Enhanced biosensing based on chemical or mechanical optical clearing

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Abstract: Both the chemicals and mechanical stress can reduce the scattering of tissue or background noise, which enhances the ability of optical biosensing. This presentation summarizes the principles and applications of tissue optical clearing.

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1. Introduction

Biomedical photonics is currently one of the fastest growing fields of life science connecting research in physics, optics and electrical engineering coupled with medical or biological applications. It allows structural and functional analysis of tissues and cells with unattainable resolution and contrast by any other methods. However, the major challenges of many biophotonics techniques are associated with the need to enhance detecting resolution even further to the sub-cellular level as well as translate them for *in vivo* studies. Advanced optical methods combined with the various contrast agents pave the way towards real molecular imaging within living cells. However, the high scattering of turbid biological tissues limits the penetration of visible and near-infrared light, and the image blurs, both the detecting resolution and contrast decrease as light propagates deeper into the tissue [1-2].

To obtain the information from deeper tissue layers, several optical imaging techniques have been developed. For instance, the limit of imaging depth to 100 μm was broken with the invention of the multiphoton microscope. Combination of multi-photon fluorescence excitation with very high collection efficiency increased the depth of imaging in scattering samples up to 2 mm. A multimodal molecular imaging technique has been introduced that combined light absorption with acoustic detection (thus termed photoacoustics) that further increased imaging depth up to a few centimeters. Alternatively, reducing scattering and absorption of tissues could also significantly enhance biosensing at tissue optical clearing [1-4].

Tissue optical clearing (TOC) method, using impregnation of tissues by optical clearing agents (OCAs), that reduces the scattering of tissue and make tissue more transparent was recently proposed for *in vivo* applications [1]. It is well known that tissues are densely packed with many types of substances, including the scatter particles with higher refractive index, i.e., collagen, elastic fibers, cells and cell compartments, and surrounding media with lower refractive index, i.e., interstitial fluid and cytoplasm. This architecture makes light travel at different speeds and angles because each component has different refractive index. Typical OCAs usually have high refractive index and, thus, penetration of OCAs into the extracellular space matches the refractive indices of the scatters and the surrounding media, and then lead to reduction of the light scattering. Most of OCAs are hyperosmotic, thus dehydrate tissues reversibly and make tissue more homogeneous for some time interval during OCA application. Both processes lead to significantly enhanced biosensing as light penetrates deeper into the tissue [1-4]. In addition, optical clearing due to mechanical stress is developed recently that maintains stratum corneum integrity, creates localized effects, and may exhibit fast rates of optical clearing [1, 5-9], which has shown to increase imaging resolution and contrast [1, 5-7]. If the chemicals induced tissue optical clearing is an exogenic method, the mechanical stress is an endogenic method.

In this presentation, we will introduce the TOC techniques by immersion liquid or mechanical stressing. And the applications of TOC will be summarized.

2. Immersion optical clearing

The chemical optical clearing method is realized by application of higher refractive indices and higher osmolality optical clearing agents (OCAs), represents a promising approach for increasing the imaging depth for optical techniques, such as linear and nonlinear spectroscopies, optical coherence tomography, and confocal microscopy. Water solutions of glycerol, glucose, ethylene glycol, polyethylene glycol, propylene glycol, polypropylene glycol and some others are used as OCAs for *in vitro* and *in vivo* studies [1-2].

The diffusion of optical clearing agents into tissues will match the refractive indexes of tissue components with extracellular fluid thus reducing the scattering of tissue. This was regarded as the major mechanism of tissue optical clearing [1-2]. However, *in vitro* experiments demonstrated that optical clearing efficacy of skin did not correlate with refractive indices of OCAs, but with molecular structure of OCAs [10], which was further supported by molecular dynamics simulation [11]. Furthermore, additional issues such as dissociation of collagen fibers [11] and tissue dehydration [12] caused by OCAs have been proposed to clarify mechanism of TOC based on experimental results. However, for *in vivo* tissue optical clearing, only the thickness of dermis and diameter of collagen fiber were reduced, but there was no dissociation of collagen fiber as observed during *in vitro* skin optical clearing studies [13]. Different views on the mechanisms of optical clearing are likely due to different experimental protocols and techniques as well as difference in studied clearing agents and the complex of mechanical and chemical properties for different tissues. Summarizing up, it is clear that the mechanisms of TOC could be due to multiple factors: OCAs increase the refractive index of interstitial fluid and enhance refractive indices match among of various substances in tissue; hyperosmotic agents induce dehydration of tissue and decrease the thickness of tissue; molecular dynamical reactions between tissue and OCAs cause dehydration of tissue or change tissue structure temporarily. Since the structure and components of tissues or OCAs different, TOC process could also be different.

