Optical transparency in live animals: a leap toward deep-tissue applications

Siyang Zheng,[a,b,†] Wenzhao Li,[a,b,†] Weiran Pang,[a,b,†] Tianting Zhong,[b,c,*] and Puxiang Lai[a,b,d,*]
[a]The Hong Kong Polytechnic University, Department of Biomedical Engineering, Hong Kong, China
[b]The Hong Kong Polytechnic University, Shenzhen Research Institute, Shenzhen, China
[c]The University of Hong Kong, Department of Electrical and Electronic Engineering, Hong Kong, China
[d]The Hong Kong Polytechnic University, Joint Research Center for Biosensing and Precision Theranostics, Hong Kong, China

Leveraging the unique features of light in biosafety, sensitivity, and resolution, optical technologies have seen a wide application in diagnosing and treating biological tissues, as well as in uncovering the mysteries and patterns of life. However, the application of light remains constrained in deep tissues, as researchers struggle to achieve the same or comparable high-resolution focusing and imaging as in superficial tissues. This limitation mainly arises from the spatial inhomogeneities of refractive index (RI) in biological tissues, where light propagation is severely hindered by scattering and absorption.[1]

There have been intensive efforts in the past few decades to overcome these challenges, including extending controllable light or information restoration to deeper tissues through optical, computational, and artificial intelligence approaches.[2,3] Excluding a focus on regulating the light-field and information transmission, researchers have, surprisingly, pioneered approaches with the biological tissue itself, making it "transparent" to light—so-called tissue clearing. The primary cause of opacity in biological tissue is the strong dispersion of light arising from the spatially varying structural components with different RIs. For example, biological tissues generally consist of water-based substances ($RI_{water} \approx 1.33$), lipids ($RI_{lipid} \approx 1.46$), proteins ($RI_{protein} \approx 1.50$), and many other constituents.[4] These molecules form various structural components, such as lower RIs of intra- and extracellular fluids, and higher RIs of cell membranes and organelles containing more lipids and proteins. For different tissues and organs, this heterogeneity is further amplified. These factors collectively lead to the strong light-scattering feature of biological tissues [Fig. 1(a)]. Intuitively, "smoothing out" these RI variations could inhibit light scattering at the root and hence enhance the transparency of tissue to light, which is called tissue clearing.

Exciting progress has been achieved along this path by mitigating the absorption and scattering effects of tissues. For example, to reduce absorption, depigmentation methods are employed, involving the removal of light-absorbing constituents such as hemoglobin and melanin from tissues, hence minimizing the impact of light transmittance.[5] To reduce scattering, tissue clearing techniques are preferred. One effective way is to increase the RI of the aqueous components with optical clearing agents, achieving an effective RI level closer to that of lipids and proteins.[6] However, most studies to date have been limited to *ex vivo* tissues. The few exceptions that have obtained live tissue transparency require an extremely high concentration (above 50%) of reagents or extended periods to take effect (more than 40 min) and lack sufficient compatibility and reversible operations that can be reliably repeated.[7] These factors undoubtedly produce a series of side effects on the organism. Hence, these few implementations have been limited to shallow tissue depths, such as the skin.[8]

Most recently, Ou et al. proposed a counterintuitive yet plain approach to the barrier and achieved biocompatible transparency of "live" tissues.[9] They first employed a food additive, tartrazine, which was rarely taken into consideration in optical clearing, to apply at multiple sites of living rodents [Fig. 1(b)]. These tissues have unprecedentedly achieved *in vivo* transparency at the millimeter depth, resulting in precise spatial resolution at the micrometer level. The selection of molecules was based on the analysis of the dielectric properties of materials using the Lorentz oscillator model and the Kramers–Kronig relationship. It was predicted that dye molecules with significant absorption resonances in the near ultraviolet spectrum (300 to 400 nm) and the blue band of visible light (400 to 500 nm), when dissolved in water, can effectively increase the real part of the RI of aqueous solutions at longer wavelengths (beyond 600 nm). This counterintuitive phenomenon can further reduce the contrast in RI between water and lipids for longer wavelengths of light, ultimately achieving optical transparency of live biological tissues. Tartrazine perfectly meets those requirements and is a common edible pigment approved by the European Food Safety Authority with good water solubility. Therefore, the solution of tartrazine can freely penetrate tissue without complex physiological or chemical processes. At lower concentrations, it can achieve a matching of RI without dehydrating or shrinking the biological tissues.

In their experiments, the research team investigated the influence of different tartrazine concentrations, optical wavelengths, and medium types on transmission. Especially *in vivo*, tartrazine solutions were locally applied to the scalp, abdominal skin, and hind limbs of shaved living mice. It was found that the tissues became more transparent, and the penetration depth and resolution of optical imaging were significantly improved. More interestingly, when the dye molecules were washed away from the surface, the tissue became opaque again. After repeating this step many times, reversible and effective clarification effects were confirmed, and the clearing process only took a few minutes. Combining tissue transparency with fluorescence imaging can achieve the goal of deep-tissue *in vivo* imaging.

