Feasibility of mapping the human brain with expansion x-ray microscopy

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Abstract: Combining synchrotron x-ray microscopy with expansion microscopy may represent a feasible approach for whole human brain connectomics at the nanoscale. Synchrotron x-ray microtomography on its own provides extremely fast imaging at high resolution, yet necessary tradeoffs between imaging throughput and resolution mean that the imaging an entire human brain with voxel sizes of less than 100 nm may still take unreasonable amounts of time with current synchrotron technology. Fast imaging with voxel sizes of 300-1000 nm is much more readily achievable in the near future. Furthermore, expansion microscopy isotropically enlarges tissue by infusion of a swellable hydrogel, facilitating resolution increases. The combination of x-ray microtomography and expansion microscopy (hereafter referred to as ExxRM) could thus push the effective voxel size down to the level needed for dense connectomics. However, because tissue volume and imaging time scale cubically with expansion factor, careful balance between design of the synchrotron x-ray optical setup and the degree of expansion will be vital. In this perspective, I will explore optimal balances between synchrotron optical engineering choices and expansion factor, propose methods to successfully implement ExxRM in the context of human brains, and estimate how much it would cost to image the human brain in this way. Imaging brains via ExxRM may represent a crucial paradigm shift in connectomics which paves the way for holistic understanding of human brain function.

Introduction

Nanoscale connectomic imaging of the entire human brain represents a long sought-after goal that could provide the foundation for dramatic advances in neurobiology, neurotechnology, and artificial intelligence.[1,2] Currently, the leading method for nanoscale connectomics is volume electron microscopy (EM). But even imaging a 1 mm^3 volume of mouse cortex over a period of 6 months required a tremendous collaborative effort by Yin et al. to develop a parallelized and fully automated transmission electron microscopy (TEM) system consisting of six instruments working in parallel.[3] Each of these six instruments cost $125,000. The mouse brain has a volume of roughly 500 mm^3, meaning that if these numbers were directly scaled, the process would take 250 years.[4] That said, Yin et al.'s TEM dataset had high resolution with 4×4×40 nm voxels, so throughput might be increased by imaging at somewhat lower resolution. As such, it is conceivable to argue that advances in EM technology may enable imaging of an entire mouse brain at sub-100 nm voxel size over the course of a few years at a cost of around $10M. EM therefore is a viable option for mouse brain connectomics. But the human brain's volume is roughly 1200 cm^3, about 2400-fold larger than the mouse brain.[5] Even if EM technology somehow advances to the point where an entire mouse brain can be imaged at 100 nm^3 voxel size in a single year for $10M, mapping a human brain with comparable parameters would take thousands of years. In my view, this provides a strong argument for the idea that a radically different approach is necessary for human brain connectomics.

Expansion light-sheet fluorescence microscopy represents a promising alternative to EM, yet this modality also falls short when considering the volume of the human brain (particularly after expansion). For instance, Lillvis et al. utilized 8-fold expansion and lattice light-sheet

microscopy (ExLLSM) to image the *Drosophila* central complex in three colors with effective 30×30×100 nm voxels over the course of 5 days.[6] But even accounting for the 8-fold expansion (512-fold volume increase), this amounts to a volume of less than 0.5 mm^3. Imaging even a 4-fold expanded mouse brain assuming these numbers would take 876 years. Lattice light-sheet microscopes are relatively inexpensive at a few hundred thousand dollars each[7] and thus might be parallelized enough to image an entire mouse brain within a year. However, the 2400-fold larger volume of the human brain relative to the mouse brain indicates that it is probably not reasonable to expect success in human connectomics through ExLLSM.

Based on these calculations, I suggest that a radically different strategy is needed for to put the goal of human brain connectomics within reach. Uniting synchrotron x-ray microtomography (XRM), expansion microscopy (ExM), a recent staining method known as Unclearing Microscopy[8] may facilitate "ExxRM" imaging of the human brain at sub-100 nm voxel size on timescales of around 1 year for a cost of around $10M. Success of this approach will necessitate overcoming some technical hurdles, but I remain optimistic that these particular challenges can be conquered. ExxRM may represent a feasible platform to acquire images suitable for dense connectomics across entire human brains.

