Computational approaches to data generated by 3D imagining of cleared nervous system tissues: A meta-analysis of reviews from 2020-21

Johanna W. Rhyner[1] ✉

ENGW3307 Project 2 Final Draft; Style: Nature Reviews Neuroscience; Word count: 4413

Abstract | Developments in neuroscientific methods, such as the rise of tissue clearing, allow researchers to examine the nervous system with a level of detail previously unimaginable. The large and complex data generated by 3D imaging of cleared tissues requires novel, innovative computational approaches. This review synthesizes trends identified in seven recent (2020-2021) reviews of tissue clearing in neuroscience, beginning with background on tissue clearing and associated labelling and imaging techniques. Computational approaches examined address four main areas: image processing, image analysis, brain atlases, and image visualization. In addition to modular options, these may be part of integrated software ecosystems or custom pipelines. This review analyzes currently available software and identifies gaps between software needs and capabilities by looking at specific neuroscientific applications.

Tissue clearing's use of chemicals to turn blocks of tissue transparent allows incredibly detailed insight into even the deepest structures of the nervous system[1] while preserving spatial resolution in three dimensions[2]. It has provided valuable insight into both normal and pathological neural circuitry[3], neuroanatomy, nervous system development, and brain microvasculature[1], and has been applied to a wide range of tissue samples, including whole brains of small animals[4]. Developments in light-sheet microscopy have increased imaging resolution capabilities[5], resulting in increasingly detailed data. Data of this size and complexity requires significant computational power and software capabilities to handle and interpret. This meta-analysis examines seven reviews of tissue clearing in neuroscience from the past two years to evaluate current computational approaches to neuroscientific tissue clearing data and identify areas with significant gaps.

[1]*Department of Psychology, Northeastern University, Boston, MA, USA.*

✉*e-mail: rhyner.j@northeastern.edu*

Tissue clearing in neuroscience

Tissue clearing, regardless of methodology, relies on the principle of refractive indices (RI). Differences in RIs of various tissue components scatter light, which is perceived as opacity[1,6] and limits the penetration depth of light microscopy[3,6,7]. Tissue clearing techniques homogenize RI values, making tissue transparent while preserving the tissue's 3D structure[1-6]. This is particularly important in neuroscience, where structure and function are often tightly coupled.

Tissue clearing has been used to study development, injury, disease, and aging in the central as well as the peripheral nervous system[3,6,7]. This has been done, for example, through the profiling of cells, circuits, and synapses[2,5,7], as well as by examining blood vessel morphology[2]. Each of these applications has unique requirements, motivating incredible diversity among tissue clearing methods. The characteristics of the tissue clearing method applied, the aims of the study, and the resources of the researchers dictate which complementary labelling and imaging techniques are used. Researchers may use existing, custom, or combined computational approaches to handle and analyze the resulting data. While this review focuses on computational methods, it will briefly examine tissue clearing, labelling, and imaging techniques as well.

Table 1 | **Tissue clearing terminology in reviews examined**

Ref	Terms
1	Hydrophobic, hydrophilic, hydrogel-based, tissue-expansion
2	Solvent, simple immersion, hyperhydrating solution, tissue transformation (hydrogel)-based clearing
3	Solvent-based, simple immersion, hyperhydation, hydrogel embedding
4	Hydrophobic, hydrophilic, hydrogel-based
5	Hydrophobic, hydrophilic, hydrogel-based
6	Hydrophobic-based, hydrophilic, hydrogel-based
7	Solvent-based, hydrogel, aqueous-based (including simple immersion, delipidation/hydration)

Classifications and methods

There is a lack of consistency in the classification of tissue clearing methods, both in standards and in terminology. J. Zhao et al. examine these in Table 2 of their review[1]. The reviews mentioned here all classify tissue clearing methods based on their chemical principles and clearing mechanisms, but differ in their exact terminology (Table 1). All reviews use hydrogel-based clearing as a category, but only J. Zhao et al. separate out tissue expansion as its own category[1]. Other categories are less consistent between reviews. Four refer to hydrophobic clearing[1,4-6], while the remaining three name this category solvent-based clearing[2,3,7]. The final category, which four of the reviews call hydrophilic clearing[1,4-6], is the most varied. Tian and Li use aqueous-based, but specify simple immersion and delipidation/hydration as subcategories[7], while both Liang and Lou and Porter and Morton use two separate categories: simple immersion and hyperhydation[2,3]. This review uses hydrophobic, hydrophilic, and hydrogel-based, as these are the most frequently mentioned terms.

