

Using sandpaper for noninvasive transepidermal optical skin clearing agent delivery

O. Stumpp

B. Chen

A. J. Welch

University of Texas at Austin
1 University Station Stop C0800
ENS 639
Austin, Texas 78712

Abstract. We present a gentle mechanical method for the noninvasive transepidermal delivery of topically applied optical skin clearing agents. Optical skin clearing reduces light scattering in highly turbid skin with the aid of hyperosmotic chemicals such as glycerol, polyethylene glycol, and solutions of dextrose. Transepidermal delivery of such agents is believed to be most patient compliant and most likely to be used in a clinical environment. Optical skin clearing has the potential to expand the current limited use of laser light in medicine for diagnostic and therapeutic applications. Light scattering limits the penetration depth of collimated light into skin. In order to increase the diffusion of topically applied optical skin clearing agents into skin, we present a gentle mechanical delivery method involving glycerol and dextrose as optical skin clearing agents and fine 220-grit sandpaper to rub the clearing agent into the tissue. Gentle rubbing causes abrasion of the superficial skin layer including the *stratum corneum*, which otherwise prevents these optical skin clearing agents from freely diffusing into skin. Results indicate very fast optical skin clearing rates. *In vivo* hamster skin turned transparent within 2 min. The $1/e$ light penetration depth increased by $36 \pm 3.75\%$ for dextrose and $43 \pm 8.24\%$ for glycerol. Optical skin clearing was reversed using phosphate buffered saline solution. Skin viability was observed 70 h post-treatment and showed scabbing and erythema on a few percent of the total optically cleared skin surface. © 2006 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2340658]

Keywords: optical skin clearing; sandpaper; transepidermal; glycerol; dextrose; optical coherence tomography.

Paper 05189SSRRR received Jul. 13, 2005; revised manuscript received May 15, 2005; accepted for publication May 17, 2005; published online Sep. 5, 2006.

1 Introduction

The feasibility of optical skin clearing has been known in the scientific community for quite a while now.¹⁻⁶ There is no doubt among scientists that the ability to reduce light scattering in highly turbid biological tissue such as skin will have a significant impact in the medical and clinical communities. Reduced light scattering permits delivery of collimated light deeper into tissue, thus allowing us to aim at deep subsurface targets.^{7,8} This in turn will open the window for many currently limited applications of light in medicine for diagnostic and therapeutic applications such as noninvasive imaging using confocal microscopy⁹⁻¹¹ and optical coherence tomography (OCT). Therapeutic applications include photodynamic therapy, photothermal coagulation of blood vessels, laser hair removal, and tattoo removal to name a few.

Optical imaging is noninvasive and has several advantages that include micron and submicron resolution as well as real-time data acquisition. Conventional optical imaging modalities such as confocal microscopy have been applied to *in vivo*

imaging. Although confocal microscopy has good spatial resolution and good depth of field, its working depth is still rather limited due to multiple light scattering in skin and cannot exceed 200 to 300 μm in general.

Interferometric imaging modalities such as OCT use near-infrared light to increase the penetration depth. OCT imaging allows cross-sectional images of *in vivo* or *in vitro* tissue in depth but this technique equally suffers from the limited penetration depth of the imaging light beam due to light scattering.

Optical skin clearing has so far been successfully applied in the laboratory environment either on *in vitro* tissues or on animals such as hamsters with a surgically implanted window model, which permits simultaneous access to epidermal and dermal sides of living skin.⁷ However, in order to bring the proven benefits of optical skin clearing from the research into the clinical environment, a successful noninvasive method has to be found to transepidermally deliver topically applied skin clearing agents under *in vivo* conditions.

Transepidermal optical skin clearing agent delivery is complicated due to the natural skin barrier formed by the *stratum corneum*.^{12,13} This barrier prevents most optical skin

Address all correspondence to Oliver Stumpp, Biomedical Engineering, The University of Texas at Austin, 1 University Station Stop C0800 Austin, Texas 78712 United States of America; Tel: 512 471 4703; Fax: 512 471 0616; E-mail: o.stumpp@mail.utexas.edu

clearing agents from freely diffusing into the tissue. The skin barrier can be breached using a variety of different mechanisms. Our laboratory as well as other groups have investigated transepidermal clearing agent delivery methods, which included penetration enhancers,^{5,14} needle-free injection gun, microneedles,¹⁵ as well as erbium:yttrium aluminum garnet (Er:YAG) laser ablation¹⁶⁻¹⁸ and using a 980-nm diode laser to irradiate the skin surface.¹⁹ All of these methods were successful to some extent. However, not all of these methods are suitable for the treatment of larger skin areas.

Most lasers require some kind of scanning mechanism or translation to treat an area larger than the laser spot size. Microneedles or a microneedle array can only treat an area as large as the array and using a needle-free injection gun is tedious and time-consuming. These are important considerations with regards to success of optical skin clearing in a clinical environment. Additionally, the incurred cost associated with the optical clearing agent delivery must not be neglected either.

Thus we present a highly effective, yet inexpensive mechanically abrasive method of transepidermal optical skin clearing agent delivery that allows free scaling of the treatment area without restrictions such as patch or array size.

2 Materials and Methods

The aim of this study was to optically clear *in vivo* hamster skin with glycerol and dextrose solution. These agents were previously identified as some of the most successful skin clearing agents.²⁰ We used nine adult Golden Syrian hamsters in the experiments. Two animals were used to refine the clearing agent delivery method using sandpaper to gauge how the rubbing force and number of rubbing passes across hamster skin affects mechanical tissue damage. The quantitative data presented in this paper is based on the remaining seven animals, of which three were used for each of the two optical clearing agents and one animal was used as a control without an optical clearing agent.

Due to the optimized rubbing protocol neither animal suffered immediate mechanical skin damage. Animals were treated and cared for according to the Institutional Animal Care and Use Committee guidelines. Animals were anesthetized using an equivalent mixture of 80 to 100 mg/kg Ketamine mixed with 7 to 10 mg/kg Xylazine, which was given as an interperitoneal injection. Animals were then shaved and residue hair removed using a Nair hair removing lotion, which was applied for 3 to 7 min until hair could be gently wiped off to expose the dorsal skin. Within minutes of cleaning the dorsal skin and removing excess Nair lotion, one or two drops of optical skin clearing agent were applied to the skin surface.

