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Review

Connectomics: comprehensive approaches for whole-brain mapping

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Abstract

The aim of connectomics analysis is to understand whole-brain neural connections. This is accomplished using new biotechnologies. Here, we provide an overview of the recent progress in connectomics analysis. The entire neural network of an organism was revealed for the first time in the nematode. *Caenorhabditis elegans* (*C. elegans*) have an advantage of their limited number of neurons and their transparency, allowing the neural network to be visualized using light and electron microscopes (EMs). It is practically impossible to adopt the same approach for mammals because of the large number of neural cells and the opacity of the central nervous system. A variety of new technologies are being developed to perform computer-assisted high-throughput image acquisition and analysis to obtain whole-brain maps for higher species, including mammals. Diffusion tensor magnetic resonance imaging and tractography and three-dimensional imaging with the EM are examples of novel approaches to connectomics. These new technologies will soon be applied not only to *Drosophila*, *C. elegans* and rodent research, but also to comprehensive connectomics analysis in a wide range of species including humans and primates. In the near future, results from connectomics analysis will reveal the neural circuitry of the whole brain and enhance our understanding of the human mind and neuropsychiatric diseases.

Key words: connectomics, connectome, brain mapping, magnetic resonance imaging, electron microscope, serial EM

Introduction

Large grants for brain connectomics analyses were recently launched all over the world, including Europe, the United States and Japan. In 2013, the Human Brain Project started in Europe. This aims to provide researchers worldwide with tools to understand how the human brain works by using unique simulation-based approaches. At the same time, President Obama in the United States emphasized the importance of brain mapping in the State of the Union Address as follows: ‘Now, if we want to make the best products, we also have to invest in the best ideas. Every dollar we invested to map the human genome returned $140 to our economy – every dollar. Today, our scientists are mapping the human brain to unlock the answers to Alzheimer’s. They’re developing drugs to regenerate damaged organs; devising new material to make batteries 10 times more powerful. Now is not the time to gut these job-creating investments in science and innovation. Now is the time to reach a level of research and development not seen since the height of the Space Race. We need to make those investments.’ (http://www.whitehouse.gov/state-of-the-union-2013) (last accessed date 3 December 2014). This statement led to the creation of the NIH-funded BRAIN (Brain Research Through Advancing Innovative Neurotechnologies) Initiative grant, which began in the United States in 2013. In Europe, the Human Brain Project, which is one of two Future and Emerging Technologies Flagship Initiatives, was launched by the European Commission in 2013 (http://cordis.europa.eu/fp7/ict/programme/fet/flagship/) (last accessed date 3 December 2014). In 2014, a brain mapping project called Brain/MINDS (Brain Mapping by Integrated Neurotechnologies for Disease Studies) officially started in Japan, aimed at understanding the mechanisms of the mind (http://brainminds.jp) (last accessed date 3 December 2014) by taking advantage of the non-human primate brain, especially the common marmoset (*Callithrix jacchus*) brain [1,2]. Comprehensive mapping studies of the non-human primate brain are essential for understanding the human brain and for developing knowledge-based strategies for the diagnosis and treatment of psychiatric and neurological disorders. The common marmoset is a small New World non-human primate that is extensively used for this purpose in biomedical research. Understanding the mechanisms of the human mind by revealing the connectomics in the brain is one of the biggest challenges of the century in the science community all over the world.

As President Obama suggested in his 2013 State of the Union Address, the current status of the connectomics project echoes the history of the human genome project (HGP) several decades ago. The HGP was an international collaborative research project to determine the sequence of the whole human genome and map all the genes under the international partnership including the United States, Europe, Japan and China. The genome project was initially proposed by the United States government in the mid-1980s, officially started around 1990, and was completed in 2003. Now, we are at the beginning of the international connectomics project to determine the neural wiring diagram of the whole human brain and map all of the functional relations of the brain through collaborations in and between Europe, the United States and Japan.

