

# Assessing molecular diffusion in tissues using optical coherence tomography

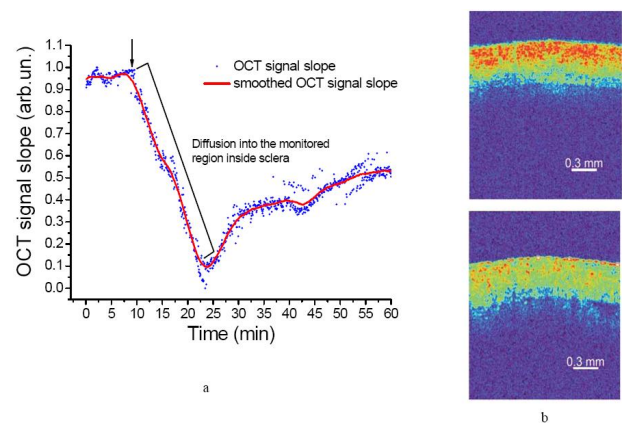
Kirill Larin, Mohamad Ghosn, and Valery Tuchin

*Advanced imaging near the surface of tissues could assist the development of novel therapeutic agents, drug delivery systems, and diagnostic tests.*

The successful management of many diseases requires long-term treatment with drugs and, as an alternative to the traditional oral route, topical drug delivery (TDD) through the skin is currently recognized as a preferred route for drug administration. However, the topical delivery of therapeutic agents to target tissues in effective concentrations remains problematic due to the low permeability of epithelial tissues and drug washout, which is when the drug is lost from the surface of the skin before it is fully absorbed.

Significant research time is currently being devoted to the development of therapeutically-effective topical formulations such as gels, creams, ointments, lotions, and the application of various permeation enhancers. The successful development of such formulations requires an understanding of the dynamics of molecular distribution in epithelial tissues. However, currently available techniques for the quantification of molecular diffusion processes in tissues are either invasive, require the isolation of tissues, or have limited resolution and sensitivity. Therefore, the development of a noninvasive biosensor capable of real-time, depth-resolved monitoring and quantification of molecular transport through epithelial tissue layers could aid the assessment of TDD systems, and lead to the development of novel therapeutic agents. Additionally, the difference in diffusion rates of macromolecules between healthy and diseased tissues could potentially be used in the development of new diagnostic methods.

We are investigating a promising molecular diffusion biosensor based on optical coherence tomography (OCT).<sup>1-5</sup> OCT has useful unique properties, such as non-destructive depth-resolved imaging at high resolution, which allow the study of



**Figure 1.** (a) Typical OCT signal slope as a function of time recorded from rabbit sclera (in whole eyeball) during a glucose diffusion experiment. The black arrow (top) indicates the time the agent was added. (b) Typical OCT images of sclera recorded before (top) topical application of glucose and after diffusion (bottom).

molecular diffusion as a function of time and depth. Diffusion of several drugs and macromolecules was monitored and quantified in the cornea and sclera of the eye, and through the skin, brain, and vascular tissues in vitro and in vivo. Molecular diffusion through tissue to the interstitial space between cells alters the tissue's morphological properties, which can then be measured optically. For example, increasing molecular concentration in tissue raises the refractive index of the interstitial fluid, which then decreases the scattering coefficient. Changes in the tissue scattering coefficient and/or refractive index are reflected in the slope of the OCT signal amplitude.

