RGB imaging of laser-excited skin autofluorescence bleaching rates

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ABSTRACT

In-vivo skin photo-bleaching (intensity decrease during irradiation) has been investigated at 405 nm cw laser excitation. Digital RGB photo-camera was used for studies of the bleaching features by analysis of fluorescent images at separated R, G and B spectral bands. Diagnostic potential based on correlations between skin pigmentation and bleaching rates is discussed.

Keywords: Skin autofluorescence bleaching, RGB imaging

1. INTRODUCTION

Fluorescence spectroscopy can provide information that is not obtainable under regular illumination, therefore it is widely used for noninvasive diagnostics. Tissue autofluorescence (AF) originates from numerous fluorescent proteins, flavins, porphyrins and chromophores with specific absorption and emission spectral bands. Laser-excited tissue AF spectra have been studied extensively over recent decades[1-4]. AF intensity decrease during irradiation (photo-bleaching) was observed by several authors[5-8]. In most cases it was characterized as exponential fading with time, indicating to decreased absorption or optical washout of fluorophores at the superficial layer of skin during irradiation. This phenomenon can be of practical interest for noninvasive skin assessment, since healthy and pathologic areas of skin show different values of the AF bleaching rates[8].

Imaging techniques allows mapping of skin chromophore distribution and other specific parameters. AF intensity decrease over the illuminated area can be recorded with camera, creating image cube where two dimensions are spatial and one temporal. AF bleaching rate maps were obtained by fitting the measured intensity values to exponential model for each single pixel, in order to see parameter distribution[9]. Multispectral imaging camera can be used to acquire AF intensity decrease at specific spectral band. RGB camera can be expressed as simple and low cost spectral imaging system for simultaneous acquisition of three spectral images – R (red), G (green) and B (blue)[10].

The aim of this study was to evaluate potential of consumer RGB camera for simple and fast multi-spectral imaging of laser-excited skin autofluorescence bleaching rates at separated R, G and B spectral bands.

2. EXPERIMENTAL

2.1. Data acquisition

The setup for imaging of laser-induced AF bleaching rates with consumer RGB camera is presented in Fig. 1a. 405 nm cw laser was used for tissue illumination and AF excitation. The laser irradiation was delivered to sample by a silica core optical fiber that was connected to collimator lens attached to the camera, thus ensuring stability and increased uniformity of irradiation. Long-pass optical glass filter (transparency edge at ~ 500 nm) in front of camera objective was used to cut the scattered laser radiation. RGB image acquisition was performed by SLR camera Canon EOS 400D with signal output to PC where each of color channel targets corresponded to different spectral region. RGB component spectral sensitivities were measured using monochromator[10] and corrected to typical skin AF spectrum at 405 nm laser excitation and long-pass filter – see Fig. 1b. G component is the most sensitive and corresponds to spectral range with maximum at 540 nm and bandwidth 520…580 nm, R relates to 570…630 nm with maximum at 600 nm and B – to

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510…540 nm with maximum at 520 nm. The fluorescence images were captured during 15 s with frame rate 3 fps to observe AF bleaching. Laser power density on the skin surface was ~20 mW/cm^2. Excited surface was ~ 1 cm^2, but only 5x5 mm area (typical spatial resolution 0.1 mm^2/px) of uniform illumination spot was used for data processing. Image acquisition was started when radiation was switched on; the collected RGB image stack was saved on PC for further processing. Ambient light was switched off to improve signal to noise ratio. All measurements were taken from the forearm skin.

