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In vivo Recognition of Vascular Structures by Near Infra-Red Transillumination †

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† Presented at the 6th International Electronic Conference on Sensors and Applications, 15–30 November 2019; Available online: https://ecsa-6.sciforum.net/

Published: 15 November 2019

Abstract: Transillumination is a very well-known non-invasive optical technique, that relies on the use of non-ionizing radiation to obtain information about the internal morphology of biological tissues. In a previous work, we implemented a laser-based illuminator operating at a wavelength of 850 nm, combined with a CMOS digital camera and narrow-band optical detection, that showed a great potential for *in vivo* imaging. A great advantage is the use of low-cost semiconductor lasers, driven by a very low current (about 11 mA, that are spatially distributed as a 6-by-6 matrix covering a 25 cm^2 area. Thanks to the strong absorption of hemoglobin at this wavelength, we have collected raw data of vascular structures that have been further processed to achieve images with much better quality. In particular, here we show that a higher contrast can be attained by expansion of grey level histograms to exploit the full range 0-255. This elaboration can be for instance exploited for the recognition of vascular structures with better resolution. Examples are reported relative to hand dorsal vein patterns and alive chick embryos blood vessels. Analyses can be successfully performed without applying any thermal or mechanical stress to the human tissue under test and without damaging or puncturing any parts of the eggshell.

Keywords: transillumination; *in vivo* imaging; chick embryo; hand dorsal vein pattern; VCSEL.

1. Introduction

Optical transillumination is a non-invasive method for imaging that allows to investigate the internal structure of thin portions of biological tissues [1–4]. It relies on the use of non-ionizing radiation, resulting thus in a totally safe diagnostic tool. The transillumination analysis consists in illuminating the sample with a light source and collecting the radiation that is transmitted through the tissue under test. The propagation of the photons is conditioned by absorption, scattering and reflection effects, taking place inside the tissue: hence, the acquired images carry important information about the morphology and the health condition of the sample. In biological tissues, absorption effects are mainly due to water, macromolecules, such as proteins and lipids, and pigments, such as hemoglobin [5,6]. In particular, in the wavelength range from 600 to 1200 nm (the so-called "diagnostic and therapeutic window") the absorption of water is much lower than that of oxygenated and deoxygenated hemoglobin. The choice of light sources emitting in this spectral region results particularly interesting when studying highly perfused tissues. As it concerns scattering, only a small amount of light is redirected but this phenomenon still prevents the formation of high-definition images comparable with the results from more complex diagnostic techniques that make use of ionizing radiations. Nevertheless, transillumination can be exploited as a first approach to perform preliminary analysis, in view of more complex and invasive tests [7,8]. Hence, it is applied

for investigation of hydrocele, hydrocephalus, caries, malignant lump and blood vessel pattern. Commercial medical devices are available: they are based on LEDs (Light Emitting Diodes) emitting visible light and they can be used only in a darkened environment [9,10]. Transillumination is also exploited for photo-plethysmo-graphy (PPG), a non-invasive optical method that allows to reconstruct a signal related to the change of blood volumes inside the blood vessels of the tissue under test. The PPG signal obtained with pulse oximetry looks similar to the arterial pressure wave, but its waveform appears distorted. This happens because the PPG analysis is carried out by applying the sensor typically to a fingertip or to an earlobe that are subjected to a mechanical pressure. This stress activates alpha adrenergic receptors which affect the arteries and veins vasoconstriction (narrowing the blood vessels).

To solve this issue and in view of the technological advancement done in the past years in the fabrication of light sources and detectors operating in the wavelength range 800-1000 nm, in previous work we proposed and demonstrated a portable Vertical Cavity Semiconductor Emitting Laser (VCSEL)-based instrumental setup for morpho-functional imaging of *in vivo* biological tissues [11,12]. We employed the optoelectronic system to acquire pictures and videos of human hands and chick embryos inside the eggshell and to extract vital sign information. In this manuscript, we show how the raw images can be further processed (by expansion and equalization of the grey level histograms) in order to obtain better quality images with higher contrast, for a more precise identification of blood vessel pattern.