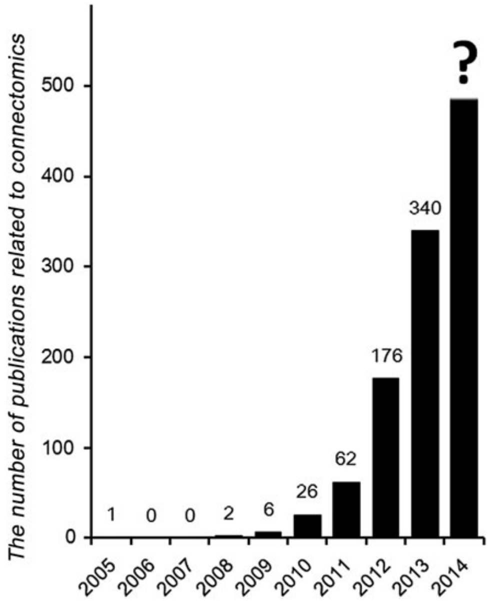

Fig. 1. The number of connectomics-related publications. According to the PubMed database, the number of publications related to connectomics analysis is increasing year by year. Papers with the keyword ‘connectome’ or ‘connectomics’ were counted.

What does connectomics stand for?

The word connectomics is relatively new and consists of two parts: one denotes the whole connection of the neural circuit (connect-) and the other refers to comprehensive analysis (-omics), similar to proteomics or to genomics. Recently, connectomics analysis has been in the spotlight as one of the new fields of research in neuroscience because it can provide structural bases for diverse functions of the human brain. As proof of the attention it has gained, an increasing number of papers have been published on connectomics over the last several years (Fig. 1). The number of publications related to connectomics was determined from a keyword search containing the words ‘connectome’ or ‘connectomics’ in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) (last accessed date 3 December 2014). The meaning of the word ‘connectomics’ was explained by Prof. Jeff W. Lichtman at Harvard University as ‘a branch of biotechnology concerned with applying the techniques of computer-assisted image acquisition and analysis to the structural mapping of sets of neural circuits or to the complete nervous system of selected organisms using high-speed methods, with organizing the results in databases, and with applications of the data’ (iBiology by Jeff W. Lichtman, Part 1: connectomics: seeking neural circuit motifs).

The overall goal of connectomics analysis is to understand the whole neural circuit in the human brain, and this is accomplished using new biotechnologies. Thus, the progress of connectomics analysis requires not only active biological investigations but also technological advancements including the development of novel microscopes, new devices and drastic improvements in the computer programs available for analysis. In fact, many novel biotechnologies were invented especially for connectomics, including the multiple-beam scanning electron microscope (SEM) and automatic tape-collecting ultramicrotome (ATUM) and its software [3,4].

Connectomics and genomics

Connectomics describes the new field of research that focuses on the comprehensive analysis of neural connections. Connectomics may appear to be a subcategory within the field of neuroscience; however, connectomics and neuroscience are independent concepts that have their own independent territories. Classical methodologies for morphological experiments in cellular and anatomical neuroscience concern normal and abnormal functions of one or some neurons, but the target of connectomics is the whole network that exists among large numbers of neurons, beyond the function of the single neuron. The desinence of the word connectomics (i.e. -omics) is the same as that of the word genomics, and the relation between connectomics and neuroscience is quite similar to that between genomics and genetics. Genetics focuses on a specific mutation, abnormality or function of one or some genes, whereas genomics focuses on whole-genome function based on the integration of all genes and is beyond the function of a single gene. Based on the many findings in the field of genetics and reports on the functions of genes, genomics achieved great progress supported by large genome-project grants in the 1990s. After the completion of whole-genome sequencing of many species around the year 2000 [5,6], new approaches for genomics emerged, including comparative genomics. This is currently referred to as the post-genome era. One of the most important factors responsible for breakthroughs in the fields of genetics and genomics was the invention of DNA sequencers. The progress of genetics was supported by the development of the gel and capillary sequencers, and that of genomics was supported by the development of a variety of next-generation sequencers that incorporated various new concepts.

Progress in the field of neuroscience was supported by the invention of light microscopy (LM) in the era of Santiago Ramón y Cajal. Cajal is well-known for his artistic drawings of the neural network that were made using LM, and he was awarded the Nobel Prize in Physiology or Medicine in 1906 with Camillo Golgi, who is the developer of the Golgi stain [7]. Just like the next-generation sequencers for genomics, new machines and procedures that are currently beyond our imagination will help to progress the study of connectomics [3]. As mentioned above, big projects for connectomics supported by governments recently started all over the world, and we are hopeful for progress in understanding the human mind via revealing the whole neural wiring diagram in the near future.