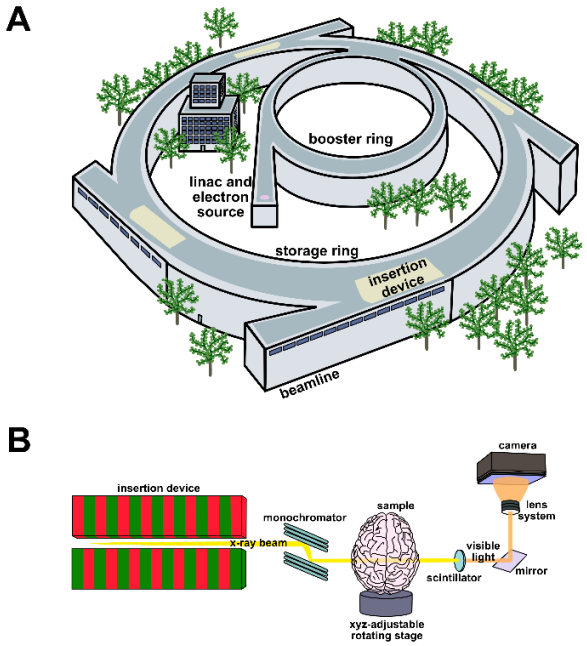

Figure 1 Principles of synchrotron XRM. **(A)** Synchrotrons generate electrons from a source, propel them through a linear accelerator (linac), raise their energy in a booster ring, and then keep the electrons circulating for long periods of time at relativistic speeds in the storage ring. As relativistic electrons move along a curved path controlled by bending magnets, they emit brilliant x-ray beams in the direction tangent to the curve in the direction of the electron movement. Insertion devices that inject the x-rays into beamlines are placed at straight sections of the synchrotron ring. These insertion devices help to stimulate emission of bright and coherent beams into the experimental stations. **(B)** Microtomography beamlines receive an x-ray beam from an insertion device and filter out a narrow band of wavelengths using a monochromator (unless a pink beam is desired, in which case this step is omitted). The beam passes through a sample on a rotating tomography stage that can be positionally adjusted to change the location of the field of view within the sample. Projection images are taken across 360° of rotation. X-rays passing through the sample are converted to visible light using a scintillator, then directed by mirrors to a lens system that magnifies the image. Finally, the light hits a detector camera and data is recorded for later 3D reconstruction.

How fast can synchrotrons image expanded brains?

Synchrotron facilities offer bright and coherent x-rays that can rapidly image large volumes of tissue at high resolutions (**Figure 1A-B**). For example, Bosch et al. employed synchrotron XRM at the Swiss Light Source (SLS) and the Diamond Light Source (DLS) to acquire 3D reconstructions of an entire mouse olfactory bulb with 325 nm voxel size.[9] In their study, tomograms of approximately 1 mm^3 volume took around 20 minutes to acquire. Another investigation by Dyer et al. used the Advanced Photon Source (APS) synchrotron to image a region of mouse cortex at 650 nm voxel size and 1.47 mm^3 volume. Imaging this volume took only 6 minutes.[10] Walsh et al. leveraged the European Synchrotron

Radiation Facility Extremely Brilliant Source (ESRF-EBS) to image a variety of whole human organs at low resolution and subvolumes within human organs at higher resolution.[11] Notably, they reconstructed a cylinder with ~57 mm^3 volume (5.4 mm diameter and 2.5 mm height) within human spleen at 1290 nm voxel size over the course of 2 hours. Ding et al. used the APS to image larval zebrafish with 743 nm voxels and 1.5 mm^3 volumes per tomogram in 20 minutes with monochromatic x-ray scans and in just 20 seconds with polychromatic "pink beam" x-ray scans.[12] Despite their much higher flux and correspondingly much shorter imaging times, the polychromatic scans unfortunately showed lower resolution due to poor signal-to-noise ratio (SNR). It should be noted that resolution represents a distinct concept from voxel size and that contrast and signal-to-noise ratio (SNR) play major roles in the final resolution. That said, voxel size can act as a rough proxy for comparisons across different imaging setups assuming that contrast and SNR are consistent. In another key example, Foxley et al. utilized the APS to image whole mouse brains (11.7×11.7×17.7 mm) over the course of 7 hours of acquisition time with 1117 nm voxels.[13] Finally, Rodgers et al. used the SOLEIL synchrotron and an alternative technique for tomographic acquisition (discussed in more detail later) to image an entire mouse brain a 650 nm voxel size in 8 hours.[14] Though the parameters for image acquisition vary considerably across different biological samples and synchrotron hardware setups (**table 1**), these examples illustrate the power of synchrotron imaging for mapping biological tissues.

