Multispectral and lifetime imaging for the detection of skin tumors

Cubeddu, R; Pifferi, A; Taroni, P; Valentini, G; Canti, G; Lindquist, C; Andersson-Engels, Stefan; Svanberg, Sune; Wang, I; Svanberg, Katarina

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Abstract

Multispectral and lifetime imaging systems have been used to detect skin cancer in humans after the topical application of δ-aminolevulinic acid ointment. Both systems allowed a clear detection of tumors based either on spectral or on temporal differences between the fluorescence signals in the neoplasia and in the healthy tissues nearby.

Key Words

(170.0110) Imaging systems; (300.2530) Fluorescence, laser-induced; (170.3650) Lifetime-based sensing; (170.1870) Dermatology.

Introduction

Since several years ago, fluorescence spectroscopy and imaging were being used for the detection of cancer based on endogenous or exogenous fluorophores. Researchers, who often have to deal with weak and relatively unspecified fluorescence emission, have always been looking for an effective selectivity criterion to discriminate neoplastic from healthy tissue. Working with exogenous fluorescent dyes, selectivity criteria have been successfully found both in the spectral and in the time domains. In the first case, two or more images are acquired with different spectral filters and a suitable algorithm, based on image subtraction and/or ratioing, leads to a pseudoinmage where areas with pixel values exceeding a predefined threshold value are marked as neoplastic ones [1,2]. This approach takes advantage of the reduction in natural fluorescence accompanied by the increase in fluorescence of exogenous markers that has been frequently observed in neoplastic tissues.

Whenever exogenous fluorophores are characterized by lifetimes different from the ones typical of the natural tissue fluorescence and when they accumulate more in pathologic areas, cancer detection can be performed, at least in principle, in the time domain as well. As an example, this is the case of some exogenous photosensitizers for the photodynamic therapy of tumors, such as Porphyrins, whose fluorescence lifetime is of about 10-15 ns, typically longer than observed in untreated tissues.

If the exogenous fluorophores are accumulated in tumors with high selectivity, a simple time gated acquisition can lead to a successful detection of pathologic areas. In some conditions, when time gating is not effective due to the limited selectivity, a difference in the exogenous fluorescence lifetimes can be exploited to discriminate the neoplastic tissue from the healthy one [3]. This can be the case for porphyrin-based compounds, which are made of a mixture of monomers, dimers and oligomers. The biochemical differences between the tumor and the healthy tissue account for a significant increase in the fluorescence lifetime in the tumor even though the fluorescence intensity is unspecific. The tumor detection can thus be carried out by a system capable to calculate the spatial distribution of the fluorescence decay time, that is a lifetime image.

The present work has been devised to evaluate the diagnostic capabilities of two imaging systems working in the spectral and in the time domains, respectively. The sensitizer used was δ-aminolevulinic acid (ALA), a precursor of the naturally occurring fluorescent agent Protoporphyrin IX, and the investigation was carried out at the Lund University Medical Laser Centre on patients affected by skin cancer.

Materials and Methods

Two patients affected by previously assessed Basal Cell Carcinomas (BCCs) (total of 5 lesions) were treated with
topical application of 20% ALA ointment 2 hours before
the fluorescence measurements.

A preliminary fluorescence investigation was carried
out using a Nitrogen pumped dye laser and an optical
multichannel analyzer (OMA). An optical fiber in contact
with the skin was used to deliver the excitation light and to
collect the fluorescence signal. Two point measurements
were performed on each BCC: the first one in the center of
the lesion, the second a few millimeters outside.

Thereafter, a multicolor and a lifetime image of each
lesion were acquired in sequence, using the experimental
setups described in the following.

**Multicolor Imaging System**

Multicolor imaging was carried out by taking three
spatially identical, but spectrally different images with the
same intensified CCD camera, using a system developed
for endoscopic applications. The images were spectrally
separated by means of dichroic mirrors in three wavelength
bands. One (named A) was in the red area, one (B) in the
blue area, and one (D) in the green-yellow area. The
fluorescence was induced by means of ultraviolet light
(390 nm) from a frequency-doubled Alexandrite laser. The
laser light was delivered by an optical fiber and the
fluorescence was detected through an endoscope. The
gated image intensifier was synchronized with the laser.
Thereby, the normal reflected light image could also be
recorded simultaneously by a color CCD camera on the
endoscope adapter. The fluorescence image was digitized
and kept in a frame grabber in a PC/486 computer.
Calculations were subsequently carried out on the digitized
image. The function

with A, B, and D defined above and \( k_1 \) and \( k_2 \) being
constants with different values for different applications,
was used to produce a pseudocolor image with a high
contrast between normal and malignant tumor tissue. The
PC computed the \( F_c \) value pixel by pixel and fed the result
via an output frame grabber to a video mixer, where the
pseudocolor fluorescence image could be mixed with the
normal image from the color CCD camera.