History of recent approaches to connectomics

In the mid-1980s, the first full connectomics analysis was completed for the nematode, *Caenorhabditis elegans* (*C. elegans*), for which the neural diagram was constructed manually [8]. Micrographs of serially sectioned samples of hermaphroditic *C. elegans* were taken using electron microscopy (EM), and their reconstruction was performed almost entirely by hand. This full *C. elegans* connectomics analysis was preceded by a series of studies in the 1970s that used manual reconstruction of electron micrographs to visualize the neural wiring within various regions of the *C. elegans* nervous system [8–12]. Due to the limited number of neurons and their transparent body, *C. elegans* proved a favorable model for this manual technique of investigating every synaptic connection among all neurons. However, full connectomics was achieved only as a result of a massive amount of effort and time. It took more than a decade for White *et al.* to completely map the whole nematode nervous system of around 300 neurons. The same procedure would be expected to take about 2.5 million years for the mouse brain (~75 million neurons) and about 33 million years for the human brain (~100 billion neurons), highlighting a need for new technologies to map the human brain in a shorter period of time. The current approach to connectomics involves the use of tracers and transgene methods, and combining them with magnetic resonance imaging (MRI) and EM technologies, to make human connectomics more plausible.

One recent innovation developed for the purpose of connectomics analysis is the expression of multiple spectral and photophysical protein variants (XFPs) such as cyan, green, orange, red and yellow fluorescent proteins on a single transgene, resulting in differential color coding of individual neurons in over 100 colors, termed the Brainbow approach [13]. Its mechanism is based on the *Cre/loxP* recombination system. For example, in the Brainbow-1.0 strategy, canonical and mutant forms of *lox* sites are inserted at the ends of different XFPs (Fig. 2) [13]. Because *Cre* recombinase promotes recombination only between identical *lox* sites, inclusion/exclusion of the open reading frames (ORFs) of

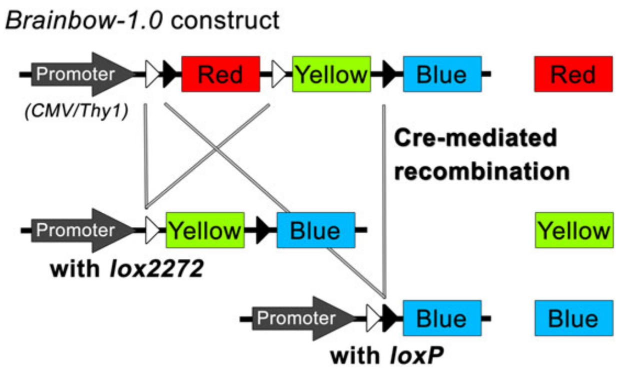

Fig. 2. The basic construct of Brainbow-1.0. A simplified version of the Brainbow-1.0 construct is illustrated, based on the original report [13].

multiple XFPs takes place in a mutually exclusive fashion. This drives stochastic expression of XFPs at varying degrees and combinations in individual neural cells. In Brainbow-2.1, two ORFs encoding different spectral variants of XFPs are placed in an opposite direction and flanked with *lox* sites, which promote both inversion and excision of this DNA cassette by the activity of *Cre* recombinase. When two of these invertible DNA cassettes were placed in tandem, three independent inversions can take place. This leads to a number of possible outcomes (larger than Brainbow-1.0) in XFP expression, all of which have equal probabilities.

The Brainbow technique can generate a broad color spectrum of over 100 different hues to label neurons. This combinatorial XFP expression allows for differential labeling of individual neurons and, in contrast to earlier fluorescence methods that made use of only two or three XFPs, the large size of the color palette drastically minimizes the likelihood of identical XFP expression in two adjacent neurons [14–17]. The Brainbow technique thus presents a novel way in which the precise projection of every neuron in the central and peripheral nervous system of the transgenic mouse can be visualized. Additionally, Brainbow technology possesses a remarkable versatility. In addition to differentiating distinct neurons, it allows researchers to distinguish mouse blastomere clones from one another in a recent study on blastomere allocation at the mouse embryo cleavage state [18]. The Brainbow technique also enables researchers to trace the lineage of neural progenitor cells [19]. Moreover, in addition to analyses of the mouse nervous system, Brainbow transgenes were recently used to draw neural wiring diagrams of the sensory nervous system of the zebrafish [20] and *Drosophila* [21,22].