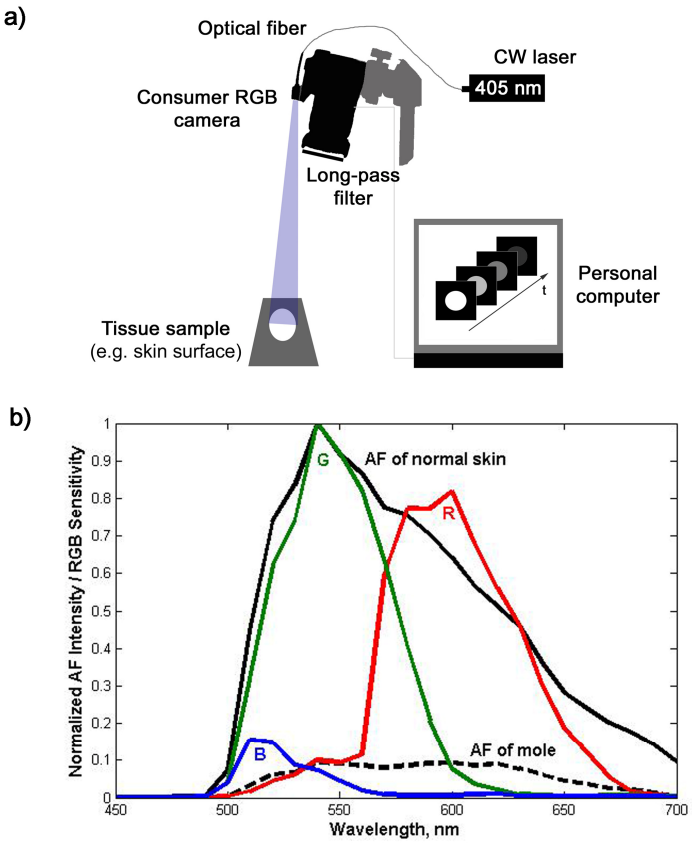

Figure 1. The experimental setup (a) and RGB channel spectral sensitivity, compared to skin AF emission spectra (b).

2.2. Data processing

Data processing was performed in *MatLab*. Color image components – red (R), green (G) and blue (B) – were analyzed separately.

Previous studies [8, 9] presented AF intensity *I* bleaching approximation with exponential function that can be expressed as:

$$I(t) = I_0 \cdot e^{-\frac{t}{\tau}}, \tag{1}$$

where τ is the bleaching rate, t - time and I_0 – initial AF intensity. Non-linear least-square regression analysis was used to calculate bleaching rate. Exponential Eq. 1 can further be transformed to linear equation as follows:

$$\ln\big(I(t)\big) = \ln(I_0) - \frac{t}{\tau}. \tag{2}$$

This allows to use linear least-squares regression analysis so speeding up the image cube processing procedure. Further, the measured AF intensity values were fitted to Eq. 2, in order to obtain the AF bleaching rate τ values. An example of fitting the measured AF intensity decrease with the model (2) is presented in Fig. 2.

