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The Medical Use of Rescaling Procedures in Optical Biopsy and Optical Molecular Imaging

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Laser-induced autofluorescences show a strong intensity distortion for endogenous NADH in the UV and synthesized markers in the NIR range because of tissue optics. Rescaling taking into account bio-optical methods results in the chromophore profile in the observed tissue region. For the *in vivo* tests an experimental NIR imager was used. NIR fluorescence of the entire body of small animals can be imaged. For first experiments an undifferentiated superficial tumor of mouse thigh was used. Corrections that are due to tissue optics must take care of a more strongly scattering of the light in the NIR range in comparison to the UV fluorescence such as in optical biopsy. For example, the diameter of the fluorescent volume is apparently larger for the same reason. Therefore, the established rescaling from the UV adapted to the NIR range is important for the interpretation fluorescence pictures in biomedicine.

KEY WORDS: Optical biopsy; optical molecular imaging; rescaling; SIDAG.

INTRODUCTION

Using optical biopsy, the analysis of laser-induced autofluorescences in squamous cell carcinoma shows a strong spectral congruence from cytokeratin and NADH in the UV range under steady state conditions [1,2]. Time-resolved measurements of fluorescence decay allows a differentiation of both sources. Taking into account the established method of rescaling [3], it is possible to construct a look-up table of intrinsic fluorescence (i.e., concentration of NADH) vs. measured fluorescence intensity for known optical parameters of the tissue under consideration.

The rescaling procedure consists of two steps. First, the signal-to-noise ratio of the CCD camera is set off in the black and white bitmap mainly by cutting off those signals, which are below the noise limit compared to the maximum intensity. In second step, tissue optics knowledge is used for rescaling fluorescence intensities by means of a Monte Carlo simulation because analytical and approximative solutions of the transport equation of light break down even for less complicated geometries. The result is the NADH profile in the tumor border region [4]. As an example for a concentration profile a native picture of a tumor of the base of a human tongue is shown (Fig. 1). Right beside it is the corresponding fluorescence and the rescaled image. The rescaling is made by using the optical parameters in relationship to the tissue region of interest.

Approaches using autofluorescence are replaced by optical molecular imaging (OMI) using of drugs such as indocyanine green (ICG) or 5-ALA to enhance the fluorescence intensity and its tissue specifity. Generally, dyes as contrasting agents for deeper tissues have to be biocompatible and absorb in the near-infrared (NIR)

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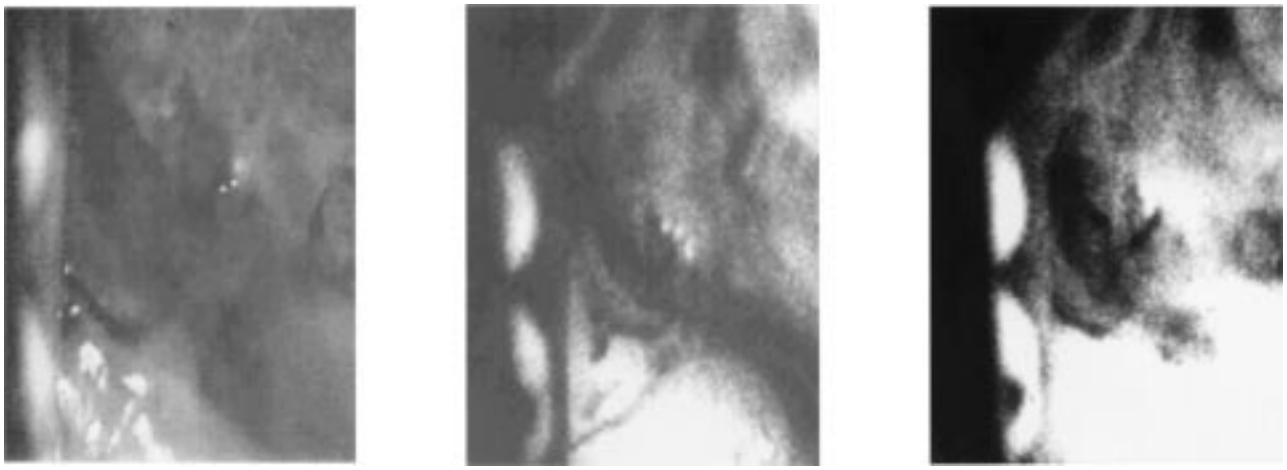

Fig. 1. Example for the rescaling method in optical biopsy of a base tumor of a human tongue. Left: Native image. Middle: Fluorescence image. Right: Rescaled image.

spectral region to ensure deep penetration of light into tissue, for example by using fluorescent probes based on the class of cyanine dyes. One of the outstanding advantages of OMI is the capability to regulate the fluorescence of dyes by specific molecular interaction, e.g. with tumor-associated proteolytic enzymes which cleave quenched dyes and restore previously quenched fluorescence. Other solutions leading to tumor-specific fluorescence comprise receptor-targeted cyanine dye-peptide conjugates.

