Clearing and Labeling Techniques for Large-Scale Biological Tissues

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Clearing and labeling techniques for large-scale biological tissues enable simultaneous extraction of molecular and structural information with minimal disassembly of the sample. We review clearing and labeling methods, and systems biology across different scales. Recent years have witnessed an explosive increase in the number of such methods and their applications, reflecting heightened interest in organ-wide clearing and labeling across many fields of biology and medicine. In this review, we provide an overview and comparison of existing clearing and labeling techniques and discuss challenges and opportunities in the investigations of large-scale biological systems.

INTRODUCTION

Biological tissues are stunningly complex, with each gram consisting of hundreds of millions of cells. Each cell can be classified into numerous genetically and functionally distinct cell types, and even within the same category, individual cells often show clear heterogeneity. In studying such a complex system, obtaining and correlating compositional (molecular) and three-dimensional (3D) spatial (structural and morphological) information across different length scales are of paramount importance. Most existing techniques, however, allow the investigation of only a small portion of the tissue at high resolution or a large area at low resolution. For example, typical histological techniques with fluorescence microscopy can yield clear images of subcellular structures only from micron-thick sections (Hoffman and Taylor, 2001); in contrast, existing large-scale 3D imaging techniques such as magnetic resonance imaging are limited in the spatial resolution and the molecular information they can provide (Huang et al., 1991; Muehllehner and Karp, 2006; Reiser et al., 2007; Van Essen et al., 2013). This challenge has been impeding both basic and clinical investigations of large tissues.

Increasing numbers of emerging clearing and labeling techniques for large-scale tissues enable the extraction of detailed 3D structural and molecular information across the millimeter-to-centimeter scale at sub-micron resolution (Fig. 1) (Dodt et al., 2007; Kim et al., 2013; Richardson and Lichtman, 2015; Susaki and Ueda, 2016). In turn, the rising interest in the study of large intact tissues in 3D is driving the development of many novel techniques from multiple laboratories. Here we review the existing clearing and labeling techniques for biological tissues, placing particular emphasis on the basic principles underlying each method. We also highlight challenges and opportunities in the development of future tissue processing techniques.

CLEARING TECHNIQUES FOR LARGE-SCALE TISSUES

The first tissue clearing technique dates back to 1910, when the German anatomist Walter Spalteholz developed an organic solution (a mixture of benzyl alcohol and methyl salicylate) to clear large tissues (Spalteholz, 1914). This pioneering work was revisited a century later when 3D fluorescence volume imaging gained in popularity (Dodt et al., 2007; McGurk et al., 2013). In turn, the rising interest in the study of large intact tissues in 3D is driving the development of many novel techniques from multiple laboratories. Here we review the existing clearing and labeling techniques for biological tissues, placing particular emphasis on the basic principles underlying each method. We also highlight challenges and opportunities in the development of future tissue processing techniques.
Clearing and Labeling Techniques for Large Tissues
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Fig. 1. Tissue clearing techniques. (A) Light scattering in biological tissues can be reduced by removal of lipid and RI matching. (B) Simple immersion in a high-RI aqueous solution renders the tissue modestly transparent by homogenizing scattering throughout the sample. (C) Delipidation and dehydration/hyperhydration followed by refractive index matching. (Top) For solvent-based clearing, the tissue is incubated in dehydrating solvent for delipidation and dehydration, and is moved to a high-RI clearing solvent where RI matching and additional delipidation occur. (Bottom) The sample is placed in an aqueous solution that contains high concentration of non-ionic detergent and denaturant, where delipidation, hyperhydration, and RI matching take place. (D) A biological sample is first transformed into a tissue-gel hybrid by hydrogel embedding (Top) or glutaraldehyde fixation (Bottom), where the gel network increases the tissue integrity. The tissue-gel hybrid then can withstand extensive delipidation by incubation in ionic detergent (SDS) assisted by electrophoresis or heating.

