HYBRiD: a simple method for clearing and visualizing mammalian bodies

A simple and affordable technique passively clears and images whole mammalian bodies or large tissues. This technique is compatible with the use of endogeneous fluoresent proteins, without the loss of signal associated with other existing methods for whole-animal clearing.

This is a summary of:
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The problem

In recent years, whole-brain tissue clearing methods — which reduce cell opacity caused by membrane lipids without damaging biomolecules and cell structures — have revolutionized the 3D visualization of the mammalian central nervous system[1]. Clearing methods include those based on the use of organic solvents (hydrophobic methods) and aqueous methods that use hydrogel scaffolds. These methods have been adapted to clear large tissues or entire mammalian bodies, although each has challenges. Hydrophobic methods tend to quench endogenously expressed fluorophores, often requiring heavy use of costly immunolabelling reagents such as antibodies or nanobodies when clearing large samples. Conversely, aqueous methods are generally less able to fully clear all tissue types. These issues present a barrier to the widespread use of these techniques in whole-animal clearing experiments.

The solution

As we were trying to clear transgenic mice using several existing methods, we realized that exchanging individual components from the two seemingly distinct hydrophobic and hydrogel-based methods could remove some key limitations and amplify the advantages of each technique.

Indeed, we found that adding a hydrogel support step (from the CLARITY protocol)[2] to the more aggressive hydrophobic DISCO clearing procedure[3,4] remarkably accelerated delipidation and preserved the fluorescence signal of endogenously expressed fluorophores. This combination, which we named hydrogel-reinforced DISCO, or 'HYBRiD', therefore allowed us to avoid the use of delicate perfusion steps or expensive antibodies for signal amplification when clearing and visualizing an entire mammalian body. We demonstrate that using this new method, the entire body of a young mouse can be passively cleared using buffer exchanges alone, while preserving the signal of commonly used fluorescent proteins such as GFP, YFP, and tdTomato.

The implications

HYBRiD provides a simple and scalable method to screen and localize fluorescence signals across the entire mammalian body and is particularly suitable for the rapid characterization of transgenic mouse models (Fig. 1). It could also offer an easy way to visualize the peripheral nervous system and vasculature spread across multiple organ systems in 3D. HYBRiD is especially useful for field applications where the perfusion of the body is not readily feasible; for example, when working with biological hazards in a controlled environment.

There are some trade-offs with the simplicity of the technique. For example, although we demonstrate that passive HYBRiD can robustly clear a whole, 3-week-old juvenile mouse in ~6 weeks, it remains to be determined how long it would take to clear an adult mouse. Also, in practice, users will still be limited by the physical dimensions of the microscope they have access to when determining the size of the sample they want to clear.

Next, we look forward to integrating automated data analysis techniques with the large data sets generated by HYBRiD. Expanding the compatibility of HYBRiD to other labelling modalities such as nucleic acids and small-molecule dyes will be another exciting frontier to fully unleash the power of whole-body image analysis.

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EXPERT OPINION

"HYBRiD is an exciting concept as the strengths of solvent-based delipidation can synergize with the tissue stabilization brought by the hydrogel. Its application to the clearing of a mouse pup while preserving endogenous tdTomato fluorescence is particularly striking as early developmental stages would be difficult to immunolabel intact or perfuse continuously with existing whole-body clearing protocols. This is an excellent novel application and showcase of the protocol." **Nicholas Renier, Paris Brain Institute, Paris, France**

FIGURE

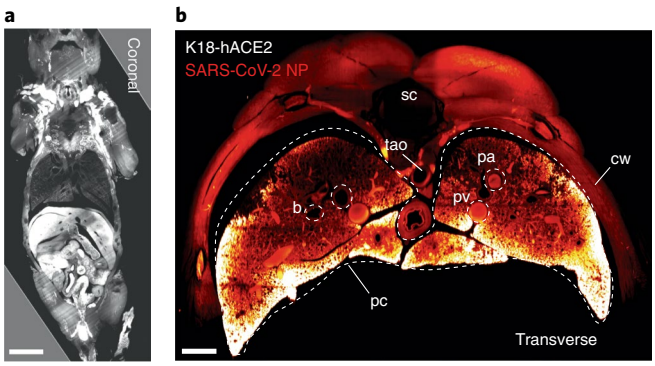

Fig. 1 | HYBRiD clearing enables visualization of mammalian bodies in 3D. a, Coronal view of a HYBRiD-cleared somatostatin (SST)-Ai9 (tdTomato) newborn mouse (500-μm digital slice), revealing the locations of SST-expressing cells across a whole mouse body. Scale bar, 2 mm. **b**, Transverse view through the chest of a HYBRiD-cleared, SARS-CoV-2 infected K18-hACE2 transgenic mouse. Red fluorescence is anti-SARS-CoV-2 nucleoprotein (NP) stained with AlexaFluor 647, showing the distribution of the virus across the mouse chest after infection. Scale bar, 1 mm. b, bronchi; cw, chest wall; pc, pleural cavity; pa, pulmonary artery; pv, pulmonary vein; sc, spinal cord; tao, thoracic aorta. © 2022, Nudell, V. et al.

BEHIND THE PAPER

We were exploring the use of different existing brain-clearing methods to visualize tdTomato expression across the mouse body without expecting to develop a new method. Soon, we realized that directly adapting brain clearing to somatic clearing would be too laborious and too expensive for a large cohort of animals. Victoria Nudell and Yu Wang bravely decided to mix and match several existing clearing protocols and carefully benchmarked all the combinations to each other and to the parental methods. Unexpectedly, we found that CLARITY and DISCO could be compatible after a few extra steps. This combination gave each method much greater tolerance and flexibility in terms of the clearing conditions, resulting in a surprisingly versatile clearing method for a wide range of tissues and fluorophores. **L.Y.**

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FROM THE EDITOR

"This tissue clearing method stood out because it combines elements of solvent-based and aqueous methods to achieve the best of both worlds: high transparency, maintenance of sample integrity and preservation of reporter fluorescence. Preserving fluorescence circumvents the need for antibodies, which can be prohibitively expensive for large samples." **Nina Vogt, Senior Editor, *Nature Methods***.