Dextrose solution was obtained by dissolving 3 g anhydrous dextrose (EM Science, Gibbstown, New Jersey) per 1 ml of distilled, ultrafiltrated water. This solution had an optical index of refraction of 1.4750. The other optical skin clearing agent was anhydrous glycerol with a similar optical index of refraction of 1.4720.

One animal served as a control. Instead of rubbing an optical clearing agent into the skin, phosphate buffered saline (PBS) solution was rubbed into the skin using sandpaper.

Upon application of either clearing agent onto the skin surface of the other remaining animals, the clearing agent was

gently rubbed into the skin using a fine 220-grit sandpaper by 3M. Skin was manually rubbed for no more than 2 to 4 min in different directions over the treatment area, which led to even distribution of the clearing agent over the treatment area. Rubbing was performed hardly without any force (approximately 15 to 30 N measured separately using a scale) in order to avoid immediate injury to the skin. Both optical skin clearing agents were highly viscous, which helped to lubricate the treated area. Lubrication also prevented tearing or other mechanical injury of the skin surface.

OCT images were taken of native, optically cleared skin as well as following rehydration with PBS to reverse the optical skin clearing procedure. Three different OCT scans were taken at each imaging site per animal for a total of nine slightly offset OCT scans for each skin condition. Care was taken to image identical tissue sites before during and after the optical skin clearing procedure. However, due to sandpaper rubbing, it was not possible to mark the skin surface for a single OCT scan but with the help of a coaxial visible aiming beam, three OCT scans were acquired within a roughly 4×3 -mm area of skin and averaged for a representative measure of the tissue optical properties. The same 4×3 -mm area of skin was then imaged throughout an experiment. The lateral and depth scan ranges were 3 mm. The system used a 1290-nm light source and had a $20\text{-}\mu\text{m}$ axial resolution. Here native skin refers to shaved and freshly depilated skin. Based on our observations, the use of a Nair depilatory does not alter the skin appearance in OCT images nor influence transepidermal diffusion of optical skin clearing agents such as those used in this study.

Quantitative data analysis involved digital reregistration of the uneven skin surface in the OCT data based on an edge-detection algorithm to obtain a flat surface, followed by averaging the linearized signal intensity across the lateral imaging range as a function of depth. A best fit exponential curve covering epidermis and dermis in depth was applied to the averaged and normalized signal intensity data from which the corresponding $1/e$ light penetration depth was derived.

Following optical skin clearing with either dextrose or glycerol, in one experiment skin was allowed to rehydrate by itself which required about 2 h. In order to reduce this time in the remaining experiments, optically cleared skin was rehydrated with PBS solution. Tissue returned to its native state within 10 to 30 min. Skin viability was determined from 70-h survival studies and judged by visual skin appearance.

3 Results

Optical skin clearing experiments were conducted with two different optical skin clearing agents. Both, dextrose solution as well as anhydrous glycerol were transepidermally delivered using gentle rubbing with sandpaper for up to 4 min. Earlier control experiments without sandpaper have shown that neither glycerol nor dextrose solutions were able to significantly penetrate into native *in vivo* skin to cause noticeable optical skin clearing. The use of a depilatory cream for 3 to 10 min did not cause immediate or subsequent skin irritation and did not enhance drug transport. Another control experiment was conducted in which saline solution was rubbed into the skin with sandpaper in the same manner as the optical skin clearing agents. Compared to the normal baseline, the $1/e$ light

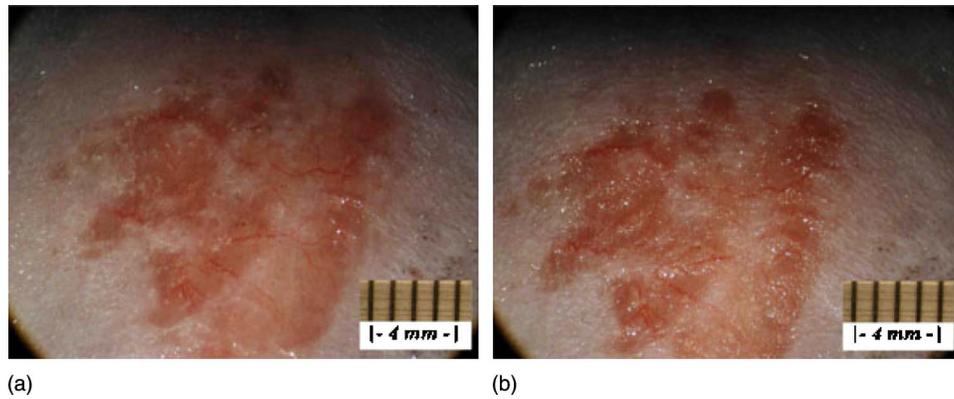


Fig. 1 Optical skin clearing of *in vivo* hamster skin with sandpaper assisted transepidermally delivered dextrose solution: (a) *in vivo* hamster skin 4 min after transepidermal delivery of optical skin clearing agent dextrose; (b) *in vivo* hamster skin 30 min after dextrose solution was removed from skin surface.

penetration depth decreased by 2.5% due to an increase in light scattering as a result of increased skin hydration.

It was found that in experiments involving the optical skin clearing agents, rubbing with sandpaper enhanced the delivery of these agents. In previous experiments where the skin barrier was altered by different methods,^{19,20} the formation of a boundary layer between skin surface and optical clearing agent was observed. This boundary layer was formed as the topically applied skin clearing agent drew tissue water to the skin surface.^{1,20} Due to the high optical skin clearing agent viscosity and the lack of mechanical stirring, the boundary layer consisting of diluted optical skin clearing agent formed near the skin surface, which subsequently lead to reduced optical skin clearing transport rates as the boundary layer added resistance due to reduced osmotic pressure. Mechanical rubbing using sandpaper prevented formation of such a boundary layer in addition to reducing the stratum corneum epidermal barrier at the same time.

