

Orthogonal polarization technique in the assessment of human skin microcirculation

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Abstract

Background The “gold standard” for the study of the *in vivo* microcirculation is intravital microscopy. The recently developed method of orthogonal polarization of light [orthogonal polarization spectral (OPS) imaging] allows for the *in vivo* transcutaneous evaluation of the microcirculation without the need for invasive surgical procedures.

Methods The application of polarized light originating from a 100 W halogen tungsten lamp is able to penetrate tissues at a depth of up to 3 mm, and generates reissued light from this depth. The evaluation of this depolarized light, from a deeper origin, may be carried out separately from the light reflected by the more superficial layers of the tissue under study because this light retains photon polarization, whereas the former light undergoes real depolarization.

Results The process of validation of the OPS technique, when compared with intravital microscopy, the “gold standard” for the *in vivo* observation of the microcirculation, has shown that it is as effective and reliable as the gold standard, reaching the same resolution level in the visualization of blood vessels, but without the need for invasive surgical procedures.

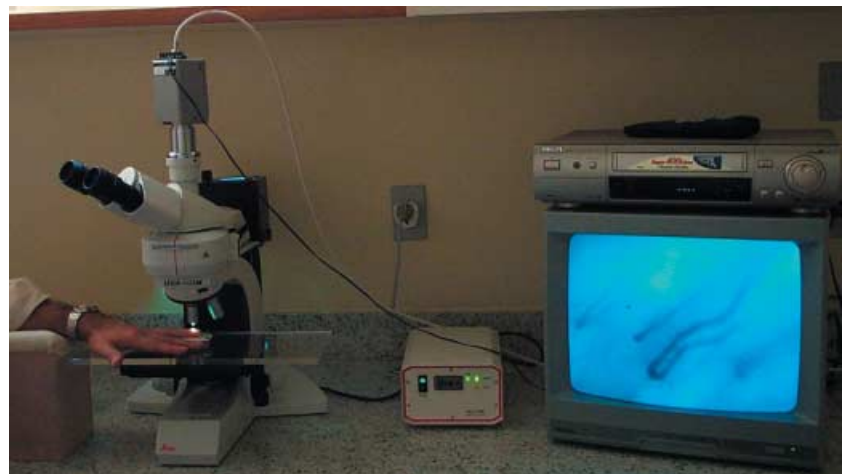
Conclusions The OPS technique is a very promising tool for dermatologists and researchers, especially in the study of vasculitis, chronic venous insufficiency, and skin tumors.

Introduction

The study of the microcirculation of the skin has received increasing interest in recent decades, but development has been limited because of the difficulty in developing an *in vivo* model for assessment.^{1,2} Another difficulty involves the performance of such an assessment in real time, thereby providing an analysis of the exact etiopathogenic mechanisms involved in several skin diseases.¹

The *in vivo* study of the cutaneous–mucosal microcirculation in the conjunctiva and on the nail bed has been made possible through a technique known as “capillaroscopy” (Fig. 1).^{1,3,4} This standard, however, presents restrictions inherent to the fact that it is limited to the peripheral regions of the integument,^{1,3–5} and that it is significant only in a few pathologic situations of systemic ailments, such as Virchowian leprosy, lupus erythematosus, dermatomyositis, systemic sclerosis, etc.^{1,4,6}

Figure 1 Capillaroscopy device. The evaluation of the microcirculation is restricted to the conjunctiva and nail bed. (Courtesy of Laboratory for Research in Microcirculation, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil)



