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Effects of refractive index mismatch between sample and immersion medium in line-field confocal optical coherence tomography

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Abstract. Line-field confocal optical coherence tomography (LC-OCT) is an optical technique based on low-coherence interference microscopy with line illumination, designed for tomographic imaging of semi-transparent samples with micrometer-scale spatial resolution. A theoretical model of the signal acquired in LC-OCT is presented. The model shows that a refractive index mismatch between the sample and the immersion medium causes a dissociation of the coherence plane and the focal plane, leading to a decrease in the signal amplitude and a degradation of the image's lateral resolution. Measurements are performed to validate and illustrate the theoretical predictions. A mathematical condition linking various experimental parameters is established to ensure that the degradation of image quality is negligible. This condition is tested experimentally by imaging a phantom. It is verified theoretically in the case of skin imaging, using experimental parameters corresponding to those of the commercially available LC-OCT device.

Keywords: Optical coherence tomography, Interference microscopy, Biomedical imaging.

1 Introduction

Line-field confocal optical coherence tomography (LC-OCT) is a recent imaging technique based on low-coherence interference microscopy [1–4]. LC-OCT uses line illumination with broadband spatially coherent light and detection with a line camera to acquire cross-sectional images of semi-transparent samples, including skin tissues [5–13]. The focus is dynamically adjusted during the scan of the sample depth, allowing the use of a microscope objective to image with high lateral resolution. By using a supercontinuum laser as a broadband light source, the axial resolution can be similar to the lateral resolution, i.e. $\sim 1\ \mu\text{m}$ at a central wavelength of $\sim 750\ \text{nm}$, measured at the surface of the sample [1, 2]. The usual experimental arrangement of LC-OCT is based on a two-beam interference microscope in the Linnik configuration (see Fig. 1). The sample to image is placed in one arm of the interferometer in contact with a glass window under a microscope objective. A reference surface of low reflectivity is placed in the other arm of the interferometer under an identical microscope objective. The Linnik interferometer is mounted on a piezoelectric-actuated (PZT) translation stage for scanning the sample depth (see Fig. 1). Immersion microscope objectives are used with an immersion medium whose refractive index is as close

as possible to the refractive index of the sample. The equality of the refractive indices ensures the preservation of the symmetry of the interferometer during the depth scan, using a single motorized displacement. This avoids defect of focus [14–18] and dispersion mismatch between the interferometer arms [19–24].

In practice, however, it is generally impossible for the refractive indices of the immersion medium and the sample to be strictly identical regardless of the depth probed, at least because of inhomogeneities in the sample [25–27]. In skin tissues, for example, the refractive index varies with depth. The superficial layer of the skin (stratum corneum) has a refractive index in the near infrared around 1.54, while that of the epidermis is around 1.41 and that of the dermis around 1.38 [28–30].

The aim of this paper is to study the main consequences on the quality of the LC-OCT images of a difference in refractive index between the sample and the immersion medium. Theoretical analyses are carried out to understand the physical phenomena that occur and to see the influence of experimental parameters such as spectral characteristics of the light detected, beam focusing and depth in the sample. Measurements are performed to validate and illustrate the theoretical predictions. A mathematical condition dependent on the value of experimental parameters is established to avoid significant degradation of image quality. This condition is tested using two different immersion

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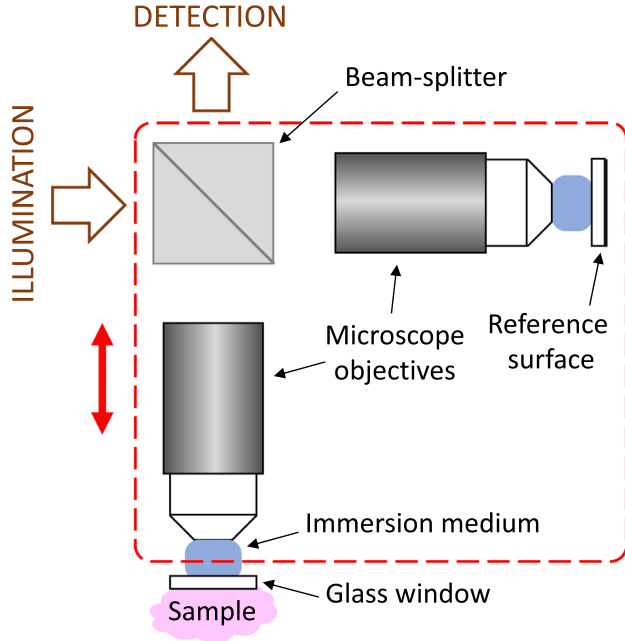


Fig. 1. Linnik interferometer implemented in LC-OCT. The components in the red dashed frames are mounted on a piezoelectric (PZT) translation stage for scanning the sample depth (motion indicated by the double red arrow).

media, both experimentally by imaging a phantom and theoretically in the case of skin imaging.

2. LC-OCT signal modeling

This section presents a theoretical model of the signal acquired in LC-OCT as a function of depth in the sample. This model will allow us to understand and predict effects of a difference in refractive index between the sample and the immersion medium. The dependence of the refractive indices with the optical frequency is ignored. This assumption is reasonable since the effect of chromatic dispersion up to first order in media of practical interest at depths less than a millimeter is not significant. In water, for example, the standard deviation of the refractive index is less than 0.002 (0.14%) over the wavelength range 0.6–1.0 μm [31].

2.1 Focal plane and coherence plane

In low-coherence interferometry, interference can be observed when the optical path difference in the interferometer does not exceed the temporal coherence length of the light source, with maximum contrast when the two optical paths are identical. In LC-OCT, the optical length of the reference arm of the interferometer determines the position of a plane in the sample arm, called the coherence plane, which corresponds to the equality of the optical paths. Interference is detected when a reflective structure in the sample is located in the coherence plane with an uncertainty of depth equal to the coherence length.

On the other hand, a reflective structure in the sample imaged by LC-OCT is in focus when it is located in the focal plane of the microscope objective placed in the sample arm of the interferometer, with an uncertainty of depth related to the depth of field.

In practice, the LC-OCT device is adjusted so that the coherence plane and the focal plane coincide at the surface of the sample pressed against a glass window (see Fig. 2a). In order to image in depth in the sample, the distance between the sample and the interferometer is reduced. After relative displacement of the interferometer a distance z closer to the sample (see Fig. 2a), the coherence plane is then at a distance z_{coh} below the sample surface and the focal plane at a distance z_{foc} below the sample surface.

The position of the coherence plane can be found by considering that the difference between the optical path lengths in the sample and reference arms of the interferometer is zero for the normally incident light beam:

$$z_{\text{coh}} = \frac{n_{\text{im}}}{n} z, \quad (1)$$

where the quantities n and n_{im} are the refractive indices of the sample and immersion medium, respectively.