3.1 Dextrose

Rubbing aided the transepidermal delivery of the clearing agent. Within 4 min, skin became highly transparent. Figure 1(a) demonstrates the significant changes of tissue op-

tical properties, which permit direct visualization of the skin vasculature within as little as 4 min after the initial application of the dextrose clearing agent. Surrounding tissue served as a control where the optical skin clearing agent wetted the skin surface. However, lacking the mechanical rubbing, the surrounding skin remained opaque.

Skin remained in the optically cleared and transparent state as long as the treatment area was kept under optical skin clearing agent occlusion. The high viscosity of dextrose solution and glycerol aided in maintaining a thin layer of the agent on the skin surface at all times.

Removal of the occluding optical skin clearing agent from the skin surface of the treatment site marked the starting point for reversal of the optical skin clearing effect. Figure 1(b) shows the same skin sample as shown in Fig. 1(a) 30 min after the topically applied skin clearing agent had been gently removed by blotting with dry paper towels. The skin is slightly less transparent. *In vivo* skin naturally rehydrates, causing a slow increase in tissue opaqueness.

Figure 2(a) shows the increased state of light scattering in the tissue and some residual areas that appear more red due to reduced light scattering. However, tissue rehydration can be achieved easily with PBS solution. Skin rehydration for

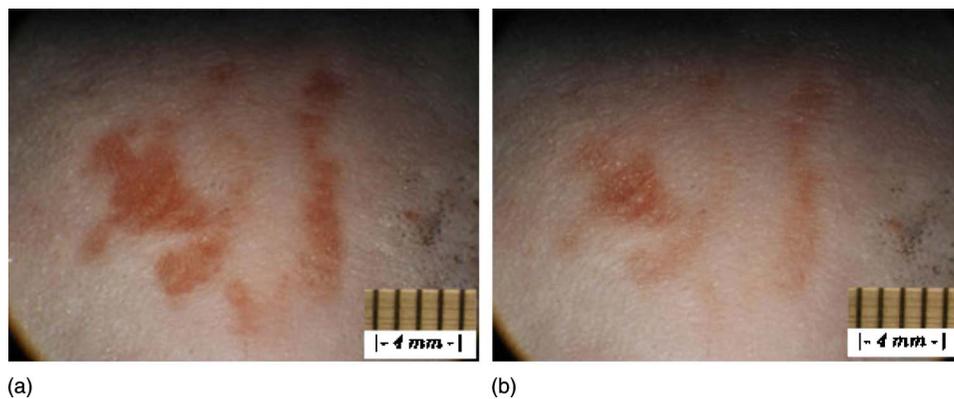


Fig. 2 Upon removal of the topically applied optical skin clearing agent, *in vivo* hamster skin returns to an opaque state: (a) *in vivo* hamster skin 100 min after removal of occluding dextrose solution; (b) *in vivo* hamster skin 2 h after removal of occluding dextrose solution, including 10 min rehydration with PBS solution.



Fig. 3 *In vivo* hamster skin 70 h after optical skin clearing experiment with dextrose shows some scabbing as a result of too much rubbing with abrasive sandpaper.

10 min followed by another 10 min skin recovery reveals an additional significant increase in tissue opaqueness. However, some red spots remain on the tissue, which eventually were covered by scabs and remained visible 70 h after the optical skin clearing experiment, see Fig. 3.

In these experiments, the state of reduced light scattering was maintained over 20 to 30 min while keeping the treatment area under clearing agent occlusion. As the optical skin clearing agent was removed from the skin surface using a dry paper towel, natural rehydration of the *in vivo* skin led to a slow increase of tissue opaqueness. Skin returned to its native state after approximately 2 h unless the rehydration process was sped up by applying PBS solution to the skin surface.

3.2 Glycerol

Anhydrous glycerol was transepidermally delivered in the same manner as discussed previously for the optical skin clearing agent dextrose. The clearing agent was also rubbed into the skin using sandpaper for no more than 4 min in order to prevent mechanical tissue damage. Then the treatment site was kept under glycerol occlusion and optical skin clearing caused a dramatic reduction of light scattering within an additional 4 min. Figure 4(a) shows the treated area and reveals

direct visualization of blood vessels through the optically cleared *in vivo* hamster skin. Here, the surrounding tissue remained opaque in spite of being exposed to the optical clearing agent. Once the occluding glycerol was removed from the skin surface, natural skin rehydration set in causing a slow increase in tissue opacity as shown in Fig. 4(b). Skin still appears red but individual blood vessels are no longer directly visible.

Recovery of natural skin optical properties is shown in Fig. 5. Only 60 min after the removal of the optical skin clearing agent and 10 min rehydration with PBS solution, treated skin returned back to almost native opaque conditions with some residual skin irritation, see Fig. 5(a). Skin scabbing in a small part of the treated skin area was visible 69 h after the optical skin clearing procedure, which is shown in Fig. 5(b).

Additional experiments, which are not presented in this paper, with the optical skin clearing agent polyethylene glycol (PEG) indicated comparable results of making hamster skin highly transparent within minutes. Natural skin rehydration after topical PEG removal was quick, causing optically cleared skin to turn turbid again faster than with glycerol. It generally took between 20 to 30 min without saline solution until skin appeared again in a native state.

3.3 Optical Coherence Tomography Imaging

Apart from the therapeutic benefits that reduction of light scattering permits, it also can significantly aid noninvasive imaging for diagnostic purposes. OCT was used as a noninvasive imaging modality based on the Michelson interferometer. This imaging technique allows obtaining cross-sectional images of tissue.