2. Materials and methods

The optoelectronic transillumination system for *in vivo* imaging features an illuminator composed of 36 VCSELs arranged in a 6-by-6 matrix covering an area of 25 cm^2. Their pumping current is driven by a custom-designed circuit. A digital CMOS camera is employed for image acquisition (Figure 1).

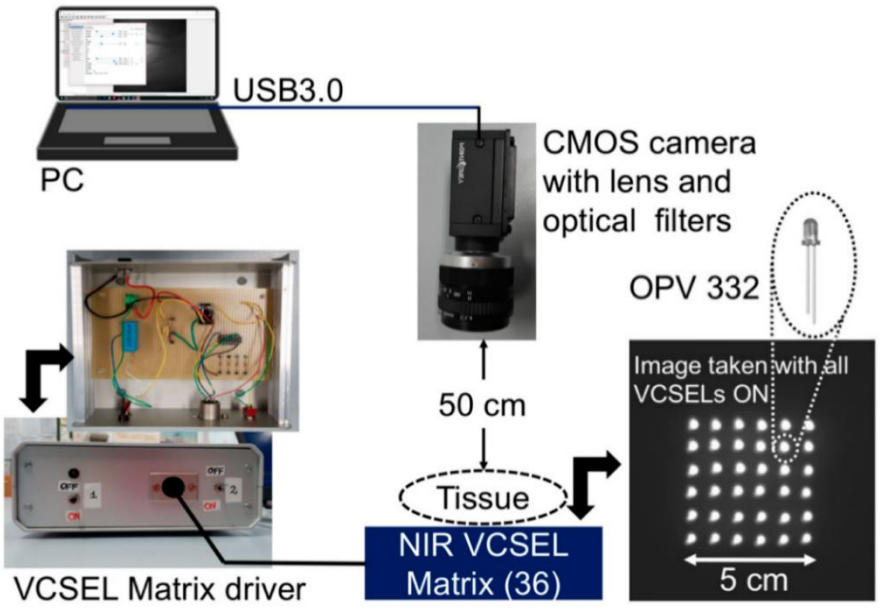

Figure 1. Portable optoelectronic configuration for transillumination-based imaging.

In particular, the employed VCSELs (OPV 332, OPTEK Technology, TTElectronics, Woking, UK) are characterized by a nominal peak emission wavelength of 850 nm, particularly interesting for our purpose as it falls within the diagnostic and therapeutic window. Each VCSEL has a small divergence angle of 4°, is driven by a DC current of 11 mA and emits an optical power of about 6 mW. The acquisition system was positioned at a distance of 50 cm from the biological tissue under examination and allows to obtain images that can be processed and subsequently archived. It consists in a CMOS camera (GS3-U3-41C6NIR-C, CMOS sensor 1”, 2048 × 2048 pixels, Point Grey Research Inc.,

Richmond, BC, Canada), a long-wavelength-pass optical filter with 780 nm cut-on wavelength (MidOpt LP780, Midwest Optical Systems, Inc., Palatine, IL, USA) and a 10-nm-bandpass optical filter centered at 850 nm (FBH850-10, Thorlabs Inc., Newton, NJ, USA). The use of the filter is fundamental for the collection of the NIR photons scattered from the biological tissue and the rejection of ambient light, thus allowing to perform the measurements in standard day-light conditions. The camera is USB 3.0-interfaced to a laptop using dedicated software (FlyCapture2, Point Grey Research Inc.). The acquired images were then processed with MATLAB.