Although the Brainbow technique allows multicolor visualization of individual neural connections, it presents a few limitations. For instance, a spectrum of 100 colors is sufficiently diverse in comparison to earlier fluorescence methods, but to view full regions of the nervous system, more colors must be produced to make up for the multiplicity of types of

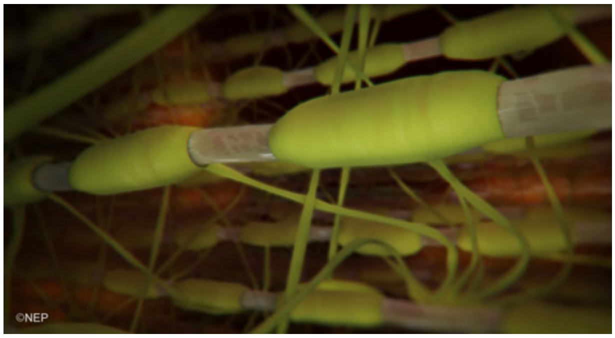

Fig. 3. An illustration of a myelinated fiber tract. DTI reflects water molecule anisotropy, which is mainly restricted by myelinated neural structures in the white matter. This is the computer graphic illustration which describes that the myelin structure from oligodendrocyte (green) wraps the neural fibers (ivory) and was kindly provided by NHK Enterprises, Inc.

neuronal cells being observed at once. In addition, a limitation concerning the low resolution of fluorescence images emerges when observing more complex neural wiring systems with LM, and this requires thinner sections to be obtained [23].

Macroscale, mesoscale and microscale connectomics

There are a lot of approaches to studying neural connectomics, and the approaches are on varying scales. MRI is a representative macroscale connectomics analysis technique that is used to detect the whole-brain wiring diagram. EM is a microscale connectomics analysis technique used to elucidate the microscopic neural connections at the synapse level. The LM approach, including the Brainbow technique, is categorized as mesoscale connectomics and aims to link the results from macroscale and microscale connectomics. In the following section, we describe and compare the main features of each approach.

Macroscale connectomics with MRI

One of the aims of connectomics is to map brain connectivity at the macro level and to unveil system-level connections. Non-invasive MRI is often chosen for macroscale connectome analysis. There are several advantages to using MRI, including the ability to perform *in vivo* longitudinal experiments, perform experiments across species, from mice to human beings and obtain and analyze data from the whole brain.

There were attempts to map structural connections with MRI by examining the presence of anatomical connections using cortical thickness measurements across the regions [24]. Diffusion MRI provides information about structural connectivity patterns by visualizing the spatial orientation

