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Fluorescence Micro-Optical Sectioning Tomography (fMOST) to Study Neural Circuits in Mice

Tomografía de Seccionamiento Micro-Óptico por Fluorescencia (fMOST) para el Estudio de Circuitos Neuronales en Ratones

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SUMMARY: Neural circuits, serving as the fundamental framework for brain functioning, intricately regulate cognitive processes, emotional responses, and behavioral patterns, thus playing a pivotal role in comprehending the complexity of the brain. However, the extreme complexity and exquisite refinement of their internal structures pose big challenges to direct observation and in-depth analysis. In this context, the Fluorescent Micro-Optical Sectioning Tomography (fMOST) has emerged as a game-changer, harnessing its exceptional high-resolution three-dimensional imaging capabilities to pave an unprecedented path for research on neural circuit. This review provides a thorough overview of the historical evolution of the fMOST technology, tracing the development from its initial conceptualization to the current state of mature applications, along with the remarkable technological leaps and breakthroughs witnessed at each step. Additionally, the review delves into several prominent fMOST technological branches, each of which excels in enhancing the imaging quality and expanding the observation scope, collectively pushing the boundaries of neuroscientific research. Crucially, this article underscores the central role of fMOST in dissecting neuronal networks and elucidating the mechanisms of neural circuits, particularly its unique contribution in exploring the potential links between the abnormalities in neural circuit and different brain diseases. By integrating the latest research findings, this review further anticipates the broad application prospects of the fMOST technology in future neuroscientific endeavors and how it would continue to guide us in delving deeper into the mysteries of the brain.

KEY WORDS: Neural circuits; fMOST; Whole-Brain imaging; Neuron three-dimensional reconstruction.

INTRODUCTION

Neurons serve as the fundamental units in realizing the functions of the brain and forming neural circuits, where different neurons are interconnected to constitute neural pathways for transmitting information, and ultimately governing human behaviors and ideologies. Abnormalities in these neural circuits may lead to different brain diseases, manifesting various dysfunctional activities of the brain (Tye, 2014). Therefore, a thorough understanding of the neural circuits is crucial for a better comprehension about biological tissue functions and diseases. Despite huge investments in neuroscience, our comprehension about the working mechanisms underlying complex functions, such as learning, consciousness, and emotions, still remains limited. This lack of knowledge is the main reason of still not having effective treatments for numerous neurological disorders. At the same time, the inherent fine and multi-scale characteristics of neuronal morphology also pose challenges to the development of imaging technology (Wang *et al*., 2021). In recent years, with advancements in the optical imaging technology, the fluorescence micro-optical sectioning tomography (fMOST) has emerged as an effective approach for studying neural circuits. fMOST integrates microscopic

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fluorescence imaging with rapid and inertia-free scanning techniques, enabling three-dimensional (3D) high-resolution fluorescence imaging at centimeter level[D1] . Its[L2] core lies in sequentially scanning a sample layer by layer through continuous optical sectioning, and capturing fluorescence signals to reconstruct a 3D structural image of the sample. This review integrates the latest research findings in this field, focusing on the development of the fMOST technology and its recent applications in the neural circuits of mouse.

2. Development of fMOST technology

The development history of the fluorescence Micro-Optical Sectioning Tomography (fMOST) technology can be traced back to the early 21st century, and it can be broadly summarized into the following key stages:

2.1 Initial Stage: Introduction to MOST Technology and Whole-Brain Imaging. In 2010, Luo Qingming first proposed the MOST (Micro-Optical Sectioning Tomography) technology and successfully applied it to the whole brain of a Golgi-stained mouse, achieving high-resolution three-dimensional imaging of the entire brain (Li *et al*., 2010). This groundbreaking achievement laid the foundation for subsequent developments in whole-brain imaging technologies.

2.2 Development Stage: Birth of fMOST Technology and Fluorescent Labeling of Images. Based upon the success of the MOST technology, Luo Qingming further developed the fMOST (fluorescence Micro-Optical Sectioning Tomography) technology (Li *et al*., 2010). This innovation enabled high-resolution three-dimensional imaging of fluorescently labeled mouse whole-brain samples, allowing researchers to trace long-range projection neurons labeled with fluorescent markers (Gong *et al*., 2013). This advancement significantly expanded the capabilities of whole-brain imaging and opened new avenues for neuroscience research.

2.3 Development of Variant and Specialization. In order to cater to diverse research needs, various fMOST variants were developed, including single-photon fMOST, two-photon fMOST, and systems utilizing structured illumination. Each variant offered unique advantages, for instance, the two-photon fMOST improved the penetration depth, while the structured illumination enhanced the resolution and contrast. These tailored approaches allowed researchers to select the most suitable imaging modality for their specific experiments.

2.4 Maturity Stage: Application of fMOST Technology in Whole-Brain Connectomics. As fMOST technology matured, its application scope was broadened to include whole-brain connectomics. Researchers employed the fMOST technology to map the whole-brain projection patterns of various types of neurons, revealing the complex connectivity between neurons (Gao *et al*., 2022) These studies provided valuable insights into the organization and function of neural circuits.

2.5 Innovation Stage: Optimization and Enhancement of fMOST Technology. In recent years, the fMOST technology has undergone continuous optimization and enhancement in terms of imaging resolution, imaging speed, and data processing. Researchers have developed multiple improved fMOST techniques, such as those using novel fluorescent probes and optimizing imaging parameters, to further improve the imaging quality and efficiency (Long *et al*., 2019). These innovations have pushed the boundaries beyond the whole-brain imaging, and paved the way for even more detailed and comprehensive studies of neural circuits and brain function. Figure 1 illustrates the basic components and operating principles of the fMOST system.