Table 1 Specifications of synchrotron tissue imaging experiments from selected references.

Reference	Smallest voxel size	Single tomogram volume	Total reconstructed volume	Imaging time per tomogram	Total imaging time
Bosch et al.	325 nm	1.39 mm^3	~5-10 mm^3	20 min.	~40-480 min.
Dyer et al.	650 nm	1.47 mm^3	1.47 mm^3	6 min.	6 min.
Walsh et al.	1290 nm	57 mm^3	57 mm^3	120 min.	120 min.
Ding et al.	743 nm	1.5 mm^3	4.5 mm^3	20 min. or 20 s*	60 min. or* 1 min.
Foxley et al.	1117 nm	5.61 mm^3	2423 mm^3	108 s	7 hours
Rodgers et al.	650 nm	special method	911 mm^3	special method	8 hours

*with pink beam acquisition

While the voxel sizes described above are not small enough for nanoscale connectomics, incorporating ExM into the sample preparation pipeline presents the possibility of attaining sufficient resolutions for tracing neurites. Expansion factors of around 4x, 10x, and 20x are respectively possible with standard ExM,[15,16] some of the newer enhanced ExM protocols,[17–19] and iterative ExM methods.[20,21] Expansion factor can also be intentionally decreased somewhat by adjusting salt concentration. It is important to note that not all ExM protocols retain lipid membranes, so selecting a recipe that preserves such cellular boundaries[18,21,22] will be vital for morphology reconstrction. Balance between expansion factor and imaging time will represent a key determinant of the success of ExxRM for whole brain imaging. Higher expansion factors increase resolution linearly while increasing imaging time cubically. Synchrotron imaging's extreme speed has the potential to keep up with such volumetric increases, though even this has certain limits.

For the task of imaging an entire human brain using a single synchrotron microtomography beamline, some calculations can help estimate how to balance between expansion factor and total imaging time. Let D equal the total imaging time, p represent the linear size of a single isotropic voxel, x represent the linear expansion factor, a equal the volume of a single tomogram, and h equal the time it takes to image one tomogram. Assume that the human brain has a volume of 1200

cm^3 (without expansion) and that voxel size influences tomogram volume through some function $a(p)$. In the comparatively "easy" case of the mouse brain, replace the pre-expansion volume with 0.5 cm^3. An equation $D = (1200\ \text{cm}^3)x^3h/a(p)$ can then be used to guess the best tradeoff between imaging time D, expansion factor x, and voxel size p. Estimates for values of $a(p)$ can be obtained from the references in **table 1**. First, consider the numbers from Bosch et al. wherein volumes of 1.39 mm^3 with 325 nm voxels were obtained over the course of 20 minutes. This would give $a(p) = a(325\ \text{nm}) = 1.39\ \text{mm}^3 = 0.00139\ \text{cm}^3$. Since Bosch et al. achieved voxel size of 325 nm, 4x expansion would be sufficient to reach effective voxel size of less than 100 nm.[9] Also consider the numbers from Foxley et al. wherein volumes of 5.61 mm^3 with 1117 nm voxels were obtained over the course of 108 seconds.[13] This would give $a(p) = a(1117\ \text{nm}) = 5.61\ \text{mm}^3 = 0.00561\ \text{cm}^3$. To reach effective voxel size of approximately 100 nm, 11-fold expansion would be required. Comparative results of this process for Bosch et al. and Foxley et al. numbers are given in **table 2**. These results indicate that attempting ExxRM with previously used methodologies would take far too long in the context of whole human brains, though they have promise for the mouse brain.

Table 2 Estimates for ExxRM total imaging times using Bosch et al. and Foxley et al. numbers.

