Evaluation of optical clearing with the combined liquid paraffin and glycerol mixture

Jingyi Wang,1 Yanmei Liang,1* Shu Zhang,1 Yueqiao Zhou,2 Haiyang Ni,3 and Yan Li3

1Institute of Modern Optics, Nankai University, Key Laboratory of Opto-electronic Information Science and Technology, Education Ministry of China, Tianjin 300071, China
2College of Precision Instrument and Opto-electronics Engineering, Tianjin University, Tianjin, 300072, China
3Department of Dermatology, Tianjin Medical University General Hospital, Tianjin, 300052, China
*ymliang@nankai.edu.cn

Abstract: By scanning biological tissues in vivo and in vitro with optical coherence tomography, it is found that liquid paraffin can enhance the percutaneous penetration of glycerol in deep layers of tissue and take synergistically optical clearing effect with glycerol. It is shown from experimental results that 30% - 50% liquid paraffin glycerol solutions have the best enhancement effect. Considering the refractive index of liquid paraffin and its medicinal value, we think liquid paraffin will play an important role in optical clearing as the penetration enhancer of glycerol in future clinical research.

©2011 Optical Society of America

OCIS codes: (110.0113) Imaging through turbid media; (170.3880) Medical and biological imaging; (170.4500) Optical coherence tomography; (170.6930) Tissue.