SIMPLE REFRACTIVE INDEX MATCHING

Matching the RI of a tissue to that of the medium reduces light scattering, the principal cause of tissue opaqueness. A tissue is a composite of biomolecules with different RIs (protein, RI~1.43; lipids, RI~1.44; water, RI~1.33), which collectively constitute the overall tissue RI of 1.4-1.5 (Bolin et al., 1989; Tuchin, 2015). Thus, simple incubation of tissues in the high-concentration solutions of formamide (ClearT, RI~1.44), formamide and polyethylene glycol (PEG) (ClearT2, RI~1.44) or fructose with α-thioglycerol (SeeDB, RI~1.49) can replace water and render the tissues modestly transparent (Ke et al., 2013; Kuwajima et al., 2013; Richardson and Lichtman, 2015). These methods exhibit weak-to-moderate clearing capabilities, but are easy to implement, reversible and economic. Each method retains different degrees of fluorescent signals: ClearT mostly quenches the signal from fluorescent proteins (FPs) as formamide disrupts the fluorophore, ClearT2 better preserves fluorescence by stabilizing proteins with PEG, and SeeDB well retains fluorescence as fructose does not disrupt FPs (Ke et al., 2013; Kuwajima et al., 2013). Most molecular probes do not penetrate well into the tissues processed with these techniques; their uses are mostly limited to the samples that are labeled in advance. These methods do not involve delipidation and preserve lipophilic dyes (e.g. DiI), which can be useful for tracing neurons in post-fixed tissues where genetic labeling is impossible.

While the concept of refractive index matching has been used to explain the clearing effect, it does not fully explain some empirical observations. For example, in human and porcine skin samples, no correlation between RI and clearing effect was observed (Choi et al., 2005; Mao et al., 2008); instead, the clearing potential (of alcohols) was related to the number of
Table 1. Comparison of tissue clearing methods. General properties and performances of major published clearing methods. The methods are categorized according to the main mode of action: RI matching by simple immersion, organic solvent-based clearing (dehydration, delipidation, RI matching), aqueous solution-based clearing (hyperhydration, delipidation, RI matching), and tissue-gel hybridization.