Each method has benefits and drawbacks, and the optimum method depends largely on the application. Generally, hydrophobic methods are faster, simpler, and result in higher transparency than other categories, but this comes at the cost of increased fluorescence bleaching, harsher lipid removal, and toxicity[1,6]. Hydrophilic methods tend to be safer, milder, and are more compatible with fluorescent labelling, but take longer[1,6]. Hydrogel-based methods allow multiplexed labelling, macromolecular preservation, and RNA detection, but are significantly more demanding in both equipment and technical operation required, and take the longest[1,6]. Hydrogel-based methods can also cause tissue expansion, which has been intentionally amplified to produce tissue-expansion methods[1].

Labelling

Labelling can either be applied before tissue clearing, in which case preserving fluorescence is the main concern, or after tissue clearing, in which case fluorescence preservation is not a concern but permeability is; these methods may also be combined[1]. Computational programs detect and analyze these signals and their distribution. For instance, ClearMap (see Table 2 and "Image analysis") can automatically map immediate early gene (IEG) expression[1,3].

Labelling can identify cell types, neuronal activity, neural circuits, and tissue morphology and molecular composition[7]. The type of labelling, and

the computational analysis performed, depends on the requirements. The properties of different labelling types are outside of the scope of this review. Broadly, pre-clearing approaches are usually genetically driven, such as via transgenic lines or viral vectors, while post-clearing approaches tend to use antibodies or chemical dyes[1,7]. The computational analyses are discussed under "Image analysis."

Imaging

Optical sectioning microscopy falls into three categories: confocal laser microscopy, multiphoton microscopy, and light-sheet fluorescence microscopy[1]. Within these, there are variations, particularly within light-sheet microscopy[5]. While optical sectioning microscopy is not the only option for imaging of tissue clearing, it is the most commonly used[1].

High-resolution microscopy produces huge volumes of image tiles when the microscope's field of view is smaller than the specimen, which is typical for cleared tissues[4]. These tiles must then be computationally stitched back together into images[1,4,5]. Furthermore, imaging of cleared tissues produces very large datasets. For instance, a single mouse brain generates anywhere from 100 GB to 30 TB of data[5]. The size of datasets produced by imaging of cleared tissues makes data management and analysis computationally expensive and challenging.

Imaging data

The data generated by imaging of cleared tissues require multiple processing steps before they can be analyzed or visualized. The 3D nature of the data complicates these steps, and though 2D approaches are adaptable, they are limited compared to 3D-specific software.

Image processing

All seven reviews examined mention data handling and analysis as a major challenge in research applications of tissue clearing, especially when large sections of tissue are of interest[1-7]. To handle tissue clearing data, Tian and Li highlight the need for powerful computational hardware, with at least 128 GB of RAM, multicore CPUs, modern GPUs, and efficient SSD storage[7]. Proper data management becomes increasingly important and increasingly difficult as data volume increases. Data of this size is often compressed to facilitate its storage and handling, requiring file formats well suited to compression and decompression[2,4]. Both Ueda et al. and J. Zhao et al. highlight KLB as an image file format commonly used for tissue clearing data, as its block-based implementation enables lossless compression, fast read and write speeds, efficient region-specific image access, and CPU-based processing[1,4]. Ueda et al. also discuss hierarchical data format version 5 (HDF5), another file format created to work with data that is significant in size and complexity[4]. Multiple file formats can also be integrated as containers for each other. The other reviews do not mention specific file formats, though Parra-Damas and Saura address the need for standardization in this area[6], indicating that there is still a significant degree of variation in formats used. A lack of standardization impedes data sharing, as data in a format not compatible with the recipient's software will need to be converted, which can be computationally expensive.

Image registration is essential to working with tiled image data. Techniques for cross-registration of image stacks include rigid affine and nonrigid B-spline registration[1]. This step is necessary for many subsequent image processing and analysis steps, such as image reconstruction[4]. While a fully automated registration algorithm has not yet been successfully implemented, Bigwarp, an ImageJ plugin (Table 3), allows for semi-automated registration. Bigwarp has other capabilities, but the registration aspect is the most emphasized in J. Zhao et al. and Ueda et al.[1,4]. Bigwarp was developed for the Fiji distribution of ImageJ[4]. Fiji is the most common ImageJ distribution, and the two are often used interchangeably[2]. For simplicity, this review refers to the software as ImageJ.

