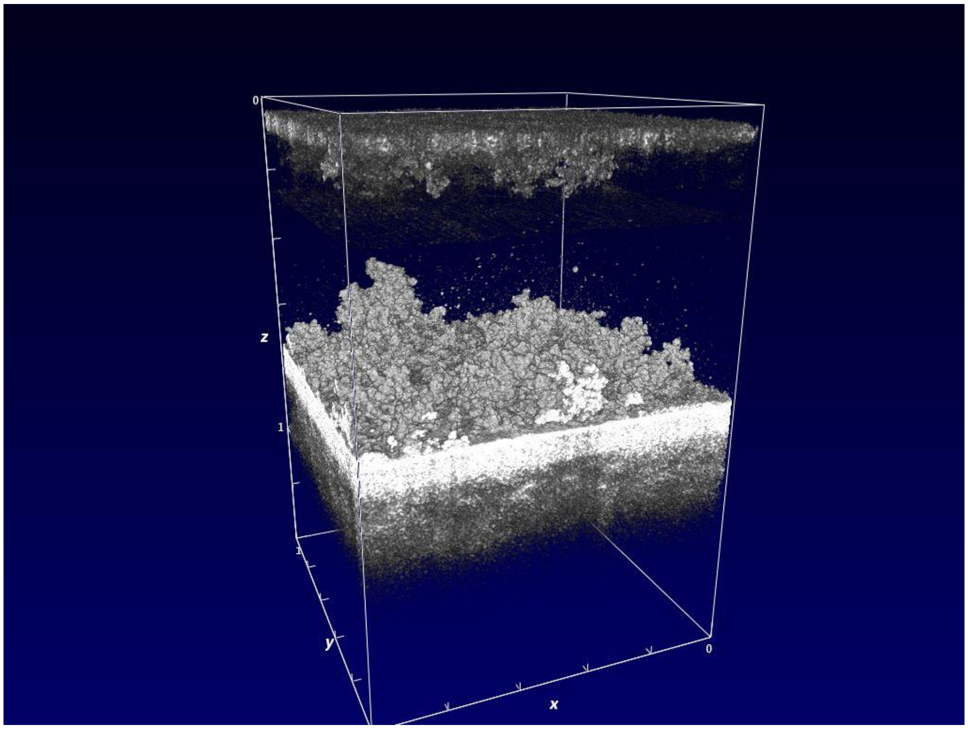
Application Note



Courtesy of Michael Wagner, Chair of Water Chemistry and Water Technology, Karlsruhe Institute of Technology

Imaging Biofilms with Optical Coherence Tomography

Optical coherence tomography (OCT) has gained much interest for imaging biofilms in recent years. Here, OCT serves as a complementary imaging method to established methods such as Confocal and Widefield Microscopy and opens up the possibility to monitor the morphology of biofilms on large scales and their dynamics in real time.

In this Application Note we discuss the restrictions that apply when imaging biofilms and the means that need to be taken to acquire biofilm images.

June 18, 2019

Part 1. Introduction

OCT is an interferometric method where the light from a broadband low-coherence light source is split into a reference arm and a sample arm. If the optical path lengths of both reference arm and sample arm are similar, light scattered back from the sample will interfere with light reflected from the reference arm mirror. This interference signal is transformed into a depth profile (A-scan) via a Fast Fourier Transformation (FFT). Subsequent A-scans can be used to generate 2D or 3D OCT images.

For biofilm imaging, samples are usually immersed in water and are often under a several mm thick layer of glass or acrylic glass. Compared to air, water and glass exhibit a larger refractive index which means that light travels slower through these media. While the geometrical path length is not affected by this, the optical path length is increased due to the increased travel time of the light. Hence, the refractive medium leads to an increased difference in path lengths between sample arm and reference arm. To obtain an OCT image this path length difference has to be compensated for. This application note will give details on the origins of the optical path length difference and how it can be compensated.

Part 2. Change in Optical Path Length

When inserting refractive media such as water, glass, or acrylic glass into the sample arm of the OCT system, the optical path length of the sample arm changes. This change in optical path length has to be compensated for in the reference arm to achieve interference between the two light beams and obtain an OCT signal. This part will detail the theoretical background of the increase in sample arm length while Part 3 will highlight the experimental realization of imaging through refractive media.