References and links

1. R. K. Wang and V. V. Tuchin, “Optical tissue clearing to enhance imaging performance for OCT,” in Optical Coherence Tomography: Technology and Applications, W. Drexler and J. G. Fujimoto, eds., (Springer, 2008).
2. V. V. Tuchin, “Optical clearing of tissues and blood using the immersion method,” J. Phys. D Appl. Phys. 38(15), 2497–2518 (2005).
3. L. V. Wang and H. Wu, in Biomedical Optics: Principles and Imaging (John Wiley & Sons, Inc., 2007), Chap. 2 & Chap. 5.
4. V. V. Tuchin, Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis, 2nd ed. (SPIE, 2007), Chap. 1.
5. E. A. Gemina, A. N. Bashkatov, and V. V. Tuchin, “Tissue optical immersion clearing,” Expert Rev. Med. Devices 7(6), 825–842 (2010).
6. V. V. Tuchin, I. L. Maksimova, D. A. Zimnyakov, I. L. Kon, A. H. Mavlutov, and A. A. Mishin, “Light propagation in tissues with controlled optical properties,” J. Biomed. Opt. 2(4), 401–417 (1997).
7. R. Graaff, J. G. Aarnoudse, J. R. Zijp, P. M. A. Sloop, J. Greve, and M. H. Koelink, “Reduced light-scattering properties for mixtures of spherical particles: a simple approximation derived from Mie calculations,” Appl. Opt. 31(10), 1370–1376 (1992).
8. E. A. Sharkov, Passive Microwave Remote Sensing of the Earth: Physical Foundations (Springer, 2003), Chap. 9.
9. I. V. Meglinskii and A. N. Korolevich, “Use of diffusion wave spectroscopy in diagnostics of blood,” J. Appl. Spectrosc. 67(4), 709–716 (2000).
10. D. J. Pine, D. A. Weitz, P. M. Chaikin, and E. Herbolzheimer, “Diffusing-wave spectroscopy,” Phys. Rev. Lett. 60(12), 1134–1137 (1988).
11. D. J. Smithies, T. Lindmo, Z. Chen, J. S. Nelson, and T. E. Milner, “Signal attenuation and localization in optical coherence tomography studied by Monte Carlo simulation,” Phys. Med. Biol. 43(10), 3025–3044 (1998).
12. G. Yao and L. V. Wang, “Monte Carlo simulation of an optical coherence tomography signal in homogeneous turbid media,” Phys. Med. Biol. 44(9), 2307–2320 (1999).
13. R. K. Wang, “Signal degradation by multiple scattering in optical coherence tomography of dense tissue: a Monte Carlo study towards optical clearing of biotissues,” Phys. Med. Biol. 47(13), 2281–2299 (2002).
14. G. Vargas, E. K. 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(2), 133–141 (1999).
15. R. K. Wang, X. Xu, V. V. Tuchin, and J. B. Elder, “Concurrent enhancement of imaging depth and contrast for optical coherence tomography by hyperosmotic agents,” J. Opt. Soc. Am. B 18(7), 948–953 (2001).
16. J. W. Fluhler, R. Darlenski, and C. Surber, “Glycerol and the skin: holistic approach to its origin and functions,” Br. J. Dermatol. 159(1), 23–34 (2008).
17. C. G. Rylander, O. F. Stumpf, T. E. Milner, N. J. Kemp, J. M. Mendenhall, K. R. Diller, and A. J. Welch, “Dehydration mechanism of optical clearing in tissue,” J. Biomed. Opt. 11(4), 041117 (2006).
18. V. V. Tuchin, Optical Clearing of Tissues and Blood (SPIE, 2006), Chap. 8.
20. J. Hirshburg, B. Choi, J. S. Nelson, and A. T. Yeh, “Reversible dissociation of collagen in tissues,” J. Invest. Dermatol. 121(6), 1332–1335 (2003).
21. J. Hirshburg, B. Choi, J. S. Nelson, and A. T. Yeh, “Collagen solubility correlates with skin optical clearing,” J. Biomed. Opt. 11(4), 040501 (2006).
22. J. Hirshburg, B. Choi, J. S. Nelson, and A. T. Yeh, “Correlation between collagen solubility and skin optical clearing using sugars,” Lasers Surg. Med. 39(2), 140–144 (2007).
23. M. H. Khan, B. Choi, S. Chess, K. M. Kelly, J. McCullough, and J. S. Nelson, “Optical clearing of in vivo human skin: Implications for light-based diagnostic imaging and therapeutics,” Lasers Surg. Med. 34(2), 83–85 (2004).
24. X. Wen, Z. Mao, Z. Han, V. V. Tuchin, and D. Zhu, “In vivo skin optical clearing by glycerol solutions: mechanism,” J Biophotonics 3(1-2), 44–52 (2010).
25. J. Jiang and R. K. Wang, “Comparing the synergistic effects of oleic acid and dimethyl sulfoxide as vehicles for optical clearing of skin tissue in vitro,” Phys. Med. Biol. 49(23), 5283–5294 (2004).
26. X. Xu and Q. Zhu, “Evaluation of skin optical clearing enhancement with Azone as a penetration enhancer,” Opt. Commun. 279(1), 223–228 (2007).
27. X. Xu and R. K. Wang, “The role of water desorption on optical clearing of biotissue: Studied with near infrared reflectance spectroscopy,” Med. Phys. 30(6), 1246–1253 (2003).
28. X. Xu and R. K. Wang, “Synergistic effect of hyperosmotic agents of dimethyl sulfoxide and glycerol on optical clearing of gastric tissue studied with near infrared spectroscopy,” Phys. Med. Biol. 49(3), 457–468 (2004).
29. M. Brezinski, K. Saunders, C. Jesser, X. Li, and J. Fujimoto, “Index matching to improve optical coherence tomography imaging through blood,” Circulation 103(15), 1999–2003 (2001).
30. V. V. Tuchin, “A clear vision for laser diagnostics,” IEEE J. Sel. Top. Quantum Electron. 13(6), 1621–1628 (2007).
31. M. G. Ghosn, V. V. Tuchin, and K. V. Larin, “Depth-resolved monitoring of glucose diffusion in tissues by using optical coherence tomography,” Opt. Lett. 31(15), 2314–2316 (2006).
32. M. G. Ghosn, N. Sudheendran, M. Wendt, A. Glasser, V. V. Tuchin, and K. V. Larin, “Monitoring of glucose permeability in monkey skin in vivo using optical coherence tomography,” J Biophotonics 3(1-2), 25–33 (2010).
33. N. Sudheendran, M. Mohamed, M. G. Ghosn, V. V. Tuchin, and K. V. Larin, “Assessment of tissue optical clearing as a function of glucose concentration using optical coherence tomography,” J. Innov. Opt. Health Sci. 3(3), 169–176 (2010).
34. F. Sharif, E. Crushell, K. O’Driscoill, and B. Bourke, “Liquid paraffin: a reappraisal of its role in the treatment of constipation,” Arch. Dis. Child. 85(2), 121–124 (2001).
35. Y. Wang, Y. Liang, J. Wang, and S. Zhang, “Image improvement in the wavelet domain for optical coherence tomograms,” J. Innov. Opt. Health Sci. 4(1), 73–78 (2011).
36. H. Ding, J. Q. Lu, W. A. Wooden, P. J. Kragel, and X. H. Hu, “Refractive indices of human skin tissues at eight wavelengths and estimated dispersion relations between 300 and 1600 nm,” Phys. Med. Biol. 51(6), 1479–1489 (2006).

