

Fluorescence diffuse tomography for monitoring tumor growth in small animals

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Introduction. Fluorescent imaging based on the specific marking of tumors is widely used in experimental oncology. The possibility to introduce genes of a particular class of fluorophores - fluorescent proteins (FPs) - into cells enabled the development of a new method: genetic marking. The fluorescence ability of FPs persists for the whole life of a cancer cell and remains after cell division. As a result, it becomes possible to estimate tumor growth rate, to study the mechanism of carcinogenesis and metastasis formation, and to investigate the safety and efficacy of intervention using novel therapeutics. Recently, a new group of FPs - red fluorescent proteins (RFPs) - was isolated, and they became useful as markers for whole-body biological imaging. The fluorescence spectrum of these proteins is in the relatively long-wave part of the spectrum (580 to 650 nm), a region that is promising for object visualization at depths up to 1 to 2 cm with millimeter resolution. Therefore, RFP-labeled tumors can be regarded as the most appropriate model for whole-body investigations.

Materials and methods. A fluorescence diffuse tomography (FDT) setup for monitoring tumor growth in small animals has been created. Low-frequency modulated light (1 kHz) from a Nd:YAG laser with second-harmonic generation at a 532 nm wavelength, which is close to the absorption maximum of RFPs, was used in the experimental setup (Fig. 1).

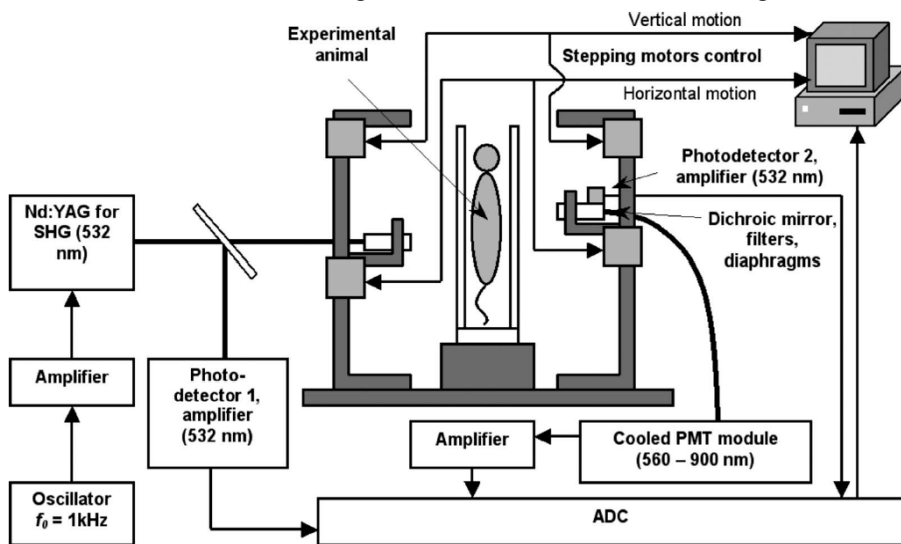


Fig. 1. Schematic of the FDT experimental setup.

The power on the investigated object was 20 mW, the beam diameter was 0.5 mm. The detector was set as close as possible to the surface of the mouse. This enabled us to collect fluorescent light with a higher numerical aperture (NA), thus providing a higher signal. The detector consisted of diaphragms, filters, an optical beam and a high-sensitivity cooled PMT module. The aperture of the detector was 0.5 mm,

and the NA of the detector was 0.22. A dichroic mirror was placed behind the input diaphragm to separate emission (540 to 650 nm) and excitation (532 nm) light transmitted through the experimental animal. The transmitted excitation light was detected by photodetector. To detect fluorescent light, we used PMT. The sensitivity of such a system is also determined by the optical density (OD) of the optical filters. Using a combination of interferometric and absorption filters allowed us to achieve an OD value of 6. We applied low-frequency modulation to increase the signal-to-noise ratio. Plane geometry was used in our FDT setup to minimize the thickness of the transilluminating tissue. Thus, the decay of the light propagated through the mouse was minimized.