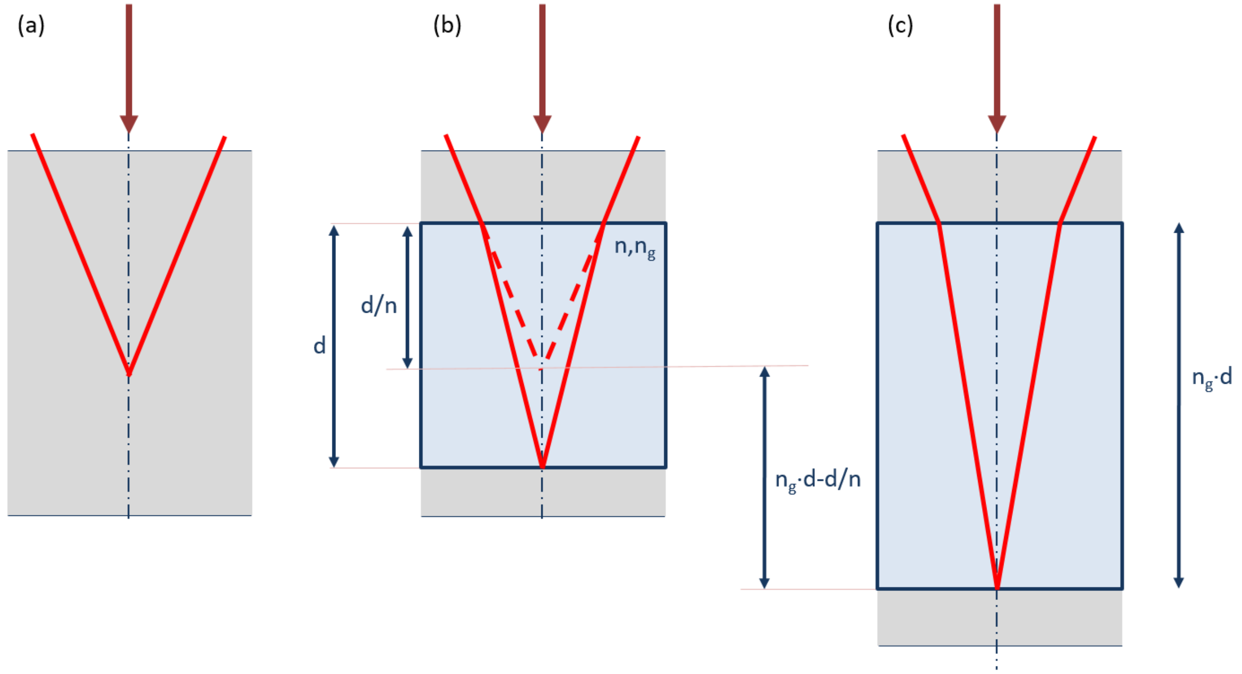


***Figure 1** (a) Shape of the focused OCT beam in air (n = 1). (b) Geometrical representation of the beam shape in a refractive medium with n > 1. The dashed line indicates the beam shape in air (cf. (a)). (c) Resulting OCT image of the geometry shown in (b). The higher refractive index increases the light travel time and hence the optical path length.*

Figure 1 shows the change of the focal position when imaging through refractive media (medium with refractive index $n > 1$). There are two changes that will lead to a change in optical path length.

Shift of the Focus

According to Snell's law light traveling from air into refractive material will be refracted towards the normal line. Hence, the interface between air and the refractive medium will bend the OCT beam so that the focus will be deeper in the sample (see Figure 1b). The additionally traveled path length is $d - d/n$, where d is the thickness of the refractive material.

Change in Optical Path Length

Due to the increased refractive index in the sample arm, the optical path length (i.e. the time it takes the OCT beam to travel to the focal position) increases. Since the medium in the reference arm is just in air its path length has to be increased to match the sample arm path length.