1. Introduction

Optical imaging technologies, utilizing the inherent optical properties of biological tissues, such as light scattering, absorption, polarization, and fluorescence, have many advantages over X-ray computed tomography (X-CT), magnetic resonance imaging (MRI) and ultrasound imaging in the aspects of safety, cost, contrast and resolution. However, the complicated morphological nature of human tissues and variations of the refractive indices with internal different components make bio-tissues become a high scattering medium for visible and near-infrared wavelengths, i.e., the therapeutic and diagnostic optical window [1–5]. Multiple scattering and absorption attenuate the effective light intensity that reaches internal tissue, diminish the detecting depth, image contrast and image definition. Therefore, they limit the clinical application of some optical imaging techniques.

It is considered that optical clearing technique can effectively reduce the multiple scattering of light and improve the effect of optical diagnostics of tissue [2,6]. Theoretical and computation models, such as Mie theory [7], radiative transfer theory [3,4,8], diffusion wave correlation spectroscopy [9,10], and Monte Carlo simulation [11–13] have been used to analyze optical features during the process that a certain agent penetrates into tissue and blood. Investigation of the theory is the basis for the development of the optical clearing technique and therefore is an important focus in optical clearing study.

G. Vargas [14] proposed the use of hyperosmotic and biocompatible agents to reduce the scattering and enhance light penetration in skin. Through being topically applied onto the skin surface or injected into the dermis, these hyperosmotic agents tend to dehydrate the skin and reduce the index mismatch between intercellular and intracellular components. Previous work
on optical clearing was mainly focused on searching the appropriate and biocompatible optical clearing agents (OCA) or improving the delivery of agents into skin so as to achieve a better optical clearing effect. Experimental studies on optical clearing of normal and pathological skin, some other tissues and blood have been reported by using glycerol [14–28], glucose [6,21], dextran [29], propylene glycol (PG) [22,25], polyethylene glycol (PEG) [6,23] and other pharmaceutical products as OCAs. However, the efficacy of optical clearing by topically applying agents is often suboptimal. The permeation of OCAs into the intact skin is slow due to the resistance of stratum corneum. Accompanying with the loss of water, the optical clearing often induces the deformation of skin and even reduces clinical safety [28]. It is thought that a non-invasive way of incorporating a chemical permeation enhancer can reduce this barrier and the risk induced by high concentration solution. Some penetration enhancers can be added in OCAs to improve the delivery of agents in skin so as to achieve a better optical clearing effect. For example, polypropylene glycol-based polymers (PPG) [23], dimethyl sulfoxide (DMSO) [25], oleic acid [25] and azone [26], as lipophilic agents, have been chosen to take synergistic effect in optical clearing process.