Experiments with FDT were performed using FPs from the RFP family: DsRed2, DsRed-Express, and Turbo-RFP. These proteins have similar positions of fluorescence maximums in the red-orange spectral region and are suitable for whole-body imaging. During the experiment, an animal was placed vertically in a container consisting of a supporting plate and a covering glass plate that was

slightly pressed to fix the animal. The distance between the plates was about 1 cm. Synchronous scanning of the object in the transilluminative configuration was provided by a single pair of a source and a detector set in motion by stepping motors. Programmable source and detector positions enable one to set the optimal law of source-detector travel. To reduce the time of investigation, an experimental animal was scanned using the following algorithm (Fig. 2): 1) large-step scanning to obtain a general view of the animal; 2) selection of the fluorescing region; and 3) small-step scanning of the selected region with different relative shifts between the source and detector to obtain sufficient information for 3-D reconstruction. One source - detector measurement took about 50 ms. Obtaining a general view of the animal with large steps (when the source and detector move synchronously, (Fig. 1b) took about 2 to 3 minutes, and detailed scanning of the fluorescent region (Fig. 1c) for 3D reconstruction took about 20 minutes.

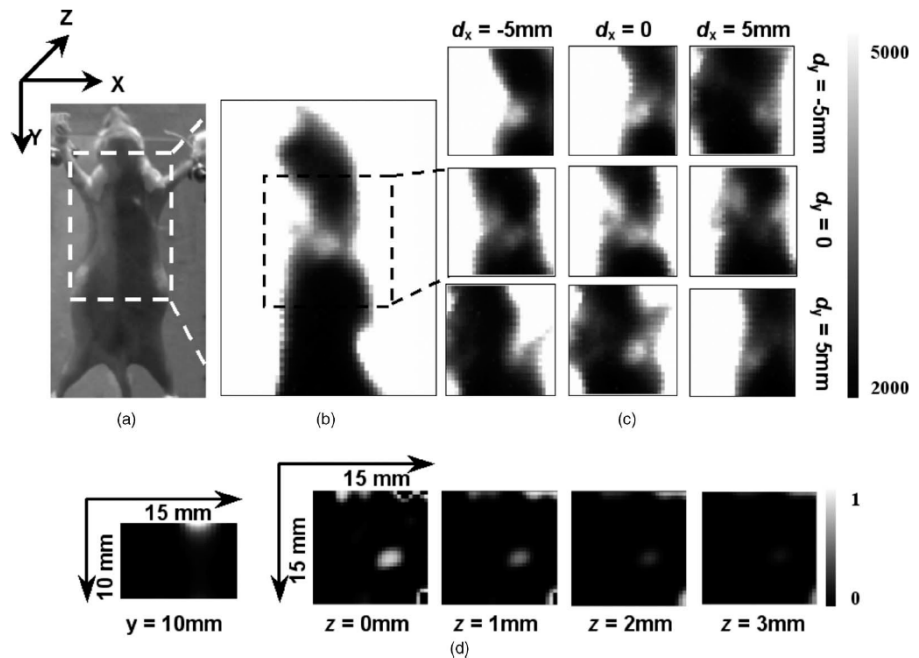


Fig. 2. Algorithm of scanning an experimental animal (a) by FDT setup: obtaining a general view of the animal (b), and scanning of the selected fluorescing region with different shifts between source and detector (c) (d_x, d_y - shifts between source and detector in the scanning plain xy). Reconstructed distribution of fluorophore concentration (d).

Results. A series of in vivo experiments were conducted using the experimental FDT setup described above. Whole-body FDT experiments were performed using HEK293 cells transfected with RFPs. A suspension of fluorescing HEK293 cells was injected subcutaneously into a female 12-week old nude mouse. Before the experiment, the anaesthetized animal was fixed on the supporting plate of the container. A suspension containing 1.7 million of cells was used in our experiments with Turbo-RFP. The FDT data of the mouse were obtained before the injection of the HEK293-Turbo-RFP suspension, then immediately after the injection, and 24 and 48 hours later (Fig. 3). The maximal intensity increase of the fluorescent signal was observed immediately after the injection. The results of subsequent imaging indicate a decrease of fluorescence intensity, possibly due to a redistribution of fluorescing cells near the region of injection. In the injection zone of the untransfected control HEK293 cells, no changes in fluorescence intensity were registered.