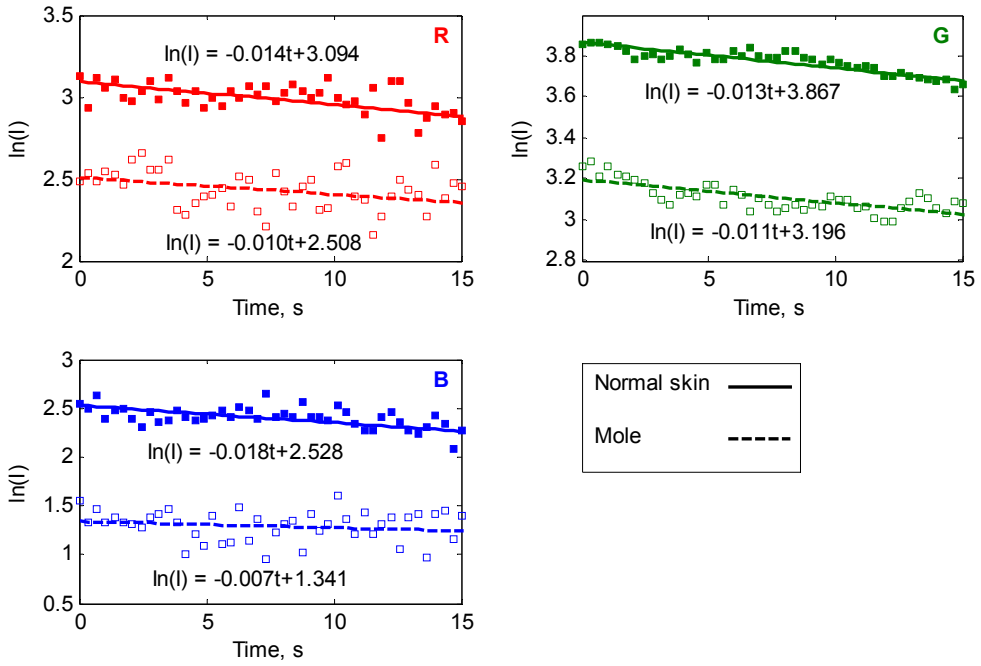

Figure 2. AF intensity changes over time (points) and model fit (line) for normal skin and mole.

3. RESULTS AND DISCUSSION

As it was expected, G channel appeared to be the most sensitive – the AF intensity values were ~ 2 and ~ 3 times higher than those at the R and B channels. AF bleaching, however, was observed at all channels. Non-uniform AF bleaching rate distribution was observed over normal skin area (II skin type) as presented in Fig. 3 (lighter color corresponds to faster bleaching). Higher τ values were found at B channel, but lower at G channel.

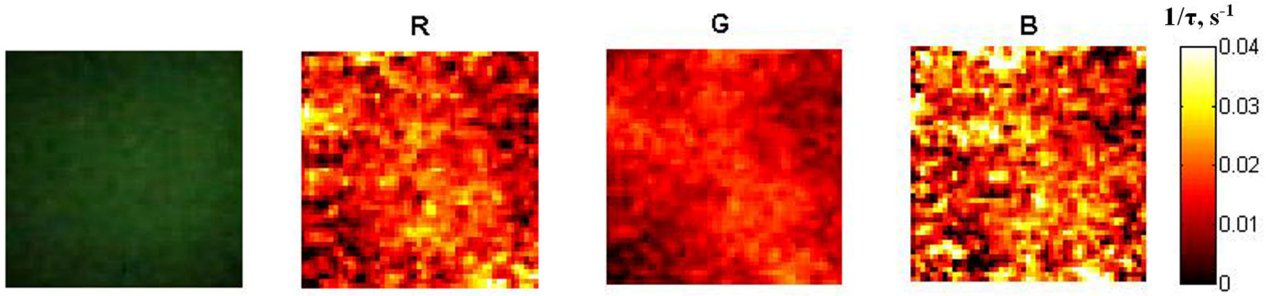

Figure 3. AF color image and bleaching rate 1/τ distribution maps at different spectral channels for normal skin (II skin type).

Measurements of the nail-skin interface showed high contrast in AF bleaching rates (Fig. 4). Dead horny layer of skin at the bases of nail appeared bright in AF color images and high bleaching rate values were observed. Opposite relation was found for skin capillary area – high AF intensity, but no bleaching.

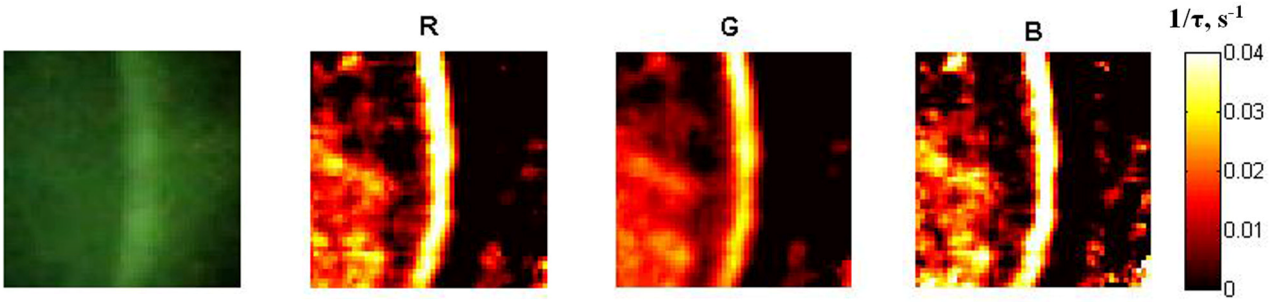

Figure 4. AF color image and bleaching rate 1/τ distribution maps at different spectral channels for nail (III skin type)