In addition, some studies have been done on better understanding of the mechanism of tissues and blood optical clearing techniques. Most researchers hold the opinion that the reduction of tissue scattering is related to the match of refractive indices of scatterers and ground matters by OCAs and tissue dehydration due to the osmotic properties of OCAs [1,2,4–6,14,15,17,18,22,24–28,30,31]. Considering the important value of OCAs, we expect to find safe and more effective agents from raw materials of clinical medicaments and cosmetics. Their optical clearing effects can be evaluated with optical coherence tomography (OCT) as Refs. [1,2,5,13–15,17,23,25,28,30–33].

After testing a lot of agents, such as ethylene glycerol and azone mixed solution, carboxymethyl cellulose (CMC) solution, methyl ρ-hydroxybenzoate (nipagin) and DMSO mixed solution, tween 80 and liquid paraffin and glycerol mixed solution, glyceryl monostearate and azone mixed solution, silicone oil, kynuric acid, and other OCAs reported in public, the combined liquid paraffin and glycerol mixed solution displayed the best optical clearing effect. The enhancement results of OCT images of rat skin in vitro and human skin in vivo by applying liquid paraffin glycerol solution are shown and some possible explanations are also given in this paper.

2. Theory and Methods

The dominant reason of limiting the imaging depth and contrast in optical imaging within therapeutic and diagnostic optical window is multiple scattering in biological tissues, which is mostly contributed by refractive index mismatch [2]. Tuchin and associates think that the major mechanism of skin optical clearing is a process of refractive index match and it is caused by the diffusion of the OCAs into tissue and water flowing out of tissue as well as the arrangement of tissue fibres in a more regular fashion [1,2,4–6,15,18,24,30–33].

Soft tissue is composed of closely packed groups of cells entrapped in a network of fibres through which water percolates. The fibres and interstitial fluid, nuclei and cytoplasm, and cytoplasm and organelles, are the major contributors to index variations. According to the classical model of scattering spheres [7,11], all scattering cores are assumed to be small spheres, the intensity of scattering may be described by the coefficient of scattering $\mu_s$. The scattering coefficient is determined by the size, density, and shape of scattering cores relative to the wavelength of incident light, and the refractive index mismatch between different material compositions and their structures, namely [7],

$$\mu_s = 3.28\pi r^2 \rho (2\pi r / \lambda)^{0.37} \left( (n_s / n_0) - 1 \right)^{2.09},$$  \hspace{1cm} (1)

where $r$ and $\rho$ are the radius and the volume density of scattering spheres, respectively, $n_0$ is the average background refractive index which is the weighted average of refractive indices of the cytoplasm and the interstitial fluid, and $n_s$ is the refractive index of scattering particles. It
can be seen that the more the value of \( n_0 \) closes to \( n_s \), the more the coefficient of scattering \( \mu_s \) approaches to zero. Equation (1) is valid for non-interacting Mie scatterers, of which meets the conditions: anisotropy scattering factor, \( g > 0.9; 5 < 2\pi r/\lambda < 50; 1 < n_s/n_0 < 1.1 \) [2]. Collagen and elastic fibres are the main components in dermis. The connective-tissue fibre whose refractive index is about 1.47 corresponds to about 55% hydration of collagen [2]. Therefore, to make the refractive indices of fibres and ground substance match, the refractive indices of OCAs should closely match that of collagen fibres.

When lack of the support of blood circulation system, it is obvious that tissue will have water loss \textit{in vitro}. Dehydration causes collagen fibres and organelles to become more closely packed, so dehydration can reduce light scattering. The dehydration has also been proven to be an important mechanism in the optical clearing process by many researchers [5,14,15,17,20], especially for the isolated tissue.

Studies done by M. H. Khan [23] demonstrated that the penetration of glycerol through the intact skin was very minimal and extremely slow. It is because glycerol is hydrophilic and penetrates the lipophilic stratum corneum poorly. In order to enhance skin penetration, the agent need to be either injected into the dermis or the stratum corneum has to be removed mechanically or thermally. To avoid the damage of skin \textit{in vivo}, a certain lipophilic substance can be added into glycerol to promote the optical clearing effect of skin and then enhance the contrast and depth of detecting signals. Liquid paraffin is a kind of high refined mineral oil used in cosmetics and pharmaceutical excipients to retain water in skin, and also has many clinical applications. It can be used to treat constipation and encopresis, and as eye ointment to relieve dryness and irritation. As the solvent of dermatology medicine and the humectant, it can plasticize the stratum corneum and improve surface smoothness of skin [34].

