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Brain maps at the nanoscale

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Abstract

Large brain tissue sections are imaged with nanoscale resolution using expansion and lattice light sheet microscopy.

Brain function depends on the coordinated action of structures that span more than nine orders of magnitude in size. Ion channels and synaptic vesicles are measured in nanometers, synapses and neurons in micrometers, and neural circuits in millimeters, while whole brains can extend over centimeters and axons can cover meters (Fig. 1a). By necessity, efforts to understand how these structures are related have had to integrate data generated at different scales. A recent study by Gao et al.[1] in *Science* takes a step toward addressing this problem, describing an imaging technique that captures high-resolution images at scales from tens of nanometers to millimeters. In a first application, the authors mapped neuronal structures in mouse cortex and across whole fly brains. Using terabyte-scale image processing and analysis tools, they could visualize details across a large span of brain dimensions.

The human brain is composed of over 80 billion neurons and probably a similar number of non-neuronal cells, which form a network of connections likely larger than the entire Internet. Out of the interaction of these cells, somehow, minds emerge. Deciphering how this occurs will require mapping the molecular identity and structure of thousands of different brain cell subtypes, as well as their synaptic wiring and coordinated activity. Complicating matters, many neurons have long-range projections that are key for their function, making it necessary to map cellular identities and connections across volumes as large as entire brains.

Imaging is an ideal tool for brain mapping because it can be noninvasive and parallelized. However, the vast spatial scale of brains pose a phenomenal challenge. The ideal imaging tool would have high resolution to reveal fine anatomical or cellular details, with contrast to report molecular signatures, while being capable of imaging whole brains in a short time, noninvasively, in awake, behaving animals.

These requirements have not yet been achieved in a single method. High-throughput electron microscopy[2] has sub-nanometer resolution, thanks to the electron beam wavelength, but requires tissue fixation and lacks molecular contrast, and it takes months to image and trace

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even small volumes (<1 mm^3). Conventional fluorescence microscopy, by contrast, is compatible with living tissue, has a high throughput and can reveal molecular content through fluorescence labeling, but it is limited to the spatial resolution of optical wavelengths, has a restricted color palette and can cause phototoxicity. Super-resolution microscopies[3–6] increase resolution by exploiting nonlinear responses of fluorophores or localizing individual molecules under sparse excitation, but imaging is slow and spatially restricted, and fluorescence bleaches quickly. Finally, vibrational imaging, using Raman scattering, has chemical sensitivity and an enhanced multiplexed color palette, but it still has limited resolution[7]. Although some of these techniques can be combined to achieve high resolution and molecular contrast (e.g., correlated fluorescence and electron microscopy), achieving reasonable speed and high resolution simultaneously remains a major challenge.

Gao et al.[1] tackled this problem in fixed samples by combining tissue expansion[8,9] with lattice light sheet microscopy[10]. Expansion microscopy[8,9] (ExM) physically increases the size of a tissue specimen while rendering it optically transparent (Fig. 1b). Infusion of a hydrogel isotropically inflates the sample, retaining the relative positions of its fluorescent tags and enhancing the spatial resolution of the imaging. The procedure also removes lipids and reduces light scattering, making 3D imaging easier. Lattice light sheet microscopy[10] (LLSM) is a high-throughput technique that projects a thin laser sheet onto the sample from the side and images the fluorescence from an orthogonal direction (Fig. 1b). The thinness of the light sheet minimizes out-of-focus background and photobleaching. With fast scanning and a high-speed camera, hundreds of planes can be imaged in a second.

Exploiting the advantages of ExM and LLSM, Gao et al.[1] create an approach with the unwieldy acronym ExLLSM to rapidly image large fixed samples with high resolution. An entire *Drosophila* brain (~$340 \times 660 \times 90\ \mu m^3$), expanded fourfold, can be imaged in days, at an effective resolution of ~$60 \times 60 \times 90\ nm^3$, with multiple fluorescent markers, creating a terabyte-scale dataset. This impressive achievement represents an increase in speed of two orders of magnitude over super-resolution microscopy. Using ExLLSM, the authors image subcellular structures in neurons, such as dendritic spines, and axonal pathways in mouse cortex, as well as presynaptic terminals across the whole fly brain.

Although ExLLSM excels in throughput and resolution, it has limitations. First, the achieved sub-100-nanometer resolution is two orders of magnitude worse than that of electron microscopy, precluding study of smaller structures such as ion channels. Other factors that compromise resolution are the required high density of fluorescent labels and the loss of fluorescence during sample expansion. Second, although ExLLSM can image a millimeter-scale fly brain, this is still far from the centimeter-scale required for whole mouse or human brains. Finally, the method can be used only in fixed samples, not in living tissues.