Brain	Assumed imaging time per tomogram	Total imaging time
Mouse, 4x, Bosch et al. numbers	20 minutes	319.4 days
Human, 4x, Bosch et al. numbers	20 minutes	2110.5 years *[unacceptable]*
Mouse, 11x, Foxley et al. numbers	108 seconds	148.3 days
Human, 11x, Foxley et al. numbers	108 seconds	973.5 years *[unacceptable]*

While the numbers above may at first appear discouraging, they are not the end of the story. Multiple approaches might be utilized to greatly enhance throughput (**table 3**). Applying the pink beam method leveraged by Ding et al. has potential to drastically reduce imaging time per tomogram.[12] Ding et al.'s study reported pink beam 20 second acquisitions for 1.5 mm^3 volumes and monochromatic beam 20 minute acquisitions for 1.5 mm^3 volumes. As such, it is intriguing to compare this with Foxley et al.'s 108 second acquisition of 5.61 mm^3 volumes by monochromatic beam. Compiling a similar setup with a pink beam could feasibly enable acquisition times of 10-20 seconds or less because pink beams have around 1000-fold greater x-ray flux than monochromatic beams.[23–25] As will be discussed in more detail later, the poor SNR associated with pink beams might be circumventable by use of clever ExM sample preparation techniques in addition to improved x-ray optical engineering and image processing methods. Pink beam imaging combined with noise reduction methods may accelerate ExxRM timelines.

Table 3 Estimates for ExxRM total imaging times using Bosch et al. and Foxley et al. numbers but assuming 20 second acquisition times through use of pink beam.

Brain	Assumed imaging time per tomogram	Total imaging time
Mouse, 4x, Bosch et al. numbers	20 seconds	5.3 days
Human, 4x, Bosch et al. numbers	20 seconds	35.2 years *[unacceptable]*
Mouse, 11x, Foxley et al. numbers	20 seconds	27.5 days
Human, 11x, Foxley et al. numbers	20 seconds	180.3 years *[unacceptable]*

Improved synchrotron sources as well as synchrotron beamline design specifically optimized for the application of ExxRM on the entire human brain may further reduce imaging

times. A recent study on mouse brain imaging by Rodgers et al. describes some promising ways to speed up image acquisition.[14] The French synchrotron SOLEIL was employed to image whole mouse brains at 650 nm voxel size over a period of 8 hours through a special type of tomographic acquisition that made the process more efficient. Rather than acquiring tomograms one at a time and concatenating them, Rodgers et al. tiled projections into concentric rings spanning the entire lateral dimensions of the mouse brain (the z-axis still required concatenation). These concentric rings were used as the basis for tomographic reconstruction. Similar strategies might be employed to accelerate imaging of expanded human brain samples. It should be noted that the 8-hour acquisition was achieved with a monochromatic beam, so using a pink beam or otherwise enhancing flux could further decrease the imaging time. Another important point is that the field-of-view is typically limited by detector array size.[14,26] This opens the possibility of still further improving imaging throughput by custom development of significantly larger detector arrays. Finally, the recent advent of 4th generation synchrotron sources such as the ESRF-EBS will reportedly enable 100-fold brighter coherent x-ray beams that may correspondingly speed up experiments.[27,28] That said, promises of 100-fold faster experiments should be considered in the context of the numbers reported by Walsh et al. (see **table 1**) where imaging time-resolution tradeoffs using the ESRF-EBS did not greatly exceed those of the other examples. But perhaps the full potential of fourth-generation sources is yet to be realized, particularly if pink beams are empoyed. Design of a custom beamline for human brain ExxRM connectomics will likely be necessary to take full advantage of available x-ray technologies.

Based on these advances and the examples discussed so far, aiming for 300 nm voxel size, 3x to 4x expansion, 10 second acquisition time per tomogram, and tomograms with volumes of ~20 mm^3 (cylinders of approximately 3 mm diameter and 3 mm height) does not seem outside the realm of possibility for a custom synchrotron microtomography beamline given current and emerging technologies (**table 4**). This may facilitate 100 nm or 75 nm effective voxel size imaging of an entire human brain within 0.51 to 1.22 years for 3x and 4x expansion respectively. These timelines are quite reasonable, particularly in the context of the importance of this research for neuroscience.

Table 4 "Wish list" of possible imaging times after a hypothetical modest investment in optimizing a state-of-the-art synchrotron beamline for imaging whole human brain by ExxRM at assumed 300 nm isotropic voxel size (100 nm or 75 nm effective voxel size after 3x or 4x expansion) with pink beam acquisition.