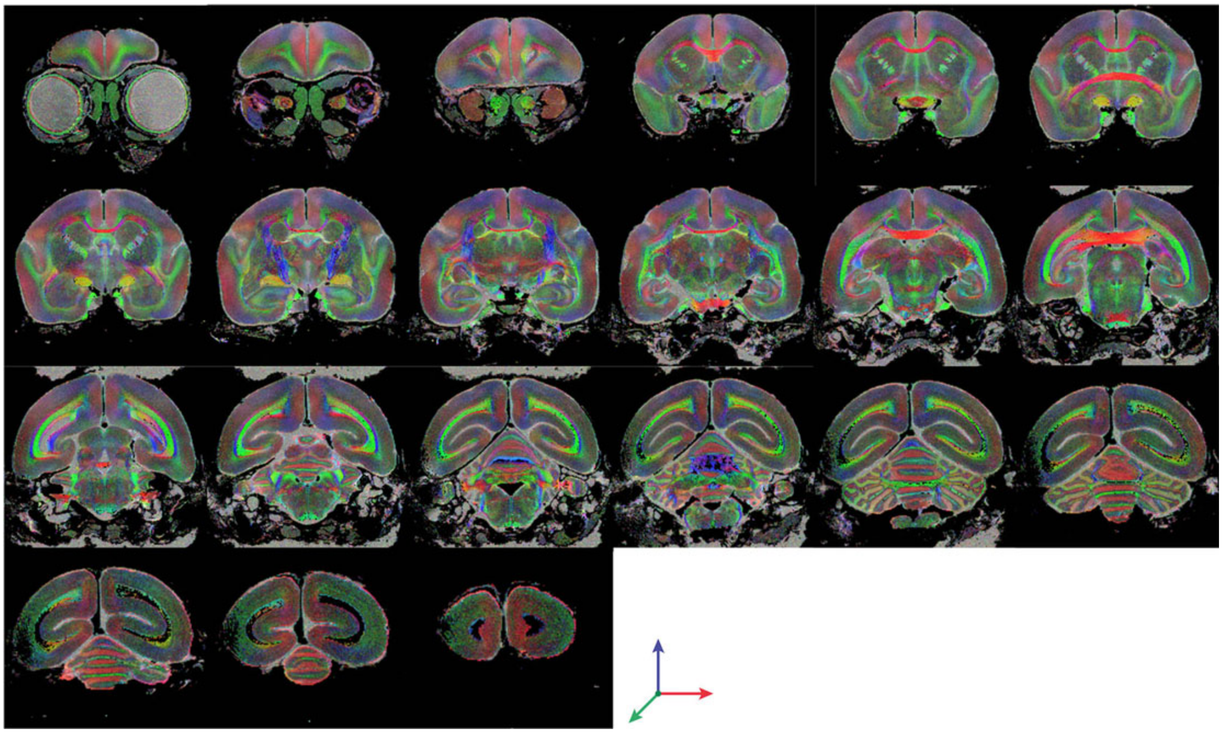

Fig. 4. Images from MRI connectomics analysis. Representative high-resolution DTI (120 μm isotropic) of the common marmoset brain overlaid with anatomical MRI. The color-coded map obtained from DTI shows white-matter structures identified through analysis of water molecule anisotropy. Colors indicate the fiber direction: green, antero-posterior; red, medio-lateral and blue, cranio-caudal.

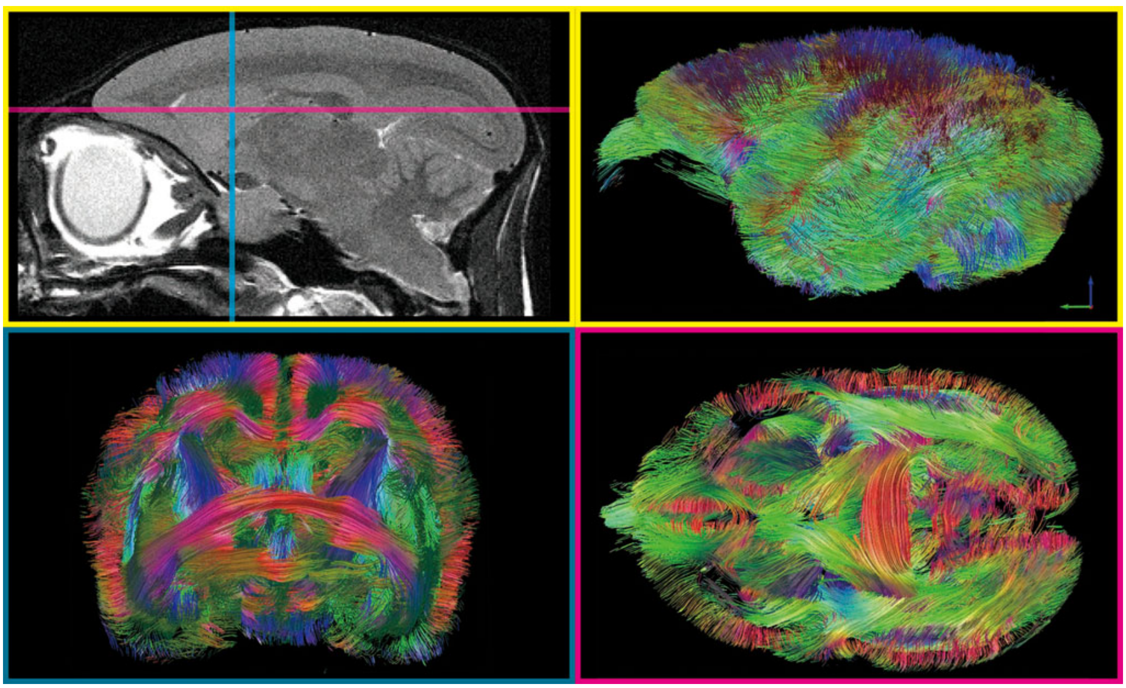

Fig. 5. The macroscale fiber connections of the whole brain identified using tractography. Tractography using DTI data can be used to identify fiber bundles in the common marmoset brain at the macroscale. Upper-left: Sagittal plane anatomical image. Upper-right: Lateral view of all fiber tracts, created using whole-brain tractography. Lower left: Fiber tracts from coronal section of the anatomical image (cyan) created using tractography. Lower right: Fiber tracts from axial section of the anatomical image (magenta) created using tractography. Colors indicate the fiber direction: green, antero-posterior; red, medio-lateral and blue, cranio-caudal.

of white-matter fiber tracts (Fig. 3) and is another approach used to map connectivity [25,26]. The most frequently used techniques are diffusion tensor imaging (DTI) (Fig. 4) and diffusion tensor tractography (Fig. 5) [27–29]. These techniques allow localization and study of the circuitries formed by major fiber tracts. Although analysis of crossing fibers presented a hurdle due to difficulties resolving multiple fiber bundle orientations inside imaging voxels [30], advanced techniques such as high angular resolution diffusion imaging (HARDI) and diffusion spectrum imaging (DSI) overcame this problem and allowed more precise evaluation of crossing fibers [31–34].