Once tiles generated by tissue clearing imaging are registered, they can be reconstructed into cohesive images by a process often referred to as "image stitching," which includes aligning the tiles properly and fusing them together[1,4]. This can be done with integrated image processing software (Table 2), such as arivis Vision4D[1,2,7], through

Table 2 | **Summary of open source and commercial software applied to cleared tissue data**

Name	Functionality	Licensing	Refs
Amira	Integrated image processing and analysis	Commercial	2,7
arivis Vision4D	Integrated image processing and analysis	Commercial	1,2,7
bioView3D	Image visualization	Open source	1,7
CATMAID	Image visualization, image annotation, data sharing	Open source	4
Cellprofiler	Cell phenotyping	Open source	2
ClearMap	Object detection and registration	Open source	1-3,6
DeepMACT	Metastasis quantification	Open source	1,2,6
iLastik	Image classification, image segmentation	Open source	4
ImageJ	Integrated image processing and analysis	Open source	1,2,4,7
Image-Pro Plus	Integrated image processing and analysis	Commercial	2
Imaris	Integrated image processing and analysis	Commercial	1,2,4,7
ManSegTool	Manual image segmentation	Open source	3
Neu Tracer	Neuronal tracing	Open source	1
NeuTube	Neuron reconstruction, neuronal tracing	Open source	1
qBrain	Cell phenotyping	Open source	5
RINZO	Nucleus distance	Open source	2
TeraFly	Image visualization, image annotation	Open source	1,4
TrailMap	Axonal mapping	Open source	1
TeraStitcher	Image stitching	Open source	1
Vaa3D	Integrated image processing and analysis	Open source	1,7
VesSAP	Vascular mapping	Open source	1,2

plugins (Table 3), like BigDataViewer[1,4,7] or BigStitcher[1,4,5] for ImageJ, or with a dedicated stitching software (Table 2), such as TeraStitcher[1]. Of these, BigDataViewer, part of the extensive and entirely open source ImageJ ecosystem[1,4], is mentioned by the most reviewers, indicating its

popularity. Notably, BigStitcher is built on BigDataViewer[4], demonstrating the highly interdependent nature of some specific software plugins. Arivis Vision4D, on the other hand, is a commercial software[1,2,7]. While it is mentioned in the same number of reviews, only J. Zhao et al. address its stitching capabilities[1]. Presumably, this software places a greater emphasis on image stitching than other commercial integrated software, but, unlike the previously mentioned ImageJ plugins, does not single out this functionality. Integrated software approaches to image data, such as arivis Vision4D and ImageJ, are further explored under "Integrated software ecosystems."

Image analysis

Once images have been processed, researchers can perform a variety of analyses, depending on their research question. While this may be supplemented with manual analysis, the bulk is ideally automated to increase efficiency and decrease tedium. One common application is the detection of objects, such as cells and/or nuclei, usually ones previously labelled. Since the detection specifications may vary significantly, researchers may opt to develop a custom computational pipeline, which could focus specifically on identifying labelled cells[6] or identifying all cells within a region, or even the whole brain[5]. ClearMap, built for iDISCO+, is a widely used open source software for automated object detection, and furthermore automatically registers the data it gathers (Table 2)[1-3,6]. The reviews emphasize different aspects of ClearMap. Liang and Luo and Porter and Morton highlight ClearMap's neuronal activity mapping capabilities, though only the latter pair discusses the registration of this information[2,3]. Parra-Damas and Saura mention it in reference to plaque quantification in Alzheimer's[6]. J. Zhao et al.'s description is the broadest, summarising the software's detection and registration capabilities[1]. Since its inception, ClearMap has been applied well beyond its original intention.

