Although the current data[1,2] are promising, it will be important to optimize delivery further, whether by phagemids, conjugated plasmids or other vehicles such as nanoparticles. One problem that could arise is that if killing is not rapid enough, or does not affect all members of the targeted population, some bacteria may survive the treatment. Among bacteria targeted with ΦNM1 carrying an RGN, ~10^{-4} survived[2]. The survivors had either not received the phagemid, had lost the phagemid or had received a corrupted phagemid. The number of bacteria that acquired resistance to infection by the phage-delivery vehicles owing to loss or mutation of their respective receptors is not evaluated in either paper, but phage resistance can be expected to add to the surviving population—and to be selected for expansion upon retreatment. For RGNs delivered by conjugation, the efficiency of killing is limited by conjugation efficiency, not RGN efficiency[1]. As Citorik et al.[1] discuss, next-generation delivery vehicles should also be designed to avoid limitations of host range, phage resistance and immunogenicity.

The increasing urgency of the problem of antibiotic resistance requires creative new approaches to drug discovery. At the same time, recognition of the importance of the gut microbiome in human health has underscored the desirability of narrow-spectrum antibiotics that minimally affect native flora. RGN antibacterials may indeed address these challenges. But their success is not guaranteed and will require further leaps in efficacy and delivery efficiency. They will have to show efficacy equivalent to that of standard-of-care antibiotics, ideally without disturbing the microbiome. This is exciting work—but it is just the beginning.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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Making biology transparent

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The molecular and cellular architecture of the organs in a whole mouse is revealed through optical clearing.

In the last several years, scientists have begun to visualize the cellular architecture of entire organs using techniques for making tissues transparent, allowing microscopes to peer into animals at depths of millimeters to centimeters. One of the more promising 'optical clearing' methods is CLARITY, which involves stabilizing specimens with an acrylamide hydrogel and then depleting them of lipids[1]. Lipids and blood scatter and absorb light, obstructing microscopy, but proteins and nucleic acids are intrinsically transparent, so spectacular images can be acquired after lipids and blood have been removed. In a recent extension of CLARITY,

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Yang et al.[2] pumped hydrogel monomers and lipid-clearing agents into animals directly through the vasculature and the cerebrospinal fluid. The advantage of their approach is speed, as large organs and even entire mice can be cleared within a few days.

Observing cells and molecules within the intact architecture of the tissues and organs they form is crucial for our understanding of the structure and function of complex biological systems. However, light absorption, scattering and sample-induced aberrations limit the optical accessibility of biological tissues. Several approaches to make tissues transparent have been developed (**Table 1**). Fixed tissues can be dehydrated and immersed in organic solvents that minimize refractive index mismatches. Such methods initially suffered from tissue-shrinking artifacts and incompatibility with transgenic fluorescent reporters, but these limitations have been largely mitigated[3]. In parallel, aqueous solutions have been developed that compensate for refractive index mismatches and that dissociate collagen fibers of the extracellular matrix while preserving fluorescent markers[4,5].

CLARITY is a relatively new approach that results in extensive optical clearing. However, its reliance on electrophoresis to remove lipids from large tissue samples introduces some complexity to the procedure. Recently developed passive methods for clearing lipids provide a simpler alternative[2,6]. For example, in the passive method of Yang et al.[2], an optimized clearing reagent delivered by transcardiac perfusion cleared large samples within a few days. The authors carefully analyzed the degree of acrylamide cross-linking required to balance the large pore sizes desirable for relatively rapid lipid clearing with undesired tissue swelling. For imaging, the resulting hydrogels were immersed in a specific solution that eliminates the mismatch in refractive indices[2,6]. Yang et al.[2] demonstrate their method by clearing a variety of mouse organs, including brain, heart, lung, intestine and kidney (**Fig. 1**) as well as the body of a whole mouse.

It is difficult to assess comprehensively how this optimized form of CLARITY compares with other emerging techniques. Generally, clearing methods should be fast, scalable, versatile and easy to use, and they should maintain tissue structure, preserve native fluorescence and generate reproducible results. As competing methods are being developed (**Table 1**), a set of benchmark experiments that quantitatively compare performance in these categories and evaluate the reproducibility of protocols would provide a valuable resource for the community.

Beyond the need to further improve clearing methods, the field faces several additional challenges. For example, spectral overlap of the available fluorophores limits the number of recordable labels in a single imaging pass. One way to circumvent this problem is to perform sequential rounds of staining and label elution, but this introduces additional time-consuming steps[1]. It is also unclear how many staining rounds can be carried out without compromising molecular integrity, antigenicity or gross sample morphology. Overall, there is a need for specifically optimized labels that are spectrally nonoverlapping or selectively activatable, and that are long-lived in the presence of clearing agents.