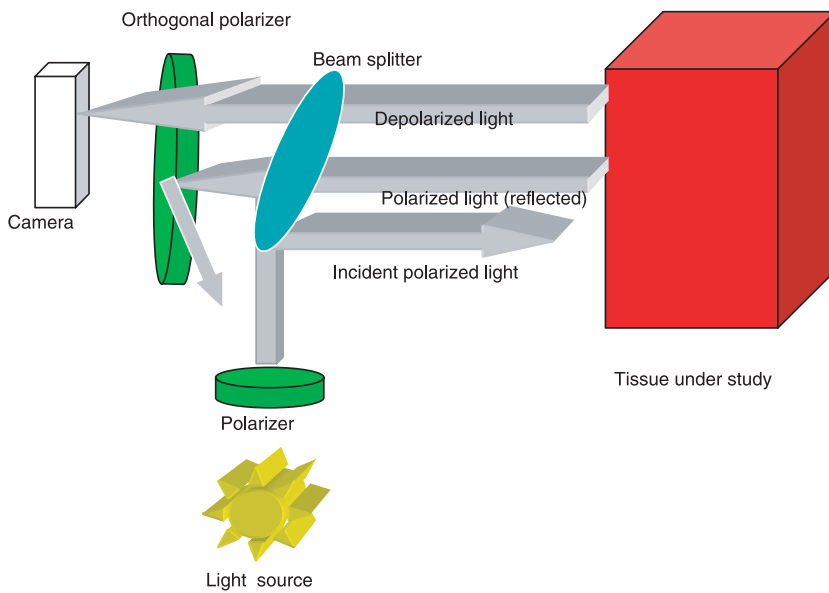


Figure 2 Physical principles of the orthogonal polarization spectral (OPS) technique

Over the years, the “gold standard” for the study of the *in vivo* microcirculation has been intravital microscopy.^{1,2,7,8} This technique allows for an accurate assessment of capillary and vascular morphology, as well as data concerning vascular physiology, such as the speed of displacement of erythrocytes, functional capillary density, and the dynamics of adherence of leukocytes to the vascular wall.⁷ Its array of actions can be enlarged considerably through the simultaneous use of fluorescent plasma markers and computer programs designed to capture and analyze images.^{9,10} Nevertheless, intravital microscopy presents certain restrictive factors, as it demands the surgical introduction of a transparent prosthesis on the dorsum of animals that have previously been immobilized in order to allow direct visualization of the microcirculation (dorsal chamber).¹¹ The preparative procedures for intravital microscopic observations have long been observed to influence the results obtained with this technique,¹¹ and such procedures make it impossible for routine use in the assessment of human skin microcirculation for obvious reasons.

In contrast, the spectral imaging technique obtained using the recently developed method of “orthogonal polarization of light” [orthogonal polarization spectral (OPS) imaging] allows for the *in vivo* transcutaneous evaluation of the microcirculation.¹² Observations are carried out in real time and with no need for any kind of invasive method (Figs 2 and 3).¹³ This paper discusses the new prospects for the use of the OPS technique in research into the assessment of the skin microcirculation.

Spectral imaging obtained by the OPS technique

The visualization of the human microcirculation *in vivo*, using reflected light, is limited to sites in which the vessels are very superficial and visible to the naked eye, such as the nail



Figure 3 Portable device that allows the inspection of individual capillaries of the cutaneous microcirculation and flow through these vessels in real time. Cytoscan® by Cytometrics®. (Courtesy of Laboratory for Research in Microcirculation, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil)

bed and conjunctiva (Fig. 1).¹⁻³ Direct visualization of the vascular bed of other regions of the skin and of visceral organs has been limited by the need for transillumination and by the use of fluorescent probes to increase contrast.^{1-3,5,6} Two innovations, however, have been introduced recently: epi-illumination and the assessment of images obtained from objects illuminated with rays of light that originate from the same direction as the observer.¹²⁻¹⁴

The utilization of polarized light and the acknowledgment that illuminated objects follow the so-called “Beer’s law” of light absorption have opened up new prospects for the study

of the microcirculation.^{13,14} When polarized light is applied to the skin surface, a large part of the radiation is simply reflected and retains its original polarization (Fig. 2).^{13,14} A small amount of light, however, is able to penetrate to deeper layers, where it undergoes multiple dispersion events before being depolarized and reissued to the surface (Fig. 2). This light may be defined as the fraction of the original light which, as a result of reflection or dispersion, returns from the illuminated object.^{13,14} Obviously, the degree of penetration of polarized light will depend on the density of the studied object and the power of the polarized radiation used in the examination, according to Beer's law.¹²⁻¹⁴

It has been observed that the application of polarized light originating from a 100 W halogen tungsten lamp is able to penetrate tissues at a depth of up to 3 mm, and generates reissued light from this depth.^{13,15} The evaluation of this depolarized light, from a deeper origin, may be carried out separately from the light reflected by the more superficial layers of the tissue under study because this light retains photon polarization, whereas the former light undergoes real depolarization.¹²⁻¹⁴

The location of a second polarizer, placed in a precise orthogonal position to the polarizer that issued the polarized bundle, allows us to reject all photons that do not undergo depolarization, i.e. that originate from the more superficial layers of the tissue.¹³⁻¹⁶ This second polarizer, also known as the crossed or orthogonal polarizer, will analyze only the light that is depolarized by passing through several photonic dispersion events in the deeper layers of the skin. The image formed when the immersion objectives are correctly placed is that of the microcirculation in contrast with an illuminated background (Fig. 2).^{15,16}