3. Results and discussion

To demonstrate the performances of the system, raw images were acquired first by transilluminating the upper limbs of human volunteers at rest: during the procedure, the subjects were seated in a comfortable position and the biological tissues were not subjected to any kind of thermal stress or mechanical constriction. Before the test, the volunteers provided their agreement to take part in the study and to publish their images. Gray scale pictures were acquired in standard ambient light conditions using exposure times of the order of hundreds of ms and allowed to visualize the vascular structure that appears darker because light is strongly absorbed by the hemoglobin present in the blood flowing in the vessels.

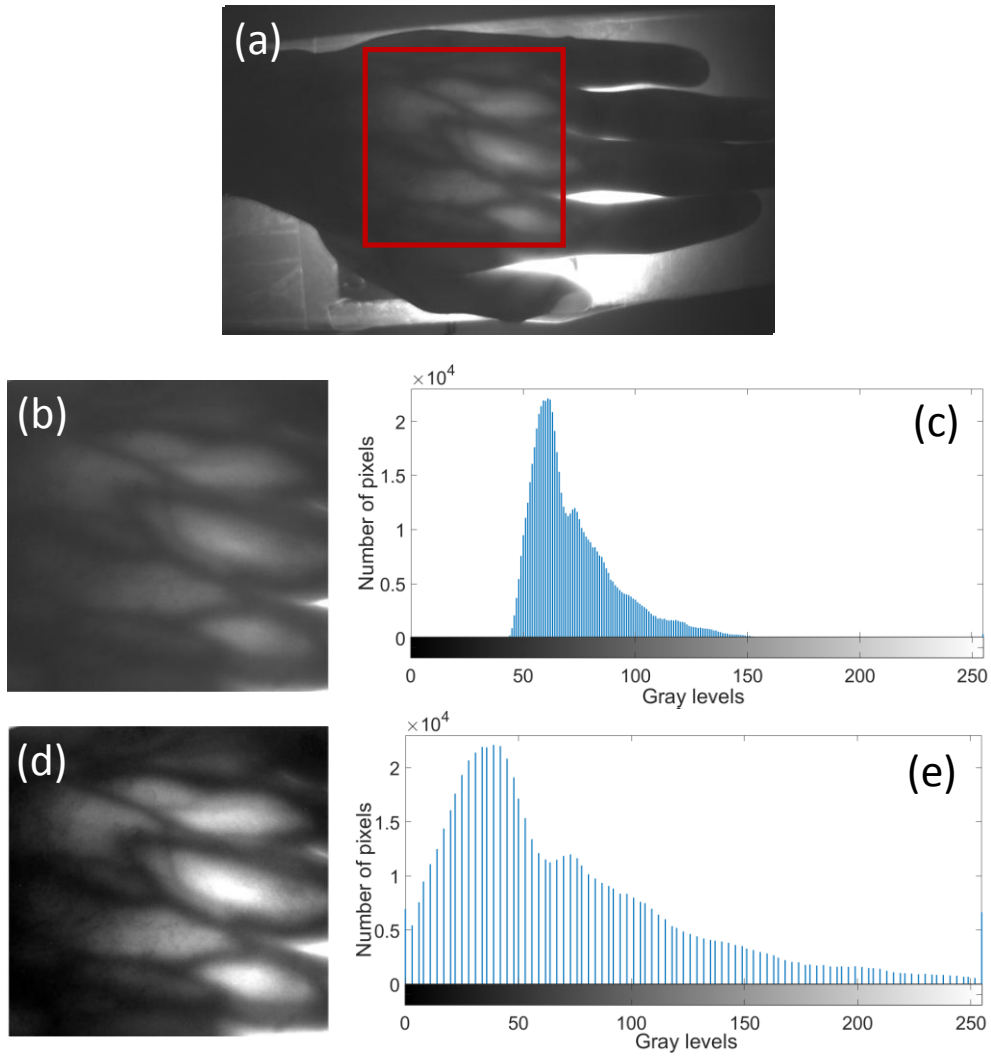

Figure 2. Transilluminated hand of a dark-skinned male subject. (a) Original image with selected region for cropping (red rectangle). (b) Cropped region. (c) Histogram of the cropped area showing distribution of the grey levels. (d) Processed higher quality image after contrast adjustment. (e) Histogram of the processed picture, showing the exploitation of the full range 0-255.