The samples produced by optical tissue clearing are often very large in size and very rich in information content. Clearing procedures open the door to imaging with maximum contrast and resolution, but an imaging modality is needed that delivers such performance at high data-acquisition speeds.

Table 1 Chemical clearing techniques for biological specimens

	Technique	Reference	Chemicals
Organic	Spalteholz	10	Benzyl alcohol Benzyl benzoate Methylsalicylate isosafrol
	Murray's Clear (BABB)	11	Benzyl alcohol Benzyl benzoate
	3DISCO	3	Tetrahydrofuran Dichloromethane Dibenzyl ether
	Clear^T	12	Formamide Polyethylene glycol
Organic/aqueous	CUBIC	8	Urea N,N,N',N'-Tetrakis(2-hydroxypropyl)ethylenediamine Triton X-100 Sucrose 2,2′,2″-Nitrilotriethanol
Aqueous	Scale	4	Urea Triton X-100 Glycerol
	SeeDB	5	Fructose α-thioglycerol
Hydrogel, electrophoretic	CLARITY (electrophoretic)	1	Acrylamide Bis-acrylamide SDS
Hydrogel, passive	CLARITY (passive)	6	Acrylamide Bis-acrylamide SDS
	PACT, PARS, RIMS	2	Acrylamide SDS
In vivo	Dermal treatment *in vivo*	9	Thiazone Polyethylene glycol

could be used to obtain a high-resolution snapshot of the specimen at the end point of a live imaging experiment, thereby producing a detailed structural reference for the entire data set. Moreover, the specimen could be stained for additional markers that were unavailable during live imaging. Combining light microscopy data generated by live and fixed-tissue imaging presents its own experimental and computational challenges, but insights gained from correlative light and electron microscopy serve as an excellent resource that should facilitate rapid implementation of these approaches.

The realization of such experiments would enable systematic identification of cell types throughout the volume monitored during live imaging, by staining for key transcription factors and other genes of interest or by high-resolution imaging of cell morphologies. With this information, one could, for example, systematically investigate the behavior and interactions of different cell populations as they build individual tissues and organs during development, or reveal the repertoire of neurotransmitters, receptors and channels in neural tissues following high-speed calcium imaging.

Finally, it is noteworthy that tissue clearing techniques are also being developed for entirely *in vivo* applications. The specimen's physiology naturally imposes tight constraints on the protocols, but a local and reversible reduction of peripheral light scattering by the skin is achievable in rodents[9]. Such *in vivo* clearing approaches have the potential to facilitate biomedical applications that are complementary to the detailed structural investigations made possible for fixed samples using CLARITY-like methods.

Light-sheet microscopy is a natural fit[7], and both custom and commercial instruments have been developed for this application. Additionally, new generations of objective lenses are corrected for the refractive index of specific clearing agents and are exceptionally well suited for light-sheet microscopy, owing to their very long working distances. These imaging approaches are being combined with computational methods to maximize image quality and to register the recorded images against a standardized reference tissue[8].

A promising, little-explored application of optical tissue clearing is retrospective augmentation of *in vivo* imaging data recorded from the same specimen. Tissue clearing

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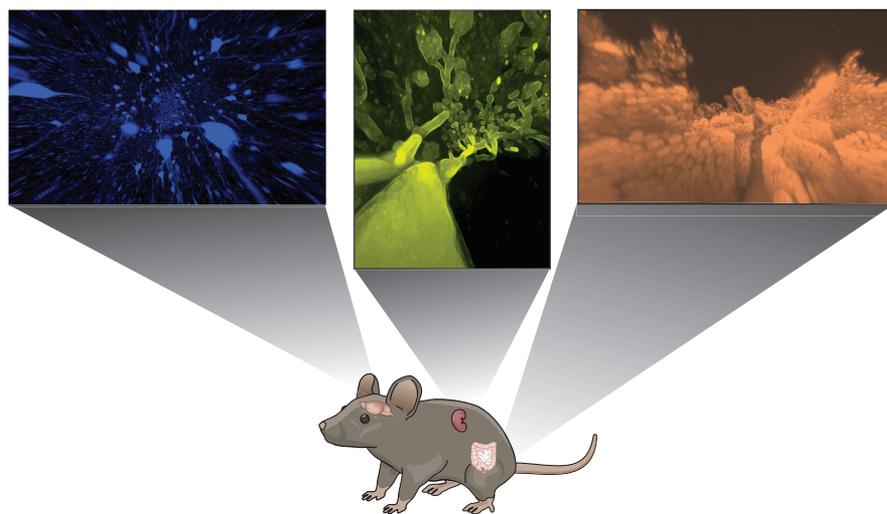
Figure 1 Whole-organ clearing with perfusion-assisted agent release *in situ*. From left to right: autofluorescent brain cells, anti-tubulin antibody stain in intact kidney tissue and acridine orange–stained intestine. (Microscopy images: Bin Yang and Viviana Gradinaru.)

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