The position of the focal plane can be calculated using geometrical optics [18, 32, 33] under the paraxial approximation:

$$z_{\text{foc}} = \frac{n}{n_{\text{im}}} z. \quad (2)$$

The paraxial approximation is applicable to microscope objectives with small numerical apertures (NA). Due to the refractive index mismatch between the sample and the immersion medium, the rays focused by the objective converge at different depths depending on their inclination. This creates uncertainty about the position of the focal plane, known as spherical aberration, which increases with the NA of the objective. LC-OCT typically employs a NA of 0.5. The formula provided in reference [27] and generalized in reference [34] can be used to calculate the distance between the marginal focus (corresponding to the most inclined rays) and the paraxial focus. For a NA of 0.5, refractive index values of $n_{\text{im}} = 1.33$ and $n = 1.40$, and a depth of 500 μm in the sample (typical maximum depth probed in LC-OCT), the distance between the two foci is less than 5 μm . This value typically corresponds to the depth of field, rendering spherical aberration insignificant and the paraxial calculation (Eq. (2)) satisfactory. Immersion microscope objectives with large apertures are equipped with a collar to correct for spherical aberration caused by the cover glass thickness variations and refractive index mismatch. In such cases, the focal plane's position corresponds to the paraxial focus's position and can thus be determined through paraxial calculation. However, the correction collar is designed only for small thicknesses and refractive index mismatches.

The comparison of equations (1) and (2) shows that the coherence plane and the focal plane do not coincide in the sample ($z_{\text{coh}} \neq z_{\text{foc}}$) if the refractive indices of the sample and the immersion medium are not equal ($n \neq n_{\text{im}}$) [15, 16]. In a homogeneous sample, the distance between

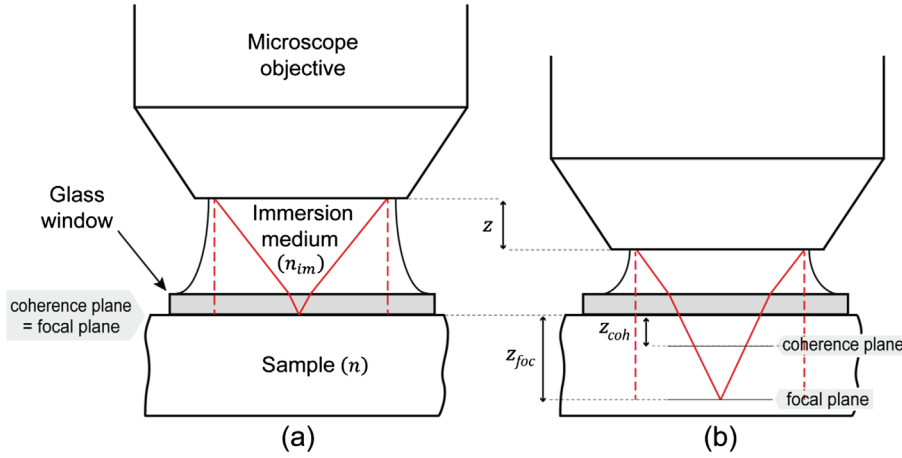


Fig. 2. Mismatch of the coherence plane and focal plane in LC-OCT. The plain red lines represent the beam in the plane of the figure, whereas the dotted red lines represent the beam in the direction orthogonal to the plane of the figure. (a) The interferometer is adjusted so that the coherence plane and the focal plane coincide at the surface of the sample. (b) When the interferometer is lowered by a distance z , the coherence plane is at a distance z_{coh} below the sample surface and the focal planes at a distance z_{foc} (here $n > n_{im}$).

the two planes ($|z_{foc} - z_{coh}|$) increases linearly with z . The consequences of the mismatch of the coherence plane and the focal plane on the LC-OCT images will be studied in the following of this paper.

2.2 Theoretical signal

Let us consider a reflective structure of the sample located at depth z_s below the sample surface. The optical intensity (or irradiance) on the detector, as a function of the depth scan (variable z , as shown in Fig. 2), is proportional to

$$\begin{aligned}
 I(z) &= \int_0^{+\infty} |A_{Ref}(\omega, z) + A_S(\omega, z)|^2 d\omega \\
 &= \int_0^{+\infty} |A_{Ref}(\omega, z)|^2 d\omega + \int_0^{+\infty} |A_S(\omega, z)|^2 d\omega \\
 &\quad + 2 \int_0^{+\infty} |A_{Ref}(\omega, z)| \times |A_S(\omega, z)| \cos[\phi(\omega, z)] d\omega. \quad (3)
 \end{aligned}$$

$A_{Ref}(\omega, z)$ and $A_S(\omega, z)$ represent the complex amplitudes of light at angular frequency ω , returning from the reference and sample arms of the interferometer, respectively. $\phi(\omega, z)$ is the phase difference between waves $A_{Ref}(\omega, z)$ and $A_S(\omega, z)$. It can be expressed as

$$\phi(\omega, z) = 2n(z_{coh} - z_s)\omega/c + \phi_0 = 2(n_{im}z - nz_s)\omega/c + \phi_0 \quad (4)$$

with ϕ_0 a constant phase difference.

$R_{ref}(\omega)$ denoting the reflectivity of the reference surface in the reference arm of the interferometer, and $S(\omega)$ the spectral intensity of the light source, we can write, ignoring a multiplication factor

$$|A_{Ref}(\omega, z)|^2 = R_{ref}(\omega)S(\omega). \quad (5)$$

Assuming that the reflectivity of the reference surface has a low dependence with ω over the spectral domain of the detector sensitivity, we have

$$\int_0^{+\infty} |A_{Ref}(\omega, z)|^2 d\omega = R_{ref}I_0, \quad (6)$$

where

$$I_0 = \int_0^{+\infty} S(\omega) d\omega, \quad (7)$$

is the optical intensity delivered by the light source.

Similarly, we write:

$$|A_S(\omega, z)|^2 = \gamma_{foc}^2(\omega, z)R_S(\omega)S(\omega), \quad (8)$$

where $R_S(\omega)$ is the reflectivity of the reflective structure considered as a planar reflector located at depth z_s in the sample. Function $\gamma_{foc}(\omega, z)$, called focus function, describes the normalized distribution of light amplitude incident into the sample as a function of depth. According to the theory of Gaussian beams, the axial distribution of light amplitude of a radially symmetrical Gaussian beam is inversely proportional to the beam width [35]. The beam radius varies with axial coordinate Z as

$$w(Z) = w_0 \left[1 + \left(\frac{Z}{Z_R} \right)^2 \right]^{1/2}. \quad (9)$$

The beam waist, defined as the minimal value of the beam radius, is related to the NA of the Gaussian beam and the optical wavelength in vacuum λ_0 as [35]

$$w_0 = \frac{\lambda_0}{\pi NA}. \quad (10)$$

The quantity Z_R , called Rayleigh length, can be written as [35]

$$Z_R = \frac{n\lambda_0}{\pi \text{NA}^2}, \quad (11)$$

n being the refractive index of the medium in which the beam propagates.

In LC-OCT, the laser beam is focused in only one transverse direction to illuminate the sample with a line of light (see Fig. 2). Function $\gamma_{\text{foc}}(\omega, z)$ is therefore inversely proportional to the square root of the beam width in the direction perpendicular to the illumination line. The maximum of $\gamma_{\text{foc}}(\omega, z)$ is reached when the beam is focused in the plane of the reflective structure, i.e. when $z_{\text{foc}} = z_S$, which corresponds to $z = (n_{\text{im}}/n) z_S$. The focus function can therefore be expressed as

$$\gamma_{\text{foc}}(z) = \sqrt{\frac{w_0}{w(Z = z_{\text{foc}} - z_S)}}. \quad (12)$$

Using the expression of the beam waist radius given in equation (9), an expression of the focus function γ_{foc} at central angular frequency $\omega_0 = 2\pi c/\lambda_0$ can be written as

$$\gamma_{\text{foc}}(z) = \left\{ 1 + 64 \left[\frac{(n/n_{\text{im}})z - z_S}{\Delta z_{\text{foc}}} \right]^2 \right\}^{-1/4}, \quad (13)$$

with Δz_{foc} the full-width-at-half-maximum (FWHM) of $\gamma_{\text{foc}}(z)$ being $\Delta z_{\text{foc}} \approx 8Z_R$.