The optical clearing effect is the determination of optical backscattering or reflectance from the internal bio-tissue, so the most prospective applications for the optical clearing technique are expected in OCT. Therefore, OCT is chosen as the detection means to evaluate the optical clearing effect in our paper.

During our experiments, OCT images are obtained by scanning the skin of rat \textit{in vitro} and human skin \textit{in vivo} at 22°C room temperature. For comparison, three OCT images are firstly obtained without applying any agent, and then about 40 micro-liter combined liquid paraffin and glycerol mixture is applied on the same scanning area, and more OCT images are thereafter obtained in succession while the mixture is infiltrating through the tissue. The purpose of our experiments is to scrutinize the optical clearing effect of this mixture with different proportion by the variation of OCT images with time elapsing. Different concentrations of liquid paraffin whose volume fractions in mixed solutions increased from 0% to 70% by 10% are studied in our experiments.

The fiber-based OCT system used in our experiments consists of a broadband Er-doped fiber light source which has a central wavelength around 1550 nm. Its lateral resolution is \( \sim 12 \) \( \mu \)m and axial resolution is \( \sim 12 \) \( \mu \)m in bio-tissue. Samples are placed on a translation platform. Light is nearly perpendicularly irradiated on the sample and the returned light is also collected perpendicularly.

3. Experimental Results and Discussions

3.1 Experimental Results In Vitro

The hairs of rat were shaved and depilated prior to all procedures. The skin was kept cooled in 0.9% NaCl solution during transportation and storage to avoid dehydration in air. The hair-depilated skin of rat is firstly scanned to observe the variation of imaging contrast and the penetration depth of the combined liquid paraffin with glycerol mixed solutions. The size of sample in each test is about 8 mm * 10 mm. The distance of OCT probe and sample platform remains unchanged after applying the agent.

The optical clearing effect of agents can be revealed by the contrast variation of the internal tissue in OCT images. Intensity ratio of regions (RIR) [35] is selected in our paper to calculate quantitatively the contrast of the internal tissue and the surface. The improvement of
contrast is evaluated by calculating the ratio of RIR of OCT images with and without the agent.

RIR is defined as follows,

$$\text{RIR} = \frac{1}{N_1} \sum_{x,y} g(x,y) $$

$$\text{RIR} = \frac{1}{N_2} \sum_{x,y} g(x,y) $$

where $N_1$ and $N_2$ are the number of pixels in the internal and surface regions, respectively, $g(x,y)$ is the gray level in the OCT image. The larger the RIR is, the higher the relative intensity value between the internal and the surface is. Further, the larger the ratio of RIR is, the better the enhanced result is. Pixels whose gray levels in the image are larger than a certain threshold can be considered as the interface pixels between the tissue and the background. A region with a given thickness below the interface pixels is selected as the surface region. Under the surface region, a region with the same thickness is selected as the internal region.

For each sample, the three OCT images without any agent applied are collected and their RIRs are averaged as the benchmark. After applying the agent, OCT images with time elapsing are collected, and their RIRs are calculated. The maximum RIR of these images is divided by the benchmark, which is regarded as the ratio of RIR of this sample. 5 samples for each concentration are tested. The average of 5 ratios of RIR for each concentration is used to evaluate its improvement of contrast.

The average ratios of RIR of different concentrations are shown in Fig. 1. The thickness of the regions is 50 $\mu$m and their width is 1 mm in calculating the RIRs. Two intervals between the internal and surface region, 300 $\mu$m and 500 $\mu$m, are used to evaluate the enhancement of image contrast at different depths. Red solid curve and blue dot curve respectively represent the average ratios of RIR at intervals of 300 $\mu$m and 500 $\mu$m. The results of liquid paraffin whose volume fractions in mixed solution increased from 0% to 70% by 10% are given. 0% concentration denotes anhydrous glycerol. 70% concentration denotes that the ratio of volume between liquid paraffin and glycerol is 7:3.