Imaging native *in vivo* hamster skin reveals the layered structure of hamster skin, composed of epidermis, dermis, and subdermal muscle fascia. Light penetration is limited in untreated skin, that is, following the topical application of an optical skin clearing agent onto shaved and depilated skin without sandpaper delivery, see Fig. 6(a), corresponding to a low imaging depth due to a shallow $1/e$ light penetration depth of approximately $350\ \mu\text{m}$. Almost identical results were obtained on skin even prior to application of either glyce-

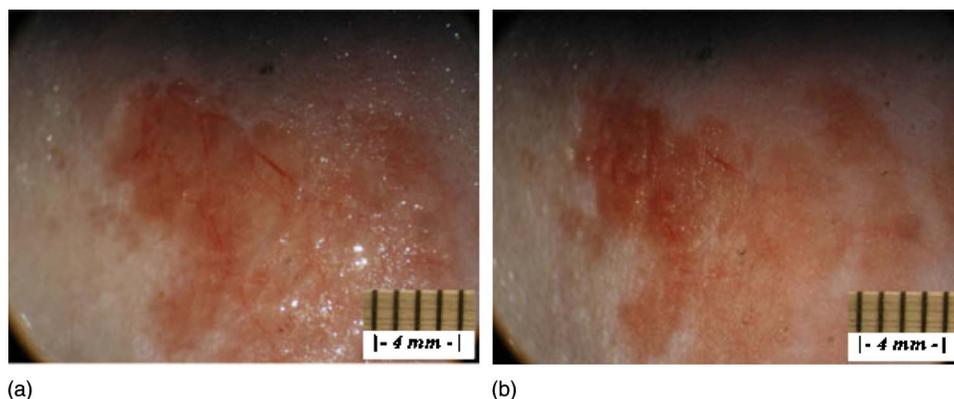


Fig. 4 Optical skin clearing of *in vivo* hamster skin with anhydrous glycerol rubbed into skin using fine grit sandpaper: (a) optically cleared *in vivo* hamster skin 8 min after initial topical application of glycerol; (b) natural skin rehydration 50 min after removal of occluding glycerol from skin surface.

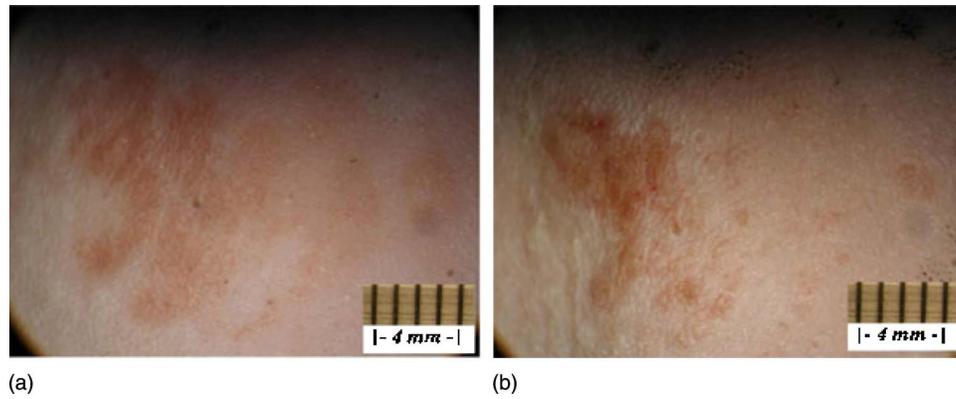


Fig. 5 Skin recovery after optical skin clearing of *in vivo* hamster skin with transepidermally delivered anhydrous glycerol: (a) *in vivo* hamster skin 70 min after removal of topically applied glycerol, including 10 min rehydration with PBS solution; (b) *in vivo* hamster skin 69 h after optical skin clearing experiment with transepidermally delivered anhydrous glycerol.

erol or dextrose solution. The reduction of light scattering allows deeper light penetration and improves OCT imaging also due to a smaller point-spread function. An example of an OCT image acquired 20 min after the application and transepidermal delivery of dextrose using the sandpaper technique is shown in Fig. 6(b). Using a false color lookup table permits better comparison between both images. Not only is the light depth penetration enhanced in Fig. 6(b), but also surface light reflection intensity is reduced due to better index matching near the surface. This is evident by less yellow image pixels in the latter image. The $1/e$ light penetration depth increased by 21%.

Once the treated skin is no longer kept under optical skin clearing agent occlusion, natural skin rehydration causes slow increase of light scattering. This led to a transient reduction of light penetration into the optically cleared *in vivo* tissue. Figure 7(a) shows previously optically cleared skin 95 min after the optical skin clearing agent dextrose was removed from the skin surface. There is less structural detail visible and the light

penetration depth is reduced to approximately 0.7 mm. Additional rehydration of optically cleared tissue using PBS solution allowed establishing normal tissue optical properties at an accelerated pace. Figure 7(b) shows two OCT depth intensity profiles with corresponding exponential best-fit curves of untreated and optically cleared skin. The $1/e$ light penetration depth of epidermis and dermis increased by roughly 44% from the native to the optically cleared state.

Other OCT images were acquired following sandpaper assisted optical skin clearing of *in vivo* hamster skin with anhydrous glycerol. Compared to OCT images with optical skin clearing agent alone but without sandpaper use, here optical skin clearing resulted in a significant penetration depth increase of the OCT signal as shown in Fig. 8(a). Blood vessels could be identified in OCT images based on the shadow they cast over tissue layers underneath. An example of imaged blood vessels is shown in Fig. 8(b). During the optical skin clearing procedure, the $1/e$ optical penetration depth increased significantly. Following the removal of glycerol after