Detection is sometimes used for simple quantification[5], but may also be part of a more complex operation, such as segmentation or phenotyping. Segmentation broadly differentiates different tissue types. Integrated software packages, such as Amira, Imaris, Image-Pro Plus, and arivis Vision4D usually include segmentation options[2]. Only one review explicitly states this, revealing a general assumption that integrated software packages address this type of analysis. Additionally, some software is specific to image segmentation. Traditionally, segmentation is done manually or with a filter based approach, but machine learning offers entirely automated options[1]. ILastik (Table 2) is a machine learning-based software that allows users to train and use the model through a GUI, making it accessible for researchers without extensive experience in machine learning[4]. Still, some software focus on enabling manual segmentation, as the accuracy of automated segmentation depends largely on the similarity between the training data and the data being analyzed. ManSegTool is a segmentation-specific software (Table 2)[3]; MaMuT is similar, but is an ImageJ plugin rather than its own software (Table 3)[4]. BigStitcher and Bigwarp (Table 3) also both have segmentation options[4]. No segmentation-specific software is mentioned in more than one review, implying that none are significantly better than segmentation included in integrated software. Whether this is a strength of integrated software, a weakness of specific software, or both is unclear.

Phenotyping is more specific than segmentation, focusing on profiling properties of cell types. It is also less commonly used in cleared tissues. Cellprofiler is a Python-based software primarily for cell profiling, though plugins lend it additional flexibility[2]. QBrain, on the other hand, is entirely specific to cell profiling, and integrates convolutional neural networks into genetic-based neuroscience methods[5]. For more detailed examination of neuronal properties, specialized software and plugins allow tracing and analysis of neurons and their parts. Neuronal tracing can be accomplished by software such as NeuTube and Neu Tracer (Table 2)[1], while plugins like Simple Neurite Tracer and Sholl Analysis provide further options for neuronal analysis (Table 3)[2]. There is no dominantly used software for phenotype analysis in cleared tissues, which may be due to

Table 3 | **ImageJ plugins specific or applicable to data generated by imaging of cleared tissues***

Name	Software Base	Functionality	Refs
BigDataViewer	ImageJ (Fiji)	Image stitching, image visualization	1,4,7
BigStitcher	ImageJ (Fiji)	Image stitching	1,4,5
Bigwarp	ImageJ (Fiji)	Image alignment, image transformation, semi-automated registration	1,4
MaMuT	ImageJ (Fiji)	Manual segmentation, cell tracking	4
Sholl Analysis	ImageJ (Fiji)	Neuron analysis	2
Simple Neurite Tracer	ImageJ (Fiji)	Neuron analysis	2

*Based on the Fiji distribution of ImageJ

lacking or varied phenotype research in this area or deficits in software capabilities.

After objects have been identified, they can be mapped anatomically. Anatomical mapping takes advantage of the structural preservation in tissue clearing and frequently includes the integration of brain atlases (see "Brain atlases") to provide reference points. ClearMap, for example, can neuroanatomically map neuronal activity (Table 2)[2,6]. Mapping software, including ClearMap, tends to be application-specific. Unsurprisingly, discussion of mapping software varies between reviews. J. Zhao et al. mention TrailMap, which uses machine learning to map axonal projections[1] and vesSAP, which focuses on vascular mapping and is also mentioned in Liang & Luo[1,2]. Ueda et al. discusses WholeBrain and Openbrainmap (Table 4), which draw on the Allen Brain Atlas (see "Brain atlases") and together form a framework for reference mapping, visualization, and data sharing, but only in one of their reviews[4]. As mapping needs tend to be more specific than other areas, this area is largely dominated by custom approaches with little overlap. Mapping can also refer to the registration of anatomical information to brain atlases, which is discussed in the "Brain atlases" section.

Image analysis in neuroscience often focuses on pathology, and certain software is specific to this purpose. DeepMACT was developed for the quantification of cancer metastasis (Table 2), although it may be adaptable to related pathological analysis[1,2]. In other cases, researchers have adapted existing software, such as ClearMap, to their research question, or developed a custom pipeline[6]. Still, Parra-Damas and Saura highlight the need for further development of computational tools for neuropathological analysis[6]. This pattern holds true for other areas of neuroscience research as well, part of a broader issue of balancing generalizability and specificity in computational approaches. The 3D nature of the data produced by tissue clearing poses additional challenges to software traditionally used in bioimage analysis, although all those mentioned here have been adapted.