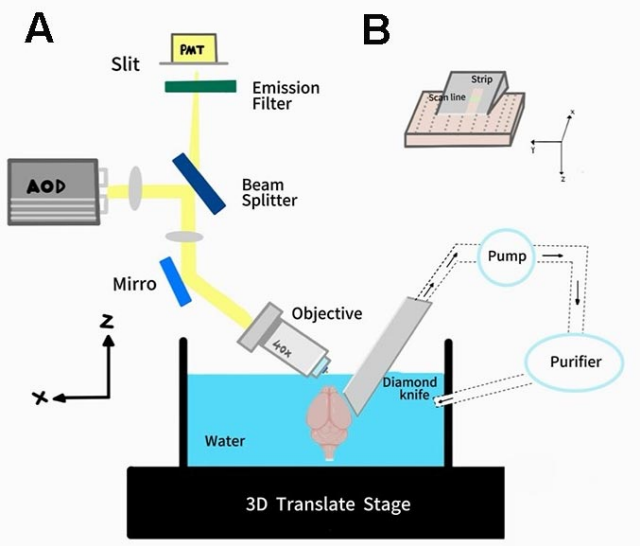

Fig. 1. The compositions and principles of fMOST system (Source from: Yang *et al*., 2013). A, Schematic representation of fMOST. The sample is mounted in a chamber filled with water, the motion of sample is controlled by a 3D translate stage. Slicing is performed by moving the sample along X-axis to generate strips, and each strip is imaged by a water-immersion objective simultaneously. Strips that have been imaged are then removed by a pump and purifier. Fluorescence imaging is achieved by a AOD-based confocal laser scanning fluorescence microscopy. AOD, acousto-optical deflector. B, Schematic representation of slicing and imaging at the same time. The thin strips will slid along the surface of the diamond knife and confocal laser scanning fluorescence imaging is performed immediately.

3. Commonly used types of fMOST techniques

In recent years, with the continuous iteration and advancement of technology, fMOST has evolved into various advanced versions, each tailored to optimize specific research requirements. This section introduces several frontier versions of the fMOST technique. Figure 2 illustrates the schematic diagrams of various microscopy tomography systems.

3.1 TDI-fMOST. The Wuhan National Laboratory for Optoelectronics successfully developed a fluorescence microscopic sectioning imaging system based on time-delay integration, known as TDI-fMOST (Yang *et al*., 2015). This system is a rapid line-scan fluorescence imaging system that not only inherits the outstanding characteristics of the MOST system but also undergoes further optimization and development. The prominent advantages of the TDI-fMOST system lie in its remarkable stability, ease of maintenance