Assuming that the NA of the microscope objectives matches the NA of the laser beam in the plane perpendicular to the illumination line, the width of the focus function can be written according to equation (11) as

$$\Delta z_{\text{foc}} \approx \frac{8}{\pi} \frac{n\lambda_0}{\text{NA}^2}. \quad (14)$$

Assuming that the reflectivity of the structure has a low dependence with ω over the spectral domain of the camera sensitivity, the optical intensity returning from the sample arm can be written as

$$\int_{-\infty}^{+\infty} |A_S(\omega, z)|^2 d\omega = \gamma_{\text{foc}}^2(z) R_S I_0. \quad (15)$$

The detected LC-OCT signal, as a function of depth in the sample (Eq. (3)) can now be written as

$$I(z) = I_0 \{ R_{\text{ref}} + R_S \gamma_{\text{foc}}^2(z) \} + 2 \times \sqrt{R_{\text{ref}} R_S} \gamma_{\text{foc}}(z) \int_0^{+\infty} S(\omega) \cos \phi(\omega, z) d\omega. \quad (16)$$

Using complex analysis, and substituting $\phi(\omega, z)$ by its expression given in equation (4), the integral in the previous equation can be written as

$$\int_0^{+\infty} S(\omega) \cos [\phi(\omega, z)] d\omega = \Re e \left\{ \int_{-\infty}^{+\infty} S(\omega) \exp [-i\phi(\omega, z)] d\omega \right\} \\ = \Re e \left\{ \exp [-i\phi_0] \hat{S}(\xi) \right\}, \quad (17)$$

where $\Re e\{-\}$ denotes the real part of a complex number and $\hat{S}(\xi)$ the Fourier transform of $S(\omega)$ with $\xi = (n_{\text{im}}z - nz_S)/\pi c$.

We suppose the spectral intensity of the light source to be described by a Gaussian-shaped function of width (FWHM) $\Delta\omega$, centered at $\omega = \omega_0$:

$$S(\omega) = S_0 \exp \left[-4 \ln 2 \left(\frac{\omega - \omega_0}{\Delta\omega} \right)^2 \right]. \quad (18)$$

The Fourier transform of $S(\omega)$ is

$$\hat{S}(\xi) = I_0 \exp \left[- \left(\frac{\pi \Delta\omega}{2\sqrt{\ln 2}} \xi \right)^2 \right] \exp [-i2\pi\omega_0\xi], \quad (19)$$

the intensity of the light source being here

$$I_0 = \int_{-\infty}^{+\infty} S(\omega) d\omega = S_0 \frac{\Delta\omega}{2} \sqrt{\frac{\pi}{\ln 2}}. \quad (20)$$

The integral in equation (16) can finally be calculated as

$$\int_0^{+\infty} S(\omega) \cos \phi d\omega = I_0 \gamma_{\text{coh}}(z) \cos [2(n_{\text{im}}z - nz_S)\omega_0/c + \phi_0], \quad (21)$$

with

$$\gamma_{\text{coh}}(z) = \exp \left\{ -4 \ln 2 \left[\frac{(n_{\text{im}}/n)z - z_S}{\Delta z_{\text{coh}}} \right]^2 \right\}, \quad (22)$$

and

$$\Delta z_{\text{coh}} = \frac{4c \ln 2}{n \Delta\omega}. \quad (23)$$

The Gaussian function $\gamma_{\text{coh}}(z)$ represents the temporal coherence function. It is maximum when $z = (n/n_{\text{im}}) z_S$, i.e. when $z_{\text{coh}} = z_S$ (the reflective structure is then located in the coherence plane). Δz_{coh} is the FWHM of $\gamma_{\text{coh}}(z)$, which can be approximated by

$$\Delta z_{\text{coh}} \approx \frac{2 \ln 2}{n\pi} \left(\frac{\lambda_0^2}{\Delta\lambda} \right), \quad (24)$$

where $\Delta\lambda$ represents the FWHM of the source spectral intensity expressed as a function of wavelength, and λ_0 the central optical wavelength.

Finally, the LC-OCT axial signal given by a planar reflector of reflectivity R_S located at depth z_S can be written as

$$I(z) = I_0 \left\{ R_{\text{ref}} + R_S \gamma_{\text{foc}}^2(z) + 2\gamma(z) \sqrt{R_{\text{ref}} R_S} \cos [4\pi(n_{\text{im}}z - nz_S)/\lambda_0 + \phi_0] \right\}, \quad (25)$$

with

$$\gamma(z) = \gamma_{\text{foc}}(z) \times \gamma_{\text{coh}}(z). \quad (26)$$

The signal expressed in equation (25) is the sum of three terms. The first term ($R_{\text{ref}}I_0$) is the intensity of light reflected by the reference surface (constant term). The second term ($R_S \gamma_{\text{foc}}^2(z)I_0$) corresponds to the intensity of light reflected by the reflective structure in the sample.

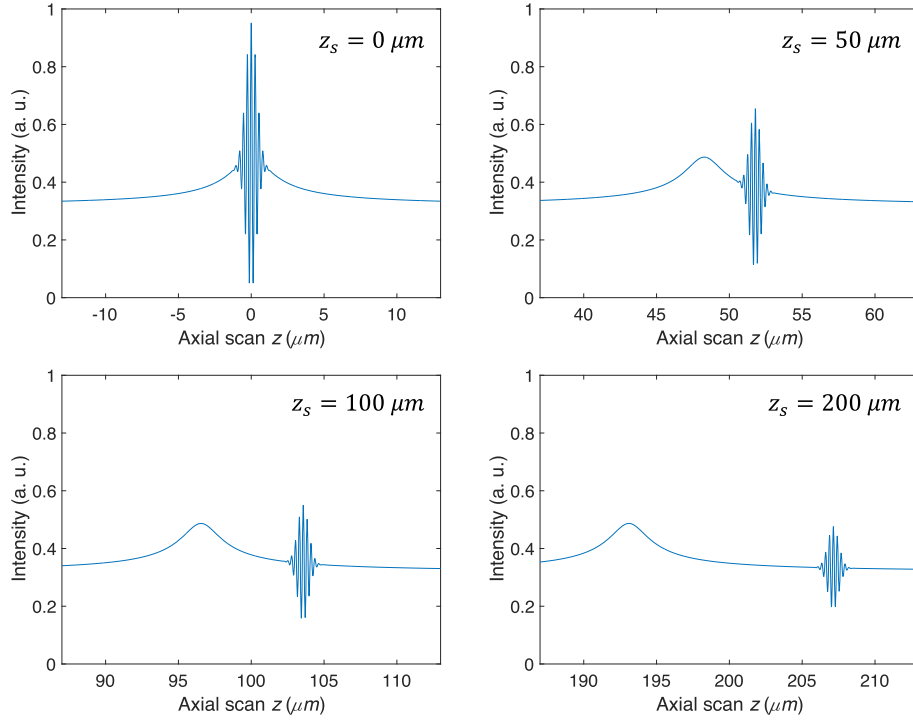


Fig. 3. Theoretical axial signal acquired in LC-OCT from a reflective structure located at different depths z_s (simulation based on Eq. (25)). The parameters of the simulation are: $\lambda_0 = 750$ nm, $\Delta\lambda = 200$ nm, $n = 1.45$, $n_{\text{im}} = 1.40$, $\text{NA} = 0.5$, $R_{\text{ref}} = 0.19\%$, $R_s = 0.1\%$, $\phi_0 = 0$.

