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# Multiple wavelength probing of skin structures in depth with elliptically polarized light

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**Abstract:** A protocol of polarization gating that combines co- and counter-elliptical measurements of *in vivo* samples illuminated at different wavelengths to selectively access sub-surface tissue layers and spectral information is proposed. To selectively access sub-surface layers, the samples were illuminated with different wavelengths (532, 570 and 633nm) and processed using subtraction (for removal of multiple-scattered components) involving images at these wavelengths. *In vivo* experiments were performed on skin abnormalities of volunteers to confirm the results of the subtraction method and access spectral information.

**OCIS codes:** 110.0113, 110.5405, 290.1350

## 1. Introduction

Polarimetry and the use of its approaches for biological tissue assessment has received considerable attention for its biomedical potential. Polarimetric methods are very sensitive to the structure of the environment, operate by excluding light that is multiply-scattered and uses the light that has kept its initial polarization [1]. The use of polarimetric imaging in more conventional techniques like microscopy or Optical Coherence Tomography (OCT) not only helps in the extraction of precise information such as on collagenous tissue structure (eye, skin, cervix), but has also been widely used for optical biopsy in dermatology [2–5]. For tissues examination, a widely used technique is the detection *via* the cross-linear imaging channel to get rid of the mirror reflections. Linear polarization gating is limited to the surface examination of tissues, thus for examining tissues deeper, the use of circular polarization was introduced [6] and was demonstrated that subtraction of images taken in co-circular illumination/detection configuration and cross-linear illumination/detection allowed for the extraction of circular polarization maintaining light and the elimination of specular reflected photons. This effect was further investigated by Monte Carlo simulations in a semi-infinite medium [7] and also verified experimentally [8]. This technique allows screening the tissue at specific depths ranging between the surface and a maximum depth defined by the maximum penetration depth of the circularly polarized photons.

In this communication, we have chosen to develop this approach because it allows for non-contact tissue examination over a large (wide-field) Region of Interest (ROI) with low-cost instrumentation. In addition and comparison to practised techniques, we introduce a novel subtraction method involving the elliptically polarized light images taken at different wavelengths to probe sub-surface volumes as well as retrieve spectral information. The extent of this method has been substantiated with *in vivo* experiments on human skin.

## 2. Materials and Methods

The experimental set-up (Fig. 1) composes of a halogen light lamp from which the incident light is first wavelength-filtered with band-pass filters (532, 570 or 633nm) and is then passed through a Polarization State Generator (PSG) composed of a linear polarizer and a quarter-wave plate, to produce elliptically polarized light. For detection, the reflected beam passes through a Polarization State Analyzer (PSA) composed of a quarter-wave plate and an analyzer, to return the state of polarization. The relative rotation angle between polarizers and quarter wave plates is tuned such as to obtain the desired polarization modes for illumination and detection. The reflected beam then passes through a magnification lens and is detected by a CMOS camera with an objective. For *in vivo* imaging, a burn scar on the dorsal side of the hand (Panel (a)) of a volunteer was observed and has been illustrated along with a white light image of the ROI. The two cross marks serve as reference targets for movement correcting the images taken *in vivo*.

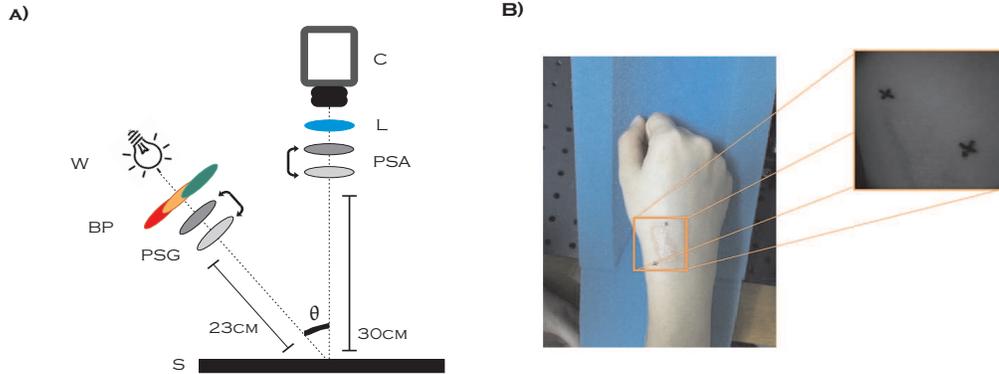


Fig. 1. A) Schematic of the experimental set-up where, W: white light source, BP: Band-pass filters, PSG: Polarization state generator composed of a polarizer and quarter-wave plate,  $\theta$ : Angle of incidence, PSA: Polarization state analyzer composed of a quarter-wave plate and analyzer, L: Magnification lens, C: CMOS camera with objective. B) Dorsal side of a hand along with a zoom-out of the burn scar which was studied for the *in vivo* experiments. Image taken in white light.