In particular, Figure 2 shows the data related to the transilluminated hand of a male dark-skinned volunteer. Figure 2(a) reports the raw image: morphological details are visible but with lower contrast with respect to pictures acquired on subjects with white skin [11,12], because of the high pigmentation of the epidermis. Hence, with the aim of obtaining higher contrast, the image was further processed using MATLAB software. The original picture was cropped (Figure 2(b)) to eliminate un-significant borders and the histogram relative to the density distribution function of the gray levels was calculated (Figure 2(c)). Figure 2(c) shows that the grey levels of the image pixels are concentrated only in a limited range of the histogram, as a consequence of the low contrast. The cropped picture was processed by contrast adjustment, a procedure that remaps image intensity values to cover the entire range of gray level 0-255 maintaining the shape of the distribution of the original image, as visible from the histogram of Figure 2(e). The modified image (Figure 2(d)) has a higher definition and tiny details of the venous tree are now recognizable.

Since the setup is portable, it was employed also to carry out in field monitoring of the growth of chicken embryos inside the eggshell. Figure 3(a), Figure 3(b) and Figure 3(c) report the original picture of the fecundated egg at day 20 of incubation, a cropped area of the image and its histogram, respectively.

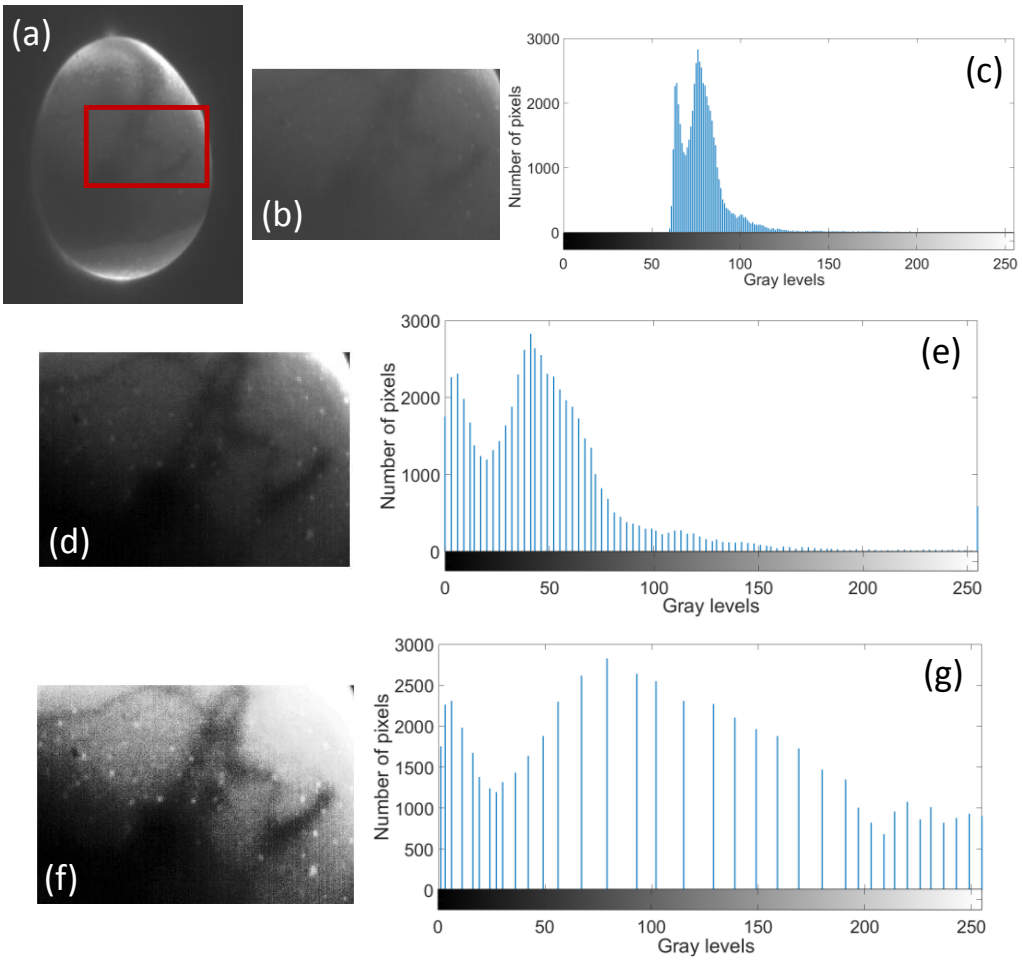

Figure 3. Transilluminated chicken embryo inside the eggshell at day 20 of incubation. (a) Original image with selected cropped area (red rectangle). (b) Cropped image. (c) Histogram of the selected area. (d) Processed picture after the step of contrast adjustment and (e) its histogram. (f) Final image after equalization (g) and its histogram.

The pixel distribution shows that only a limited range of grey levels is present in the selected area: for this reason, the blood vessels inside the eggshell are barely recognizable and the image looks very dark. Figure 3(d), with its histogram (Figure 3(e)), reports the processed image by contrast enhancement. In this case, a further elaboration step was computed, i.e. histogram equalization

(Figure 3(g)). While the expansion is limited to stretching the histogram without changing its shape, the purpose of the equalization is to change it in such a way to obtain a distribution of constant density. In the final picture (Figure 3(f)), the blood vessels are finally recognizable without uncertainty. The white small dots are due to the local egg-shell porosity.