The organization of structural connectivity can be further studied by application to network analysis [26]. Analysis of diffusion MRI data with graph and network theory can provide further information about network characteristics, such as centrality and efficiency at the whole-brain level, to reveal brain neuronal communication and architectures [35]. The resulting network architectures contain rich but complex information. Circular visualization (referred to as connectograms) was developed to retrieve information from these analyses in an effective manner [36]. The comprehensive circle map allows visual classification of connective relations and makes it possible to detect abnormalities when applied to clinical data and animal models of disease [37]. The reliability of connectogram visualization of multidimensional information obtained from MRI can be increased by combining it with anatomical tract tracing. Cross validation of macroscale MRI analysis with an accurate histological analysis should be performed in the same experimental samples. A non-human primate, for example, a common marmoset, is a promising experimental resource for cross validation.

Microscale connectomics with EM, comparing to the macro and mesoscale

EM is used to observe ultrastructure in various applications, from the diagnosis of human biopsy samples to the histological observation of many kinds of tissues and their microstructure and organelles, including the synapse, cilia, mitochondria and myelin structure. The accelerated electrons enable structure to be visualized at the subcellular level. EM has a much higher resolution than LM due to the shorter wavelength of the electron compared with that of visible light. Although diffusion MRI analysis with an extremely high resolution was recently achieved [38], the resolution of MRI is still lower than that of LM and EM. For achieving the multiscale brain mapping, we have to take advantage of these three major technologies (LM, MRI and EM). The characteristics of each technology for connectomics analyses are summarized in Table 1.

Both MRI and EM analyses require highly skilled staff and expensive machines, but this is not the case for LM. If there is a support from skilled staff, MRI can easily image a whole brain, whether small (e.g. a mouse or rat brain) or large (e.g. a human or primate brain). EM imaging also requires skilled staff, and it is much more difficult to carry out whole-brain mapping, especially for a large brain like that of a human.

MRI is usually used for live imaging (Fig. 5) and is also used on the post-mortem brain to obtain high-resolution images (Fig. 4). LM can be used to image live and dead (fixed) samples and is generally used with fluorescence time-lapse imaging for live imaging and with multicolor immunostaining of fixed samples. Because of the low penetration of electrons to water, air and other substances, observation with EM generally requires a vacuum and ultrathin sectioning of the samples along with hard fixation. Live animal imaging with EM can be performed only with creative ingenuity, including the use of high vacuum-resistant creatures [39], vacuum-resistant surface modifications designated as nano-suit [40] or the use of special EM named atmospheric scanning electron microscope (ASEM), which detects dynamic phenomena in liquid or gas [41–43].

Microscale connectomics with serial EM

Serial EM is a great technology that utilizes the EM to obtain serial images from continuous sections. There is a long history for this technology pioneered by John K. Stevens, Kristen M. Harris and their colleagues from the early 1980s [44–47]. They developed a serial reconstruction system for collecting, photographing and analyzing the

Table 1. The three major technologies used for connectomics analysis

	Light/fluorescence microscopy	Magnetic resonance imaging	Electron microscopy
Imaging performed using	Light (fluorescence)	Magnetic resonance	Electron
Resolution	High	Low	Extremely high
Skill level required for imaging	Low	High	High
Expense	Moderate	High	High
Whole-brain imaging	Difficult	Easy	Very difficult
Live imaging	Easy	Easy	Difficult