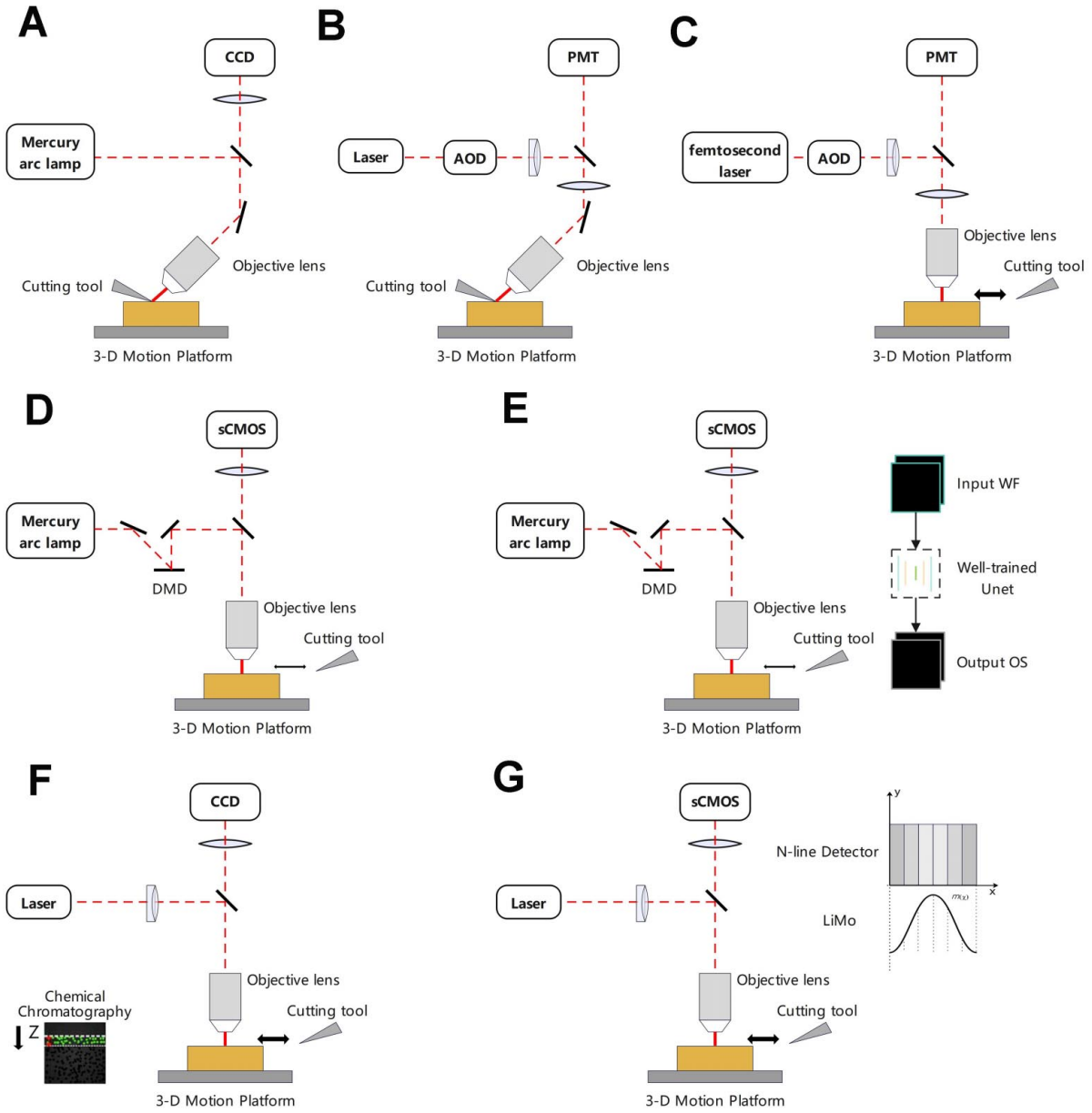

Fig. 2. Schematic diagrams of various microscopy tomography systems (Source from: Gao *et al*., 2022). A, Micro-optical Sectioning Tomography (MOST). B, One-photon Fluorescence Micro-optical Sectioning Tomography (1p-fMOST); C,Two-photon Fluorescence Micro-optical Sectioning Tomography (2p-fMOST); D, Structured Illumination Fluorescence Micro-optical Sectioning Tomography (SI-fMOST); E, Deep-learning-based fluorescence micro-optical sectioning tomography (DL-fMOST); F, Chemical Sectioning Fluorescence Micro-optical Sectioning Tomography (CS-fMOST); G, High-definition Fluorescence Micro-optical Sectioning Tomography. (HD-fMOST).

and expansion, support for multi-channel imaging, and fast imaging speed. These features enable it to provide reliable and efficient imaging results, offering researchers a comparatively more convenient operational experience.

Note that, when combined with chemical tomography techniques for imaging (Xiong *et al*., 2014; Gang *et al*., 2017), it can achieve full-brain multi-channel tomographic imaging of mouse brains at high speed, high throughput, and sub-micrometer resolution. This capability significantly enhances the imaging precision and efficiency of TDI-fMOST, allowing researchers to observe neuronal structures and their subtle synaptic changes in mouse brains with greater clarity.

From the green channel full-brain imaging data acquired by TDI-fMOST, researchers can not only clearly distinguish neuronal morphologies and projection pathways of each individual process but also visualize tiny synaptic structures such as dendritic spines and synaptic boutons. These detailed imaging data offer researchers the possibility to reconstruct the complete morphology of labeled neurons at the synaptic level, and acquire and localize the fine projection pathways of neuronal axons.

3.2 cryo-fMOST. Although the fMOST technology offers high-resolution imaging capabilities, it still has certain limitations. Firstly, gelatin and resin are two common materials used in fMOST for fixing samples. Gelatin is often employed as an embedding medium that permeates tissue samples, aiding in fixing and supporting tissue structures. However, the softness of gelatin may lead to the distortion of samples during cutting, necessitating careful handling and potentially requiring additional data processing steps to correct such distortions (Economo *et al*., 2016). On the other hand, resin is typically used for more robust embedding to maintain sample stability during sectioning by providing better mechanical support, and helping to prevent sample deformation during slicing. Nevertheless, the heat generated and chemical changes taken place during the resin polymerization may potentially cause some degree of deformation or damage to the samples (Yang *et al*., 2013), thereby affecting the accuracy of imaging.

Secondly, due to the limitations in the sample morphology and size, the fMOST technology faces difficulties in imaging multiple types of intact organs in practical applications. In order to address these issues and obtain complete three-dimensional fine structures and molecular information about biological tissues and organs, while preserving their tissue architectures and biochemical information. Deng *et al*. (2022), developed the Cryo fluorescence micro-optical sectioning tomography (cryo-fMOST) technique in 2022. The cryo-fMOST technique utilizes freezing to preserve the structural and biochemical information about samples. By integrating advanced techniques, such as an overlapping refrigerated low-temperature sample chamber, precise frozen tissue cutting technology, and a low-temperature micro-optical imaging module, this technology enables continuous high-resolution micro-optical tomographic imaging of frozen tissue organs over tens of hours. Notably, it successfully performed three-dimensional imaging and reconstruction of intact organs, such as tongue, kidney, and brain of normal mice, as well as the heart of a mouse model of myocardial infarction.

In order to further validate the multi-omics measurement compatibility of the cryo-fMOST technique, Deng *et al*, (2022) also performed sugar phosphate assays and in situ hybridization imaging on the imaged brain sections. Additionally, they developed a 3D cryo-fMOST system, which can not only improve the collection efficiency but also enhance the compatibility with subsequent biochemical experiments.

3.3 HD-fMOST. High-resolution Fluorescence Micro-Optical Sectioning Tomography (fMOST), an imaging method based on optical microscopy, aims to achieve high-resolution three-dimensional reconstruction, particularly suited for studying fine structures and functions of brain tissues. It is widely applied in many fields, such as neuroscience, pathology, developmental biology, and drug research.

This technology integrates various advanced micro-imaging and slicing techniques, utilizing genetically encoded calcium indicators (GCaMP6) for sparse labeling in conjunction with two-photon calcium imaging and high-definition full-brain imaging technology (HD-fMOST), enabling the correlation of functions with whole-brain axonal projections of L2/3 neurons in the visual cortex of live mice. The relevant experiments were conducted as follow (Zhou *et al*., 2022): Firstly, sparse labeling was done by injecting rAAV viruses containing hSyn-Cre and Cre-dependent reporter gene hSyn-DIO-GCaMP6. Subsequently, using the motion vision as a model, the responses of L2/3 layer neurons to moving gratings in awaking mice were recorded. Then, high-resolution whole-brain imaging was performed using the HD-fMOST system, and the precise locations of GCaMP6-labeled neurons throughout the brain were identified through cell matching.