4. Conclusion

We employed our house-built near infrared transillumination setup to acquire functional images of *in vivo* biological tissues and we processed the acquired data to obtain better quality and higher contrast pictures. First, the image processing sequence was tested on a male dark-skinned volunteer. In subjects with high pigmentation of the skin, it is more difficult to clearly distinguish the dorsal vein tree because of partial absorption of the light from the epidermis. By processing the original image, it was possible to obtain a better contrasted picture and to visualize also tiny details of the vessel pattern. Moreover, the setup, that is portable, was used also to perform in field monitoring of fecundated chicken eggs. Pictures of the eggs containing embryos were collected and processed by contrast enhancement and further histogram equalization. Elaborated images showed a better quality and allowed to recognize with better precision the blood vessels. Future works could focus on a more sophisticated image processing to use this transillumination system for biometric recognition and validation [13].

Author Contributions: Conceptualization, Sabina Merlo; Data curation, Valentina Bello, Elisabetta Bodo and Sara Pizzurro; Investigation, Sabina Merlo, Valentina Bello, Elisabetta Bodo and Sara Pizzurro; Methodology, Sabina Merlo, Elisabetta Bodo and Sara Pizzurro; Project administration, Sabina Merlo; Software, Elisabetta Bodo; Supervision, Sabina Merlo and Valentina Bello; Validation, Sabina Merlo, Elisabetta Bodo and Sara Pizzurro; Visualization, Valentina Bello and Elisabetta Bodo; Writing – original draft, Valentina Bello, Elisabetta Bodo and Sara Pizzurro; Writing – review & editing, Sabina Merlo.

Acknowledgments: The optical filter MidOpt LP780 was kindly donated by ImageS spa, Italy, the CMOS camera (GS3-U3-41C6NIR-C, Point Grey Research Inc.) was kindly donated by Edmud Optics as Educational Award. The authors thank the students M. Rossi Borghesano, F. Muretti, R. Catalano, E. Manferlotti for their technical support. Before, participating in the study, all volunteers gave their informed consent to be part of the test as well as for publishing pictures of their hands.

Conflicts of Interest: The authors declare no conflict of interest.

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