From Fig. 1, it can be seen that all average ratios of RIR with different concentrations are larger than 1, which means that all agents can improve the contrast. Furthermore, the optical clearing effects of the mixed solution are all better than glycerol and 40% concentration is shown the best optical clearing effect both at 300 $\mu$m and 500 $\mu$m intervals.

![Graph showing ratios of RIR with and without applying the mixed solution](image)

**Fig. 1.** Ratios of RIR with and without applying the mixed solution. Y-axis represents the ratio of RIR, and X-axis represents the volume fraction of liquid paraffin in the mixed solution.

The variation of tissue thickness can be used to reflect the deformation degree of tissue structure qualitatively. It is difficult to measure the thickness of the tissue exactly during scanning. Therefore, we kept the distance of OCT probe and sample platform unchanged after applying the agent and recorded the height variation of the interface between the tissue and
the background in OCT images, which can reflect the variation of the tissue thickness indirectly. In order to simulate the environment of the living organism and avoid dehydration in air, the sample is soaked in the physiological saline solution as Refs. [14,31].

A region is selected from the same lateral position in all images of each group. Axial positions of the surface point in this region are averaged. The variation of the average value in different images represents the change of the position of the surface, i.e., the change of the tissue thickness. Their typical results are shown in Fig. 2. Based on the above conclusion that around 40% concentration has the best optical clearing effect, only the curves of 0% and 20% - 60% concentration are given for being clearly shown. The variation trend of 10% and 70% concentration are respectively similar to 20% and 60%, so they are not given in Fig. 2. A 1 mm width region is used to obtain the average position of the surface. The starting time 0 is the time of applying the agent, and the time of the first scanning image for different concentrations are slightly different. The time interval between two scans is about 1 min.

![Fig. 2. The thickness variation of rat skin with time elapsing by applying the mixed agents of different concentrations. Y-axis represents the reduced thickness, and X-axis represents scanning time.](image)

From Fig. 2, it can be seen that tissue thickness has obvious variation during the whole scanning time for the agents whose concentrations are 20% and lower than 20%. With the increase of fractions of liquid paraffin in the mixed solution, the thickness has only slight variation within 35 min. The slopes of curves in Fig. 2 can reflect the speed of water loss. For the agents with high concentration, the water loss in the tissue is evident in the beginning, then gradually lessens and tends to stabilization. It can be explained in the following: Under the synergistic effect of liquid paraffin, the mixed solution rapidly permeate across the stratum corneum within 5 min, it leads to the dehydration of top layers of tissue. After about 10 min, the moisturizing effect of liquid paraffin begins to display, which makes the water loss and the moisture retention approximately reach the state of equilibrium. Thereafter, the dehydration becomes unobvious. After 35 min, the tissue surface is exposed in air gradually with the agent penetrating, thus the water loss happens again.

From Figs. 1 and 2, it can be concluded that adding liquid paraffin in glycerol cannot only improve optical clearing effect but also reduce water loss with time elapsing. The mixed solution within 30% - 50% concentration shows the best synthetical effect.

