COMMENTARY

Transparent live mice offer more possibilities for biomedical research

Hong Yu,[1,5] Ji-Bin Liu,[2,5] Yu-Shui Ma,[2,3,*] and Da Fu[1,2,4,*]

[1]Department of Pathology, The Affiliated Taizhou People's Hospital of Nanjing Medical University, Taizhou 225300, China
[2]Institute of Oncology, Affiliated Tumor Hospital of Nantong University, Nantong 226631, China
[3]Cancer Institute, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China
[4]Department of General Surgery, Pancreatic Disease Center, Research Institute of Pancreatic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[5]These authors contributed equally
*Correspondence: lh06076@shutcm.edu.cn (Y.M.); fd12374@rjh.com.cn (D.F.)

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Leveraging the transparency of model organisms, we can tackle a broad spectrum of challenges in life sciences and medicine. This includes revealing the molecular mechanisms underlying embryonic and tissue development. Furthermore, we can create models to study human diseases and tumors, which serve as valuable platform for drug screening and therapeutic research. In environmental science and agricultural research, transparency also allows the development of toxicological and genetic breeding models to address key issues.

The transparency of these organisms enables precise observation of cellular details, offering deeper insights into the dynamic cellular processes. This significantly enhances scientists' knowledge of diverse biological systems. In a recent issue of *Science*, a team from Stanford University demonstrated a