With continuous advancements in hardware and software, as well as the development of new genetically encoded calcium indicators (such as jGCaMP7 and

jGCaMP8), the efficiency of functional imaging in the HD-fMOST technology has been enhanced. Furthermore, the large-field-of-view and volumetric two-photon imaging, machine learning for batch matching tasks, and the application of artificial intelligence in tracking will further improve the throughput of the whole-brain functional mapping.

4. Applications of fMOST in Mouse Neural Circuits

4.1 Mapping of Whole-Brain Neuronal Projections. Utilizing the fMOST technology, researchers successfully mapped the whole-brain projection atlases of various neuronal types in mice. For instance, in 2019, Sun *et al*. (2019) employed a combination of fMOST and retrograde viral tracing to map the long-range input circuits of γ-aminobutyric acid (GABA)ergic neurons in the mouse medial prefrontal cortex (mPFC), revealing the intricate connectivity patterns of these neurons across different regions of the brain. They discovered that the long-range input circuits of GABAergic neurons primarily project to regions, such as the amygdala and hippocampus, and distinct subpopulations of GABAergic neurons exhibit unique projection patterns. These connectivity patterns are intimately linked to emotion regulation, learning, and memory behaviors in mice. Fast forwarding to 2024, Li *et al*. (2024b) demonstrated the utilization of the fMOST technology to obtain a whole-brain projection atlas of oxytocin neurons in the paraventricular nucleus of the mouse hypothalamus, providing crucial insights into the functions of these neurons under both physiological and pathological conditions. They found that oxytocin neurons not only project to traditional target areas, like the pituitary gland, but also extensively project to multiple regions of the cerebral cortex participating in the regulation of social behavior, stress response, and other physiological processes.

Having elucidated how the fMOST technology has empowered researchers to map whole-brain projection atlases of diverse neuronal types in mice and delved into the intricate links between these projection patterns and specific behavioral functions, we now shift our focus to two more specific and challenging research directions, outlining specific steps in each direction. Firstly, we concentrate on the mapping of the whole-brain histaminergic projection atlas in mice using the fMOST technology. This endeavor aims to delve deeply into how histaminergic neurons, through their unique projection networks, participate in regulating complex physiological and behavioral processes. Secondly, we explore how to integrate *in vivo* imaging techniques with the fMOST imaging to further unravel the projection patterns and dynamic changes in dCA1 neurons with distinct response modes across the entire brain.

4.1.1 Construction and analysis of whole-brain map of histaminergic projection in mouse. Histaminergic neurons form intricate connection networks with other neurons through their axons and dendrites, collectively constituting the histaminergic neuronal network. This network is believed to regulate various physiological functions, including sleep-wake cycles, attention, learning and memory, emotion regulation, and energy homeostasis (Lin *et al*., 2023). However, despite the widely recognized importance of the histaminergic system, the detailed projection patterns throughout the brain and the mechanisms underlying its functional connections with different brain regions are not yet well understood. Currently, advanced imaging

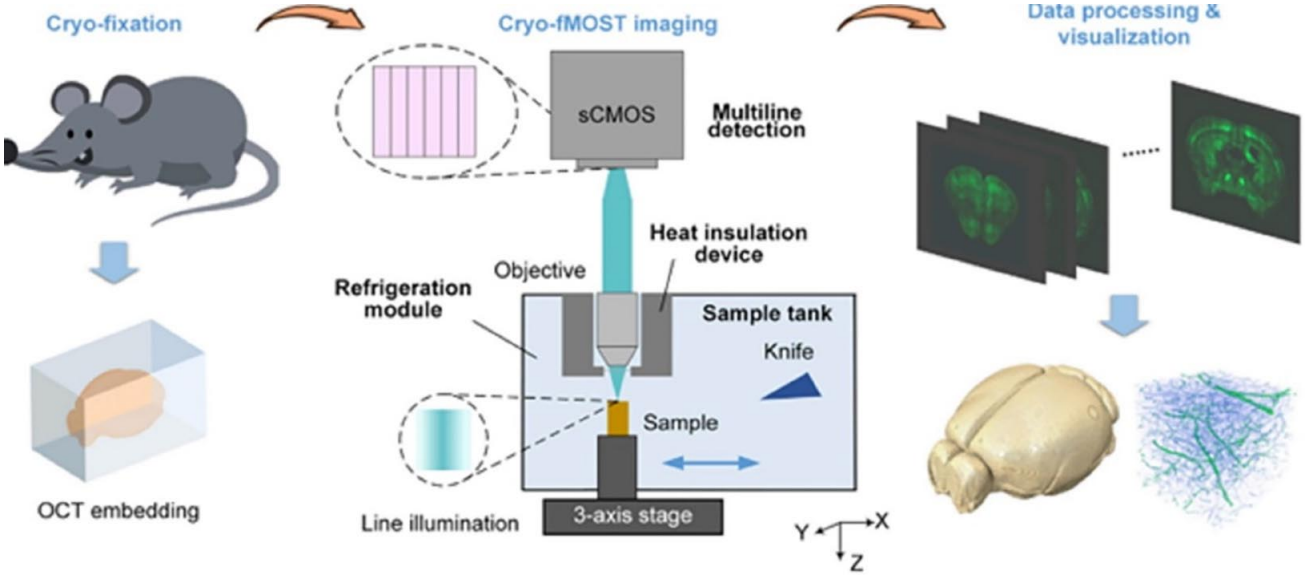

Fig. 3. Schematic of cryo-fMOST pipeline (Source from: Deng *et al*. (2022). Study on Cryo-fluorescence micro-optical sectioning tomography for volumetric imaging of various whole organs with subcellular resolution[J].

technology (fMOST) and genetic labeling methods are employed to map the whole-brain histaminergic projections in mice at the mesoscale for the first time. Its general steps are as follows:

1) Preparation of Experimental Animal. DC-CreERT2 Transgenic Mice: HDC (Histidine Decarboxylase), the key enzyme for histamine synthesis, is utilized in HDC-CreERT2 mice to enable tamoxifen-induced specific labeling of histaminergic neurons at designated time intervals.