The third term is identified as the interferometric signal, which consists of a sinusoidal modulation of period $\lambda_0/(2n_{\text{im}})$ comprised in an envelop $2I_0\gamma(z)\sqrt{R_{\text{ref}}R_s}$. Function $\gamma(z)$ is the product of the focus function $\gamma_{\text{foc}}(z)$ and the coherence function $\gamma_{\text{coh}}(z)$, given by equations (13) and (22), respectively. Plots of equation (25) are shown in Figure 3 for a reflective structure located at different depths z_s when $n \neq n_{\text{im}}$. The signal consists of a peak and interference fringes, which correspond in the simulations to $R_s\gamma_{\text{foc}}^2(z)$ and $2\gamma(z)\sqrt{R_{\text{ref}}R_s}\cos[4\pi(n_{\text{im}}z - nz_s)/\lambda_0]$, respectively. When $z_s = 0$, the focus function and the fringe envelope coincide at $z = 0$ ($\gamma_{\text{foc}}(z)$ and $\gamma(z)$ are centered at $z = 0$ when $z_s = 0$). When the depth z_s of the reflective structure increases, the distance between the focus function and the fringe envelope increases, whereas the amplitude of the interference fringes decreases.

2.3 Experimental validation

This section compares the theoretical model presented above with measurements obtained using an LC-OCT device available in the laboratory and described in reference [2]. Measurements of the axial signal acquired by LC-OCT have been performed to check the validity of the theoretical expression given by equation (25). A simple sample was made by introducing a liquid of refractive index n between the glass window of the LC-OCT device and a glass plate placed at a distance z_s below the glass window (see Fig. 4). The two interfaces between glass (fused silica) and

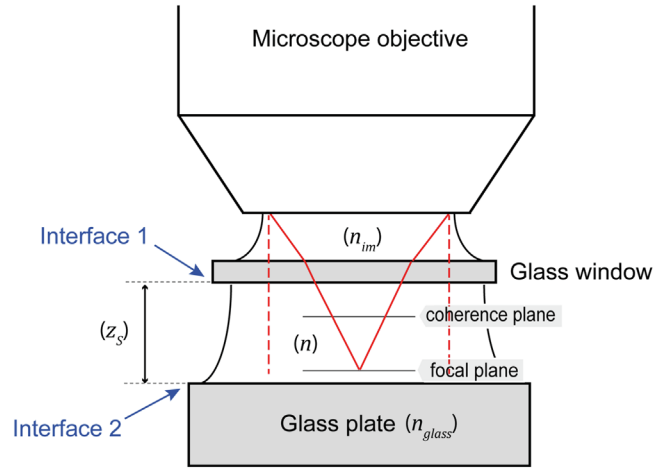


Fig. 4. Test sample with two reflecting structures: interface 1 located at $z_s = 0$, and interface 2 located at $z_s > 0$. The glass material is fused silica ($n_{\text{glass}} = 1.45$). The immersion medium is silicon oil ($n_{\text{im}} = 1.40$). The liquid placed between the two interfaces is water ($n = 1.33$), oil ($n = 1.51$) or silicon oil ($n = 1.40$).

the liquid have the same reflectivity $R_s = (n - n_{\text{glass}})^2 / (n + n_{\text{glass}})^2$. This constitutes a sample of known refractive index n with two reflecting structures of reflectivity R_s located at depth $z_s = 0$ and $z_s > 0$. Three different samples were made with pure water ($n = 1.33$), oil ($n = 1.51$), and pure silicon oil ($n = 1.40$) placed between the glass window and the glass plate. The oil was an immersion oil for

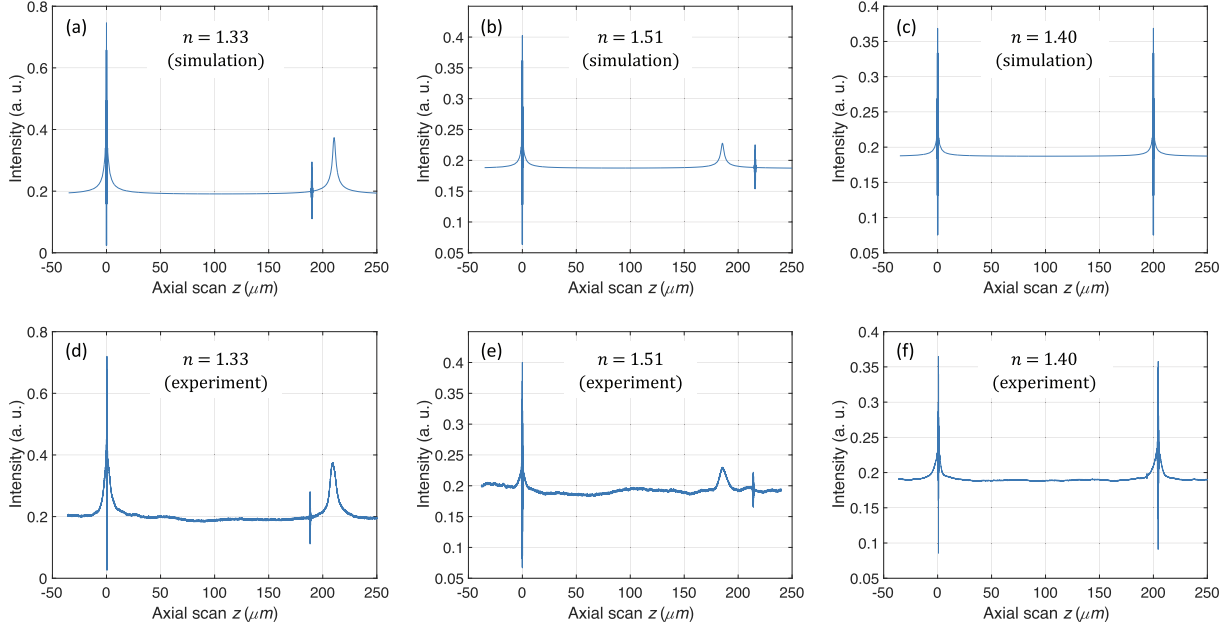


Fig. 5. Signal acquired in LC-OCT from the test sample shown in Figure 4. The parameters are: $\lambda_0 = 750$ nm, $\Delta\lambda = 200$ nm, $z_s = 200$ μm , $n_{\text{im}} = 1.40$, $n_{\text{glass}} = 1.45$, $\text{NA} = 0.5$. $R_{\text{ref}} = 0.19\%$. Three values of the refractive index n are considered (1.33, 1.40, 1.51). (a–c) Simulations based on equation (25). (d–f) Experiment. The peak corresponding to the focus on interface 2 coincides with the center of interference fringes only when $n_{\text{im}} = n$ ((c) and (f)).

microscopy (*Sigma-Aldrich*, ref. 56822). The silicon oil was provided by *Sigma-Aldrich* (ref. 378399).