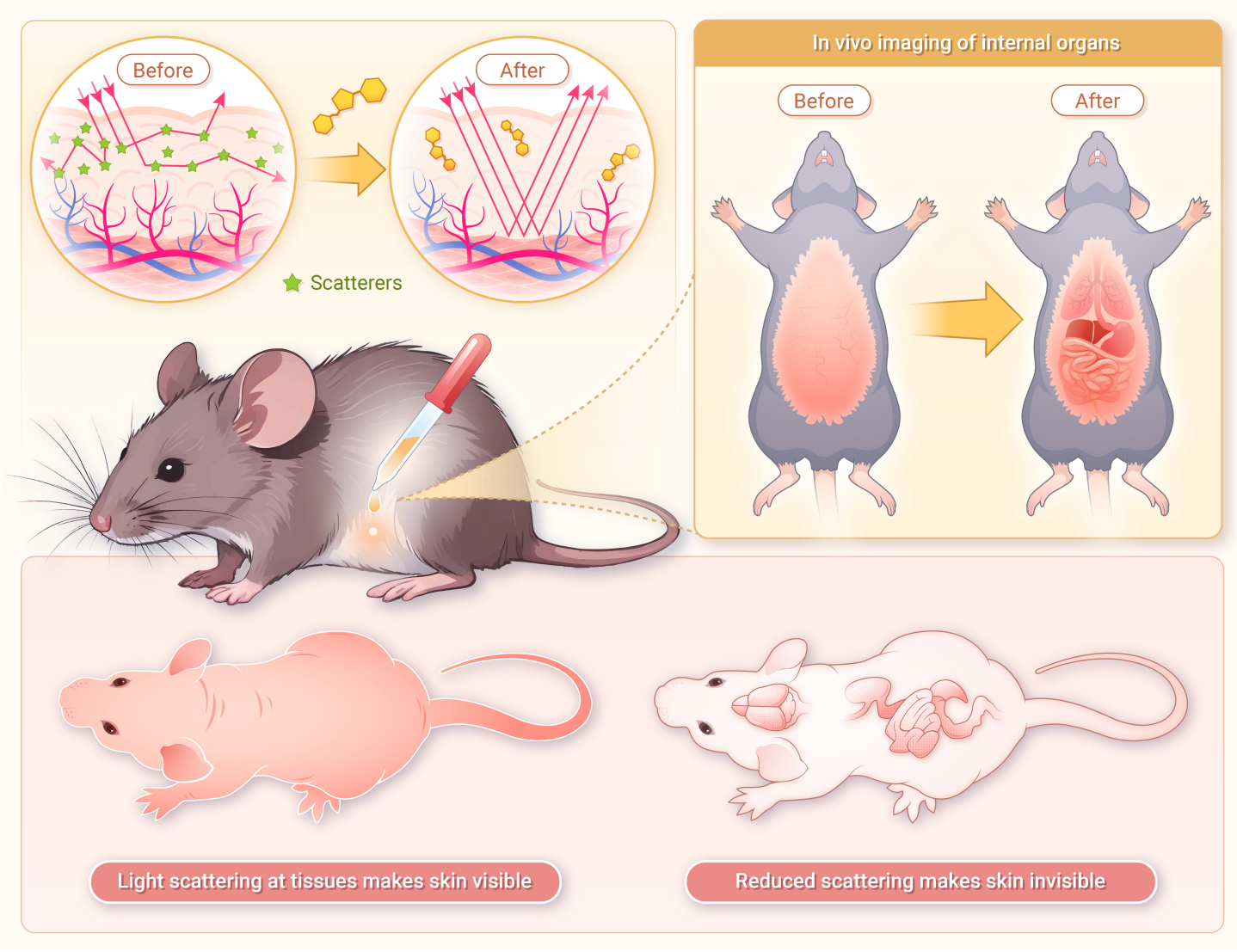

Figure 1. Schematic diagram of a new method for making live mice transparent.

remarkable transparency technique in living animals.[1] Even more astonishing is that this remarkable dye is actually just a common food coloring: tartrazine. The study describes a novel approach to rendering skin transparent, with a straightforward principle: adjusting the refractive index of cells to match that of the surrounding medium. This alignment prevents light scattering at the cell-medium interface, allowing visible light to pass through unimpeded and rendering biological tissues transparent (Figure 1).

Driven by an innate curiosity about the mysteries of life, humans spend

their entire lives striving to understand and perceive themselves. Researchers explore tissues and organs such as the brain, skin, muscle, and bone to uncover their structures and the complex connections between them. The advancement of anatomy has provided the foundation for modern medicine, and the innovations in medical technology has transformed this field dramatically. The invention of the microscope marked a turning point in the examination of living tissues. More recently, modern non-invasive anatomical techniques have revolutionized how we explore internal body structures. The development of X-ray, ultrasound, magnetic resonance imaging (MRI), and computed tomography (CT) has allowed scientists to visualize the body in unprecedented detail, surpassing the imaginations of early anatomists. Yet, this progress has only deepened scientists' desire for greater "visibility" and a more profound understanding of the human body.

In traditional anatomy, the removal of entire organs and the sectioning of living tissues have significantly influenced the exploration of internal structures and their interconnections. While some modern non-invasive imaging techniques offer excellent views of the human body's internal structures, they still struggle to delve into the more abstract issues of "structure and connection." In 1911, German anatomist Werner Spalteholz was the first to demonstrate the technique of making opaque tissues transparent. He employed chemical solvents to remove light-scattering biomolecules, achieving transparency. However, this method is incompatible with modern fluorescent markers and can damage tissue structure.

In 2012, researchers including Hans-Ulrich Dodt from the Technical University of Vienna and Ali Ertürk from the University of Munich in Germany introduced the modern transparency technology 3DISCO, an enhanced version of the Spalteholz method. 3DISCO employs a milder blend of chemical solvents to dissolve lipids, preserving the cell structure, dehydrating and solidifying the specimen into a transparent state while retaining the tissue's original structure.[2] Building on 3DISCO, Ali Ertürk surpassed the limitations of large tissue imaging by developing the ultimate DISCO method, which rendered entire organs and the bodies of rodents transparent while preserving fluorescent proteins for several months. In 2018, Zhao et al. developed a polyethylene glycol (PEG)-associated solvent system (PEGASOS), which rendered nearly all types of tissues transparent and preserved endogenous fluorescence.[3] In 2019, researchers further refined the technology, introducing vDISCO. Compared to previous transparency techniques, vDISCO increased the fluorescence signal intensity in mice by nearly 120 times. By introducing nanoantibodies into mice to label specific cell types, vDISCO achieved, for the first time, a true visualization of the state and interconnections of mouse cells, tissues, and organs without disrupting their structure, which may also be applicable for whole-body tracking of viruses, cancer cells, and even other invasive agents. Ali et al. report the detailed protocol for a pressure-driven, nanobody-based whole-body immunolabeling and clearing method, nanobody (VHH)-boosted 3D imaging of solvent-cleared organs, that renders whole mice transparent in 3 weeks, consistently enhancing the signal of fluorescent proteins, stabilizing them for years.[4] Additionally, the Transparent Embedding Solvent System (TESOS) method was developed to acquire volumetric images of uniform resolution for an adult mouse whole-body sample.[5]

On the other hand, several existing living animal models have been extensively utilized in experimental research. Zebrafish development occurs in a fully transparent state, enabling complete observation of the cardiovascular system, particularly the processes related to the immune system's development. As a result, zebrafish has become one of the most highly regarded vertebrate models in transparent-state biology.