*Continued on next page*

**Table 1.** Permeability coefficients of different drugs measured in cornea and sclera of rabbit eye

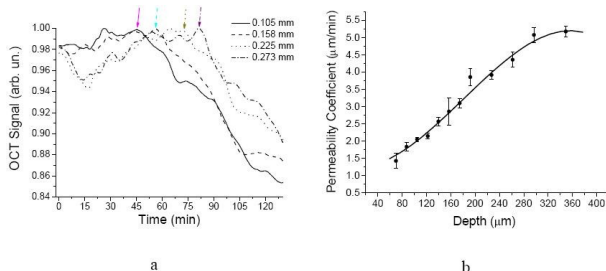
Rabbit cornea		Rabbit sclera
Agent	Permeability $\pm$ SD (cm/s)	Permeability $\pm$ SD (cm/s)
Water	$(1.68 \pm 0.54) \times 10^{-5}$ ( $n = 8$ )	$(1.33 \pm 0.28) \times 10^{-5}$ ( $n = 5$ )
Metronidazole	$(1.59 \pm 0.43) \times 10^{-5}$ ( $n = 5$ )	$(1.31 \pm 0.29) \times 10^{-5}$ ( $n = 4$ )
Ciprofloxacin	$(1.85 \pm 0.27) \times 10^{-5}$ ( $n = 7$ )	$(1.41 \pm 0.38) \times 10^{-5}$ ( $n = 3$ )
Dexamethasone	$(2.42 \pm 1.03) \times 10^{-5}$ ( $n = 7$ )	
Mannitol 20%	$(1.46 \pm 0.08) \times 10^{-5}$ ( $n = 4$ )	$(6.18 \pm 1.08) \times 10^{-6}$ ( $n = 5$ )
Glucose 20%	$(1.78 \pm 0.23) \times 10^{-5}$ ( $n = 6$ )	$(8.64 \pm 1.12) \times 10^{-6}$ ( $n = 14$ )

SD: standard deviation.  $n$ : number of samples.

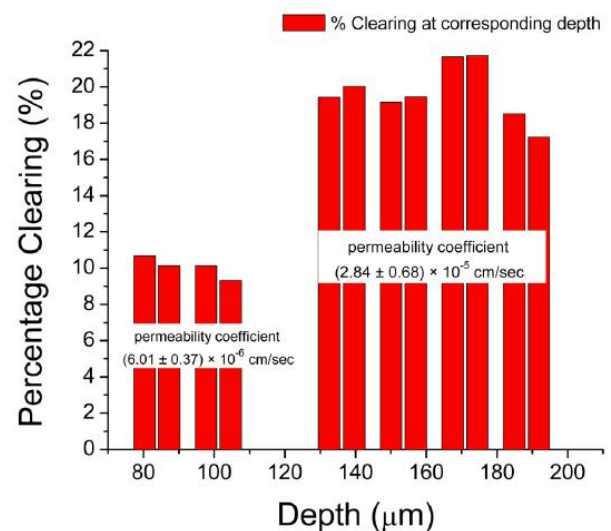
### Diffusion studies

Typical results obtained from molecular diffusion studies in epithelial tissues are shown in Figure 1. The propagation of glucose molecules (20% concentration) through rabbit sclera changed the local scattering coefficient, which was reflected in the slope of the OCT signal. Increasing local glucose concentration in deep tissues resulted in a decrease of the OCT signal slope, and vice versa during the diffusion process. The permeability coefficients for several molecules and drugs in the sclera and cornea of rabbit eyes were measured noninvasively and are summarized in Table 1.<sup>3</sup>

Figure 2(a) shows a typical OCT signal measured at depths of 105, 158, 225 and 273  $\mu\text{m}$  away from the rabbit sclera surface during a mannitol diffusion experiment. The arrows on each of the OCT signals depict the time the drug action reached that particular depth and is manifested as sharp decrease in the OCT signal. Figure 2(b) shows typical permeability coefficients of glucose measured at different depths in a sclera and demonstrates that the glucose diffusion rate inside sclera is nonlinear. This nonlinearity is due to molecular diffusion through at least two layers:



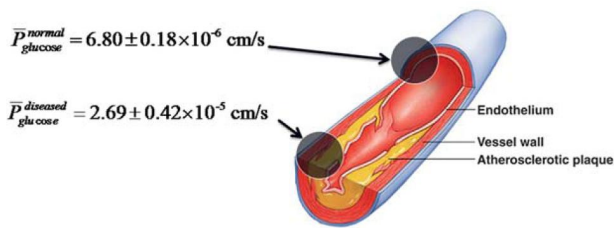
**Figure 2.** (a) OCT signal as a function of time recorded at different depths during a mannitol diffusion experiment in sclera. Arrows indicate the mannitol reaching different depths. (b) Glucose permeability coefficients measured at different depths in a sclera.