| Technique | Reagents | Clearing properties | Labeling properties |
|-----------|----------|---------------------|---------------------|
| **RI matching by simple immersion: aqueous-based clearing** | | | |
| Clear² | 95% formamide | − | Medium | Young adult mouse brain | 2-3 days | − | + | Yes (small) | − | Kuwajima et al., 2013 |
| Clear² | 50% formamide, 20% PEG | − | Medium | Young adult mouse brain | 2-3 days | ++ | + | No change | + | Kuwajima et al., 2013 |
| SeeDB | 80.2% fructose, 0.5% tri-glycerol | − | Weak | Young adult mouse brain | Several days | ++ | ++ | Yes (small) | − | Ke et al., 2013 |
| **Dehydration, delipidation and RI matching: solvent-based clearing** | | | |
| BABB | BABB | − | Strong | Adult mouse brain | 2-3 days | (half day) | − | Yes (small) | − | Doct et al., 2007 |
| 3DISCO | DBE, DOM | − | Very strong | Young adult mouse brain | 1-3 days | + | (1-2 days) | - | Shrinkage; hard and brittle | - | Entuk et al., 2012a, 2012b |
| IDISCO | DBE, DOM | − | Very strong | Adult mouse brain | 1-3 days | + | (2-4 days) | - | Yes (large) | 28 | Reiner et al., 2014 |
| **Hyperhydration, delipidation and RI matching: aqueous-based clearing** | | | |
| ScaleA² | 4M urea, 10% glycerol | 0.1% TX-100 | Medium | Adult mouse brain | 2 weeks | ++ | - | Expansion; soft and fragile | - | Hama et al., 2011 |
| ScaleS | 4M urea, sorbitol | 0.2% TX-100 | Strong | Old mouse brain | Several days | ++ | + | No change; firm and sectionable | Yes (limited) | 5 | Hama et al., 2015 |
| CUBIC | 4M urea, aminoalcohols | 15% or 0.1% TX-100 | Very strong | Neonatal marmoset brain | 1-2 weeks | + | - | Expansion | Yes (small) | 3 | Sussi et al., 2014 |
| CUBIC-Persion | 4M urea, aminoalcohols | 15% or 0.1% TX-100 | Very strong | Adult mouse brain | 2 weeks (whole body) | - | - | Yes (small) | 2 | Tainaka et al., 2014 |
| **Tissue-gel hybridization followed by delipidation and RI matching** | | | |
| Electrophoresis-assisted delipidation | | | |
| CLARITY | SDS, FocusClear | 4% SDS | A4P4 or A0.5P4 A0.0125 | 1.45 | Very strong | Adult mouse brain; 500-μm thick post-mortem human brain | 2-4 weeks | ++ | - | Yes (large); multi-round ISH (small) | 11 | Chung et al., 2013; Tomer et al., 2014 |
| SE-CLARITY | SDS, custom RI matching solution | 200 mM SDS | A4P4 | 1.46 | Very strong | Adult mouse brain | 1-3 days | ++ | - | Yes (large); Not tested | 3 | Kim et al., 2015 |
| ACT-PRESTO | SDS, RIMS⁺ (or CUBIC-mount) | 4% SDS | A4P0 | 1.43-1.48 | Very strong | Adult rabbit brain (modest transparency) | 2-3 days | ++ | - | Yes (large); ISH 75 | Lee et al., 2016 |
| **Passive delipidation** | | | |
| PACT | SDS, RIMS | 8% SDS | A4P0 | 1.38-1.48 | Very strong | Adult mouse brain and whole-body | ≥1 month | ++ | - | Minimal expansion | Yes (large); smFISH (small) | 8 | Yang, et al., 2014 |
| PARS | SDS, RIMS | 8% SDS | A4P0 | 1.38-1.48 | Very strong | Adult mouse brain and whole-body | 1-2 weeks | ++ | - | Minimal expansion; not tested | Yes (large); Not tested | 6 | Yang, et al., 2014 |
| **EDC-CLARITY** | SDS, FocusClear | 4% SDS | A4P4/80.0 or A0.5P0.1 M EDC | 1.45 | Very strong | Adult mouse brain | 2-4 weeks | ++ | - | Shrinkage (during hybridization and stringency wash) | Multiplexed ISH using DNA-based amplification (large) | - | Sylvestrak et al., 2016 |
| SWITCH | SDS, custom RI matching solution | 200 mM SDS | G1P4; pH 3.4% GA, pH 7.1% GA | 1.47 | Very strong (mild browning) | Adult rat and young marmoset brains | 4 days-2 weeks | − | − | Minimal expansion; hardening | Yes (large); multi-round ISH (+20) | 86 | Murray et al., 2015 |

*The largest brain tissue used in the original studies.

¹Time required for clearing a whole mouse brain or hemisphere (not including staining).

²Qualitative evaluations based on recent comparative experiments (Economou et al., 2016; Hama et al., 2015).

³Largest indicates millimeter-scale samples such as whole organs. Note that immunostaining compatibility was not demonstrated in the original ScaleS paper (alternative protocol termed AbScale is required for immunostaining). CLARITY demonstrated in situ hybridization in 500-μm brain blocks. smFISH was demonstrated in PACT-processed 100-μm brain slices. In EDC-CLARITY method, specific RNAs were detected in 2-mm block of mouse cortex by DNA-based signal amplification method (Hybridization Chain Reaction).
hydroxyl groups in the medium (Mao et al., 2008). Likewise, a high-RI zinc iodide solution was ineffective in clearing brain tissues (Ke et al., 2013), and 85% glycerol solution did not clear the tissue as efficiently as FocusClear, although the two solutions have approximately same RI (Chung et al., 2013). These indicate that chemical properties of the medium, as well as physical properties such as RI, strongly influence tissue transparency; comprehensive investigations on the mechanism of tissue clearing will be necessary to rationalize and refine existing tissue clearing protocols in the coming years.