Although these technological advancements have successfully achieved complete transparency in model organisms like mice, they rely on dissected animal models or early-stage transparent zebrafish embryos. No fully transparent living mouse model has yet been successfully developed. In addition, the design and screening molecules that can absorb UV light to achieve higher color transparency is also an attractive research area. Currently, researchers can induce transparency by manipulating genes to remove pigments from living tissues or reduce the light-scattering capacity of molecules within tissues. For instance, mutating the motif gene in zebrafish completely eliminates melanocytes. Furthermore, theoretically, transparency can be attained by genetically editing molecules related to align their refractive index with that of the cytoplasm, thereby reducing light scattering.

In this paper,[1] based on a deep understanding of optical principles, researchers predicted that dye molecules with a specific structure, when dissolved in water and absorbed by biological tissues, could enhance the refractive index of the tissue fluids by absorbing light of specific wavelengths, perfectly matching the refractive indices of surrounding fats and proteins, thereby effectively guiding light through biological tissues to achieve transparency. After evaluating the optical properties of various dyes, the research team determined that tartrazine matched the prediction. On the scalp of mice, the skin became transparent within minutes as the dye molecules diffused, revealing the blood vessels on the brain's surface; When applied to the abdomen, the internal organs became visible; Applying it to the legs allowed direct observation of muscle contractions. More importantly, the dye's effect is temporary and reversible on biological tissues, with the skin returning to its normal opacity after rinsing. Additionally, researchers found that residual tartrazine is excreted in the urine within 48 hours, with no apparent long-term effects on the living animals.

In summary, the application of this biocompatible dye enables the safe and non-invasive observation of deep tissues and organs within living animals in a reversible manner, facilitating the understanding of their structure and function. This technology is expected to enhance current optical imaging methods, aiding in the discovery of fundamental life processes in the fields of life sciences and medical research. Additionally, this approach has broad future applications, including enhanced visibility in venous blood draws, more convenient laser tattoo removal, and potentially aiding in the early detection and treatment of cancer, such as by enhancing light penetration for the removal of cancerous and pre-cancerous cells using lasers. However, to date, this tissue transparency effect has only been tested in laboratory animals. Researchers note that since human skin is approximately 10 times thicker than that of mice, further testing is required to determine the necessary dye dosage for skin penetration, and caution against direct application to humans.

REFERENCES

1. Ou, Z., Duh, Y.S., Rommelfanger N.J., et al. (2024). Achieving optical transparency in live animals with absorbing molecules. Science **385**: eadm6869. DOI: 10.1126/science.adm6869.
2. Ertürk, A., Becker, K., Jährling, N., et al. (2012). Three-dimensional imaging of solvent-cleared organs using 3DISCO. Nat. Protoc. **7**: 1983–1995. DOI: 10.1038/nprot.2012.119.
3. Jing, D., Zhang, S., Luo, W., et al. (2018). Tissue clearing of both hard and soft tissue organs with the PEGASOS method. Cell Res. **28**: 803–818. DOI: 10.1038/s41422-018-0049-z.
4. Cai, R., Kolabas, Z.I., Pan, C., et al. (2023). Whole-mouse clearing and imaging at the cellular level with vDISCO. Nat. Protoc. **18**: 1197–1242. DOI: 10.1038/s41596-022-00788-2.
5. Taylor, K.L., Lister, J.A., Zeng, Z., et al. (2011). Differentiated melanocyte cell division occurs in vivo and is promoted by mutations in Mitf. Development **138**: 3579–89. DOI: 10.1242/dev.064014.

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AUTHOR CONTRIBUTIONS

D.F. and Y.S.M. provided the conception, revision and supervision. H.Y. and J.B.L conducted the literature research and wrote the initial manuscript and made the figure. All authors have read and approved to publish the article.

DECLARATION OF INTERESTS

The authors declare no competing interests.