Overall Increase of Sample Arm Length

Taking the shift of the focus and the change in optical path length in consideration, the overall increase of the sample arm length Δl is, as illustrated in Figure 1c:

$$\Delta l = n_\mathrm{g} d - {}^{d}\!/_{n}$$

Table 1 details the refractive indices for water and common materials used for flow cells. Note that the refractive index will change with the wavelength of the used OCT system.

Table 1 Table of refractive indices for water, PMMA, and different kinds of glass.

Material	λ = 900 nm		λ = 1050 nm		λ = 1310 nm	
	n_p	n_g	n_p	n_g	n_p	n_g
Water 24°C	1.327	1.340	1.324	1.340	1.320	1.343
Water 37.6°C	1.324	1.341	1.321	1.339	1.316	1.339
PMMA	1.485	1.497	1.484	1.492	1.482	1.489
Quartz	1.452	1.465	1.450	1.463	1.447	1.462
N-BK7	1.510	1.523	1.507	1.521	1.504	1.519
N-LAK22	1.640	1.659	1.638	1.655	1.634	1.651
N-SF11	1.760	1.798	1.754	1.786	1.748	1.775
N-SF57	1.818	1.861	1.812	1.847	1.805	1.834

Increase of Sample Arm Length for Multiple Layers of Refractive Material

If often happens that the OCT beam does not only travel through air and the medium the sample is immersed in but rather through air, a protective layer, and the medium the sample is immersed in. An example for the latter case is biofilm in a flow cell where the OCT beam travels through air, a glass or PMMA window, and finally water. In these cases, the individual layers can be treated separately. Figure 2 details the case of an air/refractive medium/air system. Here, the same formula as above applies.

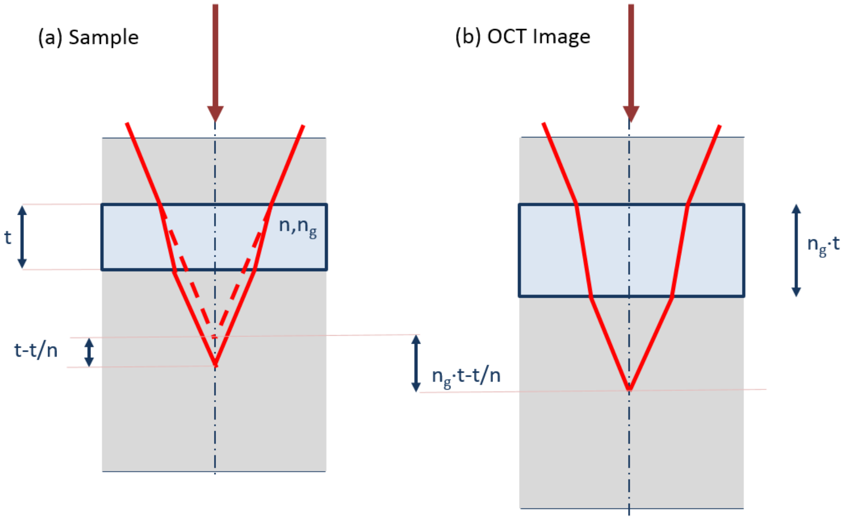


Figure 2 ***Change of sample arm length when imaging through multiple layers of refractive material. (a) Geometrical representation of the beam shape when passing through a layer of a refractive medium with n > 1. The dashed line indicates the beam shape in air. (b) Resulting OCT image of the geometry shown in (a). The higher refractive index increases the light travel time and hence the optical path length.***

Part 3. Experimental Realization

3.1. Adjusting Focus and Reference Arm Length

In Part 2 of this Application Note it was shown that the focus shift down and the reference arm length increases when imaging through refractive media. Since both the focal position and the reference arm length have to be adjusted it is helpful to follow the following routine:

1. The video monitor image can help with the coarse focus adjustment. Once this is achieved, adjust the focus until autocorrelation signals appear (Figure 3b). When the sample is in focus the objective will collect the most backscattered light from the sample. Hence, interference between light different layers of the sample will be the strongest in focus. Note that autocorrelation signals will always appear at the top of the OCT image and will not move when changing the reference arm length.
2. Adjust the reference arm length in order to move the sample image into the visible depth range of the OCT image (Figure 3c). If the travel range of the reference arm is not sufficient see chapter 3.2.
3. Move the sample image towards the top of the OCT image. This guarantees the highest sensitivity during the OCT measurement (compare the intensity levels of Figure 3c and d).

