jacobian matrix,  $L$  the identity matrix and  $\delta\mu_a$  is the update in absorption coefficient. Eq. (3) is iterated until the forward model matches the experimental data within a pre-set threshold.

## 2.2 Fluorescence tomography

The fluorescence light emission can be modeled with two coupled diffusion equations, one describing the excitation photon density,  $U^x$ , and the second the fluorescence photon density,  $U^f$ .

$$-\nabla \cdot D(\mathbf{r})\nabla U^x(\mathbf{r}) + \mu_a(\mathbf{r})U^x(\mathbf{r}) = q(\mathbf{r}) \quad (4)$$

$$-\nabla \cdot D(\mathbf{r})\nabla U^f(\mathbf{r}) + \mu_a(\mathbf{r})U^f(\mathbf{r}) = U^x(\mathbf{r})\eta(\mathbf{r}) \quad (5)$$

Again, the image formation is posed as a least square problem. But here the fluorescence yield,  $\eta(\mathbf{r})$ , is sought by minimizing  $\Theta$ .

$$\Theta = \min_{\eta} \{ \|y - F(\eta)\|^2 + \lambda \|\eta - \eta_0\|^2 \} \quad (6)$$

Eq. (6) can now easily be linearised, since the forward operator  $F(\eta)$  is linear with the fluorescence yield, and an update equation can be derived

$$(J^T J + \lambda L^T L) \delta\eta = J^T (y - F(\eta)) - \lambda L^T L \delta\eta \quad (7)$$

The change in fluorescence yield  $\delta\eta$  can now be found in a one-step-iteration, which makes the fluorescence problem computationally easier than the optical tomography.

## 3 Experimental Setup

### 3.1 Dot pattern projection for surface rendering

A dot pattern matrix is projected onto the mouse surface, see Fig. 1. An image is recorded for a flat surface and with the mouse present. By evaluating the movement of the dots made by the presence of the mouse, and knowing the angle of illumination, the height in every dot-pixel can be retrieved. Thereafter the shape of the mouse is known.

### 3.2 White-light transillumination imaging

White-light transillumination measurements were made at wavelengths ranging from 650 nm to 890 nm measuring the optical properties. A Xenon-Mercury arc lamp was used together with a liquid crystal tunable filter and a CCD-camera. A total of 6 sources were used, positioned on the ventral end of the mouse around the injected tumour.

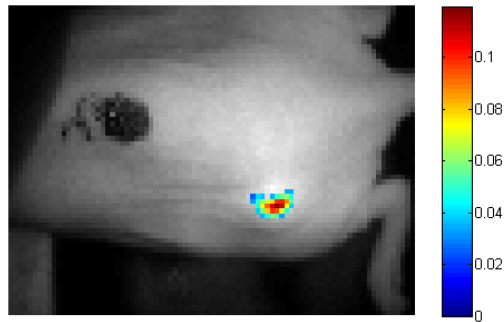


Fig. 2. Reconstructed fluorescence image of the m-THPC in an animal of drug-light interval of 4 hours.

### 3.3 Fluorescence imaging

Fluorescence measurements of the FosPeg drug were made at excitation at 652 nm and emission at 720 nm. A high power laser diode was used as light source. Here a total of 20 sources, placed around the injected tumour, were used.

## 4 Method

### 4.1 Animal handling

Female NMRI nu/nu mice were intravenously injected with a sensitizer dose of 0.15mg/kg m-THPC concentration. The tumor model was a murine HT29 colon cancer inoculated in both the left and right hind thigh. Five different drug-light interval were investigated; 2h, 4h, 8h and 18h. In total 25 animal were used.

### 4.2 Optical tomography

The finite-element mesh was made by the tool iso2mesh. For solving all diffusion equations, and for calculating the inverse solutions, the TOAST toolbox was used. Initially, the background optical properties was found by using the white-light transillumination data. With the optical properties known, the fluorescence yield was found from the the fluorescence data.

## 5 Results

A preliminary fluorescence image is shown in Fig. 2. It displays the relative fluorescence yield of the m-THPC drug in an animal of drug-light interval of 4 hours. The image is cropped to only the region of the tumour inclusion.

## 6 Summery and Outlook

In summery, the m-THPC biodistribution can non-invasively *in vivo* be imaged with florescence tomography. It know remains to find how well fluorescence tomography can quantify the m-THPC concentration, which will be assessed by comparing to chemical extraction.