This study is remarkable from several aspects. First, it defies the stereotype that introducing highly absorptive molecules goes against the optical clearing of biological tissues. Despite the seemingly counterintuitive selection, tartrazine, a dye with strong absorption near the ultraviolet regime, successfully enhances the transparency of live tissue to longer-wavelength visible light. Second, the reagents and procedures are exceptionally safe, accessible, and repeatable. The biocompatible water-soluble dye circumvents the adverse effects on biological activities typically associated with conventional tissue-clearing methods, and the application only requires gentle massaging of the tissue. Finally, achieving such biocompatible and reversible transparency with significant depth in living organisms is unprecedented.

However, challenges remain. The permeating depth of the dye and the transparency wavelength range could be further improved. The method still cannot absolutely overcome the RI heterogeneity of tissues

*Address all correspondence to Tianting Zhong, tianting.zhong@connect.polyu.hk; Puxiang Lai, puxiang.lai@polyu.edu.hk

†These authors contributed equally to the work.

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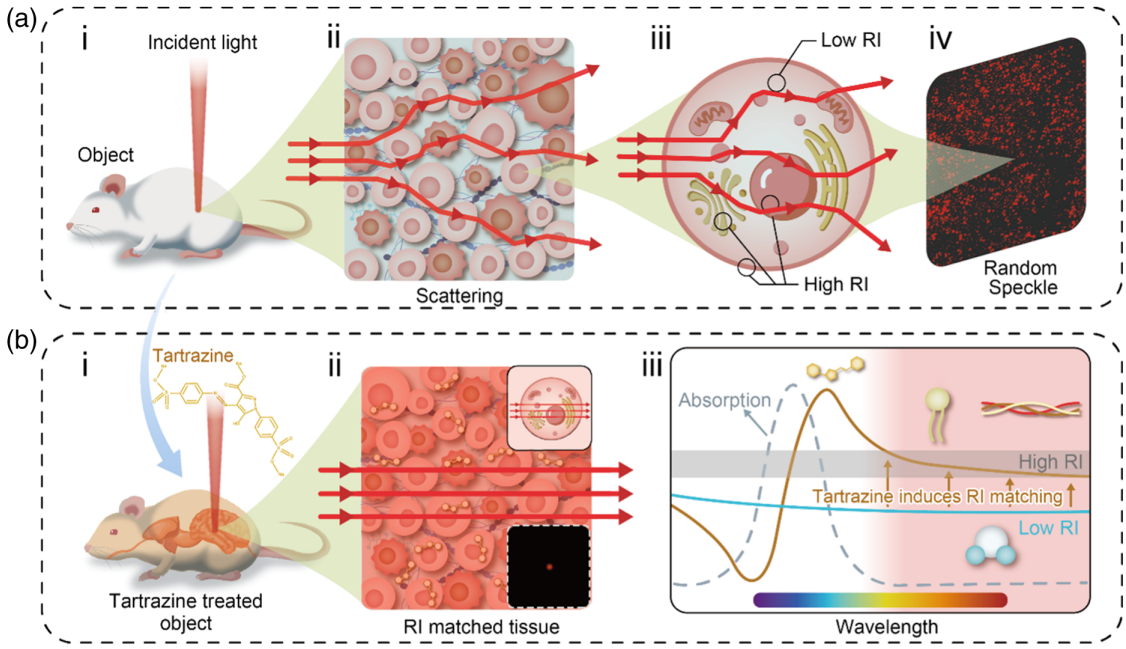

Fig. 1 Illustration of live tissue clearing based on the utilization of tartrazine. (a) (i) Light propagation in an untreated mouse. (ii)–(iv) Due to the mismatch of RIs, light propagation through tissues and cells experiences strong scattering, resulting in seemingly random speckles outside the tissue sample. (b) (i) Light propagation in a mouse treated with tartrazine. (ii) With improved RI homogenization, light propagation through tissues and cells exhibits considerably suppressed scattering, enabling controllable light delivery (e.g., optical focusing) through the tissue sample. (iii) Mechanism of optical clarification achieved by tartrazine-like absorbing molecules. The gray dashed line is its absorption spectrum, and the golden solid line is its simulated RI curve; at longer wavelengths, it can effectively reduce the contrast in RI between water and lipids, realizing RI matching.

due to their diverse components, resulting in some residual scattered light. Moreover, this method is currently only applicable to small rodents; its application scenario needs to be expanded. The procedure of selecting dye molecules in this study can be theoretically extended and combined with artificial intelligence and computing simulations.[10] This could potentially lead to the discovery of more optimum molecules suitable for different wavelengths or various tissues such as bones. Technically, this method could also be integrated with other manners such as fiber, microneedles, and ultrasound induction[11,12] to further increase the penetration depth into tissue.

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