**DELIPIDATION AND DEHYDRATION/HYPERHYDRATION FOLLOWED BY REFRACTIVE INDEX MATCHING**

Lipids are a major source of light scattering in biological samples because of their high RI and their tendency to form granular structures (main reason for tissue’s milky appearance) (Tuchin, 2015). As such, many clearing techniques remove lipids from the sample to achieve optical homogeneity and transparency. The methods that involve delipidation step can be sub-divided into two groups, one that uses organic solvents and the other that utilizes urea and detergents. Each technique typically involves dehydration and hyperhydration, respectively.

Clearing methods based on organic solvents, such as 3DISCO, dehydrate a tissue to render the tissue optically homogeneous (RI of dehydrated proteins is > 1.5) and match the RI with the solvents (RI = 1.56). The solvent-based methods have better optical clearing capability and are generally faster than the simple immersions; they yield more transparent samples than Clear	extsuperscript{2}, Clear	extsuperscript{2} and SeeDB (Hama et al., 2015), and benzyl alcohol and benzyl benzoate (BABB)-based method and 3DISCO can clear a whole mouse brain in 2-3 days (Dodt et al., 2007; Ertürk et al., 2012a). The organic solvents rapidly quench FPs, however, severely restricting time for imaging and storage. For example, 3DISCO uses dibenzyl ether (DBE) and tetrahydrofuran (THF) to better retain the fluorescence emissions, but the half-life of green fluorescent protein (GFP) signal in the final clearing solution was still only 1-2 days (Ertürk et al., 2012b). As an alternative, in IDISCO, molecules of interests are immuno-labeled with antibodies conjugated with Alexa Fluor that are relatively stable in organic solvents, to circumvent the FP quenching problem (Renier et al., 2014). Additionally, the dehydrated and delipidated tissues are substantially shrunken in organic solvents, which may be advantageous for imaging a large volume due to the reduced size, albeit at a reduced resolution (Hama et al., 2015; Richardson and Lichtman, 2015). In these methods, the toxic organic solvents must be carefully handled.

Aqueous-based solutions containing non-ionic detergents (e.g. Triton X-100) and denaturing agents (e.g. urea) can also clear tissues effectively with less of the quenching and toxicity problems. In these methods, the detergents remove lipids, while the urea solution partially denatures endogenous proteins, facilitates hyperhydration of biomolecules (Hua et al., 2008; Richardson and Lichtman, 2015), and matches the overall tissue RI to ~1.38 (Hama et al., 2011). ScaE2, ScaS, CUBIC and CUBIC-perfusion are the examples of such methods. CUBIC protocol uses basic imidazole-alcohol-based cocktails in addition to urea and Triton X-100 to enhance its clearing capability (Susaki et al., 2014). A modified CUBIC clearing reagent—used in CUBIC-perfusion—elutes hemochromophores from red blood cells, the principal light absorber in the blood, to further increase tissue transparency (Tainaka et al., 2014). Perfusing the CUBIC solution for two weeks can clear a whole body of infant and adult mouse while preserving FPs (Susaki et al., 2015; Tainaka et al., 2014). These methods are beneficial due to the use of nontoxic reagents, ease of implementation, and their strong clearing capability, but the samples can be damaged by hydration-induced expansion (urea can expand samples considerably as shown in ScaE2—1.26x linearly—which can distort micro- and macrostructures of the tissues; Hama et al., 2011) and high concentration of detergents. ScaS uses sorbitol, a mild tissue-permeant sugar alcohol with dehydrating property, to counterbalance the tissue expansion caused by urea (Hama et al., 2015). In addition, ScaS incorporates ≤ 0.2% of Triton X-100 to avoid compromising tissue integrity and attenuating fluorescence signals (Hama et al., 2015). As such, ScaES preserves the ultrastructure (evaluated by electron microscopy) that is damaged in other techniques (Hama et al., 2015).