Tamoxifen Induction: Tamoxifen is administered to the mice at specific time intervals to induce the expression of the Cre recombinase, thereby marking histaminergic neurons.

2) Preparation Tissue Sample.

Perfusion and Fixation: After deep anesthesia, mice undergo perfusion to remove blood, followed by the fixation of the brain tissue with 4 % paraformaldehyde.

Dehydration and Clearing: The brain tissue is processed with appropriate dehydrants and clearing agents to facilitate subsequent optical imaging.

3) fMOST Imaging.

Optical Sectioning: The entire mouse brain is subjected to continuous optical sectioning using the fMOST system. This technique combines high-resolution optical imaging with automated sectioning, enabling continuous high-resolution imaging of the entire brain.

Fluorescent Labeling: Histaminergic neurons genetically labeled are marked with specific fluorescent dyes prior to imaging for identification during the imaging process.

4) Acquisition and Processing of Data

Image Acquisition: High-sensitivity cameras are used to capture images of each optical slice layer.

Image Reconstruction: The acquired images are then reconstructed in three dimensions (3D) to form a 3D image of the entire mouse brain.

Neuron Tracing: Image analysis software is employed to trace the axonal projections of histaminergic neurons for reconstructing their precise structures throughout the brain.

5) Data Analysis

Quantitative Analysis: Quantitative analyses are performed on the distribution areas.

Structural Feature Analysis: Characteristics of histaminergic neuronal cell bodies and fibers, including their number, density, and distribution, as well as the 3D structural features of histaminergic projections, are analyzed from the perspectives of both whole-brain and individual neuron.

6) Results and Validation

Whole-Brain Atlas Construction: The reconstructed 3D structures of histaminergic projections are presented as a

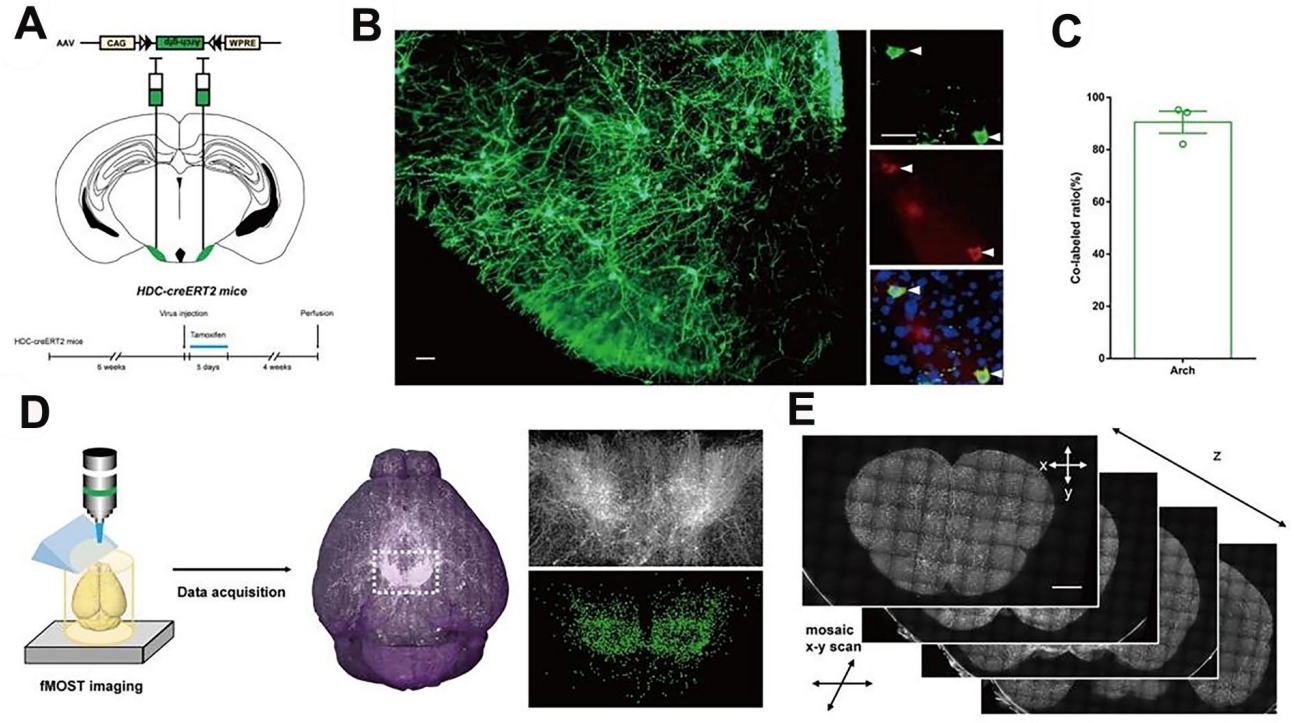

Fig. 4. Virus-labeled histaminergic neurons and downstream circuits in HDC-ArchGFPTMN mice (Source from: Lin *et al*., 2023). A, Experimental scheme and timeline for generation of HDC-Arch-GFPTMN mice. AAV-CAG-FLEX-ArchT-GFP was injected into each side of the TMN of HDC-CreERT2 adult mice; B, Representative immunohistochemical pictures of expression specificity in HDC-Arch-GFPTMN mice; GFP and HDC double-positive neurons are indicated by arrowheads.(Scale bars: 50 mm.); C, Expression specificity analysis of GFP in HDC-ArchGFpTMN mice; D, Main steps of data generation, acquisition in fMOST imaging; E, Processing in fMOST imaging.

whole-brain atlas for showcasing their projections in different brain regions.