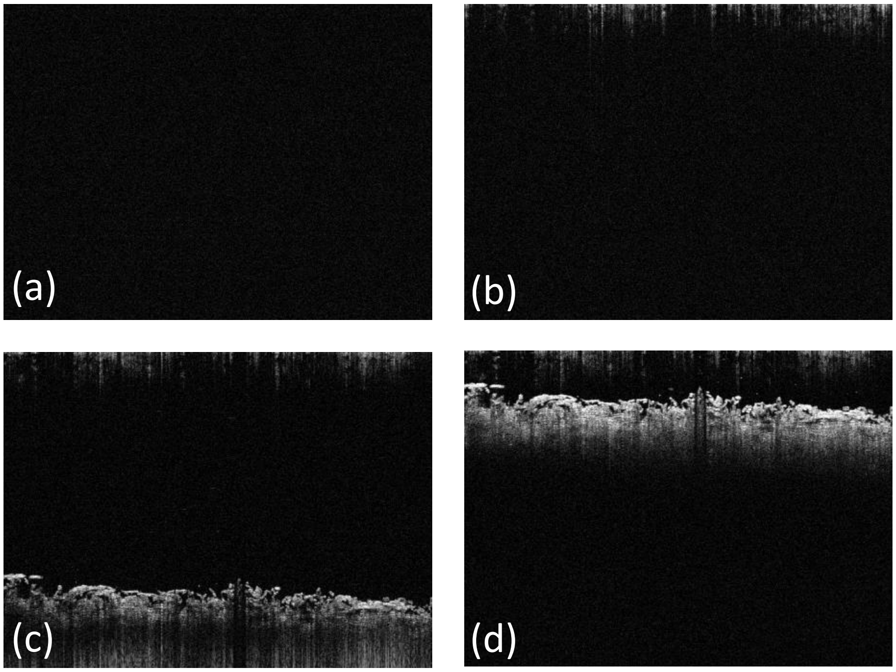


Figure 3 ***(a) Beginning of the measurement, neither the focus nor the reference arm length are adjusted. (b) Autocorrelation signals appear when the sample is in focus. (c) Adjusting the reference arm length moves the image of the sample into the visible depth range. (d) Moving the sample image to the top guarantees the highest sensitivity.***

Dispersion Compensation

Imaging through refractive media often leads to dispersion artifacts. That is, the refractive index of the medium is different for the different wavelengths of the broadband OCT light source and hence the different wavelengths travel at slightly different speeds through the refractive medium. This results in slightly different optical depth results for the reconstructed OCT image and therefore to an axially (i.e. in depth) blurred OCT image. Figure 4a shows an example of such a blurred OCT image.

There are two common ways to compensate the wavelength dependent refractive index. First, the exact same amount of refractive medium that is present in the sample arm can be inserted in the reference arm. This equalizes the travel times and hence reduces the dispersion artifacts. However, this does not account for additional dispersion caused by the sample itself. For example, in a flow reactor as described above, the biofilm is imaged through several mm of glass and water. These cause a high amount of dispersion, which deteriorates the axial resolution at the position of the biofilm. Hence, software dispersion compensation is often the preferred method to sharpen the OCT image (see Figure 4b). Please refer to chapter 4.6 of the ThorImageOCT manual for more details on the software dispersion compensation.