**Figure 3.** Optical clearing and permeability coefficient at different depths in a rabbit sclera after topical application of 40% glucose solution.

in the epithelium, diffusion is slower while in the stroma diffusion is faster. This is partly due to the dissimilar collagen organization in each tissue. The cloudiness of tissue fluids, or turbidity, caused by suspended particles could decrease the effectiveness of OCT in some diagnostic and therapeutic procedures. Hence, transformation of a turbid medium into a transparent one with minimal or no alteration of its composition has been the subject of number of studies.<sup>6</sup> An example of the assessment of optical clearing of 40% glucose in rabbit sclera at different tissue depths is shown in Figure 3. The permeability coefficients are higher in the deeper tissues, which also clear a higher proportion—around 20%—of glucose molecules compared to the shallower tissues.

Continued on next page



**Figure 4.** Glucose permeability coefficients measured in normal (upper right) and diseased (lower left) aorta samples.

### Diagnostic methods

Advances in clinical diagnostic methods are often governed by innovations in imaging and measurement technologies. Early diagnosis is unquestionably crucial for the successful treatment of many devastating diseases. In our pilot study, we quantified the permeability of several compounds in normal and diseased animal and human arteries.<sup>5</sup> The results show a dramatic difference in the rate of molecular permeability between healthy and diseased samples (see Figure 4), information that could be used in a diagnostic context.

Moreover, purely structural OCT imaging of arteriosclerotic lesions at early stages was not able to effectively differentiate between normal and diseased areas, whereas functional OCT methods demonstrated superior contrast and sensitivity. The application of this novel technique could make the early diagnosis of vascular abnormalities possible and bring us closer to fully understanding the pathology of major cardiovascular diseases.

*The studies were supported in part by grants from W. Coulter Foundation and Office of Naval Research (KVL) and Federal Agency of Education of RF No 1.4.06, RNP.2.1.1.4473 and by CRDF BRHE grant RUXO-006-SR-06 (VVT).*

### Author Information

**Kirill Larin and Mohamad Ghosn**  
 Biomedical Optics Laboratory  
 University of Houston  
 Houston, TX

**Valery Tuchin**  
 Optics and Biomedical Physics  
 Saratov State University  
 Saratov, Russia

### References

1. M. Ghosn, V. V. Tuchin, and K. V. Larin, *Depth-Resolved Monitoring of Glucose Diffusion in Tissues by Using Optical Coherence Tomography*, **Optics Lett.** **31** (15), 2006.
2. K. V. Larin and M. Ghosn, *Influence of experimental conditions on drug diffusion in cornea*, **Quantum Electron.** **36** (12), pp. 1083–1088, 2006.
3. M. Ghosn, V. V. Tuchin, and K. V. Larin, *Non-Destructive Quantification of Analytes Diffusion in Cornea and Sclera by Using Optical Coherence Tomography*, **Invest Ophthalmol Vis Sci** **48** (6), pp. 2726–2733, 2007.
4. K. V. Larin, M. G. Ghosn, S. N. Ivers, A. Tellez, and J. F. Granada, *Quantification of glucose diffusion in arterial tissues by using optical coherence tomography*, **Laser Phys. Lett.** **4**, pp. 312–317, 2007.
5. M. G. Ghosn, E. F. Carbajal, N. Befrui, A. Tellez, J. F. Granada, and K. V. Larin, *Permeability of Hyperosmotic Agent in Normal and Atherosclerotic Vascular Tissues*, **J. Biomedical Optics** **13** (1), p. 010505(3), 2008.
6. V. V. Tuchin, **Optical Clearing of Tissues and Blood**, SPIE Press, 2005. PM 154