**TISSUE-GEL HYBRIDIZATION FOLLOWED BY DELIPIDATION AND REFRACTIVE INDEX MATCHING**

Native biomolecules and their contextual information can be lost during harsh clearing conditions that involve organic solvents or high concentrations of detergents. In CLARITY and its variants, a gel is embedded throughout a tissue to effectively transmute the sample into a tissue-gel hybrid, wherein the gel network provides an extra support for the biological structures and bolsters tissue integrity (Chung et al., 2013; Kim et al., 2015; Murray et al., 2015; Tomer et al., 2014; Yang et al., 2014). The tissue-gel hybrid can undergo extensive delipidation by strong ionic detergent, sodium dodecyl sulfate (SDS), while well-preserving structures and molecules covalently linked to the gel network. Delipidation can be achieved by slow but simple incubation in the SDS solution for several weeks (Tomer et al., 2014), but can also be expedited by electrophoresis (Chung et al., 2013; Tomer et al., 2014), a newly devised electrophoresis strategy termed stochastic electrotransport (Kim et al., 2015), thermal energy (Murray et al., 2015), or perfusion of SDS solution (Yang et al., 2014). Delipidated samples are immersed in commercially available RI-matching solutions (e.g. FocusClear or Easy-Index) or custom-made solutions (e.g. RIMS, 2,2’-thiodiethanol (TDE), 80% glycerol, CUBIC-mount or other unnamed recipes) for full optical clearing (Aoyagi et al., 2015; Chung et al., 2013; Costantini et al., 2015; Kim et al., 2015; Lee et al., 2016; Yang et al., 2014). CLARITY, PACT, PARS, stochastic electrotransport and SWITCH all exhibit very strong clearing performance, owing to the effective lipid removal (Chung et al., 2013; Hama et al., 2015; Kim et al., 2015; Murray et al., 2015; Tomer et al., 2014; Yang et al., 2014). These methods do not quench FPs (with the exception of SWITCH, which uses heat to expedite clearing) and use mostly nontoxic chemicals. The clearing time varies depending on how lipids are removed, with the passive incubation being the slowest (more than a month for a mouse brain) and the stochastic electrotransport the fastest (2-3 days for a mouse brain). Notably, delipidation increases hybrid porosity, facilitating the diffusion of molecular probes (e.g. antibodies) for labeling.

The original CLARITY employs electrophoresis for lipid removal, but this requires a special electrophoresis chamber and the protocol is difficult to implement. Also, the strong electric field can compromise the molecular and structural integrity of the tissue, as the electric field exerts forces on the charged endogenous biomolecules (e.g. nucleic acids and proteins) as well as the SDS micelles. Three CLARITY-based approaches have been developed with the motivation to address these problems: (1) low-density tissue-gel hybrid formation followed...
by passive delipidation (passive CLARITY, PACT and PARS),
(2) high-density dialehydrate-tissue-gel formation followed by
thermally facilitated delipidation (SWITCH) and (3) stochastic
electrotransport-assisted active delipidation.

Passive CLARITY, PACT and PARS use low concentrations
(even zero) of fixatives and hydrogel monomers (acrylamide
and bis-acrylamide) to allow users to clear large samples sim-}
ply and easily by incubating in SDS solution within a reasonable
timeframe (weeks) (Tomer et al., 2014; Yang et al., 2014). Re-
ducing tissue-hydrogel hybrid density effectively facilitates
the diffusion of bulky SDS micelles, but at the cost of structural
and molecular integrity, requiring special attention for optimizing
the hydrogel composition and clearing condition.