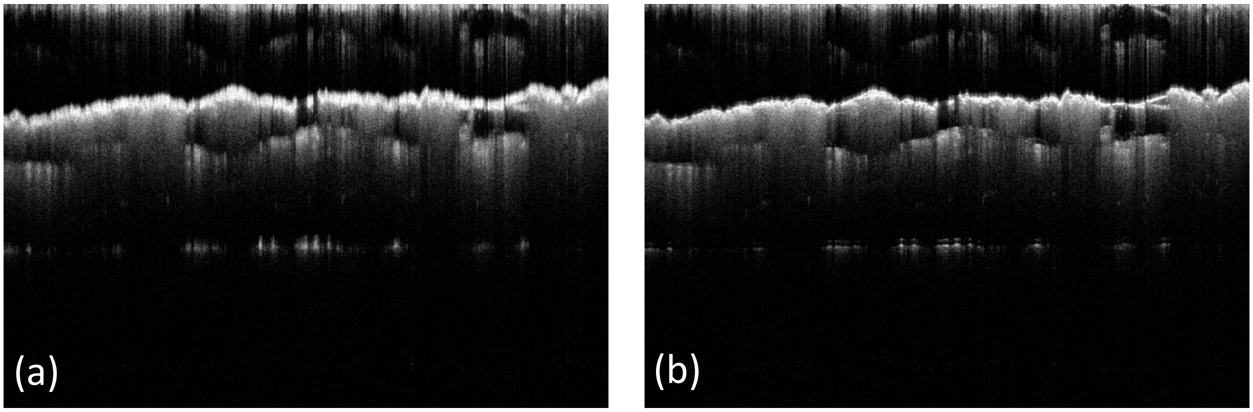


***Figure 4** (a) Axially blurred OCT image caused by dispersion. (b) Reconstructed OCT image using software dispersion compensation.*

3.2. Extension of the Reference Arm

The OCTG scanner of Thorlabs SD-OCT systems typically come with a 15 mm travel range of the reference arm. When imaging through tens of mm of refractive material this is often not enough travel range to move the sample image into the visible depth range. In these cases, it is necessary to extend the reference arm length beyond the maximum travel range. Thorlabs offers two extensions; the SRA10 and SRA30 extend the reference arm length by 10 and 30 mm, respectively. For more details on these items and quotes please get in touch with Thorlabs' OCT Support Team (oct-support@thorlabs.com).

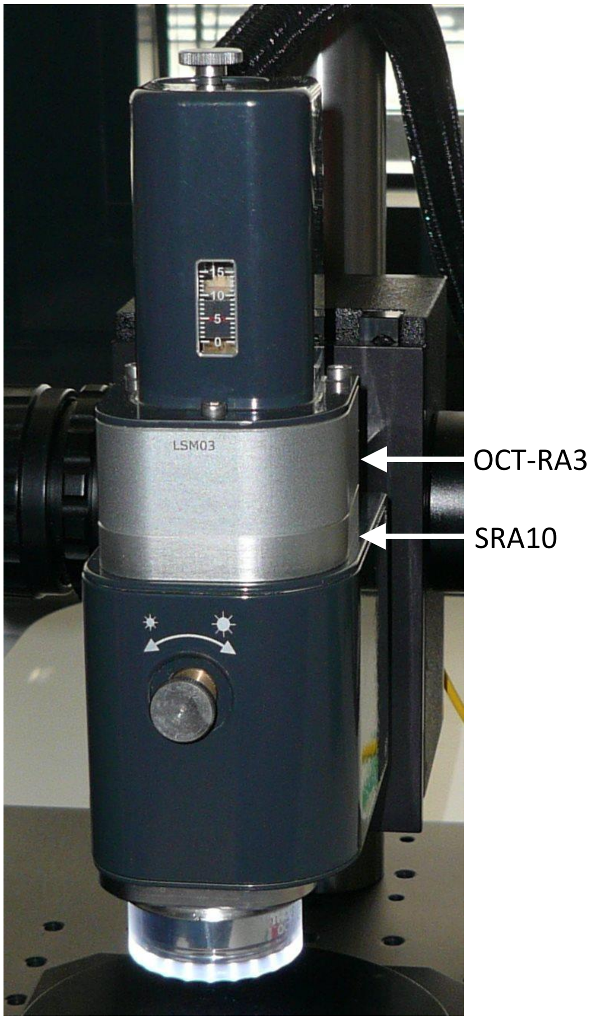


Figure 5 ***Extension of the reference arm length using an SRA10. The SRA10 is mounted between the scan head and the reference arm adapter (here OCT-RA3).***