Expansion factor	Assumed volume per tomogram	Assumed imaging time per tomogram	Total imaging time
3x	10 mm^3	20 seconds	2.05 years
3x	20 mm^3	20 seconds	1.03 years
3x	10 mm^3	10 seconds	1.03 years
3x	20 mm^3	10 seconds	0.51 years
4x	10 mm^3	20 seconds	4.87 years
4x	20 mm^3	20 seconds	2.44 years
4x	10 mm^3	10 seconds	2.44 years
4x	20 mm^3	10 seconds	1.22 years

Recommended methodologies for ExxRM

ExxRM's success will require developing methodological strategies to mitigate technical challenges. Approaches that ensure stability of expanded tissues under brilliant x-ray illumination for long durations will be crucial. Obtaining sufficient contrast to resolve subcellular features will

also be vital. ExM cubically dilutes the amount of cellular material per unit volume, so creative staining techniques will be needed. In addition, multicolor imaging would greatly benefit the usefulness of whole brain connectomics data, yet rapid polychromatic pink beam imaging does not allow the usual edge subtraction methods that XRM can use to map the positions of distinct stain materials. These challenges must be conquered to translate ExxRM.

Expanded tissues are known for their fragility and synchrotron x-rays are known for their harshness, so strategies for solving the problem of sample degradation are needed. Newer expansion recipes that use high monomer concentrations have displayed substantially greater physical sturdiness than earlier generations of ExM hydrogels.[18,21] But under the long durations of exposure to high flux pink beam x-rays required for imaging whole expanded human brains, even fixed tissues without expansion may degrade, so further advances in ExM sample preparation will be necessary. The issue could be partially ameliorated by cutting human brain samples into smaller volumes. But this only goes so far. As such, it will probably be necessary to develop a new method of stabilizing expanded samples, perhaps infusing them with a rapidly crosslinkable or crystalizing substance and thus irreversibly locking all biomolecules into place **(Figure 2A-B)**. The chosen substance would need (i) to undergo an inducible crosslinking or crystallization reaction that does not cause distortion of the expanded tissue, (ii) to not shrink or distort the expanded tissue while it diffuses into the hydrogel, and (iii) to be invisible to x-rays at the pink beam energy range used for imaging and to contrast sharply with the stained tissue itself (the nature of this staining will be discussed in the next paragraph). The first of these might be achieved through some form of rapid photopolymerization. So long as the expanded tissue's structure can be modified to gain enough rigidity that it does not shrink upon addition of exogenous charged molecules, one might subsequently induce further polymerization or mineralization inside the gel, replacing water with some kind of ordered crystalline material or a hard plastic. This material would need to be composed of low-Z elements to prevent beam hardening in thick samples. Though the material would need to be invisible to x-rays, it would not require translucency to visible light, so there likely exists a wide range of available options to explore. Keeping the sample at low temperatures during imaging may also help if heat from the x-rays is problematic, though this would not help prevent the direct radiation damage from ionization.[29] Such methods may facilitate sufficient stabilization to prevent tissue degradation even with very long exposures to extremely brilliant synchrotron x-rays.

Another engineering hurdle in ExxRM is how to attain sufficient feature contrast for capture of clear images even with relatively noisy pink beam scanning. Because expanded tissues experience cubic dilution of target biomolecules, contrast from traditional stains such as osmium tetroxide almost entirely vanishes during x-ray microtomographic imaging (Collins et al., unpublished data). But an elegant solution to this problem has come in the

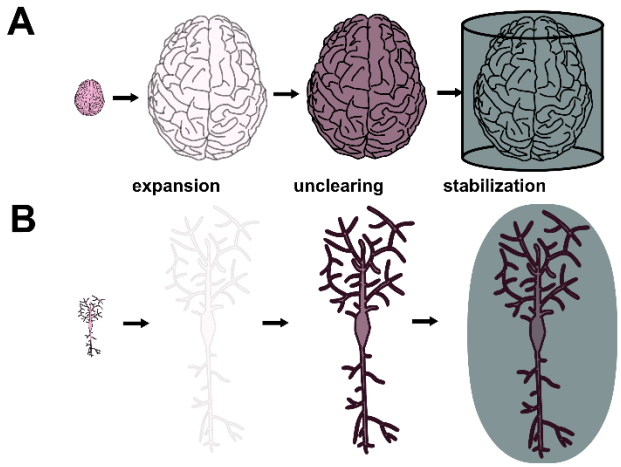

Figure 2 Proposed sample preparation technique for ExxRM. **(A)** Macroscale view of a human brain undergoing expansion, unclearing, and stabilization. **(B)** Nanoscale view of a neuron within the brain undergoing expansion, unclearing, and stabilization. Considerable amplification of signal density after unclearing is visible.