Based on discussed above, the TOC efficacy depends not only on the type of OCA but also on its treatment time. The longer time tissue is immersed, the more transparent tissue becomes. Sometime, it needs several weeks, even several months to make a mouse brain transparent when it immersed into an OCA like Sca*l*e [14]. Comparing with other soft tissues, it is more difficult to induce skin clearing by topical treatment with OCAs because the barrier function of *stratum corneum* restricts the penetration of OCAs into the dermis. Even though the direct exposure of skin dermis to OCAs or dermal injection of OCAs can also make skin clear, it is difficult for OCAs at low concentration to produce good skin optical clearing efficacy, while OCAs at high concentration could induce edema, suppuration, or even scarring. In order to develop effective and safe way to breach the SC integrity and accelerate the permeability of OCAs into dermis, various physical methods and chemical penetration enhancers were used to enhance the delivery of OCAs into tissue, which improves the tissue optical clearing efficacy [1, 15-17].

3. Mechanical optical clearing

For the tissue optical clearing method caused by delivering exogenous hyperosmotic chemicals, the dehydration is an important mechanism [12]. And other nonchemical techniques for water redistribution, such as application of mechanical compression should also induce tissue optical clearing, which has been proposed recently [1, 6-8].

Localized mechanical compression can also induce an optical clearing effect in tissue. As tissue is compressed, water and blood are displaced laterally from regions undergoing high compressive strain (relative thickness change), decreasing local water volume fraction and thus reducing refractive index mismatch between constituents and lowering scattering and absorption. Comparing with the immersion optical clearing with exogenous OCAs, the mechanical optical clearing is a safe and noninvasive alternative because no foreign material is delivered to the tissue. Moreover, the stratum corneum is undisturbed, so the barrier function of stratum corneum and epidermis is maintained. Such a technique may circumvent the drawbacks of chemical optical clearing and provide a fast controllable technique to enhance the light penetration in the tissue [6-8].

It should be noticed that mechanical tissue optical clearing is mainly from the probe contact pressure [9]. With the development of the research, some specialized devices were developed. Besides uniform compression over a large area of tissue, other works used local mechanical compression to induce smaller ($\sim$1mm^2) compression zones, creating higher local pressure gradients within the compressed tissue. Tissue Optical Clearing Device (TOCD) consist of an array of pins within a chamber was an effective tool. Light may be delivered through these pins for either diagnostic or therapeutic applications. Vacuum pressure is applied to stretch the tissue between the pins, creating a reaction force that compresses the tissue beneath the pins, increasing light transmission [6,7].

4. Optical clearing enhances biosensing

Furthermore, many new original studies are elaborated using immersion optical clearing in combination with microscopy imaging, ultramicroscopy, etc., which have demonstrated a great power not only for tissue structural and functional imaging with higher resolution. Chiang's group introduced a kind of OCAs called FocusClear [18]. By direct immersion of a 500 μm tissue section in the solution, the biological structures, including animal and plant cells and organisms can become transparent. Combined with fluorescence label and LSCM, it allows one to visualize the microstructure and vascular net-work with subcellular-level resolution, and then obtain 3D visualization of microvasulature. With the development of various optical clearing methods, such as, Scale [14], dibenzyl ether (DBE) in combination with THF, Clarity, et al., it has come true to obtain high resolution neuron structure using various microscopy imaging techniques, which produces significant breakout for the research of neuroscience [19-22]. In addition, immersion optical clearing shows enormous potential for *in vivo* optical imaging, diagnosis and therapy. With the skin and cranial optical clearing windows, laser speckle contrast imaging (LSCI)

can be applied to image dermal or cortical blood flow at high resolution [23-25]. Optical coherence tomography (OCT) also shows a great potential for disease diagnosis.