continuous EM sections from the synapses and dendrites. Their effort makes it possible to observe the subcellular structure with extremely high resolution; much higher than can be obtained with LM or MRI. In addition, this EM-based approach enables quantitative three-dimensional evaluation of the precise location of synapses, vesicles, buttons, mitochondria, endoplasmic reticulum, ribosomes, cilia, myelin and other organelles in a micro-domain of the brain [48–50]. Serial EM has been widely applied to many projects to analyze the microstructure of cells and tissues in three dimensions with high resolution. An important characteristic of serial EM is that the serial image acquisition is carried out not only by a transmission electron microscope (TEM), but also by SEM. For general TEM observation in biology, the culture cells and tissues are fixed, dehydrated, embedded into plastic and ultrathin sectioned. In contrast, SEM analysis focuses on detecting the detailed surface information of the specimen through the critical point drying. The progress of SEM technology means that it is now possible to obtain high-resolution images with adequate contrast from the sectioned surface of biological samples. Primary challenges for the serial EM were carried out by using TEM; however, a new hybrid technique of SEM and TEM was recently developed [51]. The introduction of automated transmission-mode scanning electron microscopy (tSEM) for large volume reconstruction was reported. In addition, another group utilized TEM camera array (TEMCA) system, which is the combination of commercially available TEM and a large scintillator with four high-speed charge-coupled device (CCD) cameras for acquisition of large field view of serially sectioned EM samples efficiently [50].

As for the SEM-based techniques, there are two independent procedures used to obtain serial EM images of the surface from the resin-embedded tissue. One, designated as ATUM, is to collect all serial ultrathin sections on tape or glass, and the other is to carry out sectioning in a vacuum chamber for continuous observation on the block surface. There are three major SEM-based approaches for serial EM: the ATUM system with SEM (ATUM-SEM), the serial block-face SEM (SBF-SEM) and the focused ion beam-SEM (FIB-SEM). The characteristics of these approaches are summarized in Table 2. ATUM-SEM is a new procedure for serial EM that was developed by Prof. Jeff W. Lichtman and his group [4,52]. The block of the brain prepared for EM is embedded in plastic and sectioned to around 30 nm thickness, and the sections are automatically collected on a continuous plastic flexible tape using a conveyor-belt device called ATUM (Fig. 6). The ATUM device can generate a series (as many as several tens of thousands) of brain slices without any loss of the sample. The sections on the flat tape are placed on a silicon wafer, coated with a conductive material and observed with the SEM at high resolution. The ATUM-SEM has some advantages and some disadvantages. One advantage of this system is the collection of sections on a stable plastic tape, meaning that it is easy to image each sample multiple times with different resolutions. This also makes it possible to carry out immunostaining (post-embedding immuno-EM) on the tape. There are many options to increase the conductive property of the section, including carbon coating, colloidal silver, carbon double-stick tape and increased conductivity of tape, and this helps to increase the image quality by allowing the escape from unwanted charge-up. For the progress of connectomics, a

Table 2. The three major serial-EM approaches for connectomics analysis

	Automatic tape-collecting ultramicrotome scanning electron microscopy	Serial block-face scanning electron microscopy	Focused ion beam scanning electron microscopy
Abbreviations	ATUM-SEM	SBF-SEM	FIB-SEM
Sectioning performed by	Diamond knife	Diamond knife	Ion beam
Sectioning performed in	Automatic tape-collecting ultramicrotome	SEM chamber	SEM chamber
Fate of sections	Placed on the tape	Lost in chamber	Disappear
Imaging performed by	SEM	SEM	SEM
Location of tissue when imaged	Sections on the tape	Block surface	Block surface
Potential for re-observation	High	Impossible	Impossible
XY resolution	Extremely high	High	High
Z resolution	High	High	Extremely high
Size of imaged area	Wide	Moderate	Extremely narrow
Ease of three-dimensional reconstruction	Difficult	Easy	Easy
Potential for staining	Moderate	Impossible	Impossible

SEM, scanning electron microscope.

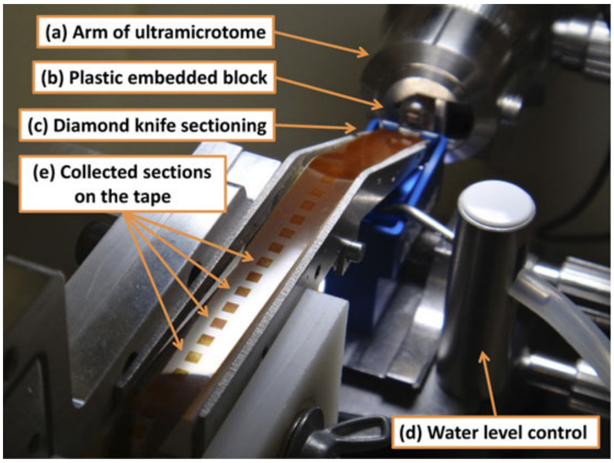

Fig. 6. A photograph of the tape collection in ATUM. The ATUM device with an ultramicrotome automatically collects serial ultrathin sections on a tape with a regular interval. The arm of the ultramicrotome (a) holds the plastic embedded tissue block (b) and makes ultrathin sections with a diamond knife (c). The system can generate several tens of thousands of sections, and the water level of the knife's water boat is automatically maintained (d). The partially submerged conveyor belt collects the ultrathin sections on to the surface of tape (e).