### 3. Main Results and Conclusions

A scar on the dorsal side of a volunteer's hand was observed at two different wavelengths,  $633\text{nm}$  (Fig. 2) and  $532\text{nm}$  (Fig. 3). It can be observed that the scar is quite clearly outlined in images with higher ellipticity (Panel F in Fig. 2 and 3). When comparing Panel F in both Fig. 2 and 3, it is evident that we are accessing different volumes. It also implies the fact that with  $633\text{nm}$ , corresponding to the red wavelengths, there is almost no absorption and thus you see a deeper volume, whereas with  $532\text{nm}$ , corresponding to the green wavelengths, you only see the superficial layers.

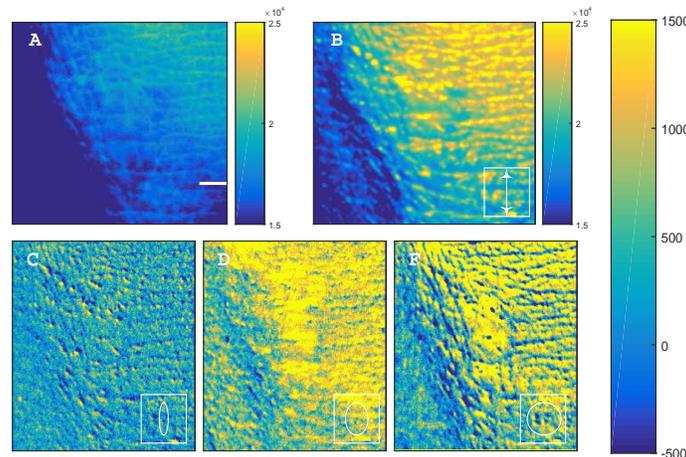


Fig. 2. Contrast for linear and elliptic channels for *in vivo* measurements on dorsal side of a human hand containing a scar. Panel A is the Cross-linear image. Panel B is the Co-linear image. Panel C-F are the contrasts for the subtraction of elliptic (co and counter) channels  $10^\circ$ ,  $30^\circ$  and  $45^\circ$  respectively. Scale bar (Panel A),  $0.5\text{mm}$ . Wavelength,  $633 \pm 10\text{nm}$ . Image exposure time,  $500\text{ms}$ . Behaviour of the polarized light (linear/elliptical) is represented at the bottom-right corner of each panel. Panels A and B are raw images and have their own colorbar. Panels C-F have a common colorbar represented at the right border of the image.

In this communication, we testify the feasibility of depth examination by tuning the state of polarization and compare systems of image subtraction at different wavelengths. Depending on optical properties of the medium, the exact depth

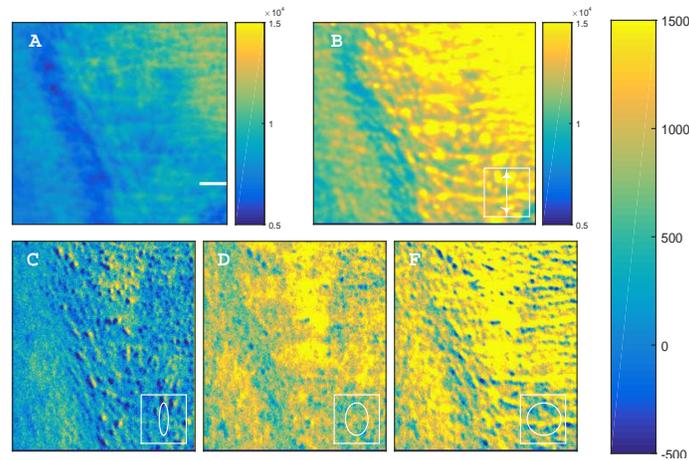


Fig. 3. Contrast for linear and elliptic channels for *in vivo* measurements on dorsal side of a human hand containing a scar. Panel A is the Cross-linear image. Panel B is the Co-linear image. Panel C-F are the contrasts for the subtraction of elliptic (co and counter) channels  $10^\circ$ ,  $30^\circ$  and  $45^\circ$  respectively. Scale bar (Panel A),  $0.5\text{mm}$ . Wavelength,  $532\pm 10\text{nm}$ . Image exposure time,  $500\text{ms}$ . Behaviour of the polarized light (linear/elliptical) is represented at the bottom-right corner of each panel. Panels A and B are raw images and have their own colorbar. Panels C-F have a common colorbar represented at the right border of the image.

and extent of these subsurface volumes can be calculated. With advancement in the set-up and better signal processing, this method should allow for imaging deeper volumes at specific/user-defined depths. This method could be very valuable in the field of non-invasive blood flow-sensing, detection of low-lying tumours or other skin abnormalities.

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