Multicolor Imaging and Contrast Enhancement In Cancer-tumor Localization Using Laser-induced Fluorescence In Hematoporphyrin-derivative-bearing Tissue

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Published in:
Optics Letters

DOI:
10.1364/OL.10.000056

1985

Multicolor imaging and contrast enhancement in cancer-tumor localization using laser-induced fluorescence in hematoporphyrin-derivative-bearing tissue

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Received September 17, 1984; accepted November 15, 1984

Simultaneous imaging of laser-induced fluorescence in three selected wavelength bands from hematoporphyrin-derivative-bearing tissue has been performed, permitting considerable contrast enhancement for cancer-tumor localization.

Hematoporphyrin derivative (HPD), when injected intravenously, has the property of being selectively retained in cancer tissue. The presence of this compound in tumors can be used in two ways: for detecting malignancies through the specific HPD fluorescence released following UV- or blue-light excitation or for photochemical destruction of the tumors through HPD-assisted singlet oxygen release following laser irradiation at 630 nm. This strongly developing field of research was recently reviewed by Dougherty.[1]

Fluorescence-monitoring systems for endoscopic applications[2,3] normally detect the total red fluorescence in a selected wavelength band. In a recent investigation,[4,5] we demonstrated that, by monitoring the full laser-induced fluorescence (LIF) spectrum and by forming simple functions of fluorescence intensities in selected wavelength bands, it is possible to achieve a strong contrast enhancement in cancer-tumor detection, which is particularly valuable when a high level of natural HPD-nonrelated LIF is present. In this Letter we show how such procedures, which were used in point measurements, can be extended to the practically and clinically more interesting case of instantaneous imaging of the tissue area under consideration.

In our experiments we use the experimental arrangement illustrated in Fig. 1. For inducing fluorescence we use a nitrogen laser emitting at 337 nm in 10-nsec pulses at 10 Hz. The pulse energies used are about 1 mJ. By using cylinder lenses, the laser beam is shaped to a 20-mm-long, 1-mm-wide line at the position of the object. A fluorescence-free quartz plate is used for defining the object plane; the object is placed from below against the quartz plate. The LIF is directed by means of a plane mirror toward a multimirror arrangement consisting of three plane, individually adjustable first-surface aluminum mirrors, mounted as close as possible. A common achromatic lens, in our case a Nikon f = 80-mm/3.5 telelens, is used to form three individual images of the streak of LIF released in the object. By adjusting the mirrors, the three images (reduced by a factor of 4) are put side by side, and a Tracor Northern TN-1223-4IG intensified linear-array detector is placed at the threefold image line position. The array detector consists of 1024 diodes arranged in a strip of dimensions 25 mm × 2.5 mm. An interference-filter arrangement, selecting 5–10-nm-wide bands at 630, 600, and 488 nm, is placed in front of the array, allowing spatially resolved LIF to be detected in these three bands (488 nm represents a wavelength in the desired blue-green region, for which a filter was available). A dual-mirror arrangement was used previously in our laboratory for simultaneously imaging two radicals excited by different laser wavelengths in connection with combustion experiments.[6] A multimirror arrangement can also be used for quasi-2-D imaging.[7] In the present work a multimirror arrangement is used instead to produce the wavelength decomposition of the LIF necessary for contrast enhancement in LIF imaging measurements on solids.

The array detector is connected to a TN-1710 ID-ARSS mainframe, which is coupled to a minicomputer with floppy-disk data-storage capability. Although single-pulse measurements with a good signal-to-noise ratio were possible, we normally accumulated data from 50 pulses. Because of the gated action of the image intensifier in the detector arrangement, measurements could be made in full ambient illumination.

We used an experimental rat cancer system, as described previously.[4] A tumor on one hind leg of white Wistar-Furth rats, induced through subcutaneous in-

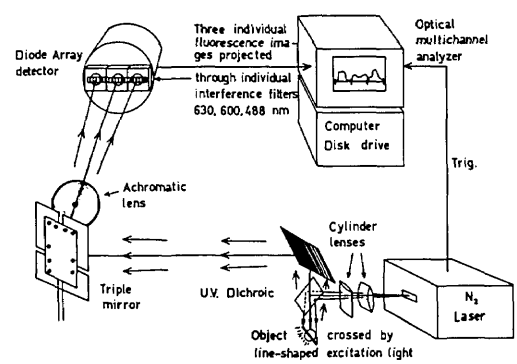

Fig. 1. Experimental arrangement for spatially resolved multicolor LIF measurements.

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jection of cancer cells, was allowed to grow to a diameter of 5–20 mm. One to three days before the animals were killed and studied, they were intravenously injected with a HPD solution (Photofrin I, ORD Inc., Cheektowaaga, New York), normally at 5 mg/kg of body weight. In our measurements the tumor capsule and the surrounding muscle fascia were exposed by removing the hairy skin, which is strongly fluorescent.