form of a recently developed technique called unclearing microscopy.[8] For this technique, M'Saad et al. biotinylated primary amines (found on proteins, phosphatidylethanolamine lipids, etc.) throughout expanded samples, treated the sample with streptavidin horseradish peroxidase (streptavidin-HRP) fusion protein, and then stained with ionic silver reagents (from the EnzMet™ HRP Detection Kit) or with 3'3-diaminobenzidine (DAB). This triggered enzymatic deposition of enough chromogenic silver or DAB to make 20-fold expanded HeLa cells visible to the naked eye despite their 8000-fold increase in volume relative to the unexpanded state. Phase contrast light microscopy subsequently revealed subcellular features such as mitochondrial cristae, nuclear pore complexes, and nuclear membrane. Unclearing thus facilitates physical reconstruction of the structures that are pulled apart by ExM, filling in the gaps left by the expansion process. Silver stain unclearing could enable absorption XRM of expanded tissues since silver has excellent x-ray attenuation at relevant beam energies. But phase contrast XRM may represent a better option since it circumvents issues of beam hardening in thick samples and can decrease the necessary dose of radiation per unit time by using x-ray wavelengths which are not absorbed as strongly by the tissue. Furthermore, phase contrast XRM is most sensitive to differences in sample density[30] and DAB staining forms dense localized precipitates (which can be imaged via phase contrast XRM),[31] so this approach might provide superior contrast in the context of ExxRM. Here, ExM's sample dilution might prove advantageous because it could generate strongly distinct densities between DAB-stained cellular features and the rest of the stabilized hydrogel. It should be noted that, for phase contrast XRM, the previously mentioned stabilization approach would need to fill the space between cellular features with a substance that differs substantially in density from the DAB (or similar stain). Combining Unclearing Microscopy with phase contrast XRM could provide excellent feature contrast for ExxRM connectomics.

Multicolor ExxRM is not strictly necessary for connectomics, yet having the ability to mark several colors would greatly enhance the value of the data since synapses and key biomolecules could then be tagged within the 3D reconstruction. Unfortunately, multicolor imaging is much more difficult to achieve with rapid polychromatic pink beam scanning since one cannot simply image with a monochromatic beam at an energy corresponding to an absorption edge of a target tag material. That said, methods involving specially designed scintillator detectors have been proposed for facilitating absorption XRM in multiple colors even with the use of pink beam.[32] Since phase contrast XRM relies primarily on density differences,[30] one might utilize substances exhibiting highly distinct densities as molecular tags. I speculate that metallic nanoparticles may have sufficiently different density relative to DAB that they could be computationally labeled after data collection. Another possibility for labeling is to simply employ large enough metallic nanoparticles that they appear as distinctive black dots (perhaps around 100 nm diameter). It might be difficult to induce reliable diffusion of these metallic nanoparticles into tissue, though post-expansion pre-stabilization staining could help overcome the problem since the expanded gel is more porous than pre-expansion tissue. As such, it may be possible to develop special labeling or optical engineering techniques that allow polychromatic beams and effective 2-3 color ExxRM imaging to coexist.

Though some trial and error will doubtless prove necessary for optimizing ExxRM techniques, the technologies to do so lie within fairly close reach. Sample stabilization under long duration and brilliant x-rays could be accomplished by first treating the expanded hydrogel to preclude shrinkage and distortion and then by switching out the water for some kind of sturdy substance such as an ordered crystal or hard plastic (composed of low Z elements to prevent x-ray absorption). Strong cellular feature contrast could be achieved through applying Unclearing

Microscopy and optimizing for phase contrast XRM approaches. Unclearing by HRP's enzymatic deposition of DAB may facilitate excellent results since this would behave as a powerful way to counter cubic dilution of signal from expansion. These methodological directions may pave the way to successful ExxRM.