ExLLSM can already image brain structures across five orders of magnitude. Further expansion and light multiplexing could push the technique into wider spatial scales. Potential applications include surveying the location, size, orientation and morphology of each neuronal and non-neuronal class. Extensive neuronal tracing with ExLLSM could be used to reconstruct connectivity. Moreover, the technique could be applied to investigate brain structures during neural development or disease states, or under genetic or

pharmacological perturbations. Finally, to correlate structure, function and behavior, it will be important to combine ExLLSM with in vivo imaging of neural activity, as multi-modal 'hybrid' microscopies may do in the future[11]. The ingenuity behind these emerging microscopies for studying the brain—and the sheer beauty of many of the images—would surely keep pioneers such as Cajal smiling today.

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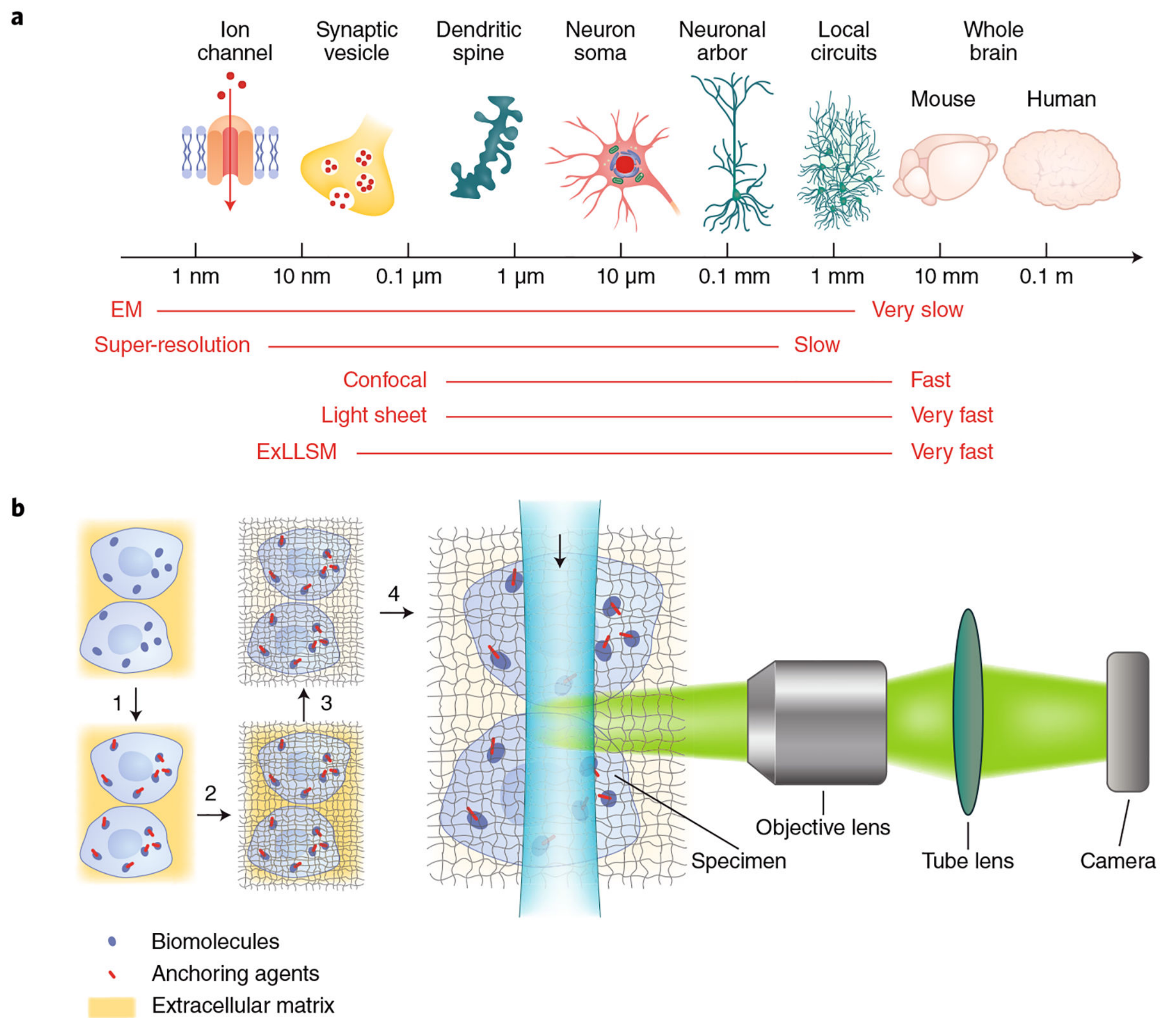

Fig. 1 |. Combination of expansion microscopy with lattice light sheet microscopy enables brain-wide imaging with nanoscale resolution.

a, Brain structures spanning more than nine orders of magnitude in dimension, and spatial range coverages of different imaging tools. EM, electron microscopy; ExLLSM, expansion lattice light sheet microscopy. **b**, Workflow of expansion lattice light sheet microscopy. Not to scale. Anchoring chemical agents are attached to biomolecules or to labels in the sample (1), followed by in situ polymer synthesis (2) and the sample undergoes chemical denaturation (3) and expansion (4). The enlarged sample is then imaged with a lattice light sheet microscope. Adapted from ref.[9], Springer Nature.