Lower intensity bleaching rates were observed for a mole comparing to normal skin (Fig. 5). Pigmented skin area has significantly lower AF intensity than normal skin creating high intensity gradient on the mole-normal skin border, therefore even little movements can occur as artifacts in maps of bleaching rates. Decreased measurement time could reduce movement possibility increasing image quality. Nevertheless mean values of bleaching rates τ correlated to results achieved before[9] – showing slower bleaching in mole comparing to normal skin (Fig.2). As in previous cases, higher τ values were found at the B channel, but lower at the G channel.

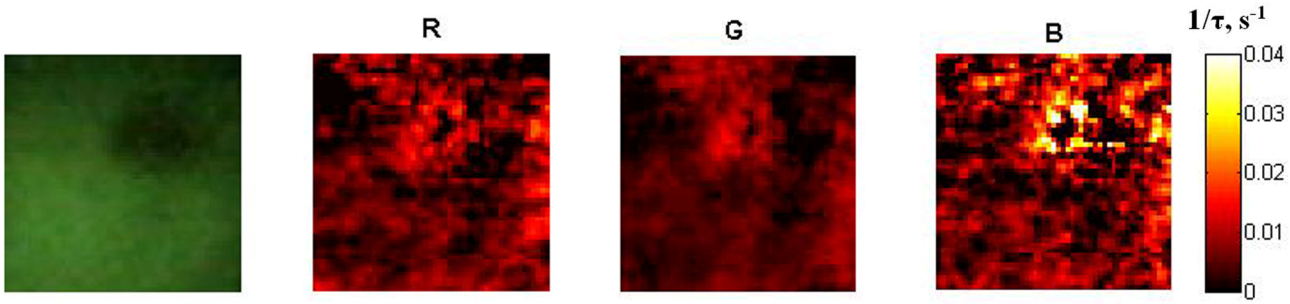

Figure 5. AF color image and bleaching rate 1/τ distribution maps at different spectral channels for mole (III skin type)

To demonstrate differences in AF intensity and bleaching rate values acquired at separated R and G channels, the corresponding ratio maps are presented in Fig. 6. It could indicate that skin has not uniform AF spectrum over the whole irradiated area; the bleaching rates also are varying at two different spectral bands (R and G).

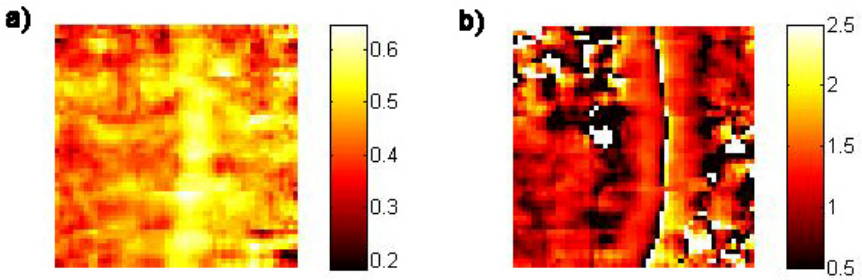

Figure 6. Ratio maps for skin-nail interface: AF intensity ratio R/G (a) and AF bleaching rate ratio R/G (b).

4. CONCLUSIONS

Consumer RGB camera proved to be useful for simple and fast multi-spectral imaging of laser-excited skin autofluorescence bleaching rates, acquiring three spectral images at once.

Exponential AF intensity decay model can be transformed to linear equation using logarithmic intensity scale, so allowing to use of linear least-squares regression analysis instead of the non-linear one and to speed up the data processing.

Differences in bleaching rates at separated RGB channel maps were observed, but further studies are needed to describe its nature. As AF bleaching character is affected by laser power density, this dependence should be thoroughly studied.

Decrease of the laser-excitation and image acquisition time (< 10s) might reduce sample movement artifacts and enable more reliable clinical measurements. More advanced illumination setup could enlarge the excitation area.

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