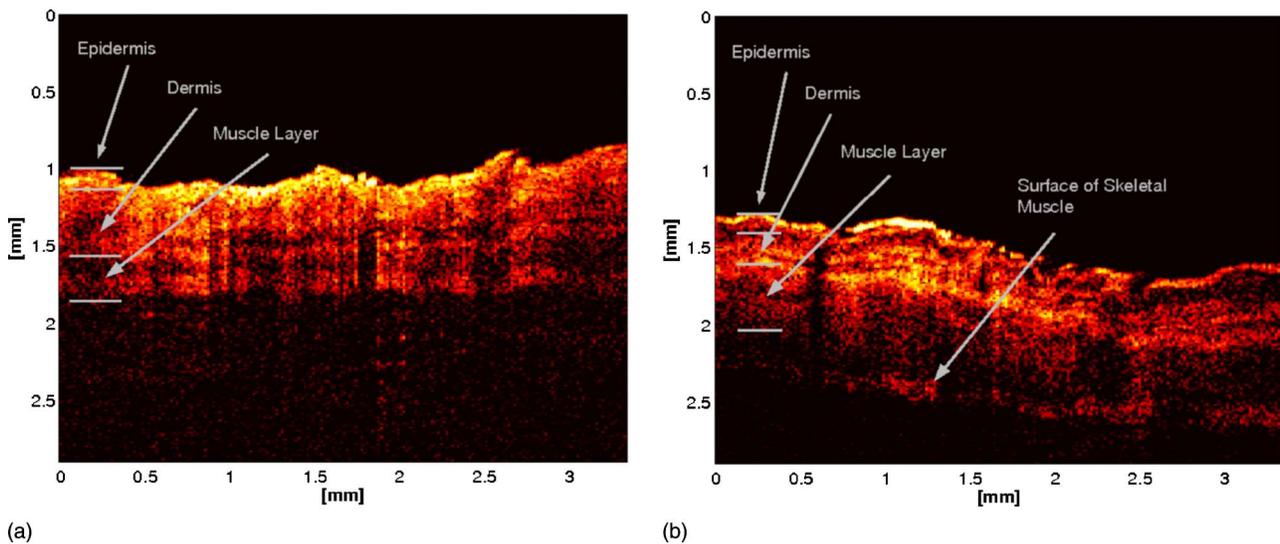


Fig. 6 OCT images of native and optically cleared *in vivo* hamster skin: (a) OCT image of *in vivo* hamster skin (control) following topical application of anhydrous glycerol alone; (b) OCT image of optically cleared *in vivo* hamster skin 20 min after sandpaper-aided dextrose application.

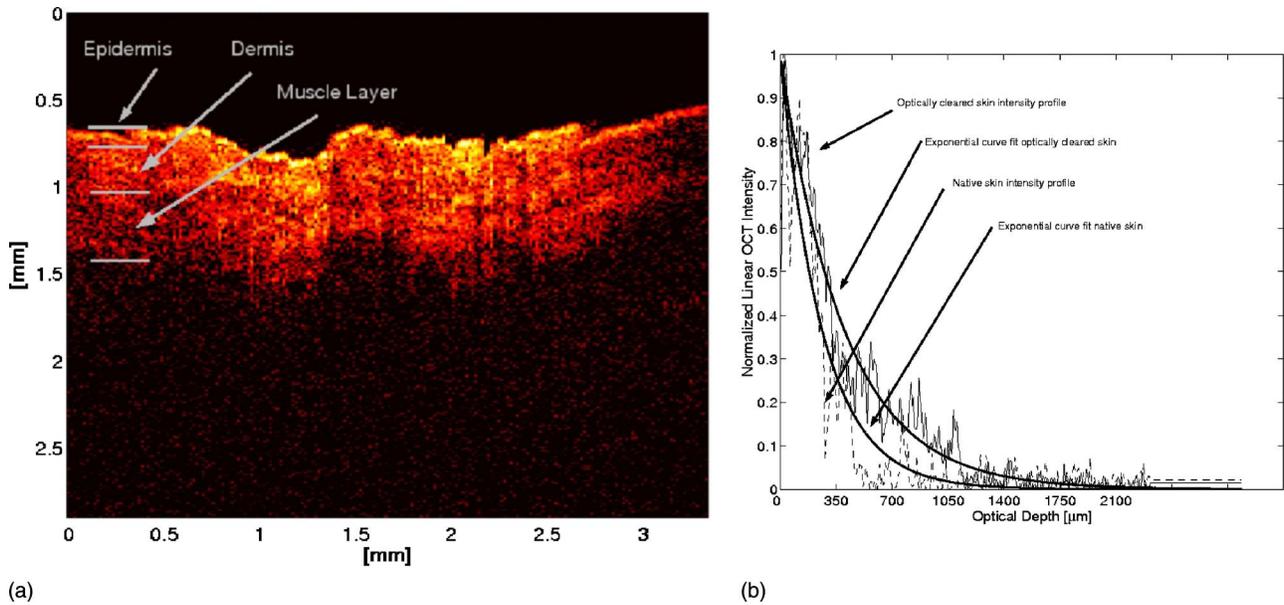


Fig. 7 Effect of natural and topical skin rehydration on OCT imaging of *in vivo* hamster skin: (a) effect of natural rehydration is limiting OCT penetration depth 95 min after optical skin clearing agent removal; (b) OCT intensity profiles of native and optically cleared hamster skin.

20 min caused slow natural rehydration of optically cleared skin leading to a reduction of the light penetration depth.

Following rehydration, OCT imaging revealed similar penetration depths as was previously shown for the native tissue state, cf. Fig. 6(a) and again, strong scattering gives rise to high OCT signal strength near the skin surface. This effect is also reflected in the dynamic change of the optical $1/e$ penetration depth shown in Fig. 9(a) based on averaged data from three different animals. Based on the OCT images of one animal shown here the optical penetration depth varied roughly between 0.5 mm to 0.8 mm, respectively, which depended partially on the tissue site and state of opacity but

differed greatly from the optical penetration depths ranging from 1 to 1.5 mm in the optically cleared skin samples. Figure 9(a) summarizes the optical skin clearing results with glycerol based on a total of nine different OCT scans from three animals, while Fig. 9(b) shows similar statistical data obtained with dextrose as the optical skin clearing agent.

Based on digital photographs such as shown in Figs. 3 and 5(b), the visible skin conditions, scabbing and slight erythema, were measured by their area with respect to the total skin area previously optically cleared with the optical clearing agents dextrose and glycerol. Results are shown in Table 1.