The signal acquired by a pixel of the LC-OCT camera as a function of the depth scan is shown in Figure 5 (experiment and simulation). Due to the reflection of light on the two interfaces, the signal consists of the sum of two peaks and two packets of interference fringes. The LC-OCT device is adjusted so that the coherence plane and the focal plane coincide at interface 1. Therefore, the interference fringes and the peak of light produced by the interface 1 coincide at $z = 0$ ($\gamma(z)$ and $\gamma_{\text{foc}}(z)$ are centered at $z = 0$ when $z_s = 0$). The interference fringes and the peak of light generated by light reflection on interface 2 located at $z_s = 200$ μm do not coincide when the refractive index of the sample differs from the refractive index of the immersion medium ($n \neq n_{\text{im}}$), as predicted by the simulation. Depending on the value of n , the interference fringes are detected before or after the focus. Simulations are in good agreement with measurements. Note, however, that the amplitude of the interference fringes produced by interface 2 is slightly lower in the experiment. This can be explained by the transmission factor of interface 1 ($T = 1 - R_s$), not taken into account in the simulation, which reduces the amplitude of the detected signal from interface 2. The presence of chromatic dispersion mismatch in the interferometer when $n \neq n_{\text{im}}$, not taken into account in the simulation either, also contributes to these discrepancies. Other factors can decrease interference contrast in practice, such as the existence of stray light that does not contribute to interference, defects in the beam splitter that is not perfectly 50/50 across the entire spectral range of detector sensitivity, and imperfections in the interferometer setting.

3 Effects of refractive index mismatch between sample and immersion medium

The aim of this section is to study the consequences on LC-OCT image quality of the shift between the focus function and the fringe envelop, resulting from a difference in refractive index between the sample and the immersion medium. A degradation of the signal amplitude and image resolution will be highlighted and quantified. Conditions to ensure that degradation is not significant will be established.

3.1 Degradation of signal amplitude

The signal in the LC-OCT images correspond to the envelop of the interference fringes, which is obtained by digital processing of the acquired signal $I(z)$ expressed in equation (25) [1, 2, 36]. The processed LC-OCT axial response given by a reflective structure of reflectivity R_s located at depth z_s in the sample is therefore proportional to $\sqrt{R_s} \gamma(z)$. Theoretical plots of $\gamma(z)$ (Eq. (26)) are shown in Figure 6 for different values of the structure depth z_s with a mismatch of the refractive index between the sample and the immersion medium. The deeper the structure, the weaker the signal amplitude.

We will now establish an explicit expression of the signal attenuation by simplifying the expression of function $\gamma(z)$. Typical values of the experimental parameters in LC-OCT are $\lambda_0 = 750$ nm, $\Delta\lambda = 200$ nm, $\text{NA} = 0.5$ and $n_{\text{im}} = 1.40$ [1–5]. Considering a sample with $n = 1.45$ yields $\Delta z_{\text{foc}} \approx 10$ μm (Eq. (14)) and $\Delta z_{\text{coh}} \approx 1$ μm (Eq. (24)). Since

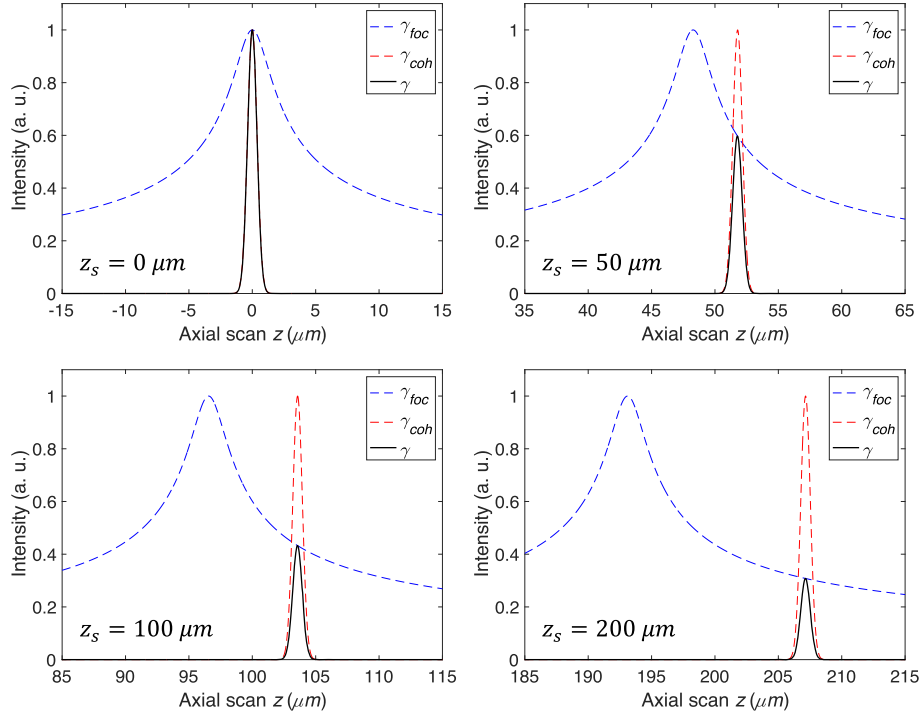


Fig. 6. Simulation of $\gamma(z)$ (Eq. (26)) for different values of the reflecting structure depth z_S . The parameters of the simulation are $\lambda_0 = 750$ nm, $\Delta\lambda = 200$ nm, $n = 1.45$, $n_{\text{im}} = 1.40$, $\text{NA} = 0.5$.

$\Delta z_{\text{coh}} \ll \Delta z_{\text{foc}}$, function $\gamma(z)$ can be approximated by the coherence function $\gamma_{\text{coh}}(z)$ multiplied by the value of $\gamma_{\text{foc}}(z)$ at the maximum of $\gamma_{\text{coh}}(z)$, i.e. when $z_{\text{coh}} = z_S$, which corresponds to $z = (n/n_{\text{im}}) z_S$. The fringe envelope $\gamma(z)$ can therefore be considered as a Gaussian function multiplied by an attenuation factor $\alpha < 1$ given by

$$\alpha = \gamma_{\text{foc}}(z = (n/n_{\text{im}}) z_S) = \left[1 + \left(8\varepsilon \frac{z_S}{\Delta z_{\text{foc}}} \right)^2 \right]^{-1/4}, \quad (27)$$

with

$$\varepsilon = 1 - \left(\frac{n}{n_{\text{im}}} \right)^2. \quad (28)$$

Assuming that $\Delta n = |n - n_{\text{im}}| \ll 1$, an approximate expression of the signal attenuation is

$$\alpha = \left[1 + \left(2\pi \frac{\Delta n z_S \text{NA}^2}{n_{\text{im}}^2 \lambda_0} \right)^2 \right]^{-1/4}. \quad (29)$$

Equation (29) is plotted in Figure 7 as a function of the depth z_S of the reflective structure for different values of the refractive index mismatch Δn . As can be seen, the attenuation may be significant for values of Δn of only a few percent. With the following condition:

$$\frac{\text{NA}^2}{\lambda_0} \frac{\Delta n}{n_{\text{im}}^2} z_S \leq 1, \quad (30)$$

the attenuation of the signal is less than 2.5 ($\alpha \approx 0.4$). Equation (30) can be seen as a condition for keeping signal attenuation low.