To find the optimal duration of optical clearing effect, 12 samples with the mixed agent of different concentrations are further scanned, respectively. For each sample, the RIRs of OCT images every five minutes are calculated, and divided by the benchmark. For each concentration, 12 groups of ratios of RIR are obtained, whose average at the same time are calculated. The results of 30% - 50% concentrations are shown in Fig. 3. The intervals between the surface and the internal region in Fig. 3(a) and Fig. 3(b) are also 300 µm and 500 µm, respectively.
The average ratios of RIR with time elapsing at 30%, 40% and 50% are shown with blue dash curve, red solid curve and green dot curve, respectively. From these curves, it can be seen that the contrast of internal and surface region are all improved after the agents applied and the improvement of 40% concentration is the best, which coincides with that of Fig. 1. It reaches optimal effect after 10min after applying 40% concentration solution. To show the best and worst optical clearing effect, the maximal and minimal ratios of RIR of 40% concentration in 12 groups are given in Fig. 3. Those of 30% and 50% concentration are similar to those of 40% concentration, so they are not given in Fig. 3. From these bars, although the agents have the optical clearing effect statistically, there are one or two exclusions in 12 samples at some time points. From Fig. 3(a) and Fig. 3(b), it can be also seen that the contrast at 300 µm and 500 µm intervals have the similar variation trend with time elapsing for 30%, 40% and 50%, respectively.

A group of typical OCT images obtained by applying 40% concentration solution are given in Fig. 4. Figure 4(a) is the OCT image without the mixed solution. Figures 4(b)–4(e) are taken every 10 min after applying the agent. From these OCT images, it can be seen that the detected depth is improved and the internal tissue is more clearly identified than Fig. 4(a). Its contrast is indeed enhanced and more fine details of tissue structure can be distinguished with the elapsing of time. It can be further seen that the signal intensity of the internal region is gradually increased from 10 min to 20 min and turn to be stable during 20 - 40 min.

In order to indicate the variation of signals of surface and internal regions with time elapsing, intensity as a function of depth is calculated. In order to reduce the interference of surface curvature, only a 1.3 mm width region with the flat surface is selected (as shown in a yellow rectangle in Fig. 4(a)). The intensities of signal with the same axial position in this
region are averaged. The averaged intensities in this region varying with depth in Fig. 4(a)–4(e) are shown in Fig. 5(a). The intensities varying with depth at an arbitrarily selected position (as shown in the yellow line in Fig. 4(a)) are given in Fig. 5(b). The red solid curve represents the signal without the agent. The green dot curve, blue dot-and-dash curve, pink solid curve and dark dot curve represent the signal after applying agent 10 min, 20 min, 30 min and 40 min, respectively.

Fig. 5. Intensity of OCT signals with scanning depth in Fig. 4. (a) is the average intensity in a region shown in the yellow rectangle of Fig. 4(a). (b) is the intensity of an arbitrarily selected position shown in the yellow line in Fig. 4(a).

From Fig. 5, it can be seen that the intensity of the surface are effectively reduced by applying the mixed solution and the intensity of the internal signal is improved simultaneously.

3.2 Experimental Results In Vivo

Different positions of human skin, such as palm, back of hand, wrist, arm, finger, and etc., are scanned in vivo by our OCT system by applying the mixed solution of 30% - 50% concentrations to evaluate the optical clearing effect, and we find the similar results of contrast enhancement.

To find the optimal duration in vivo, 8 groups of OCT images of human finger are scanned at different concentrations, respectively. As the method of Fig. 3, the ratios of RIRs of OCT images in vivo are calculated at 30% - 50% concentrations. The results are shown in Fig. 6. The intervals between the surface and the internal region of Fig. 6(a) and 6(b) are 500 µm and 700 µm, respectively.

Fig. 6. Ratios of RIR of human finger with time elapsing at 30%, 40% and 50% concentrations. (a) is the results at 500 µm interval, (b) is the results at 700 µm interval.

The average ratios of RIR with time elapsing at 30%, 40% and 50% are shown with blue dash curve, red solid curve and green dot curve, respectively. It can be seen from Fig. 6, 30% - 50% concentrations all can enhance the contrast of OCT images in vivo. Their improvement is much better than that of rat skin. Because of the possibly existed variation of scanning
position as a result of human body wobbling during repetitive scans and individual difference, the fluctuation of the curves in Fig. 6 is larger than those of in vivo. Different with the experimental results in vitro, the ratios of RIR at 30% concentration is higher than those of other two concentrations. Considering the results of Fig. 6(a) and 6(b), the time that reaches the best optical clearing effect is around 10 - 30 min for 30% concentration. These differences between in vivo and in vitro need to be further investigated in future.