EXPERIMENTS AND RESULTS

For the *in vivo* studies of the new dyes an near infrared imager was used. The imager consists of a 740-nm cw diode laser (1,5 W, CeramOptec) and a cooled CCD-camera (Hamamatsu). The near infrared fluorescence at 790 nm of an entire mouse body can be imaged with a specific interference filter in relationship to the curves in Fig. 4. On the left a native image of a mouse is shown, on the right the fluorescence image (Fig. 2).

Figure 3 shows an undifferentiated superficial tumor of mouse thigh. The images are taken 20 h after administering 2 μM/kg NIR96010 (IDF/Schering), which is synthesized *bis*-1,1′-(4-sulfobutyl)indotricarbocyanines with the substituent R_1.

To provide high hydrophilicity, two different aminosugar derivates, D-glucamine and D-glucosamine, were used for synthesis. The plasma protein binding decreases in agreement with increasing hydrophilicity. NIR96010 is considered to be the most hydrophilic compound because of its lowest plasma protein binding. The

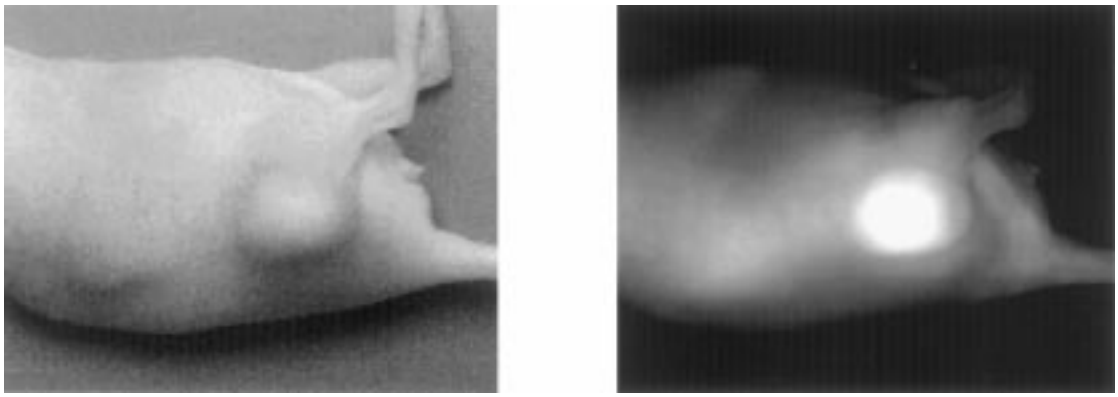

Fig. 2. Native (left) and fluorescence image (right) of superficial tumor of a mouse thigh.

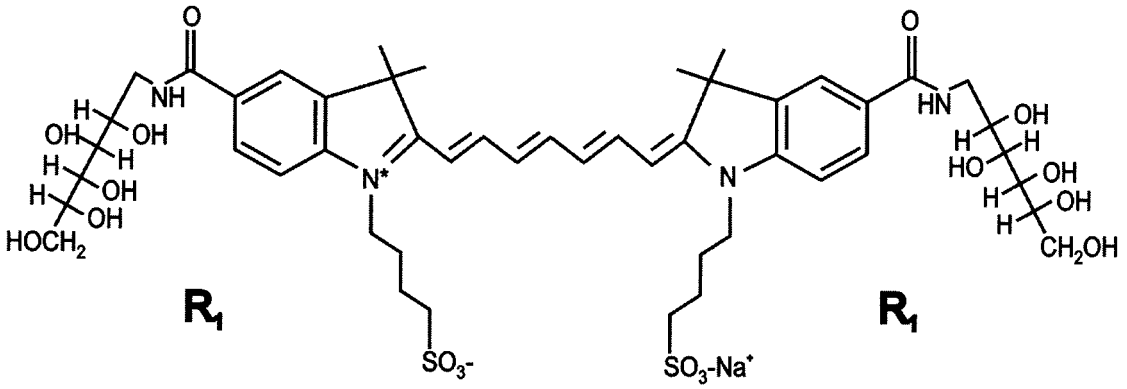

Fig. 3. Chemical structure of hydrophilic cyanine dye SIDAG.

absorbance and fluorescence spectrum of NIR96010 in plasma show a maximum for 755 nm and 790 nm, respectively (Fig. 4).

In Fig. 2 on the left, a white-light image of a tumor-bearing mouse (F9 teratom) is shown, and the right image shows a NIR fluorescence image demonstrating a high tumor-to-normal tissue fluorescence contrast after intravenous injection of the dye SIDAG. SIDAG served as a model compound and was taken from a set of synthesized indotricarbocyanines of high hydrophilicity (Fig. 3) [5]. As a result of the imaging experiment, a contrast ratio of approx. 10 was achieved at 20 h after injection of 2 μmol compound per kg body weight (Fig. 2). Due to the high hydrophilicity of the dye, its pharmacokinetic properties are fundamentally different to ICG, exhibiting extravasation from the vasculature and preferential retention in tumor tissues [7]. The absorbance and fluorescence spectra in plasma show a maximum at 755 nm and 790 nm, respectively (Fig. 4). In contrast to SIDAG, ICG does not lead to comparable contrast (data not shown) as it is completely bound to plasma proteins, predominantly distributed in the intravasal space and rapidly cleared from the tissue by the liver with a short tissue half-life.