**Lifetime Imaging System**

The lifetime imaging system is essentially made of a
Nitrogen pumped dye laser, emitting 1 ns pulses at 405 nm,
and an intensified CCD video camera, whose light
amplifier can be gated with a gate rise time of 2 ns. A
precision delay generator and some electronic devices
complete the system. The images were acquired and
processed by high performance image boards in a personal
computer.

In the present experiment, four images per sample were
acquired, after the delays of 0, 5, 10, and 20 ns (with
respect to the excitation pulses). The images were
processed according to the algorithm described in the
following.

Based on a simplified model, the measured fluorescence signal \( l(t) \) can be assumed to be roughly
monoexponential. Image acquisition corresponds to time
integration and spatial sampling, which lead to the
following matrix representation of the irradiance \( H_{ij} \):

\[
H_{ij}(d_k) = k \int_{t_0}^{t_\infty} l_{ij}(t) dt = C \tau_{ij} l_{ij}(0) \exp \left( -d_k / \tau_{ij} \right),
\]

where subscripts \( i,j \) refer to image pixels, \( d_k \) is the delay (0,
5, 10 or 20 ns), \( \tau_{ij} \) is the decay time matrix, \( l_{ij}(0) \) is the
matrix of the corresponding fluorescence intensity
immediately after the excitation pulse, and \( C \) is a system
dependent constant. The spatial distribution of the decay
time \( \tau_{ij} \) can be calculated by doing a simple linear
regression:

\[
\frac{1}{\tau_{ij}} = \frac{n \sum d_k \ln H_{ij}(d_k) - \sum d_k \sum \ln H_{ij}(d_k)}{n \sum d_k^2 \left( \sum d_k \right)^2},
\]

where \( n \) is the number of delays.

Then, the amplitude matrix is given by:

\[
A_{ij} = \frac{H_{ij}(d_0)}{\tau_{ij} \exp (-d_0 / \tau_{ij})}.
\]

Finally, \( \tau_{ij} \) and \( A_{ij} \) can be plotted in gray shade or
pseudocolor images.

**Results and Discussion**

Figure 1 shows two typical OMA spectra taken in the
center of the lesion (Figure 1a) and 4 mm outside (Figure
1b). In both cases, the short wavelength rise is caused by the
cut-off filter, which is used to remove the scattered
laser light. The blue-green signal is due to the natural
tissue fluorescence and is significantly lower in the
spectrum acquired from the lesion than 4 mm outside it. In
the center of the lesion the dominant peak, which
corresponds to the induced Protoporphyrin IX emission,
is centered at 635 nm. No contribution to the spectrum comes
from porphyrins in the case of healthy tissues. The
reduction in natural fluorescence and the presence of the
exogenous signal are typical findings in cancerous tissues,
which can be profitably used for tumor detection in the
spectral domain.

Figure 2 shows a white light image of the same lesion
as considered above, taken with the fluorescence camera
(Figure 2a) and the corresponding multicolor image
(Figure 2b). In the white light image, two areas can be
identified as darker than the background: the one on the
left is a benign nevus, while the one on the right is the
BCC, as it could be easily assessed by a visual examination of the patient. In the multicolor image, the BCC emerges from the background with sharply defined margins, while the nevus still remains darker than the healthy skin, allowing the detection of the malignant lesion and its discrimination from the benign one.

Figure 3 shows images of the same lesion as considered before, taken by means of the time resolved imaging system. Figures 2 and 3 show a left-right inversion and an unlike magnification since they have been recorded through completely different optical devices (endoscope and macro lens, respectively). Figure 3a is a map of the fluorescence lifetimes calculated using the four delayed images, while Figure 3b shows the spatial distribution of the corresponding amplitudes. It is well known that the Protoporphyrin IX emission is characterized by a long lifetime ($\tau > 15$ ns), while the other components of tissue fluorescence are short living ($\tau \leq 5$ ns). The bright spot in Figure 3a corresponds to the lesion and the light gray area surrounding it coincides with the region over which the ALA was applied. On the other hand, the healthy skin (including the nevus) is dark. As evident from Figure 3a, the estimated lifetimes are in the range of 8 to 18 ns. All these pieces of information indicate that the porphyrin emission dominates the image.

The Protoporphyrin IX is induced over all the area covered by the cream, but significantly longer lifetimes are detected in the lesion. This is in agreement with previous results. Actually, in a systematic study performed with
experimental tumors in mice sensitized with Hematoporphyrin Derivative, it has been observed that a significant lengthening of the porphyrin fluorescent lifetime takes place in the tumor [4]. The distribution of fluorescence amplitudes reported in Figure 3b again allows a clear localization of the tumor area. In this case, the detection of the lesion is based on the fact that porphyrins are induced at a higher concentration in the neoplastic tissue than in the surrounding healthy skin, leading to a bright spot, which demarcates the pathologic tissue.

In conclusion, based on these preliminary findings, both the multicolor and the lifetime imaging systems allow a reliable detection of basaliomas in patients sensitized with ALA. Further studies are in progress to confirm the results obtained so far and investigate more in detail the diagnostic potential of the two techniques.

References