Validation and Comparison: The accuracy and reliability of the fMOST technique are verified by comparing its results, such as the electrophysiological recordings and immunohistochemical staining, with those obtained from other techniques.

Figure 4 provides evidence of successful viral labeling of histaminergic neurons in HDC-ArchGFPTMN mice and outlines theprocess for visualizing these neurons and their circuitry using the cryo-fMOST imaging.

4.1.2 Mapping Whole-Brain Projection Atlas of Mice using fMOST Technology Combined with *in vivo* Imaging

In order to investigate the projection atlas of the functionally defined neurons in the dorsal CA1 (dCA1) region, we successfully mapped the projections of dCA1 neurons with distinct response patterns by combining *in vivo* imaging and fMOST imaging techniques (Li *et al*., 2024a).

1) Experimental Animals and Virus Injection. Male C57BL/6J and DAT-Cre mice aged 6-13 weeks were used in the experiments. At the age of 6 weeks, the mice were injected with viruses. After 6-7 weeks of viral expression, the mice were sacrificed for fMOST imaging.

2) dCA1 Surgery. In order to map the projection atlas of the functionally defined dCA1 neurons, a mixture of AAV2/8-hSyn-Cre-WPRE and AAV2/9-syn-FLEX-jGCaMP7b-WPRE or AAV2/9-hSyn-FLEX-GCaMP6s-WPRE viruses was utilized. Wild-type C57BL/6J mice received injections of 20-40(nl) of the viral mixture into their right dCA1 region (coordinates relative to bregma: AP -2.06 mm, ML 1.35 mm, DV -1.3 mm). One week later, a GRIN lens (1.0×8.2 mm, NEM-100-40-10-520-DS, GRIN-TECH GmbH) was implanted above dCA1.

3) fMOST Imaging, Cell Reconstruction, and Image Registration. Imaging of the mouse brain samples was performed using the HD-fMOST system, which integrates the linear illumination modulation (LiMo) microscopy with the thin-layer tissue slicing technology. The embedded brain was imaged in a water bath containing propidium iodide (PI) at a voxel resolution of 0.325×0.325×1.0 mm^3. The HD-fMOST system automatically executed the imaging and slicing cycles until the acquisition of the whole-brain data was complete. The raw data were saved in 16-bit depth and TIFF format with LZW compression (Fig. 5). Experiments were performed using combination of viral expression-based labeling, *in vivo* deep brain calcium imaging and HD-fMOST imaging, providing a basis for proposing a strategy to link the whole-brain projection groups to the neurodynamics of individual neurons.

4) Data Analysis. The projection strength matrix for each brain region was calculated using ln (axon length + 1). The probability of projection for each functional cluster was determined by dividing the number of neurons projected to the target brain region by the total number of neurons in the corresponding functional cluster. The hierarchical clustering was performed based on the projection intensity matrix using the Euclidean correlation metrics and Ward's linkage.

5) Combining Whole-Brain Projection Mapping with Neuronal Calcium Dynamics. Using the somatosensory cortex as an example, the whole-brain projection map of S1BF neurons, activated by mechanical whisker stimulation, was mapped by combining two-photon calcium imaging and HD-fMOST. Sparse labeling of L2/3 neurons in S1BF was achieved by injecting AAV-CaMKII-Cre and AAV-hSyn-Flex-jGCaMP7b. Two weeks after viral expression, the imaging of the neuronal dynamics began, converting the calcium dynamic responses of L2/3 neurons into mechanical stimulation.

4.2 Relationship Between Neural Circuits and Diseases. In recent years, the fMOST technology has also been applied extensively in exploring the relationship between neural circuits and diseases. For instance, by performing fMOST imaging on a mouse model of the Alzheimer's disease (AD), Ma *et al.* (2024), observed a significant shortening in dendrite lengths and a decrease in synaptic numbers among efferent neurons projected from the prefrontal cortex to the telencephalon. Further research revealed that the acetylcholine deficiency led to the disruption of calcium homeostasis in these neurons, subsequently impairing their structural and functional integrity. Monitoring of the neuronal activity indicated that the firing frequency and patterns of these neurons also underwent abnormal changes. This study offers a novel perspective on elucidating the pathogenesis of AD and provides a basis for developing new therapeutic strategies. Additionally, Bo *et al.*, investigated the influence on the cardiac function by modulating the activity of glutamatergic neurons in the primary motor cortex. Bo *et al.* (2024), further elucidating the role of neural circuits in the physiological regulation.