Image visualization

Visualizing 3D images poses a unique challenge, one complicated by the size of the images and not entirely mitigated by compression. Visualization is a standard component of integrated image processing and analysis platforms, such as Imaris (Table 2)[1,7]. Of all the visualization software examined in these reviews, bioView3D (Table 2) is the most specific to 3D visualization[1,7]. TeraFly provides image visualization options, in addition to image annotation (Table 2)[1,4]. BigDataViewer, an ImageJ plugin, also has some image stitching capability, and is the foundation for the stitching-specific BigStitcher (Table 3)[1,4,7]. BigDataViewer is mentioned the most often in

Table 4 | **Packages and libraries relevant to tissue clearing data***

Name	Software Base	Functionality	Ref
ImgLib2	ImageJ	Generic image processing	4
Openbrainmap	R	Image visualization, data sharing	3,4
WholeBrain	R	Brain mapping, atlas integration	3,4

*All packages and libraries mentioned here are open source

these reviews, and seems to be commonly used. This is likely because, as part of the ImageJ ecosystem, it is open source and easy to integrate with other image processing and analysis software, while retaining the necessary specificity to perform well. Some other software have visualization capability to support their primary purpose. The R-based Openbrainmap integrates image visualization into its mapping framework[3,4]. Similarly, CATMAID, while primarily for data collaboration, facilitates this by including data visualization capabilities[4]. Variations in visualization capabilities aside, the additional features or integration capabilities offered by each data visualization software dictate the software's suitability.

Brain atlases

Tissue clearing images and analytic data may be registered to a brain atlas. Brain atlases combine detailed anatomical data from the whole brain, creating a sharable reference to enable subsequent data integration and mapping for image processing and analysis[1,2,4,5]. Researchers may elect to generate their own brain atlases, or use and/or contribute to an existing brain atlas. Two commonly used atlases are the Allen Brain Atlas[1,2,4-6] and CUBIC-Atlas[1-5]. The use, contribution to, and sharing of atlases facilitates the consolidation of data vast not only in size, but in scope. Their importance is underscored by the mention of brain atlases in all but one of the reviews.

Outside of atlases, but related in purpose, software frameworks with a collaboration focus can also facilitate data sharing. The only one mentioned in the reviews examined here is CATMAID, a toolkit to facilitate the sharing of annotated image data[4]. Importantly, data in atlases is shared after it has been processed and analyzed, whereas CATMAID is intended for collaboration during processing and analysis[4]. It is unclear how widely CATMAID is used, but based on the reviews examined here, atlases are a much more common method of data sharing.

Applications of computational approaches

Integrated software ecosystems prioritize ease of deployment, while custom pipelines can be tailored to specific use cases but require significant computational ability. Furthermore, commercial and open source integrated software options differ in their strengths and weaknesses. The differences between the two types of integrated software ecosystems and the possibilities offered by open source pipelines are clearest in their application. To further elucidate the differences between commercial and open source integrated software platforms, this review will discuss a comparative application of Imaris and ImageJ. Subsequently, the SHANEL computational pipeline will be used to examine custom pipeline approaches.

Integrated software ecosystems

All commercial software examined here are integrated software systems, though the reverse is not true: ImageJ and Vaa3D are both open source integrated software ecosystems (Table 2). Presumably, this difference is because wide-ranging and flexible software is more marketable, but open source software often arises out of a specific need. This is especially clear when looking at the variety of plugins available for ImageJ, a selection of which are noted in Table 3.

Gautier and Ginsberg compared ImageJ and Imaris for endosome quantification[8]. They found that accuracy of quantification was higher in 3D reconstructions in Imaris, whereas ImageJ 3D reconstructions overcounted small particles and particularly struggled to account for overlapping objects[8]. This echoes Liang and Luo, who state that ImageJ is not as capable as commercial software or custom pipelines[2]. It should be noted that endosome counting is a highly specific operation, and ImageJ may be more successful in other applications.

ImageJ's image segmentation function uses global thresholding, making it less accurate when there is a low signal-to-noise ratio and a lack of landmarks[8]. Imaris, on the other hand, uses local thresholding, allowing it to better filter out noise[8]. Thus, when the signal-to-noise ratio is high, ImageJ is likely to be more successful. Furthermore, the incorporation of noise-reduction plugins might improve ImageJ's performance on noisy images. However, when accurate detection is important in a low signal-to-noise ratio image, Imaris is a better option. Depending on the specificity of the need, computational capabilities, and funding, a custom pipeline might offer an alternative solution.