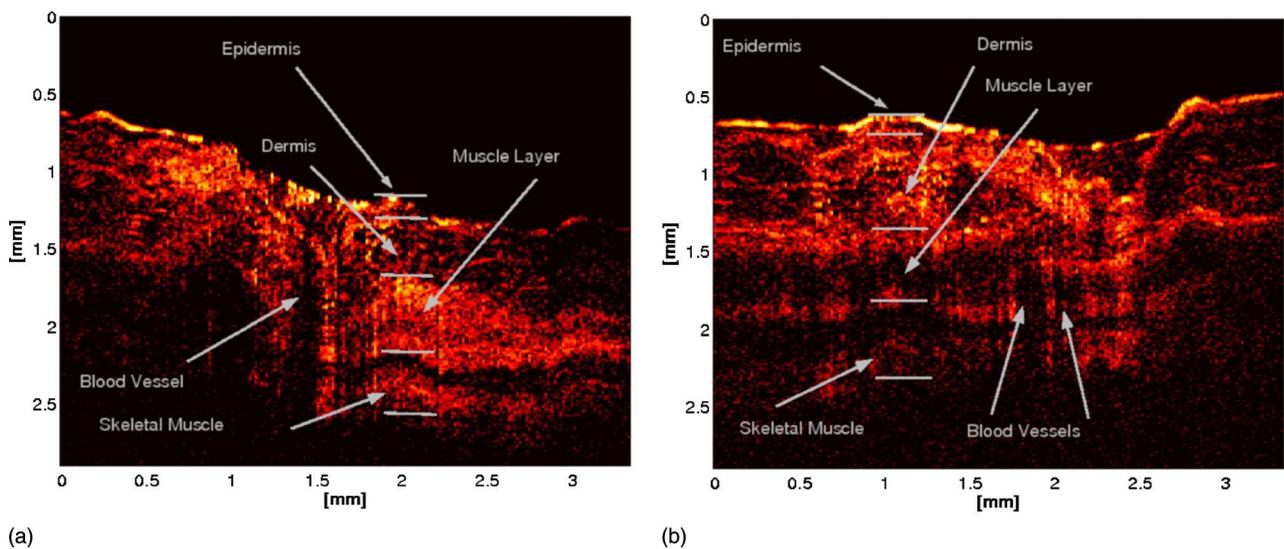


Fig. 8 OCT images of *in vivo* hamster skin optically cleared with anhydrous glycerol: (a) OCT image of *in vivo* hamster skin 16 min after topical application of anhydrous glycerol with sandpaper; (b) OCT image of *in vivo* hamster skin showing blood vessels following optical skin clearing with anhydrous glycerol and sandpaper.

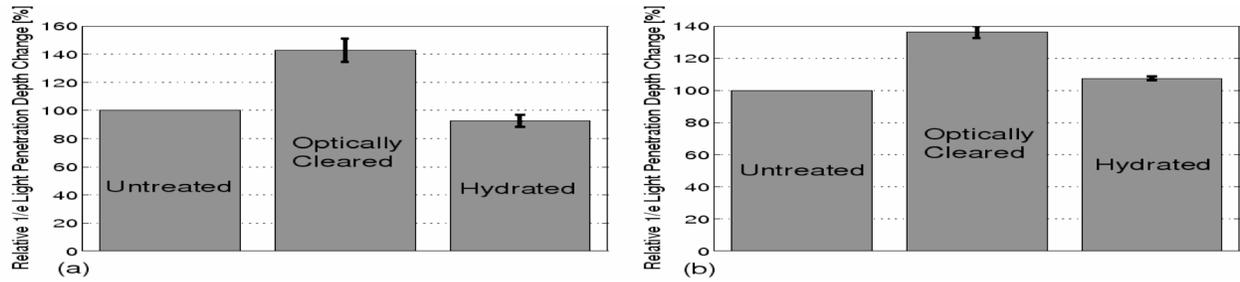


Fig. 9 Dynamic changes of light penetration depth into *in vivo* optically cleared hamster skin with glycerol and dextrose: (a) $1/e$ light penetration depth at 1290 nm as a function of time for hamster skin optically cleared with glycerol and sandpaper; (b) $1/e$ light penetration depth at 1290 nm as a function of time for hamster skin optically cleared with dextrose and sandpaper.

4 Discussion

Using a topically applied highly viscous optical skin clearing agent such as dextrose solution or anhydrous glycerol in conjunction with gentle sandpaper rubbing allows noninvasive, transepidermal delivery of these agents, which results in drastically reduced light scattering in *in vivo* hamster skin. In order to avoid mechanical damage to skin, rubbing was time limited not to exceed 4 min. During this time, the topically applied skin clearing agent was evenly distributed over the treatment site by gently rubbing in different direction and orientation across the skin. Optical skin clearing could generally be observed within as little as 2 min, but continued rubbing allowed us to obtain better results.

Transepidermal optical clearing agent delivery is believed to be enhanced by the use of gentle sandpaper rubbing due to the microscopic damage to the stratum corneum. Individual abrasive particles dislodge superficial corneocytes and make small grooves in the stratum corneum, which subsequently fill with optical skin clearing agent. Due to such microscopic grooves in the stratum corneum the effective thickness of this diffusion barrier is reduced and the available surface area for diffusion artificially increased. Additionally, as mentioned in Sec. 1, prolonged rubbing disturbs the otherwise naturally forming boundary layer near the skin surface consisting of diluted optical skin clearing agent, which helps to reduce the resistance to diffusional agent transport.

Control experiments in which either optical skin clearing agent was topically applied following skin shaving and depilation did not result in optical skin clearing. Additionally, the use of a depilatory cream was not found to have any influence on skin irritation or optical skin clearing. As anticipated, skin can become superhydrated when PBS solution is rubbed into the skin with sandpaper. As a result, light scattering is increased and the optical $1/e$ penetration depth is slightly decreased. Compared to other transepidermal optical

skin clearing agent delivery modalities, such as localized Er:YAG laser ablation,²¹ the rate and extent of optical skin clearing was much more evident using the sandpaper delivery method. Removal of the stratum corneum was easily achieved using an Er:YAG laser. However, topical application of an optical skin clearing agent alone did not result in the substantial visible reduction of light scattering that was observed following the use of sandpaper. Similar to Er:YAG laser ablation or the use of a 980-nm diode laser,¹⁹ tape stripping is a well-known method to remove the stratum corneum. However, optical skin clearing results are expected to be similar to those following Er:YAG laser ablation, while the experiments presented here do show improved clearing results superior to the removal of stratum corneum alone.

In comparison to tape stripping,²² using sandpaper might be a faster way of sufficiently breaching the stratum corneum natural skin barrier. A direct comparison of tape stripping performed on a hairless guinea pig with the experiments outlined in this study on hamster skin is hardly adequate due to the significant differences in skin thickness of the two animal models. However, tape stripping is done on reasonably dry skin and for the duration of tape stripping no topical optical skin clearing agent can be applied. On the other hand, using sandpaper to deliver topically applied optical skin clearing agents is thought to be a faster and more effective method because the hyperosmotic agent is in contact with the skin from the very beginning. We also believe that gentle rubbing enhances diffusional optical skin clearing agent transport across the breached natural skin barrier based on comparison of optical skin clearing results with Er:YAG or 980-nm diode laser with the sandpaper results.