4.3 Exploration of Neural Circuit Plasticity. The plasticity of neural circuits is one of the crucial mechanisms that enables the nervous system to adapt to environmental changes. The fMOST technology provides robust support for exploring the plasticity of neural circuits. For instance, Yang *et al*. (2022) employed the fMOST technology in conjunction with the optogenetics and chemogenetics methods, to investigate the input-output connectivity patterns of GABAergic neurons in the zona incerta (ZI) of mice. Their study revealed the topological connection and functional subdivisions of ZI across different brain regions. These

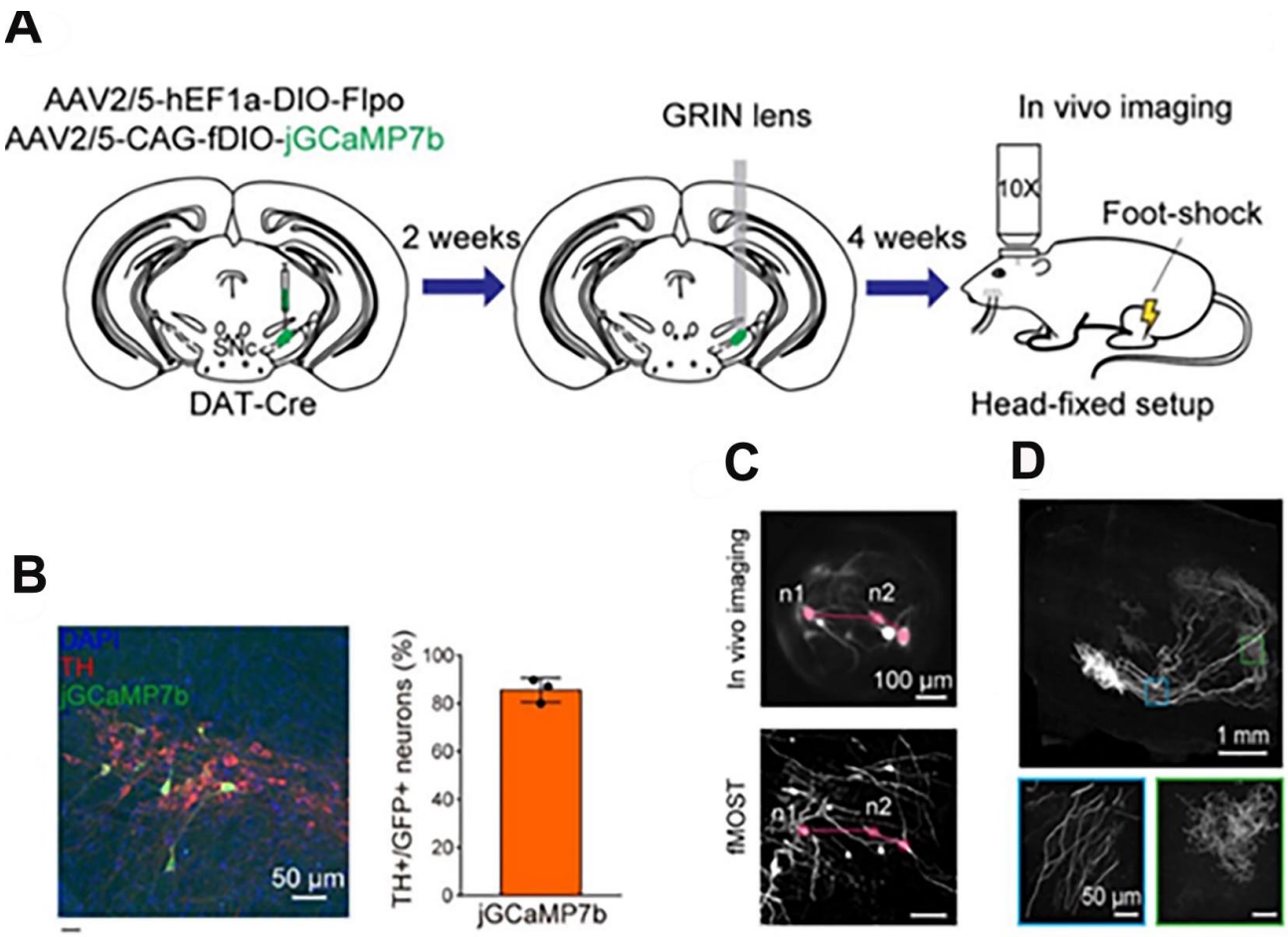

Fig. 5. Projection map of functionally defined neurons in the dorsal hippocampus (dCA1) (Source from: Li *et al*., 2024a). A, Schematic diagram of viral mixture injection and GRIN lens implantation in SNc and *in vivo* imaging in head-fixed mice with foot-shock delivered; B, An example image showing jGCaMP7b expression (Green) and TH immunostaining (Red) in SNc of DATCre mice (Left). The percentage of TH+ neurons in jGCaMP7b + labeling neurons (Right, n = 3 mice); C, Cell registration for neurons between *in vivo* imaging and HD-fMOST imaging. Lines indicate the distribution pattern of representative neurons; D, Whole-brain projection of sparsely labeled DA neurons in SNc (Top), and enlarged view of boxed regions indicated in the top (Bottom).

research efforts not only deepened our understanding about neural circuit structure but also provided significant clues for exploring the molecular mechanisms underlying the plasticity of neural circuits.

5. Summary and Outlook

Apart from successfully imaging the brain of mouse, the fMOST technology has also made notable progress in some other fields:

5.1 Application of Micro-Optical Sectioning Tomography in Stroke. Stroke is a disease of the central nervous system with high rates of incidence, mortality, and disability. Current research on stroke pathogenesis, post-stroke pathophysiological changes, and neurovascular protection remains insufficient. Traditional three-dimensional visualization techniques for vessels, such as micro-computed tomography (micro-CT) angiography and synchrotron radiation microtomography, either suffer from low imaging resolution or can display only the vascular network while losing neuronal information, thus making it difficult to synchronously study the neural and vascular networks (Plouraboue *et al*., 2004; Urao *et al*., 2016). Leveraging its high-resolution 3D visualization capabilities, the fMOST technology can reveal post-stroke microcirculation changes, neural projection pathway reconstruction, and neuroplasticity processes, offering a new perspective for understanding the stroke pathogenesis and pathophysiological changes. Furthermore, combined with immunolabeling techniques, fMOST enables co-imaging of vessels and neurons (Renier *et al*., 2014), opening up new research avenues for neurovascular protection after stroke. However, challenges remain in applying fMOST to stroke research, including data

storage and computation bottlenecks for larger brain atlases like non-human primates, stability and maintenance of instruments for long imaging sessions, inability to directly perform continuous dynamic observations (as fMOST is currently an ex vivo technique), limiting comprehensive understanding about real-time stroke progression, and need for advanced algorithms to completely eliminate non-specific staining interferences in complex pathological states like blood-brain barrier disruption. Future developments in fMOST would focus on enhancing the data processing efficiency, optimizing imaging protocols, enhancing *in vivo* observation capabilities, and refining atlas analysis software to play a more profound role in research on stroke and broader brain disease, towards advancements of medical research.