Custom pipelines

When existing software options do not satisfy the scientific need, researchers may develop their own custom pipeline. Due to lack of standardization[6] and the unique challenges associated with 3D images, this happens frequently in tissue clearing. S. Zhao et al. developed a custom pipeline in conjunction with SHANEL, a new tissue clearing technique, to accommodate the terabytes of data they produced[9]. Many of the integrated softwares mentioned here are sufficient for relatively smaller tissue clearing data, but are difficult to scale to the amount of data the SHANEL group generated. Their approach employed convolutional neural networks (CNNs), and was able to demonstrate improved
performance in key areas: detection, segmentation, and quantification[9]. While adapting software built for analysis of 2D images allows for faster deployment, creating custom approaches based on 3D data that leverage machine learning improves capability and accuracy. Fortunately, most researchers make their code openly available. Unfortunately, this software usually lacks a user interface and may be difficult to navigate without prior machine learning experience. It may also be less generalizable, and need to be adapted if not used as originally intended. With further developments in computational approaches to tissue clearing, the gap between software needs and capabilities will close.

Conclusion

Tissue clearing has the potential to provide unparalleled insight in the field of neuroscience, particularly in the examination of circuitry on a whole brain level. In order to realize its full potential, tissue clearing must be paired with powerful computational approaches. Custom pipelines that leverage machine learning, especially deep learning, currently perform the best. The SHANEL group's deep learning approach to data analysis outperformed both Imaris and ImageJ, and was able to process massive data volumes with speed and accuracy[9]. As advances in tissue clearing, labelling, and microscopy produce increasingly large and complex data, machine learning is becoming increasingly indispensable. Yet the SHANEL group's deep learning algorithms and other existing custom approaches lack the breadth of features and ease of use necessary to be broadly applicable by researchers with varying research goals and levels of computational expertise. In this ImageJ has the advantage, due to its intuitive GUI and wide array of plugins, including, but far from limited to, those listed in Table 3.

Currently available ImageJ software options do not perform as well as commercial options[8] or custom approaches[9]. However, the open source nature of ImageJ makes it easy for researchers to introduce new plugins tailored to the needs of tissue clearing data. To be successful, these new plugins should leverage machine learning. Due to the unique requirements of 3D imaging data, further research is needed to compare the various machine learning approaches available. CNNs are commonly used to analyze images and have been applied to tissue clearing data.[9] Future reviews should evaluate the strength, weaknesses, and

optimal implementations of this and other machine learning approaches to the processing and analysis of tissue clearing data. Synthesized review of current machine learning options will provide the necessary foundation for practical testing of these options and future advances in computational approaches to 3D imaging data in neuroscience and beyond.

Published online 16 April 2021

1. Zhao, J., Lai, H. M., Qi, Y., He, D. & Sun, H. Current status of tissue clearing and the path forward in neuroscience. *ACS Chem Neurosci* **12**, 5-29 (2021).
2. Liang, X. & Luo, H. Optical tissue clearing: illuminating brain function and dysfunction. *Theranostics* **11**, 3035-3051 (2021).
3. Porter, D. D. L. & Morton, P. D. Clearing techniques for visualizing the nervous system in development, injury, and disease. *J Neurosci Methods* **334**, 108594 (2020).
4. Ueda, H. R. *et al.* Tissue clearing and its applications in neuroscience. *Nat Rev Neurosci* **21**, 61-79 (2020).
5. Ueda, H. R. *et al.* Whole-brain profiling of cells and circuits in mammals by tissue clearing and light-sheet microscopy. *Neuron* **106**, 369-387 (2020).
6. Parra-Damas, A. & Saura, C. A. Tissue clearing and expansion methods for imaging brain pathology in neurodegeneration: from circuits to synapses and beyond. *Front Neurosci* **14**, 914 (2020).
7. Tian, T. & Li, X. Applications of tissue clearing in the spinal cord. *Eur J Neurosci* **52**, 4019-4036 (2020).
8. Gautier, M. K. & Ginsberg, S. D. A method for quantification of vesicular compartments within cells using 3D reconstructed confocal z-stacks: Comparison of ImageJ and Imaris to count early endosomes within basal forebrain cholinergic neurons. *J Neurosci Methods* **350** (2020).
9. Zhao, S. *et al.* Cellular and molecular probing of intact human organs. *Cell* **180**, 796-812.e719 (2020).

Acknowledgements
The author thanks R. M. Shansky for the introduction to the topic of tissue clearing, C. Musselman for the helpful notes and guidance, and the unnamed author(s) of the Nature Reviews format guide, which was used to format this review.

Author contributions
J. W. R. was the sole contributor to this work.

Competing interests
The author declares no competing interests.

Peer review information
The author thanks A. Panzone and H. Murphy for their contribution to the peer review of this work.