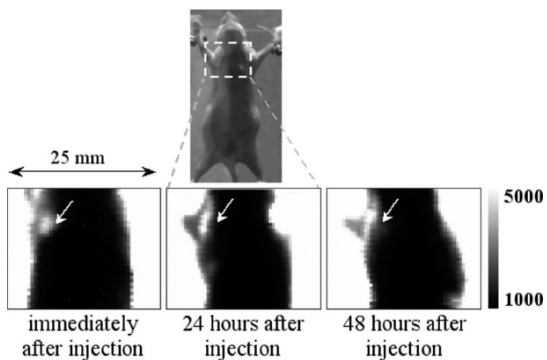


Fig. 3. Fluorescent images obtained in a transillumination configuration of source and detector of a nude mouse after injection of HEK293-Turbo-RFP cells.

The results of 3-D reconstruction in our in vivo experiments are shown in Fig. 4. As is clear from the results of the reconstruction, the concentration of the fluorophore is higher on the surface of the animal, namely in the injection area.

Discussion. The results of our experiments demonstrate the ability of the FDT method to detect fluorescence-marked cells in a small animal body, to perform 3-D tumor reconstruction, and to carry out monitoring experiments.

These results may be used to investigate tumor growth and metastasis formation mechanisms, and to estimate therapeutic responses.

The use of the normalized Born approach in the reconstruction algorithm may significantly improve the quality of a reconstruction. But detecting green light propagated through biotissue is complicated by the high intensity of light that is scattered in the supporting plate and propagated around an experimental animal. Moreover, the dynamic range of the “green” detector should be very high to detect the excitation light propagated through the experimental animal in areas of different thickness. By using an immersion liquid, the intensity of stray light will be decreased and the thickness of the transilluminating medium will be constant, so the detection of excitation light will be possible.

Another limiting factor for the detection of FP emission with low intensity is autofluorescence. This is not an acute problem if one works with nude mice. But even a small background can be a limiting factor for detecting small concentrations of fluorophore. The most effective way to overcome this limitation is to add spectral resolution measurements or lifetime measurements using the frequency-domain technique. Moreover, using of additional spectral resolution can essentially improve the reconstruction algorithm. Also described FDT setup can be easily modernized for reception additional spectral resolution measurements.

References.

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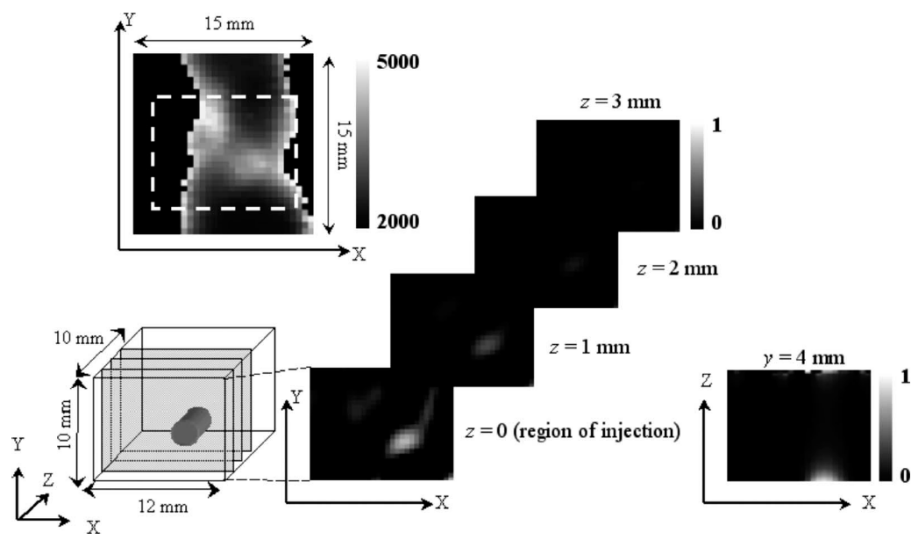


Fig. 4. Results of the 3-D reconstruction of the fluorophore concentration