A group of typical OCT images before and after applying 30% liquid paraffin glycerol solution is given in Fig. 7. Figure 7(a) is the OCT image without the agent. Figures 7(b)–7(g) are taken in 5 min - 30 min every 5 min after applying agent. Compared Figs. 7(b)–7(g) with Fig. 7(a), it can be seen that the intensity of internal signal are obviously increased. After liquid paraffin glycerol solution is applied on the skin surface, a high osmotic pressure occurs, leads the agent to penetrate through the stratum corneum rapidly. Along with the mixed agent penetrating, refractive index mismatch in tissue is lessened, and the profile of papillary layer in dermis becomes more noticeable.

Fig. 7. OCT images of human finger. (a) an OCT image without the agent, (b) 5 min, (c) 10 min, (d) 15 min, (e) 20 min, (f) 25 min and (g) 30min OCT images after topical application of 30% liquid paraffin glycerol solution on the same area, respectively.

From Fig. 7, it can be seen that the optical clearing effect of the mixed agent maintains almost changeless in 10 - 30 min in vivo. We think the blood flowing in blood-containing layers maintains the circulation of water, so that dehydration in vivo is not obvious as in vitro.

Macroscopically speaking, optical scattering mainly occurs at the tissue interfaces whose refractive indices mismatch, so the most obvious scattering layers are thus the surface of skin and the boundary between epidermis layer and dermis layer. After OCAs are applied, the scattering light on the boundaries can be reduced effectively, thereby enhancing transmittance and decreasing diffuse reflectance. From all the experiments, we find the mixed agent can effectively reduce the signal intensity reflected from the surface and improve the intensity of internal signal.

The refractive indices of epidermis and dermis of human skin in vivo are around 1.42 and 1.37 [36], and that of stratum corneum which is a part of epidermis is ~1.5. In our experiment, the refractive indices of liquid paraffin and glycerol, being measured with Abbe refractometer under visible light, are 1.4691 ± 0.0003 and 1.4689 ± 0.0003, respectively. It has been proven that glycerol can reduce the gradient of refractive indices between sub-layers in tissue. Therefore, adding liquid paraffin in glycerol will not alter the refractive match effect of glycerol, and can be expected to show a similar effect as glycerol alone.

From these results, we further prove that OCAs combined hydrophilic agents with lipophilic agents can enhance optical clearing effect and improve the speed of percutaneous penetration simultaneously. The stratum corneum, as a protective layer of the skin, is a dense medium with poor penetration to foreign molecules. The speed of glycerol alone to diffuse through the stratum corneum is slow. Only lipophilic agents have the ability of membrane penetration and can be used as the carrier of drug delivery. Liquid paraffin, as a kind of lipophilic agent, can enhance the percutaneous penetration of glycerol in deep layers of tissue and take synergistically optical clearing effect with glycerol, and block the evaporation of water and compensate the water loss induced by glycerol and thus reduce the tissue deformation. In addition, glycerol, as a kind of hydrophilic agent, has better optical clearing
effect for the tissue under the stratum corneum than lipophilic agents as said in [23,26]. Under their co-operation, the optimum optical clearing effect is obtained.

4. Conclusions

OCAs can reduce the multiple scattering of light and improve the effect of optical diagnosis of tissue, whose components need be selected by synthetically considering their refractive indices, safety, biocompatibility, and so on. From the experimental results in vivo and in vitro, it is shown that liquid paraffin has good synergistic effect with glycerol in optical clearing. Because of its excellent comprehensive characteristics, such as appropriate refractive index, being lipophilic, capability of preserving moisture, and some medicinal values, we definitely believe that liquid paraffin will play an important role in optical clearing and medical treatment in future clinical research.

Acknowledgments

This research is supported by the National Natural Science Foundation of China (Grant No. 60637020 and 60677012) and the Tianjin Foundation of Natural Science (No. 09JCZX18300).