To illustrate the measurement technique we first chose a 10-mm-diameter tumor with a central necrosis from an animal that had received only one tenth of the normal HPD injection. This example, which is illustrated in Fig. 2, is not a typical tumor case but was selected because of the strongly structured LIF. In Fig. 2a a typical LIF spectrum of HPD-bearing tissue is shown, with the characteristic HPD contribution in the 610–700-nm region. The spectral positions of the fluorescence bands used are also indicated, and designations (A, A′, B, and D, following Ref. 4) of the fluorescence signals are shown. In Fig. 2b the response function of the system in different spectral bands is shown. The recording was made on a paper exhibiting a whitish fluorescence. The paper was placed in the object plane and was moved during the exposure to wash out any structure in the paper. The three profiles result from the laser-beam light distribution, some vignetting in the lens, and the variation in response along the detector. From the curves, multiplicative correction functions (having the value 1 at the center and increasing values farther out in the wings) are determined, which when applied reduce the recorded data to the idealized flat-response case. In Fig. 2c raw recordings of the tumor fluorescence are shown. Excess red fluorescence is shown in both the 630- (A′) and 600-nm (D) bands, whereas the blue response (B) seems flatter. The curves multiplied by the correction functions are shown in Fig. 2d. To lift off the specific HPD fluorescence A from the background, which is also included in the measured A′ curve, we subtract the D curve (600 nm), multiplied by a factor k, from the A′ curve, as illustrated in Fig. 2e. The factor k is obtained by comparing imaging measurements of this kind with corresponding pointwise spectrally fully resolved measurements (as described in Ref. 4) for the same samples. Such sequential measurements, with the array detector in the focal plane of a spectrometer, were performed with a laser spot size of 2 mm on eight points along the line where the imaging was performed (the spectrum in the top of the Fig. 2 is the recording from the central, necrotic part of the tumor). In Fig. 2f the results of sequential measurements of A and B are given, showing good agreement with the imaging considering the nature of the sample and problems with reproducible positioning of the sample. In this example, contrast in the A curve is very good, and further contrast enhancement using the B curve (with reduced tumor fluorescence) is not needed.

In Fig. 3 we show some examples of the result of contrast enhancement by properly subtracting 600- from 630-nm curves. Although the correct multiplicative factor to be used in the subtraction can be found with the procedure indicated above, an alternative, straightforward procedure for contrast enhancement can be adopted by simply multiplying the D curve by

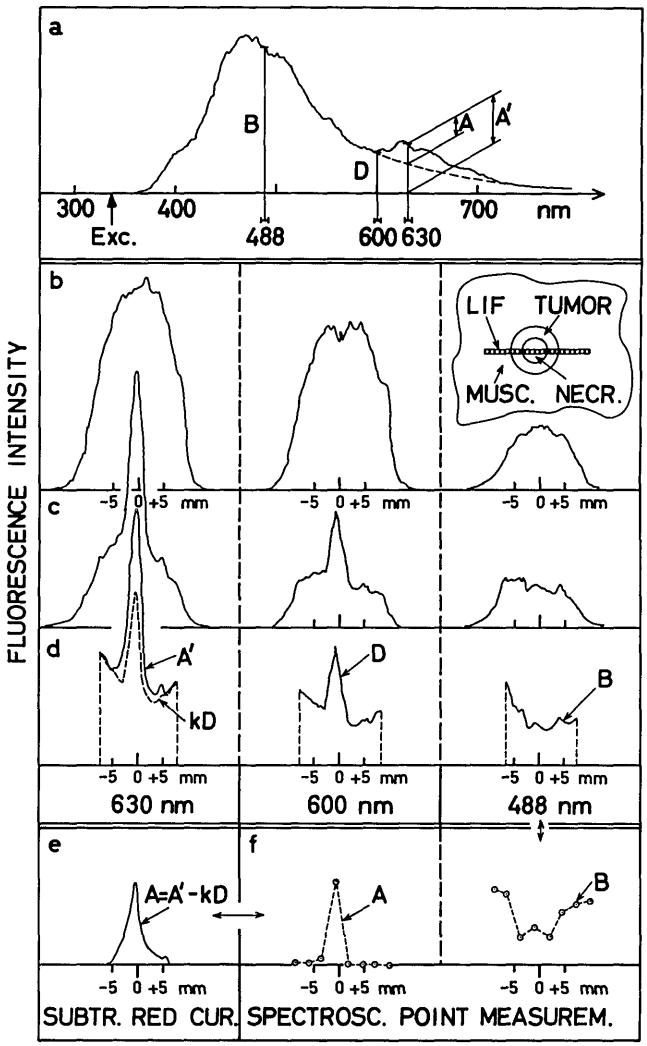

Fig. 2. Illustration of spatially resolved LIF measurements across a tumor of a rat injected with 0.5 mg/kg of body weight of HPD two days earlier. The figure insert showing the tumor and the image line has the correct scale and pertains to all three columns. a, Fluorescence spectrum at the center of the tumor. b, Response functions in the different bands (recording of the fluorescence from a paper). c, Raw data from tumor recording. d, Corrected tumor data. e, Curve obtained (A) after subtraction of properly multiplied 600-nm curve (D) from 630-nm curve (A′). f, Data obtained in corresponding pointwise, spectrally resolved measurements.

a factor k chosen to fit the A′ and D curves in the nontumor region. The tumor region then shows up in the subtraction because of its excess 630-nm light. The curves in Fig. 3 were obtained in this way. For the cases in the upper row, 5 mg/kg of body weight of HPD was injected, whereas only 0.5 mg/kg was used for the cases graphed the lower row. Clearly, the higher dose produces more-distinct monitoring of the tumor. It should be noted that, owing to the strong sloping background associated with the intense blue fluorescence, the light detected through the 630-nm filter is frequently stronger in the muscle region; thus the results obtained by a simple one-filter technique would be exactly opposite what might be expected. However, the simple procedure illustrated above readily reveals the specific spectral characteristics of tumor tissue.