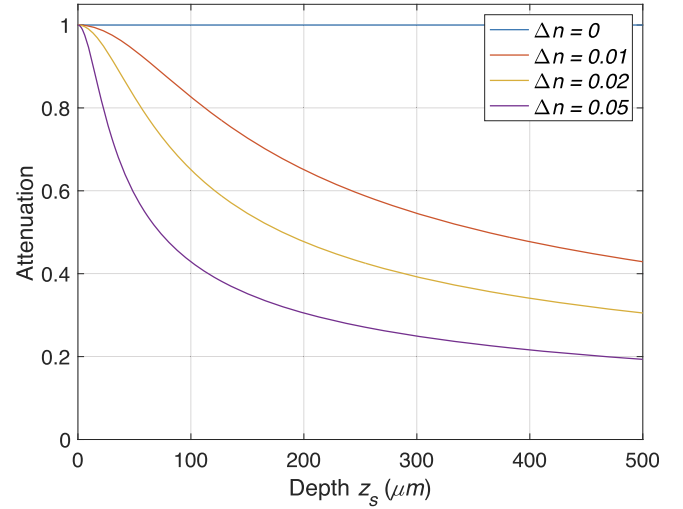


Fig. 7. Signal attenuation α as a function of depth in the sample for various values of Δn . Simulation based on equation (29) with $\lambda_0 = 750$ nm, $\Delta\lambda = 200$ nm, $\text{NA} = 0.5$, $n_{\text{im}} = 1.4$.

3.2 Degradation of lateral resolution

If the focal plane of the microscope objective does not coincide with the coherence plane, the image of the structures in the vicinity of the coherence plane appears blurred because of the defect of focus, which degrades the lateral resolution. If $n_{\text{im}} \neq n$, the degradation increases with depth and it is obviously all the more important as the depth of field of the objectives is small (i.e. NA is high). Inhomogeneities

of the refractive index within the sample also induce a distortion of the optical wavefront, which introduces optical aberrations. Considered alone, this effect yields to an effective lateral resolution degraded compared to its diffraction-limited theoretical value. Although less significant than the effect of focus defect, those aberrations cannot be easily corrected.

As seen previously, a reflective structure located at z_S is detected with a maximum signal when it is located in the coherence plane, i.e. when $z_{\text{coh}} = z_S$, which corresponds to $z = (n/n_{\text{im}})z_S$. We consider that a defect of focus is not visible provided that the structure lies within the depth of field Δz_{foc} , i.e. when the distance Δz between the coherence plane and the focal plane is such that

$$\Delta z = |z_{\text{coh}} - z_{\text{foc}}| \leq \Delta z_{\text{foc}}, \quad (31)$$

with Δz_{foc} defined as the width of the focus function (Eq. (14)).

Since $z_{\text{foc}} = (n/n_{\text{im}})z$, Δz can be expressed as a function of the refractive indices of the sample and immersion medium and depth in the sample as

$$\Delta z = \left| 1 - \left(\frac{n}{n_{\text{im}}} \right)^2 \right| z_S. \quad (32)$$

Assuming that $\Delta n = |n - n_{\text{im}}| \ll 1$, a condition for a focus defect not to be noticeable is

$$\frac{\text{NA}^2}{\lambda_0} \frac{\Delta n}{n_{\text{im}}^2} z_S \leq \frac{4}{\pi} \approx 1. \quad (33)$$

It is interesting to notice that the conditions for no significant loss of signal (Eq. (30)) and no significant defect of focus (Eq. (33)) are identical. The maximum depth of a structure that can be imaged without significant signal loss and resolution degradation can therefore be estimated from equation (33) alone. Figure 8 shows the imaging depth that can be reached without degradation of image quality as a function of the refractive index mismatch Δn for different values of the microscope objective NA.

4. LC-OCT imaging

In this final section, we propose to illustrate the image degradation due to a difference in refractive index between the sample and the immersion medium by imaging a phantom with a known refractive index using two different immersion media. Finally, we propose to see theoretically whether the condition of non-degradation of image quality (Eq. (33)) is verified for skin imaging using two different immersion media.

4.1 Image of a phantom

A phantom was fabricated by embedding titanium dioxide (TiO_2) particles in a polydimethylsiloxane (PDMS) matrix (Sylgard 184 silicone Elastomer Kit, *Neyco*, France) whose refractive index is 1.41 at the wavelength of 750 nm [37]. The PDMS matrix was fabricated using a ratio of 10:1 by

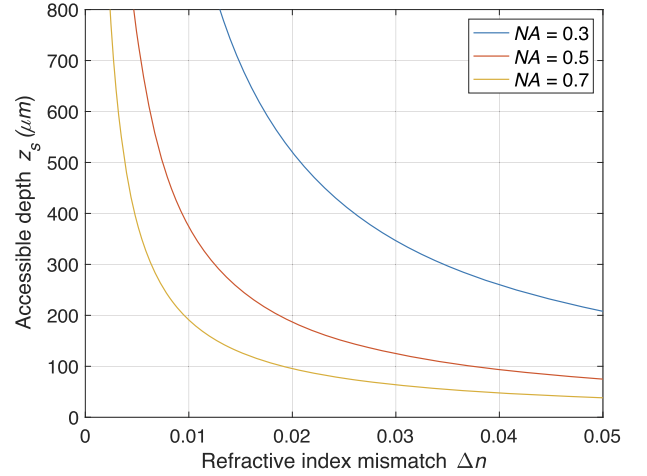


Fig. 8. Depth (z_S) in the sample that can be probed without significant loss of both signal amplitude and lateral resolution, as a function of refractive index mismatch. Simulation based on equation (33), with $n_{\text{im}} = 1.40$ and $\lambda_0 = 750$ nm, for different values of the microscope objective NA.

weight of PDMS pre-polymer and curing agent (Sylgard 184). The phantom was prepared by first mixing the curing agent with a powder of TiO_2 particles (*Sigma-Aldrich*). The mixture was placed in an ultrasonic bath for 30 min to prevent particles aggregation, and then mixed with the PDMS pre-polymer. The obtained mixture was poured on a 40-mm diameter petri dish. Air bubbles were removed using a vacuum pump for 1 h, and the phantom mixture was finally cured for 1 h30 min at 80 °C. The concentration of TiO_2 particles in the PDMS is 0.40% by weight, and their diameter is less than 5 μm . This results in a refractive index of the phantom that is similar to that of the PDMS matrix, i.e. $n \sim 1.41$.

LC-OCT images of the phantom in vertical section were acquired, using two different immersion media: silicon oil and water. The images are shown in Figure 9. The refractive index of the phantom ($n \sim 1.41$) being close to that of the silicon oil ($n_{\text{im}} = 1.40$), focusing and signal amplitude are maintained throughout the scan depth when silicon oil is used as the immersion medium (Fig. 9a). In this case, equation (33) is fully satisfied since $\Delta n \sim 0$. When water is used as the immersion medium ($n_{\text{im}} = 1.33$), the mismatch in refractive indices ($\Delta n \sim 0.08$) leads to a focusing defect and a signal loss that increases with depth (Fig. 9b). In this case, equation (30) is satisfied (i.e. image quality is not degraded) for a depth $z_S < 70$ μm (taking $n = 1.41$, $\text{NA} = 0.5$, $\lambda_0 = 750$ nm and $\Delta\lambda = 200$ nm), which seems to agree with the observation of the acquired image.