Cost estimates for whole brain ExxRM

While the power of the synchrotron facility comes with a high price tag, ExxRM may still represent the most overall cost-effective option for human brain connectomics. Consider the costs associated with the DLS as an illustrative example. The DLS is a third-generation facility and is currently one of the better synchrotrons in terms of its ability to produce bright and coherent x-rays. Building the DLS and its first seven beamlines from 2003-2007 cost about $316M, its later upgrades cost $144M and $134M, and its yearly operational costs have increased from $28M in 2007-2008 to $81M in 2019-2020 (**table 5**). Based on these data points, construction of a new synchrotron beamline costs approximately $10M and yearly maintenance may cost roughly $500K. This provides a framework for estimating the cost of a dedicated human brain ExxRM connectomics beamline.

Table 5 Costs associated with the Diamond Light Source[33] as a case study on how much money is needed to build and maintain a state-of-the-art synchrotron facility.

Facility	Diamond Light Source
Initial costs	$316M: synchrotron, first seven beamlines, surrounding buildings, construction started in 2003 and completed in 2007
Upgrade 1 costs	$144M: fifteen more beamlines, detector development program, construction started in 2004 and completed in 2012
Upgrade 2 costs	$134M: ten more beamlines, not stated when construction started but was completed in 2021
Maintenance and operational costs	$28M in 2007-2008, $48M in 2012-2013, $81M in 2019-2020

Compact Light Source (CLS) technology should be considered as well before continuing. CLS instruments produce x-ray beams that fall somewhere between laboratory x-ray microscopes and synchrotrons in terms of brightness and coherence.[34] CLS instruments are furthermore small enough to fit into a single room and are inexpensive enough that a large number of them could potentially be constructed in parallel. At first glance, CLS technology seems a more economically viable alternative to synchrotron beamlines, yet it still probably is not a good option at this time. Existing CLS instruments are not likely suitable for human brain (or even mouse brain) ExxRM connectomics in the foreseeable future because their optical engineering requirements and mediocre level of x-ray flux preclude rapid tomography at submicron resolution, particularly when a large field of view is desired.[35] There is a small possibility that future advances in CLS technology could change this situation, yet this seems fairly unlikely, so synchrotron-based imaging remains the best route.

Building an entire synchrotron solely for ExxRM connectomics is probably less efficient than establishing an agreement with an existing synchrotron to construct a connectomics beamline. While one might envision additional parallelization through custom design of the beamline to split the beam to land in multiple sample chambers, splitting the beam would divide the photon flux and therefore increase imaging times for no net gain in speed. As such, parallelization would likely require an additional insertion device and thus an entire new beamline for each new sample chamber. Insertion devices consist of a series of precisely engineered magnets built into straight

sections of the synchrotron's ring. These magnets, known as undulators or wigglers depending on the type of insertion device, stimulate directed emission of a brilliant x-ray beam out from the storage ring.[36] Yet even if we assume $10M total plus yearly maintenance costs for each connectomics beamline, imaging multiple human brains over the course of a year or a single human brain in just a few months remains a reasonable proposition. For a project as important as mapping the human brain at sufficient resolution for dense neuronal reconstruction, price tags in the range of tens of millions of dollars may not be out of reach.

Conclusion and outlook

Connectomics needs a technological paradigm shift if it is to feasibilize dense mapping of one or more human brains. Though it comes with some technical challenges, ExxRM may represent a key strategic shift that drastically reduces human brain connectomics timelines from centuries or millennia down to 1-2 years (for image acquisition). Data storage and early processing steps (e.g. tomographic reconstruction) will of course require data centers and high-performance computing, but this field is rapidly advancing and will likely be able to handle the challenge. Assuming 1 byte per voxel, the amount of storage needed for a 4-fold expanded human brain with 300 nm physical voxel size (75 nm effective voxel size) is about 2.84 exabytes. Segmentation of the human brain dataset will probably represent a vastly more difficult problem as well as require substantially more compute resources, so further advances in this area will need to occur in parallel with ExxRM development. Realizing the benefits of connectomics in the form of complete computational models of the brain will take additional extensive research. Precisely correlating gene expression and electrophysiological properties with neuronal morphology (i.e. "cell type") may represent a major step towards bridging the divide between structural data and functional activity. Nonetheless, the allure of having a holistic anatomical picture of the brain may serve as a driving force in the meantime. ExxRM has the potential to transform the dense connectomics field, enabling anatomical imaging of the entire human brain with sub-100 nm voxel size and high contrast in around 1 year for a price of roughly $10M.

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