In contrast, SWITCH takes the opposite approach of trans-
foming a tissue to an even denser and more robust hybrid
structure that allows using harsh conditions to expedite clearing.
In SWITCH, glutaraldehyde (GA), a bifunctional crosslinker, is
infused throughout the tissue, crosslinking biomolecules much
more strongly than formaldehyde used in other methods. This
significantly increases the heat-, chemical- and physical re-
sistance of the sample (Hopwood, 1972; Murray et al., 2015;
Sung et al., 1996), allowing for efficient passive clearing at high
temperatures of 70–80°C without compromising the molecular
and structural integrity of the sample. This approach is simple
and rapid (adult mouse hemisphere cleared in 12 h at 80°C)
(Murray et al., 2015). However, fluorescence is lost under the
harsh condition, which necessitates the additional labeling.

Stochastic electrotransport is a new electrokinetic method us-
ing a rotational electric field to selectively facilitate the disper-
sion of highly electromobile molecules, such as SDS micelles
(Kim et al., 2015). This technique also uses a temperature-
controlled circulating system to prevent Joule heating from
damaging the sample and nanoporous membranes to minimize
the electrolysis of SDS (Kim et al., 2015). When applied to gel-
embedded tissues to remove lipids, stochastic electrotransport
can substantially accelerate the clearing process (∼10 times
faster than the original CLARITY method) without damaging the
tissue sample or quenching the FP signals (Kim et al., 2015).
Stochas-
tic electrotransport requires even more complex electrophoresis
device for its implementation than CLARITY does, but the device
and the associated buffers are commercially available.

**LABELING TECHNIQUES FOR LARGE-SCALE TISSUES**

How can we observe and extract useful structural and contex-
tual biological information from the cleared tissues? Labeling
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within one day. Stochastic electrotransport-assisted whole-mount labeling is compatible with the tissues cleared with CLARITY, CUBIC and SWITCH (Kim et al., 2015; unpublished observations).

**INCREASING CONCENTRATION GRADIENT AND MODULATING PROBE-TARGET BINDING REACTION KINETICS**

SWITCH enhances probe penetration by increasing the concentration gradient of the probes (i.e. using high concentration of probes) and controlling kinetics of probe-target binding reactions inside a tissue (Murray et al., 2015). Using high concentration of probes can lead to high level of nonspecific binding and saturated signals at the surface; to tackle this issue, SWITCH uses a set of buffers (SWITCH-Off & SWITCH-On) that reversibly modulate chemical reaction kinetics. Specifically, SWITCH-Off buffer inhibits the binding reaction between the labeling probes and their targets, and SWITCH-On buffer facilitates the reaction. In SWITCH-Off buffer, molecular probes (e.g. antibodies) are first dispersed throughout the tissue. The buffer is then "switched" to SWITCH-On buffer, enabling the binding reaction to occur. In this way, SWITCH enhances both probe penetration and labeling uniformity—myelinated fibers throughout a mouse hemisphere could be uniformly stained in 9 days. Furthermore, SWITCH transforms a tissue into a robust dialehyde-tissue-gel, which can support more rounds of labeling and elution. With this method, 22 rounds of antibody labeling were demonstrated in a single tissue (Murray et al., 2015).

**DISCUSSION AND CONCLUSION**

Understanding complex biological systems in native 3D context has long been an unmet goal in biology and medicine, mainly due to the physical constraints imposed by light scattering and slow molecular diffusion. Recent advances in clearing and labeling techniques significantly improved scalability and throughput, making tissue-level investigations more accessible than ever before; the techniques have been applied to a wide range of biological systems, from a small mouse embryo to mouse whole organs, marmoset brains, postmortem human brain tissues and even plant organs (ClearSee and PEA-CLARITY demonstrated clearing of plant tissues; see Kurihara et al., 2015; Palmer et al., 2015; Warner et al., 2014). While more creative methods will be developed and introduced to the field, immediate improvements can be made by combining strengths of the existing methods. For example, the concept of SWITCH can be combined with other immersion-based clearing methods to facilitate uniform molecular delivery into the tissue, or with stochastic electrotранsport to further accelerate clearing and labeling.