Using sandpaper rubbing allowed free scaling of the treatment site and it was easy to optically clear an area as large as approximately 18×6 mm (glycerol) or 11×12 mm (dextrose). The images shown in Figs. 1–5 indicate both optical skin clearing agents have the ability to make *in vivo* hamster skin entirely transparent. This allowed direct visualization of the skin vasculature.

Skin remained in the optically cleared state as long as there was a layer of optical clearing agent covering the treatment site. Removal of such layer initiated a slow, natural rehydration process during which skin returned to its natural, opaque state. Without external influence this process took more than 2 h. However, faster rehydration could be initiated by applying saline solution to the optical clearing agent-free treatment

Table 1 *In vivo* skin condition 70 h after optical clearing treatment with both agents. Numbers denote area affected in relation to entire optically cleared skin surface area.

	Dextrose	Glycerol
Scabbing	5.8%	11.7%
Erythema	23.6%	11.7%

site. Saline solution is not viscous and therefore difficult to keep in contact with the skin surface. An effective rehydration method was found where a saline soaked paper towel was placed onto the skin surface. The towel remained in contact with the skin for approximately 10 min during which additional saline solution was applied onto the towel.

It is noted that although the optical skin clearing result could be regarded as homogenous, there were some adverse skin reactions in small local areas of the treated skin. Figures 3 and 5(b) show some localized scabbing 70 h after the treatment. This effect is attributed to inadvertently induced mechanical skin damage due to excessive sandpaper rubbing. In these experiments, the skin was rubbed manually, which causes uneven pressure distribution and potentially more rubbing in certain areas than others.

It is encouraging that there was skin that could be optically cleared and then returned to a natural opaque state without other obvious adverse skin reactions. Scabbing may be reduced by limiting the rubbing time to even less than 4 min. Also, using more dilute solutions of optical skin clearing agents may prevent lethal osmotic stress on cells within the epidermis. Additional experiments suggested that such adverse skin reactions could be minimized by more gentle and evenly distributed rubbing with sandpaper, but could not be avoided entirely.

Obtained OCT images nicely illustrated the image enhancing effect when light scattering is reduced in *in vivo* skin. The ability to image deeper and to resolve better structural image detail is of tremendous value to clinicians as Refs. 9 to 11 indicate. The benefits of optical skin clearing for improved OCT depth imaging and image contrast were reported by several scientists in the literature.^{3,5,23,24}

It is noted that OCT images do not show a flat skin surface. Because the data was acquired on living and breathing animals, there were some potential motion artifacts present. Furthermore, with a lateral imaging range of 3 mm, the natural shape and curvature of the animal cannot be eliminated from the OCT data. However, while imaging through an inclined surface may slightly reduce the measured optical penetration depth, the overall change of optical skin properties as a result of transepidermal optical skin clearing agent delivery was far greater. Data processing involved digital reregistration of the skin surface via an edge detection algorithm resulting in a flat skin surface followed by averaging the OCT signal intensity as a function of depth across the entire lateral imaging range. The resulting data was accurately described by a best-fit exponential curve that was used to denote the $1/e$ penetration depth. With nine different OCT measurements per skin condition (i.e., untreated, optically cleared, and rehydrated) any surface artifacts can be assumed to be averaged out. Statistically, the optical penetration depth in the optically cleared skin is significantly higher compared to the untreated and rehydrated skin.

It is interesting to note that for native hamster skin and rehydrated skin, Figs. 6(a) and 7(a) show strong OCT signals near the skin surface. This is caused by large amounts of backscattered light, which at the same time limits light penetrating into deeper layers. Contrarily, once the *in vivo* hamster skin is optically cleared, OCT signal intensity is much more homogeneously distributed throughout the skin cross section in depth.

Although there is no apparent difference in optical *in vivo* hamster skin clearing with either clearing agent, there are subtle differences in the OCT images. In OCT images obtained on skin that was optically cleared with glycerol, the return signal from the muscle layer underneath skin was slightly stronger compared to data obtained on dextrose cleared skin. This was quantified by determining the average $1/e$ light penetration depth as shown in Fig. 9 where glycerol led to a $43 \pm 8.24\%$ light penetration increase versus dextrose with $36 \pm 3.75\%$. However, the upper skin layers in glycerol cleared skin do not appear contiguous. Dispersed throughout an apparently layered structure, there are seemingly empty spaces from which no OCT signal is obtained. This is most likely caused by near-perfect optical index matching. Furthermore, optical tissue clearing with glycerol has been shown to result in significant physical tissue shrinkage, while dextrose did not induce such a high degree of dehydration.²⁰

Also, glycerol cleared skin appeared thicker in OCT images compared to dextrose cleared skin. Physically, skin thickness does not change except for shrinkage effects due to dehydration; in which case, the former skin is physically thinner. OCT imaging however is depth sensitive on an optical depth scale. Thus, if skin that is physically thinner than another skin sample appears optically thicker in OCT imaging, then its bulk index of refraction must be accordingly higher. However, depth scaling assumed a constant optical index of refraction of approximately 1.4 in both cases because it is difficult to determine a depth-resolved optical index of refraction gradient.

5 Conclusion

We have shown an effective method for transepidermal delivery of optical skin clearing agents. Hamster skin became optically transparent within 2 min allowing direct visualization of the vasculature. Using gentle rubbing with fine grit sandpaper served to reduce stratum corneum skin barrier function and effectively prevented formation of a concentration gradient between topically applied agent and interstitial water collecting near the skin surface—optical skin clearing agent interface. Compared to previous experiments involving transepidermal optical skin clearing agent delivery, the advantages of using sandpaper have been demonstrated in this paper.

Skin recovery was monitored for up to 70 h and showed some areas of treated skin with residual scabbing and erythema. Future investigations will determine whether these skin reactions are caused by mechanical skin damage from excessive rubbing with sandpaper or are the result of the high concentration optical skin clearing agents used. Optical skin clearing results with glycerol, dextrose solution, or PEG resulted in comparable reduction of light scattering. Using OCT imaging revealed the significant benefits of imaging into deeper skin layers with enhanced image contrast.