Therefore, the OPS technique allows the *in vivo* assessment, in real time, of the microcirculation situated up to 3 mm in depth in the skin.¹³⁻¹⁶ To achieve this, the luminous energy must have an appropriate intensity, two light polarizers must be positioned orthogonally, and an appropriate objective should be available. The tissue is illuminated with linearly polarized light at a wavelength of 538 nm reflected through a polarizer oriented orthogonally to the plane of the light. As polarization is preserved in reflection, only photons scattered from relatively deep in the tissue contribute to the images. Groner *et al.*¹⁶ reported the application of the OPS technique in the study of the microcirculation for the first time in 1999. OPS technology has been incorporated into a portable device, which allows the inspection of individual capillaries of the cutaneous microcirculation and flow through these vessels in real time (Fig. 3).¹⁴

Validation of the OPS technique in comparison with intravital microscopy

Intravital microscopy is the standard method for assessing the microcirculation in experimental studies involving animals.¹⁷

Laemmel *et al.*¹⁸ compared intravital microscopy and the OPS technique in the cremaster muscle and cutaneous pedicles of mice. The animals were studied according to several parameters that had already been established as standards for microcirculation:^{17,18} detection of the speed of the displacement of erythrocytes in arterioles and venules, functional capillary density (number of flowing capillaries per unit area), diameter of the dermal papilla, and capillary diameter.¹⁷⁻²⁰

The microcirculation analysis was carried out in six capillary entanglements and seven areas of venules randomly selected and demarcated for assessment by both techniques.¹⁸ The speed of blood flow was estimated by the Cap-Image® program, one of the software packages used to analyze dynamic capillaroscopy.²¹ The functional capillary density was evaluated by counting the number of capillaries with circulating erythrocytes in a given area, randomly chosen, divided by the area of observation.^{17,18}

The comparison of the two methods demonstrated that the OPS technique provided an image definition similar to that of intravital microscopy. Moreover, the measurements carried out using the two techniques provided similar values, with an average difference of the same vessel of 0.2 µm, a relative average difference of less than 1.5%, and a repeatability coefficient of 3.6 µm.^{14,17-19} It was also possible to observe the vasomotor responses to epinephrine using both techniques, with no loss of image quality or time required for the detection of the response. The speed of displacement of erythrocytes proved to be equally trustworthy in both techniques.¹⁷⁻¹⁹

In conclusion, the process of validation of the OPS technique, when compared with intravital microscopy, the "gold standard" for the *in vivo* observation of the microcirculation, has shown that it is as effective and reliable as the gold standard, reaching the same resolution level in the visualization of blood vessels (Fig. 4), but without the need for invasive surgical procedures. This enables its utilization in *anima nobili*.¹⁷⁻²⁰

The use of the OPS technique in the assessment of the microcirculation in solid organs

The OPS technique has been used in the assessment of the microcirculation of several solid organs over the past few years (Table 1).²²⁻²⁶ Microcirculation disorders are a part of the pathophysiology of several illnesses, such as multiple organ failure during hemorrhagic shock, endotoxemia, and, in particular, tissue injuries with ischemia and late tissue reperfusion after transplants, large-scale invasive surgeries, and acute myocardial infarction.^{22,23} De Backer²³ reported that patients with severe sepsis presented a decrease in capillary density and a decrease in the proportion of perfused capillaries. The severity of these alterations was more pronounced in nonsurvivors than in survivors, and was related to the development of multiple organ failure. These



Figure 4 Evaluation of the skin microcirculation by the orthogonal polarization spectral (OPS) technique allows the detection of the speed of the displacement of erythrocytes in arterioles and venules, the functional capillary density (number of flowing capillaries per unit area), the diameter of the dermal papilla, and the capillary diameter. (Courtesy of Laboratory for Research in Microcirculation, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil)

alterations could be reversed by vasodilators drugs, either topically applied or administered intravenously.²³

Langer *et al.*²² studied the microcirculation of the hepatic surface in mice after induced lobular hepatic ischemia, comparing intravital microscopy with the OPS technique (Table 1). The image quality was similar in both techniques, and the OPS technique did not require the use of fluorescent contrast, which was necessary when performing intravital microscopy.^{17,18} Likewise, Tugtekin *et al.*²⁴ studied the microcirculation of the intestinal villi in pigs exposed to the intravenous endotoxin of *Escherichia coli* (lipopolysaccharide B) using the OPS technique, and confirmed the reliability of the technique for the intestinal microcirculation (Table 1).