Optical clearing techniques and devices based on mechanical compression could provide major improvements to optical imaging modalities. Research demonstrated use of mechanical compression for image contrast enhancement of optical coherent tomography [6,7], or for tissue optical clearing to observe pathological changes in tissue [8, 26]. Topical pressure is applied to temporarily squeeze blood out of the illuminated tissue volume, and the influence of oxy-hemoglobin on the reflection spectra is effectively reduced. After a short optical clearing time the carotenoid absorption becomes easily discernable in a 460–500 nm spectral window and its optical density can be calculated with high accuracy. Thus, a non-invasive rapid determination of skin carotenoid levels can be used to monitor skin carotenoid concentration changes over time in response to carotenoid containing natural or supplemental diets, and is easily adaptable for applications in clinical and field setting.

In summary, localized mechanical compression induces tissue optical clearing in a non-invasive manner without use of exogenous chemicals, which also demonstrate enhance biosensing in some aspects. However, the mechanical pressure makes it impossible for monitoring blood flow. For *in vitro* optical clearing, chemical optical clearing technique is still an irreplaceable method for imaging deeper tissue.

5. References

[1] V. V. Tuchin, Optical Clearing of Tissues and Blood, PM 154, SPIE Press, Bellingham, WA, 2006

[2] D. Zhu, K. V. Larin, Q. Luo, V. V. Tuchin, "Recent progress in Tissue optical clearing", Laser & Photonics Reviews, 1-26, /DOI 10.1002/lpor.201200056 (2013)

[3] E. A. Genina, A. N. Bashkatov, V. V. Tuchin, "Tissue optical immersion clearing", Expert Rev. Med. Devices 7(6), 825–842 (2010)

[4] K.V. Larin, M.G. Ghosn, A.N. Bashkatov, E.A. Genina, N.A. Trunina, and V.V. Tuchin, "Optical clearing for OCT image enhancement and in-depth monitoring of molecular diffusion", IEEE J. Select. Tops. Quant. Electr. 18(3) 1244–1259 (2012)

[5] C. Drew, T. E. Milner, C. G. Rylander, "Mechanical tissue optical clearing devices: Evaluation of enhanced light penetration in skin using optical coherence tomography". J. Biomed. Opt.,14(6):064019-1-6(2009)

[6] A Gurjarpadhye, W. C. Vogt, Y. Liu, C. G. Rylander, "Effect of localized mechanical indentation on skin water content evaluated using OCT". Int. J. Biomed. Imaging., 817250:817250 (2011)

[7] Izquierdo-Roman A, Vogt WC, Hyacinth L, Rylander CG. "Mechanical tissue optical clearing technique increases imaging resolution and contrast through ex vivo porcine skin". Laser. Surg. Med., 43(8):814–823(2011).

[8] Igor V. Ermakov and Werner Gellermann, "Dermal carotenoid measurements via pressure mediated reflection spectroscopy", J. Biophotonics 5, No. 7, 559 –570 (2012)

[9] C. Li, J. Jiang, K. Xu, "The variations of water in human tissue under certain compression: studied with diffuse reflectance spectroscopy", J. Innovative Opt. Health Sci. 6(1), 1350005(2013)

[10] Z. Mao, D. Zhu, Y. Hu, X. Wen, Z. Han, "Influence of alcohols on the optical clearing effect of skin in vitro", J. Biomed. Opt., 13(2), 021104, 2008

[11] J. M. Hirshburg, K. M. Ravikumar, W. Hwang, and A. T. Yeh, "Molecular basis for optical clearing of collagenous tissues," J. Biomed. Opt. 15(5): 055002 (2010).