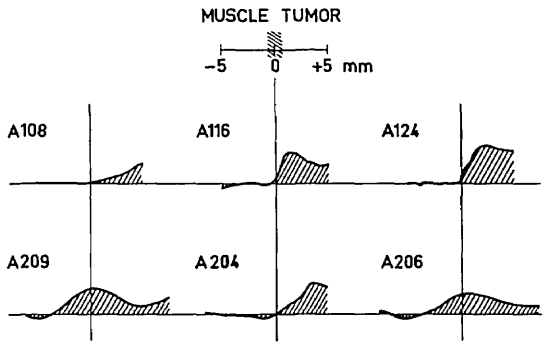

Fig. 3. Examples of contrast enhancement at tumor edges obtained by fitting D and A′ curves in the (distant) nontumor region with subsequent subtraction of the D curve. Rats were injected with 5 mg/kg of body weight of HPD one to three days before the investigation for data shown in the curves in the upper row and with 0.5 mg/kg for data in the lower row. Intensity scales differ; only contrast (and noise) is considered.

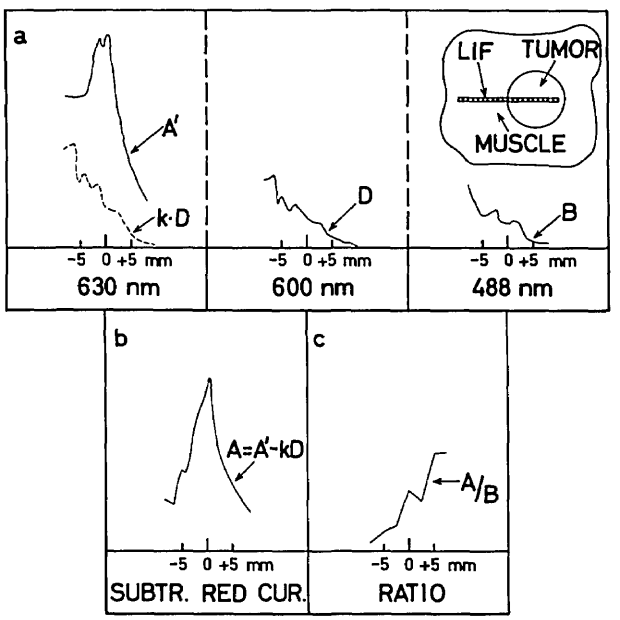

Fig. 4. Recordings of fluorescence along a tissue line extending from muscle to blood-stained tumor; 5 mg/kg of body weight of HPD was injected one day before the investigation. a, Corrected fluorescence curves. b, The A curve obtained by subtraction of red curves. c, A/B ratio curve.

In a last example, we illustrate how full advantage of the red and blue fluorescence curves can be taken in difficult cases in which there is a considerable HPD content in the tissue surrounding the tumor and/or there is signal reduction from the tumor owing to strong superficial tumor vascularization. In Fig. 4a, the corrected fluorescence distributions are given. The properly subtracted curve yielding the A distribution is shown in Fig. 4b. As can be seen, the blue fluorescence becomes weak in the tumor. A reduction by a factor of 2–5 is normal, but here the reduction is much stronger because of heavy blood staining in the tumor surface. This results in a lower specific HPD signal (A) in the central part of the tumor as well. However, by dividing A by B as discussed in Ref. 4, the spectral shape of the curve rather than specific, in this case attenuated, intensities is monitored, and the tumor appears as an increase in the A/B ratio (Fig. 4c). Object distance, surface topography, illumination fluctuations, and spatial nonuniformities have no influence on A/B measurements.

As is illustrated in this Letter, spatially resolved, simultaneous LIF monitoring in properly chosen wavelength bands can be achieved, permitting substantial contrast enhancement in cancer-tumor detection. Clearly, these techniques can be extended to 2-D monitoring by using a rectangular illumination field and a vidicon or matrix detector, and adaption for fiber-optical systems for human lung and bladder applications can also be made.[8] An advanced imaging system based on different principles has been described by Profio *et al.*[3,9] It is obvious that a multicolor, computerized LIF system has wide applications also outside the field of medicine, e.g., for industrial inspections of surface cleanliness.[10,11]

The authors are grateful to J. Ankerst for valuable discussions and for providing the properly prepared test animals used in the investigations. The assistance by E. Sjöholm in the measurements was very valuable. The support by the Lund HPD working group is gratefully acknowledged.

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