Acknowledgments

Funding for this research was provided through the National Science Foundation (Grant No. BES9986296) and Albert W. and Clemmie A. Caster Foundation.

References

1. V. V. Tuchin, I. L. Maksimova, D. A. Zimnyakov, A. H. Mavlyutov, and A. A. Mishin, "Light propagation in tissue with controlled optical properties," *J. Biomed. Opt.* **2**(4), 401–417 (1997).
2. G. Vargas, K. F. Chan, and A. J. Welch, "Effects of tissue optical clearing agents on fluorescence signal from a subsurface," *Lasers Surg. Med. Suppl.* **12**, 3 (2000).
3. G. Vargas, K. F. Chan, J. K. Barton, H. G. Rylander III, and A. J. Welch, "Use of an agent to reduce scattering in skin," *Lasers Surg. Med.* **24**, 133–141 (1999).
4. G. Vargas, K. F. Chan, S. L. Thomsen, and A. J. Welch, "Use of osmotically active agents to alter optical properties of tissue: Effects on the detected fluorescence signal through skin," *Lasers Surg. Med.* **29**, 213–220 (2001).
5. R. K. Wang, X. Xu, V. V. Tuchin, and J. B. Elder, "Concurrent enhancement of imaging depth and contrast for optical coherence tomography by hyper-osmotic agents," *J. Opt. Soc. Am. B* **18**(7), 948–953 (2001).
6. B. Choi, L. Tsu, E. Chen, T. S. Ishak, S. M. Iskandar, S. Chess, and J. S. Nelson, "Determination of chemical agent optical clearing potential using in vitro human skin," *Lasers Surg. Med.* **36**(2), 72–75 (2005).
7. G. Vargas, M. Ducros, S. Dozier, and A. J. Welch, "Selective photo-coagulation of cutaneous blood vessels: Evaluation of vessel damage by color doppler optical coherence tomography," *Proc. SPIE* **3907**, 136–142 (2000).
8. J. Maier, S. Walker, S. Fantini, M. Franceschini, and E. Gratton, "Possible correlation between blood glucose concentration and the reduced scattering coefficient of tissues in the near infrared," *Opt. Lett.* **19**, 2026–2064 (1994).
9. M. Goldgeier, C. Alessi Fox, J. M. Zavislan, D. Harris, and S. Gonzales, "Non-invasive imaging, treatment, and microscopic confirmation of clearance of basal cell carcinoma," *Dermatol. Surg.* **29**(3), 205–210 (2003).
10. Z. Tannous, A. A. Torres, S. Gonzales, "A non-invasive guide for Mohs micrographic surgery facilitated by aluminum chloride, an excellent contrast enhancer," *Dermatol. Surg.* **29**, 839–846 (2003).
11. E. Ruocco, G. Argenziano, G. Pellacani, and S. Seidenari, "Non-invasive imaging of tumors," *Dermatol. Surg.* **30**, 301–310 (2004).
12. H. Schaefer, A. Zesch, and G. Stuttgen, *Skin Permeability*, Springer Verlag, Berlin (1982).
13. H. Schaefer and T. E. Redelmeier, *Skin Barrier: Principles of Percutaneous Absorption*, Karger, Basel CH, Switzerland (1996).
14. Y. He and R. K. Wang, "Dynamic optical clearing effect of tissue impregnated with hyperosmotic agents and studied with optical coherence tomography," *J. Biomed. Opt.* **9**(1), 200–206 (2004).
15. S. Henry, D. McAllister, M. Allen, and M. R. Prausnitz, "Microfabricated microneedles: A novel approach to transdermal drug delivery," *J. Pharm. Sci.* **87**, 922–925 (1998).
16. S. L. Jacques, D. J. McAuliffe, I. H. Blank, and J. A. Parrish, "Controlled removal of human stratum corneum by pulsed laser to enhance percutaneous transport," U.S. Patent No. 4,775,361 (1988).
17. J. S. Nelson, J. L. McCullough, T. C. Glenn, W. H. Wright, L. H. Liaw, and S. L. Jacques, "Midinfrared laser ablation of stratum corneum enhances in vitro percutaneous transport of drugs," *J. Invest. Dermatol.* **97**, 874–879 (1991).
18. J.-Y. Fang, W.-R. Lee, S.-C. Shen, Y.-P. Fang, and C.-H. Hu, "Enhancement of topical 5-aminolaevulinic acid delivery by erbium:YAG laser and microdermabrasion: a comparison with iontophoresis and electroporation," *Br. J. Dermatol.* **151**, 132–140 (2004).
19. O. Stumpp, T. E. Milner, J. Neev, and A. J. Welch, "Enhancement of transepidermal skin clearing agent delivery using a 980 nm diode laser," *Lasers Surg. Med.* **37**(4), 278–285 (2005).
20. O. Stumpp, "Transepidermal delivery and diffusion of optical skin clearing agents for reduction of light scattering in biological tissue and its clinical applications," Ph.D. Thesis, University of Texas at Austin (2005).
21. O. Stumpp, A. J. Welch, H. S. Gill, and M. R. Prausnitz, "OCT analysis of microneedle and Er:YAG surface ablation for enhanced transdermal delivery of hyper-osmotic agents for optical skin clearing," in *Ophthalmic Technologies XIV*, *Proc. SPIE* **5319**, 121–129 (2004).
22. R. J. McNichols, M. A. Fox, A. Gowda, S. Tuya, B. Bell, and M. Motamedi, "Temporary dermal scatter reduction: Quantitative assessment and implications for improved laser tattoo removal," *Lasers Surg. Med.* **36**(4), 289–296 (2005).
23. G. Vargas, "Reduction of light scattering in biological tissue: implications for optical diagnostics and therapeutics," Ph.D. Thesis, University of Texas at Austin (2001).
24. R. K. Wang and V. V. Tuchin, "Optical coherence tomography: Light scattering and imaging enhancement, Chapter 13 in *Coherent-Domain Optical Methods: Biomedical Diagnostics, Environmental and Material Science*, Vol. 2, pp. 3–60, Kluwer Academic Publishers, Boston, (2004).