[12] T. Yu, X. Wen, V. V. Tuchin, Q. Luo, D. Zhu, Quantitative analysis of dehydration in porcine skin for assessing mechanism of optical clearing, J. Biomed. Opt., 16(9), 095002(2011)

[13] X. Wen, Z. Mao, Z. Han, V. V. Tuchin, D. Zhu, "In vivo skin optical clearing by glycerol solutions: mechanism", Journal of Biophotonics, 3(1), 44-52 (2010)

[14] H. Hama, H. Kurokawa, H. Kawano, R. Ando, T. Shimogori, H. Noda, K. Fukami, A. Sakaue-Sawano, and A. Miyawaki, "Scale: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain," Nat. Neurosci. 14: 1481–1488 (2011).

[15] X. Wen, S. L. Jacques, V. V. Tuchin, D. Zhu, "Enhanced optical clearing of skin in vivo and OCT in-depth imaging", J. Biomed. Opt., 17(6), 066022 (2012)

[16] C. Liu, Z. Zhi, V. V. Tuchin, Q. Luo, and D. Zhu, "Enhancement of skin optical clearing efficacy using photo-irradiation," Lasers Surg. Med. 42(2): 132–140 (2010).

[17] Z. Zhi, Z. Han, Q. Luo, and D. Zhu, "Improve optical clearing skin in vitro with propylene glycol as a penetration enhancer," J. Innovat. Opt. Health Sci. 2(3), 269–278 (2009)

[18] H. H. Lin, J.S.Y. Lai, A.L. Chin, Y.C. Chen, and A.S. Chiang, "A Map of Olfactory Representation in the Drosophila Mushroom Body," Cell, 128, 1205–1217 (2007).

[19] H. U. Dodt, U. Leischner, A. Schierloh, N. Jährling, C.P. Mauch, K. Deininger, J.M. Deussing, M. Eder, W. Zieglgnsberger, and K. Becker, "Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain," Nat. Methods 4(4): 331-336 (2007).

[20] Ertürk, C.P. Mauch, F. Hellal, F. Förstner, T. Keck, K. Becker, N. Jährling, H. Steffens, M. Richter, M. Hübener, E. Kramer, F. Kirchhoff, H.U. Dodt, and F. Bradke, "Three-dimensional imaging of the unsectioned adult spinal cord to assess axon regeneration and glial responses after injury," Nat. Med. 18: 166-171 (2012).

[21] Ertürk, K. Becker, N. Jährling, C. P. Mauch, C. D. H, J. G. Egen, F. Hellal, F. Bradke, M. Sheng, H.U. Dodt, Three-dimensional imaging of solvent-cleared organs using 3DISCO, Nat. Protoc. 7,1983-1995 (2012)

[22] K. Chung, J. Wallace, S. Kim, S. Kalyanasundaram, A. S. Andalman, T. J. Davidson, J. J. Mirzabekov, K. A. Zalocusky, J. Mattis, A. K. Denisin, S. Pak, H. Bernstein, C. Ramakrishnan, L. Grosenick, V. Gradinaru, & K. Deisseroth, "Structural and molecular interrogation of intact biological system", Nature, Volume:497, 332–337(2013)

[23] J. Wang, R. Shi, D. Zhu, "Optical clearing method induced switchable skin window for dermal blood flow imaging", J. Biomed. Opt., 18(6), 061209 (2013).

[24] J. Wang, Y. Zhang, T. Xu, Q. Luo, D. Zhu, "An innovative transparent cranial window based on skull optical clearing", Laser Phys. Lett. 9(6), 469-473(2012)

[25] D. Zhu, J. Wang, Z. Zhi, X. Wen, Q. Luo, Imaging dermal blood flow through the intact rat skin with an optical clearing method, J. Biomed. Opt.,15(2), 026008 (2010)

[26] Yu.P. Sinichkin, N. Kollias, G. Zonios, S.R. Utz, and V.V. Tuchin, Reflectance and fluorescence spectroscopy of human skin *in vivo*, Chapter 13 in *Handbook of Optical Biomedical Diagnostics*, 2nd edition, ed. by V.V. Tuchin (Ed.), SPIE Press, 2014