large area of observation is desirable. The ATUM-SEM system can be used to image an area of several square millimeters (high XY resolution), which is the largest area possible among the several serial-EM approaches. The major factors limiting the size of the imaged area are the infiltration of the fixative used for sample preparation, the maximum width of the diamond knife, the width of the collecting tape and the file size of the tiling images. The Z resolution is limited to 20–40 nm (average, 30 nm) owing to the minimum interval of the diamond-knife sectioning and the solidity of the plastic within which the brain is embedded. A disadvantage of the ATUM-SEM is the rotation of sections or unequal interval between sections on the tape, which makes it difficult to acquire sequential images automatically.

The SBF-SEM system is another SEM-based serial-EM imaging strategy used to acquire images of the top surface of microtome-sectioned plastic inside the SEM chamber [53]. Each section created with the diamond knife exposes a new surface for imaging, and thousands of sectioning repetitions create a series of surfaces for imaging. One advantage of this system is the automatic image acquisition and three-dimensional reconstruction carried out by the software built into the SEM. The possibility of the misalignment along the XY axes is limited; therefore, it is easy to find the same position in each section. This allows accurate automation of image acquisition, tiling, three-dimensional reconstruction and neural tracing. The disadvantage of the SBF-SEM system is that all sections are lost after sectioning. It is impossible to image an area of interest a second time at a different resolution, or to apply post-embedding immuno-EM to the surface of the section. There is a limited chance to enhance the conductivity of the block itself to allow the escape of unwanted charge-up. The maximum area that is possible to observe using a recent version of SBF-SEM is several 100 μm square, and this is restricted by the width of the diamond knife, the size limit of the loaded block and the maximum area that can be imaged by the microscope. The Z resolution is similar to the ATUM system; however, the thickness of the section depends on the quality of the conductive plastic within which the sample is embedded.

The third approach for serial EM is the FIB-SEM [54]. Similar to the SBF-SEM system, images are obtained from the surface of the sectioned block inside the SEM chamber. The unique characteristic of the FIB-SEM is that sectioning is carried out using a FIB, and FIB-SEM is, therefore, the best solution for studying hard tissues such as teeth and bone. The diamond knife used in the ATUM and SBF-SEM systems would not be able to cut these hard tissues smoothly and may become damaged by the tissue. An additional advantage of FIB-SEM is that the FIB can perform extremely thin sectioning (as thin as 4 nm), and the Z resolution is, therefore, higher than in the diamond-knife-sectioning approaches. The automated image acquisition and easy three-dimensional reconstruction without XY dislocation is another merit of the FIB-SEM. A disadvantage of the FIB-SEM, similar to the SBF-SEM, is the loss of the sections after sectioning, leaving no chance for re-observation or post-embedding immuno-EM and limited alternatives for enhancing the conductivity. An additional disadvantage of the FIB-SEM is that it has the smallest area of observation of all three approaches at 10 μm square area at best, even for the latest machine.

Application for LM/EM correlative analysis

In a small number of laboratories, the localization of protein or RNA is routinely visualized with different levels of resolution using both LM and EM for exactly the same sample [55–65]. This approach gives important information regarding microstructure and molecular localization in a mutually complementary manner. The combination of the serial-EM technology and the LM/EM correlative analysis that we used helps to increase the evidence for understanding physiological phenomena in future studies. It is quite important and meaningful to develop novel correlative technologies in order to connect the mapping results obtained from microscale EM and mesoscale LM.

Conclusions and perspectives

Several international connectomics projects recently began, and connectomics is attracting increasing attention. The study of connectomics reveals the structure and function of human brain and can shed light on the mechanisms underlying neuronal diseases. The recently launched Japanese connectomics project (Brain/MINDS) aims to create a structural and functional map of the non-human primate brain by studying neural connectomics on various scales (macro, meso and micro). This project will utilize the latest technological innovations and involve international collaboration with Europe and the United States. For the structural connectomics aspect of the Brain/MINDS project, specific brain areas associated with disease in the common marmoset will be analyzed using MRI, LM and EM [1]. We hope the challenges for connectomics described here will be solved in the near future to further progress our understanding of the human brain and mind.

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