A common challenge in all tissue clearing and labeling techniques is the lack of quantitative measurement on the loss of molecular integrity during tissue processing. In most reports, a few microscopic images from a small portion of the sample are used to demonstrate the retention of nucleic acids and proteins. However, this cannot convincingly demonstrate the molecular integrity of the tissue; at the very least, quantitative biochemical measurements on the loss of biomolecules should be accompanied as complementary measures. Most simple immersion, hyperhydration, and solvent-based clearing methods published thus far did not quantitatively measure the loss of biomolecules, even though significant loss of proteins as well as lipids is likely to occur during the incubation in organic solvents, urea, and detergents. Only EDC-CLARITY (a recently published variation to CLARITY that uses carbodiimide-based chemistry to improve RNA preservation and detection in the tissue-gel hybrid network) provides comprehensive and quantitative measurements of RNA retention (Sylwewstrak et al., 2016). Likewise, we believe that future tissue clearing protocols should set and follow rigorous experimental standards for measuring the loss of molecular information, as developing more preservative tissue processing techniques will become more important in the coming years (Pallotto et al., 2015).

Decreased or quenched FP signal and increased autofluorescence after tissue processing present another challenge. Essentially all tissue clearing and labeling techniques expose the sample to the condition that partially or fully quenches FP signal, and alter biochemical properties of the tissue that sometimes result in increased autofluorescence (e.g. arising from Schiff bases formed by aldehyde fixation). While minimizing such alterations is a way to approach the problem, amplifying the fluorescence signals of target molecules could also effectively address the issue. For example, recent advances in molecular amplification tools, such as SunTag (protein, up to 24-fold) and hybridization chain reaction (mRNA, approximately 200-fold), can be synergistically combined with tissue clearing to enable deep and stable imaging (Choi et al., 2010; 2014; Tanenbaum et al., 2014). Most recently, EDC-CLARITY leveraged hybridization chain reaction to detect microRNAs and mRNAs in clarified mouse and human tissues (Sylwewstrak et al., 2016). Such signal amplification would enable imaging of low-copy-number molecules with long working distance objective lenses, which have limited numerical aperture.

High-throughput imaging of a large volume of cleared tissues is a significant challenge, as conventional scanning-based microscopy (e.g. confocal or two-photon laser scanning microscopy) would take many weeks to months for sub-cellular resolution imaging of a cubic centimeter-sized tissue. This issue is now addressable with emerging light-sheet microscopy (LSM) (Keller and Ahrens, 2015). LSM is advantageous in that its configuration not only allows for fast imaging by simultaneous illumination of an entire plane, but also reduces the energy load of excitation light on the specimen, thereby minimizing photobleaching and phototoxic effects. Among the various LSM techniques, COLM (CLARITY-Optimized Light-sheet Microscopy), the LSM method optimized for CLARITY-processed large-scale tissues, can achieve imaging of an entire mouse brain in several hours—two orders of magnitude faster than conventional scanning-based microscopy (Tomer et al., 2014). Recently developed SPED (SPHERical-aberration-assisted Extended Depth-of-field) light sheet microscopy further increases the imaging speed by extending the detection depth of field, which eliminates the requirement to move heavy detection objectives (Tomer et al., 2015).

The resulting terabytes of massive volumetric images is already calling for the extensive innovation in data management and analysis. At the same time, we must find ways to turn the vast amount of data from organ-wide experiments into new biological insights and discoveries. Clearing and labeling methods for large-scale biological tissues will continue to evolve in the coming years, and so will imaging and analysis technologies. With concerted and collaborative efforts, molecular, chemical, and optical profiling of large-scale biological systems will find many more applications in the near future.

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