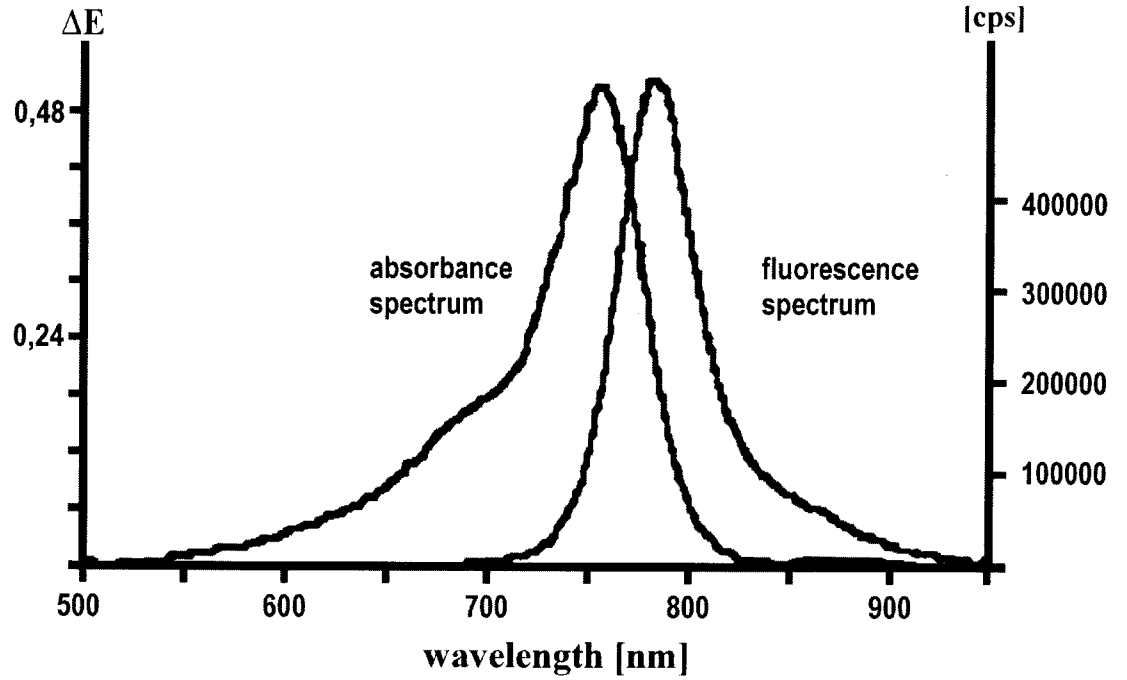

Fig. 4. Absorbance and fluorescence spectrum.

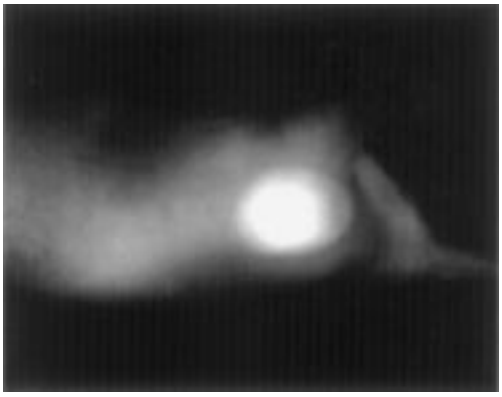

Fig. 5. Fluorescence image from Fig. 2 after rescaling procedure.

DISCUSSION

In the in vivo model discussed herein, contrast-enhancement relied mainly upon a more distinct perfusion of tumors compared to normal tissues, nonetheless providing imaging results suitable for studies on image rescaling.

To improve the image of the concentration of the NIR-fluorphore in the mouse, a new robust rescaling principle can be developed as a first approach. For tissue-optics related corrections it is to be considered that the light scattering in the NIR range is stronger compared to the UV fluorescence used in optical biopsy. Nevertheless, the crucial point is the knowledge of the optical parameters μa, μs, and g (anisotropie) and the use of inverse Monte Carlo methods. The diameter of the fluorescent volume is apparently larger for the same reason. Furthermore, the signal-to-noise ratio can be improved by suppressing parasitic fluorescences [4]. (Figure 5) shows the first result in rescaling the above fluorescence image of the mouse thigh.

With the modified rescaling principle for the NIR-spectral region rescaled images of NIR-fluophores can be obtained. The concentration profile of NIR-fluophores in the border region of tumors can be received just like the NADH profile in optical biopsy and can be used as a first step of the three-dimensional reconstruction of the distribution of the fluophore [6]. Based on this principle, the enhancement of border sharpness and local resolution provides a chance to identify and analyse small tissue abnormalities, such as early-stage tumors. The method is suitable in combination with non-specifically accumulated dyes, as shown in this paper, and might improve further using targeted or smart fluorescent probes of NIR fluorescence.

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