4.2 Skin imaging

LC-OCT has been designed so far for imaging of skin tissues [1, 5]. The superficial skin layer (stratum corneum) has a refractive index in the near infrared around $n = 1.54$, and a thickness of 10–30 μm . The rest of the epidermis has a mean refractive index around $n = 1.41$ and a thickness of 100–200 μm . The refractive index of the dermis, the deepest

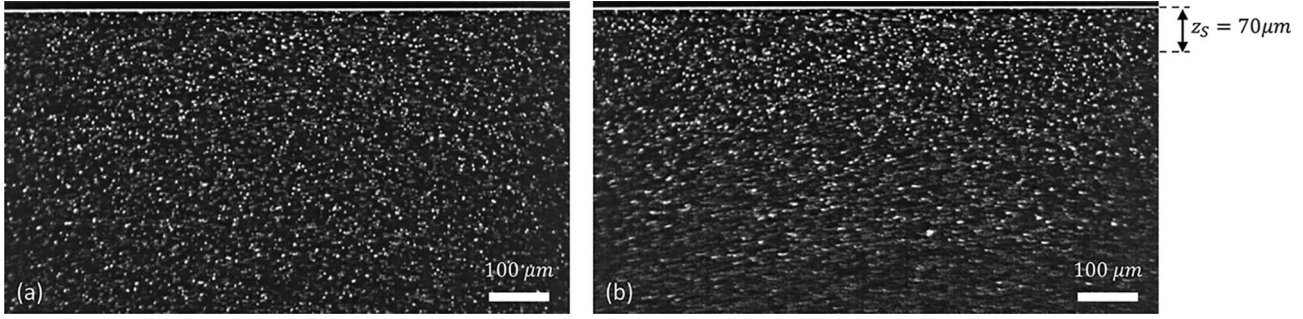


Fig. 9. LC-OCT images (vertical sections) of TiO_2 particles embedded in PDMS ($n \sim 1.41$), using an immersion medium of refractive index $n_{\text{im}} = 1.40$ (a) and $n_{\text{im}} = 1.33$ (b).

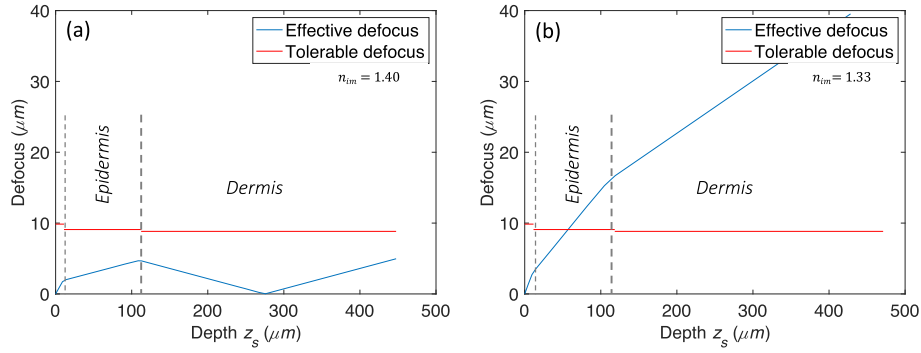


Fig. 10. Theoretical defocus ($\Delta z = |z_{\text{coh}} - z_{\text{foc}}|$) in the different skin layers with an immersion medium of refractive index $n_{\text{im}} = 1.40$ (a) and $n_{\text{im}} = 1.33$ (b) compared to the tolerable defocus (Δz_{toc}). Calculations based on equations (32) and (14) with $\text{NA} = 0.5$, $\lambda_0 = 750$ nm and $n = 1.54/1.41/1.38$ (stratum corneum/epidermis/dermis).

layer, is around $n = 1.38$ [28–30]. Due to variations in skin refractive index, a mismatch between the coherence plane and focal plane is unavoidable during depth scanning. In this section, we propose to calculate the defocus $\Delta z = |z_{\text{coh}} - z_{\text{foc}}|$ that occurs as a function of the imaging depth in skin tissues. The simulation was carried out using equation (32) with silicon oil (Fig. 10a) and water (Fig. 10b) as the immersion medium. The epithelium thickness was considered to be $120 \mu\text{m}$, with a $20 \mu\text{m}$ stratum corneum. The tolerable defocus, considered to be depth of field Δz_{toc} , was calculated using equation (14) with $\text{NA} = 0.5$ and $\lambda_0 = 750$ nm. The simulation shows that image quality is maintained in all skin layers when silicone is used as the immersion medium ($n_{\text{im}} = 1.40$). This corresponds to the LC-OCT device commercialized by Damae Medical. If water is used instead of silicon oil as the immersion medium ($n_{\text{im}} = 1.33$) for skin imaging, the condition for preserving image quality is not satisfied at depths larger than $z_s \sim 70 \mu\text{m}$. This depth corresponds to about half of the thickness of the epidermis in the skin model considered in this simulation.

5 Conclusion

A theoretical model of the signal acquired in LC-OCT has been described. The model shows that a difference in refractive index between the sample and the immersion medium causes dissociation between the coherence plane

and the focal plane, resulting in a decrease in signal amplitude and a degradation of the image's lateral resolution. Measurements were carried out to validate and illustrate the theoretical predictions. A mathematical condition (Eq. (33)) linking various experimental parameters was established to ensure that image quality degradation is insignificant. This condition was tested experimentally by imaging a phantom. It was verified theoretically in the case of skin imaging, with experimental parameters corresponding to those of the LC-OCT device marketed by Damae Medical. The mathematical condition expressed in equation (33) can be used to define the experimental parameters of an LC-OCT device, including the NA of the microscope objectives, the center optical wavelength, the maximum imaging depth and the refractive index of the immersion medium, in order to image with optimum quality a sample whose refractive index is known.

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Conflicts of Interest

The author declares no conflicts of interest.

Data availability statement

This article has no associated data generated and/or analyzed.