Uhl *et al.*²⁵ used the OPS technique in neurosurgical procedures in order to study the human cerebral microcirculation (Table 1). Conditions such as intracerebral aneurysm and subarachnoid hemorrhage could be studied *in vivo* to identify the extent of the vascular damage and the existence of microvasospasms on the arterial bed. One patient presenting with multiform cerebral glioblastoma also had the tumor microcirculation investigated using the OPS technique in the same study. Christ *et al.*²⁶ suggested that the peripheral microcirculation could be monitored noninvasively during open-heart surgery in neonates using this technique.

In reality, the use of the OPS technique for the assessment of solid organs requires the introduction of the OPS probe into the physical vicinity of the organ under study, because the depth of penetration of reissued depolarized light is no greater than 3 mm.^{14,22–24} Nevertheless, the OPS technique

Table 1 Applications of the orthogonal polarization spectral (OPS) technique

Anesthesiology	Tissue reperfusion
Orthopedic surgery	Rotator cuff surgery
	Knee surgery
	Necrotic tissue detection
	Lipid emboli detection
Cardiac/vascular surgery	Emboli detection
	Anastomosis patency
	Inflammatory response
	Ischemia reperfusion injury
Critical care	Shock monitoring
Gastrointestinal	Crohn's disease
	Bowel resections
Gynecology	Cervical cancer detection
Laparoscopic and endoscopic	Necrotic tissue detection
	Cancer monitoring
Neurosurgery	Detection of microvasospasms
Transplant surgery	Reperfusion of organs

offers great practical advantages when compared with intravital microscopy, as its probe is small enough to be introduced through a small incision,^{17,18,22,25} and it can be used to assess antivascular tumor treatment *in vivo*.²⁷

Prospects regarding the use of the OPS technique in the assessment of the microcirculation of the skin

The skin is an organ with special characteristics that presents a wide range of opportunities for the use of the OPS technique. Its extreme ease of access allows for an extensive assessment of the dermal microcirculation using the OPS technique.²⁸ The variation in skin thickness allows some areas, such as the face, thorax, dorsum, and limbs, to be examined easily by the OPS technique: the skin in these regions is not very thick and presents abundant vascularization. Because of the thickness of the corneal layer and the existence of the stratum lucidum, the palmar–plantar surfaces present greater technical difficulties for transcutaneous visualization. Mucosal surfaces are ideal areas for the method because they lack a corneal or granular layer and are richly vascularized.^{28–30}

The blood supply to the skin and mucosa is composed of three distinct and interrelated segments: the arterial segment, the arterial and venous capillary segment, and the venule segment. Two important dermal plexuses exist, one on the superficial or papillary dermis and the other on the deep dermis, next to the interface with the subcutaneous tissue.^{31,32} From the superficial plexus the capillary loops are formed and projected next to the dermal–epidermal junction in order to perfuse the epidermis through diffusion. The vascular plexus is extremely important in the stability of the dermal elastic

and collagen fibers, having a direct impact on the function of dermal fibroblasts, the cells that produce collagen fibers.³² The deep plexus features a connection with large-caliber vessels of the muscle structure, as well as with the superficial plexus, in addition to issuing tributary vessels that perfuse the skin annexes, such as the pilous follicle and the eccrine and apocrine sweat glands.^{31,32} Almost all of the skin microcirculation, however, is found within the papillary dermis, approximately 3–4 mm below the surface, and therefore within the reach of the OPS technique.^{28–30}

The visualization of the dermal microcirculation is influenced, however, by the amount of melanin present at the dermal–epidermal junction. The same Beer's law that permits the application of the OPS technique, thanks to the peculiar optical density of hemoglobin present on the vascular bed, makes it difficult to view images in individuals with a large amount of melanin in their skin, as the absorbance of both molecules is similar.^{14,15,23} Therefore, there is a restriction on the use of the OPS technique in the study of the skin microcirculation in individuals with phototypes IV, V, and VI (mulattoes and the black population) according to the classification proposed by Fitzpatrick.^{15,16,23}

The OPS technique has, until now, seldom been used in dermatologic studies in humans;^{33–36} moreover, its use has been limited to evaluations of the gastrointestinal mucosa²⁴ and the cerebral^{22,25} and cardiac²⁶ circulation. Klitzman *et al.*³⁶ used OPS to study angiogenesis related to the scar process in mice. Zhao *et al.*³³ reviewed the noninvasive methods for skin blood flow imaging in the microcirculation, and reported the OPS technique to be promising. Erdmann *et al.*²⁹ reported the OPS technique to be useful in plastic surgical procedures.