5.2 fMOST Technology in Cardiovascular Research.. The coronary artery disease incidence has been rising in recent years, posing a significant threat to human health. In 2022, Tang *et al*. (2022) used a Tie2 Cre CX40-LSL-GFP dual lineage tracing system combined with tissue clearing and fMOST to clearly analyze the developmental process of coronary arteries in neonatal mice. They discovered a novel mode of coronary artery generation in neonates and further found that these arteries persist into adulthood, playing a crucial protective role in heart injuries, such as myocardial infarction. This finding provides new research directions for vascular repair and treatment of damaged hearts, and also offers insights into the cardiovascular regenerative medicine.

5.3 Weakly Supervised Iterative Neuron Recognition Method. Huang *et al*. (2020), proposed a 3D deep network technology based on weakly supervised learning, successfully achieving automatic reconstruction of complex neuronal structures utilizing the fMOST (fluorescence micro-optical sectioning tomography) system. This technology leverages a 3D deep residual Convolutional Neural Network (CNN) coupled with a weakly supervised learning framework, enabling accurate detection and tracking of neuronal structures from optical microscopy images characterized by high noise and low signal-to-noise ratios, without the need for extensive manual annotations. Experimental results demonstrate that WSINet achieves state-of-the-art automatic recognition accuracy on both the fMOST dataset (Gong *et al*., 2013), and the public BigNeuron dataset (Peng *et al*., 2015), setting a new benchmark for the development of automatic neuron recognition technologies.

However, the fMOST system brings not only technological innovations, but also a series of challenges. Breakthroughs in imaging systems have facilitated large-scale applications of brain imaging systems, generating up to 10PB of neuronal image data annually, posing significant challenges to information processing (Cyranoski, 2017). At the mesoscale, the dataset of a single image of the mouse brain may reach up to 10TB, while a human brain dataset may exceed 35.4PB (Economo *et al*., 2016). Identification of neuronal morphological features from such a vast and complex brain dataset is incredibly difficult, placing unprecedented demands on its processing, storage, and analysis capabilities. Furthermore, cross-species research, particularly towards humans and non-human primates, exacerbates data processing difficulties due to increased brain volumes and data complexity. The key challenges to popularize the fMOST technology and its in-depth application lie in ensuring data accuracy while enhancing processing speed, reducing technical barriers and costs. With continuous maturation and improvement of the fMOST technology, and advancement of big data processing and artificial intelligence technologies, we have reason to believe that fMOST would demonstrate its unique value and potential in more fields. On the one hand, with the continuous improvement of the resolution and speed of imaging, fMOST would be able to capture finer and more dynamic changes in neural activities, providing deeper and more comprehensive information for analyzing the functions of neural circuits. On the other hand, combined with advanced data processing and analysis techniques, fMOST would play a greater role in research on complex neurological diseases, such as neurodegenerative diseases, psychiatric disorders, and drug addiction, providing scientific evidence for early diagnosis, precise treatment, and prevention of these diseases.

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RESUMEN: Los circuitos neuronales, que constituyen el marco fundamental del funcionamiento cerebral, regulan de forma intrincada los procesos cognitivos, las respuestas emocionales y los patrones de comportamiento, desempeñando así un papel fundamental en la comprensión de la complejidad del cerebro. Sin embargo, la extrema complejidad y el exquisito refinamiento de sus estructuras internas plantean grandes desafíos para la observación directa y el análisis profundo. En este contexto, la Tomografía de Seccionamiento Microóptico Fluorescente (fMOST) se ha convertido en una tecnología revolucionaria, aprovechando sus excepcionales capacidades de imagen tridimensional de alta resolución para allanar el camino a la investigación sobre circuitos neuronales. Esta revisión ofrece un panorama exhaustivo de la evolución histórica de la tecnología fMOST, rastreando su desarrollo desde su conceptualización inicial hasta el estado actual de sus aplicaciones maduras, junto con los notables avances tecnológicos observados en cada etapa. Además, la revisión profundiza en varias ramas tecnológicas destacadas de la fMOST, cada una de las cuales destaca por mejorar la calidad de la imagen

y ampliar el alcance de la observación, ampliando así los límites de la investigación neurocientífica. Fundamentalmente, este artículo subraya el papel central de la fMOST en la disección de las redes neuronales y la elucidación de los mecanismos de los circuitos neuronales, en particular su contribución única a la exploración de los posibles vínculos entre las anomalías en los circuitos neuronales y diferentes enfermedades cerebrales. Al integrar los últimos hallazgos de investigación, esta revisión anticipa las posibilidades de aplicación de la tecnología fMOST en futuras investigaciones neurocientíficas y cómo podría seguir guiándonos en la profundización de los misterios del cerebro.

PALABRAS CLAVE: Circuitos neuronales; fMOST; Imágenes cerebrales completas; Reconstrucción tridimensional de neuronas.

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