References

- Dubois A., Levecq O., Azimani H., Siret D., Barut A., Suppa M., Del Marmol V., Malvey J., Cinotti E., Rubegni P., Perrot J.L. (2018) Line-field confocal optical coherence tomography for high-resolution noninvasive imaging of skin tumors, *J. Biomed. Opt.* **23**, 106007.
- Dubois A., Levecq O., Azimani H., Davis A., Ogien J., Siret D., Barut A. (2018) Line-field confocal time-domain optical coherence tomography with dynamic focusing, *Opt. Express* **26**, 33534–33542.
- Ogien J., Levecq O., Azimani H., Dubois A. (2020) Dual-mode line-field confocal optical coherence tomography for ultrahigh-resolution vertical and horizontal section imaging of human skin in vivo, *Biomed. Opt. Express* **11**, 1327–1335.
- Ogien J., Daures A., Cazalas M., Perrot J.-L., Dubois A. (2020) Line-field confocal optical coherence tomography for three-dimensional skin imaging, *Front. Optoelectron.* **13**, 381–392.
- Latriglia F., Ogien J., Tavernier C., Fischman S., Suppa M., Perrot J.L., Dubois A. (2023) Line-field confocal optical coherence tomography (LC-OCT) for skin imaging in dermatology, *Life* **13**, 2268.
- Monnier J., Tognetti L., Miyamoto M., Suppa M., Cinotti E., Fontaine M., Perez J., Orte Cano C., Yélamos O., Puig S., Dubois A., Rubegni P., Marmol V., Malvey J., Perrot J.-L. (2020) In vivo characterization of healthy human skin with a novel, non-invasive imaging technique: line-field confocal optical coherence tomography, *J. Eur. Acad. Dermatol. Venereol.* **34**, 2914–2921.
- Pedrazzani M., Breugnot J., Rouaud-Tinguely P., Cazalas M., Davis A., Bordes S., Dubois A., Closs B. (2020) Comparison of line-field confocal optical coherence tomography images with histological sections: validation of a new method for in vivo and non-invasive quantification of superficial dermis thickness, *Skin Res. Technol.* **26**, 398–404.
- Ruini C., Schuh S., Sattler E., Welzel J. (2021) Line-field confocal optical coherence tomography—practical applications in dermatology and comparison with established imaging methods, *Skin Res. Technol.* **27**, 340–352.
- Ruini C., Schuh S., Gust C., Kendziora B., Frommherz L., French L.E., Hartmann D., Welzel J., Sattler E. (2021) Line-field optical coherence tomography: in vivo diagnosis of basal cell carcinoma subtypes compared with histopathology, *Clin. Exp. Dermatol.* **46**, 1471–1481.
- Suppa M., Fontaine M., Dejonckheere G., Cinotti E., Yélamos O., Diet G., Tognetti L., Miyamoto M., Orte Cano C., Perez-Anker J., Panagiotou V., Trepant A.L., Monnier J., Berot V., Puig S., Rubegni P., Malvey J., Perrot J.L., del Marmol V. (2021) Line-field confocal optical coherence tomography of basal cell carcinoma: a descriptive study, *J. Eur. Acad. Dermatol. Venereol.* **35**, 1099–1110.
- Donelli C., Suppa M., Tognetti L., Perrot J.L., Calabrese L., Pérez-Anker J., Malvey J., Rubegni P., Cinotti E. (2023) Line-field confocal optical coherence tomography for the diagnosis of skin carcinomas: real-life data over three years, *Curr. Oncol.* **30**, 8853–8864.
- Cinotti E., Bertello M., Cartocci A., Fiorani D., Tognetti L., Solmi V., Cappilli S., Peris K., Perrot J.L., Suppa M., Del Marmol V., Rubegni P. (2023) Comparison of reflectance confocal microscopy and line-field optical coherence tomography for the identification of keratinocyte skin tumours, *Skin Res Technol.* **29**, e13215.
- Perez-Anker J., Puig S., Alos L., García A., Alejo B., Cinotti E., Orte Cano C., Tognetti L., Lenoir C., Monnier J., Machuca N., Castillo P., Gibert P.R., Rubegni P., Suppa M., Perrot J.L., Del Marmol V., Malvey J. (2022) Morphological evaluation of melanocytic lesions with three-dimensional line-field confocal optical coherence tomography: correlation with histopathology and reflectance confocal microscopy. A pilot study, *Clin. Exp. Dermatol.* **47**, 2222–2233.
- Dubois A. (2017) Focus defect and dispersion mismatch in full-field optical coherence microscopy, *Appl. Opt.* **56**, D142–D150.
- Dubois A., Moneron G., Boccara A.C. (2006) Thermal-light full-field optical coherence tomography in the 1.2 micron wavelength region, *Opt. Comm.* **266**, 738–743.
- Labiau S., David G., Gigan S., Boccara A.C. (2009) Defocus test and defocus correction in full-field optical coherence tomography, *Opt. Lett.* **34**, 1576–1578.
- Safrani A., Abdulhalim I. (2011) Spatial coherence effect on layer thickness determination in narrowband full-field optical coherence tomography, *Appl. Opt.* **50**, 3021–3027.
- Abdulhalim I. (2012) Spatial and temporal coherence effects in interference microscopy and full-field optical coherence tomography, *Ann. Der Physik* **524**, 787–804.
- Hitzengerger C.K., Baumgartner A., Drexler W., Fercher A.F. (1999) Dispersion effects in partial coherence interferometry: Implications for intraocular ranging, *J. Biomed. Opt.* **4**, 144–156.
- Dubois A., Grieve K., Moneron G., Lecaque R., Vabre L., Boccara A.C. (2004) Ultrahigh-resolution full-field optical coherence tomography, *Appl. Opt.* **43**, 2874–2882.
- Zeylikovich I. (2008) Short coherence length produced by a spatial incoherent source applied for the Linnik-type interferometer, *Appl. Opt.* **47**, 2171–2177.
- Abdulhalim I. (2009) Theory for double beam interference microscopes with coherence effects and verification using the linnik microscope, *J. Mod. Opt.* **48**, 279–302.
- Lychagov V.V., Ryabukho V.P., Kalyanov A.L., Smirnov I.V. (2012) Polychromatic low-coherence interferometry of stratified structures with digital interferogram recording and processing, *J. Opt. A Pure Appl. Opt.* **14**, 015702.
- Federici A., Dubois A. (2015) Full-field optical coherence microscopy with optimized ultra-high spatial resolution, *Opt. Lett.* **40**, 5347–5350.
- Schmitt J.M., Kumar G. (1996) Turbulent nature of refractive-index variations in biological tissue, *Opt. Lett.* **21**, 1310–1312.
- Tuchin V.V., Utz S.R., Yaroslavsky I.V. (1994) Tissue optics, light distribution, and spectroscopy, *Opt. Eng.* **33**, 3178–3188.
- Tearney G.J., Brezinski M.E., Southern J.F., Bouma B.E., Hee M.R., Fujimoto J.G. (1995) Determination of the refractive index of highly scattering human tissue by optical coherence tomography, *Opt. Lett.* **20**, 2258–2260.
- Ding H., Lu J.Q., Wooden W.A., Kragel P.J., Hu X.H. (2006) Refractive indices of human skin tissues at eight wavelengths and estimated dispersion relations between 300 and 1600 nm, *Phys. Med. Biol.* **51**, 1479–1489.
- Lai J.C., Zhang Y.Y., Li Z.H., Jiang H.J., He A.Z. (2010) Complex refractive index measurement of biological tissues by attenuated total reflection ellipsometry, *Appl. Opt.* **49**, 3235–3238.
- Kono T., Yamada J. (2019) In vivo measurement of optical properties of human skin for 450–800 nm and 950–1600 nm wavelengths, *Int. J. Thermophys.* **40**, 1–14.
- The International Association for the Properties of Water and Steam (September 1997). *Release on the refractive index of ordinary water substance as a function of wavelength, temperature, and pressure (IAPWS R9-97)*. <http://www.iapws.org/relguide/rindex.pdf>
- Visser T.D., Oud J.L., Brakenhoff G.J. (1992) Refractive index and axial distance measurements in 3-D microscopy, *Optik* **90**, 17–19.
- Hell S., Reiner G., Cremer C., Stelzer E.H.K. (1993) Aberrations in confocal fluorescence microscopy induced by mismatches in refractive index, *J. Microsc.* **169**, 391–405.
- Wang X., Zhang C., Zhang L., Xue L., Tian J.-G. (2002) Simultaneous refractive index and thickness measurements of bio-tissue by optical coherence tomography, *J. Biomed. Opt.* **7**, 628–632.
- Saleh B.E.A., Teich M.C. (1991) *Chapter 3: Beam optics. Fundamentals of photonics*, John Wiley & Sons, New York.
- Larkin K.G. (1996) Efficient nonlinear algorithm for envelope detection in white light interferometry, *J. Opt. Soc. Am. A* **13**, 832–843.
- Schneider F., Draheim J., Kamberger R., Wallrabe U. (2009) Process and material properties of polydimethylsiloxane (PDMS) for Optical MEMS, *Sensors Actuators A: Phys.* **151**, 95–99.