Milner *et al.*²⁸ reported the noninvasive assessment of the skin microcirculation through the surface of burn wounds by the OPS technique. This study allowed the inspection of individual capillaries of the cutaneous microcirculation and blood flow through these vessels in real time. Two distinct microcirculatory patterns were seen. Superficial burns showed small visible dermal capillaries, and the flow of individual erythrocytes through these capillaries was clearly visible in real time.²⁸ Conversely, deep burns showed large thrombosed vessels coursing in a “criss-cross” manner.²⁸ There were marked differences between the mean optical densities of normal skin and superficial burns (65.8 ± 15.6 and 64 ± 14.6 , respectively) and deep burns (131.2 ± 31.1). These findings indicate that the OPS technique may be useful in the assessment of the cutaneous microcirculation in burns.²⁸ Milner³⁷ was also able to predict the outcome of postburn scars using the OPS technique.

Genzel-Boroviczeny *et al.*³⁸ used this new technology to examine the quantitative changes in the microvessels, and thus the noninvasive assessment of tissue perfusion, in term and preterm infants. Blood pressure, heart rate, hematocrit, and body and incubator temperature were studied. The vessel

diameter ranged from 6 to 24 μm , and the vessel density from 219 to 340 cm/cm^2 , with no change between days 1 and 5 and no difference between term and preterm infants. Red blood cell velocity increased in preterm infants from the first day after birth [median, 528 $\mu\text{m}/\text{s}$; 95% confidence interval (CI), 486–564 $\mu\text{m}/\text{s}$] to day 5 (median, 570 $\mu\text{m}/\text{s}$; 95% CI, 548–662 $\mu\text{m}/\text{s}$; $P = 0.001$), and correlated with the decrease in median hematocrit from 44% (CI, 40–60%) to 39% (CI, 37–43%) ($P = 0.006$).

Lindeboom *et al.*^{39–41} performed several studies to analyze the microcirculation mucosal parameters in smokers and nonsmokers, and also evaluated 10 patients with oral squamous cell carcinoma of the tongue, comparing the images with those obtained on the contralateral side as controls. The carcinomas were characterized by chaotic and dilated vessels accompanied by numerous areas of hemorrhage. The OPS technique may possibly play a future role in both the detection of early oral mucosal vascular aberrations and the effect of antitumor agents on the tumor microvasculature.⁴⁰

Lupi *et al.*³⁵ performed an evaluation of the skin microcirculation in female patients with gynoid lipodystrophy using the OPS technique. The study showed clinical improvement after the topical use of caffeine, mainly amongst nonsmokers who exercised regularly. The microcirculatory parameters evaluated were the functional capillary density, i.e. the number of flowing capillaries per unit area, diameter of the dermal papilla, and capillary diameter. After 1 month of treatment with a topical solution containing caffeine 7%, treated patients showed a statistically significant decrease in thigh circumference in more than 80% of cases and a reduction in hip circumference in 67.7%. The microcirculatory parameters, however, did not change significantly after treatment.³⁵

In summary, the OPS technique is a very promising tool for dermatologists and researchers, especially in the study of vasculitis, chronic venous insufficiency,³⁴ and skin tumors.⁴⁰ When reflected light is used, as in capillaroscopy, it is quite difficult to obtain good image contrast and detail because of surface scattering and the turbidity of the surrounding tissue.⁵ In OPS imaging, the phenomenon of cross-polarization mitigates these effects.²² When the light is absorbed by hemoglobin, an image of the illuminated hemoglobin-carrying structures in negative contrast is created. This “virtual backlighting” technology allows the visualization and measurement of real-time images of the microcirculation without the use of fluorescent dyes or transillumination.¹² This method has been validated for the quantitative measurement of microcirculatory parameters in an animal model compared with intravital fluorescence microscopy.³⁵

The OPS technique also enables the monitoring of the microcirculatory response to therapy, and thus may become a valuable clinical tool once a standardized and automated analysis routine is established.

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