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Technology Offer

Device and method for creating an optical tomogram of a living microscopic sample

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Abstract

This technology describes a method and device for generating optical tomograms of microscopic samples using an advanced imaging process. The method involves rotating and displacing a sample within an optical microscope's focal plane to capture a series of images from multiple angles. These images are subsequently processed to create a detailed three-dimensional model of the sample. The innovation reduces motion stress on living samples and minimizes the time required for tomogram acquisition. The device incorporates a rotatable sample stage and a precise imaging setup that enables high-resolution tomography suitable for applications in biological research, particularly on fragile, live samples.

Background

Conventional methods for 3D microscopic imaging, such as selective plane illumination microscopy (SPIM) and light sheet fluorescence microscopy (LSFM), are based on optical fluorescence and require labeled or stained specimens. Instead or in addition to the fluorescence imaging, it is advantageous to have alternative modalities using the same or similar sample mounting. However, some tomographic, high-resolution methods involving significant sample rotation are also restricted by mechanical limitations, which hinder efficient data acquisition and stress the sample. This innovation addresses these issues by introducing a smooth, continuous movement system that supports rapid image capture while maintaining minimal physical disruption to the specimen and that is compatible with existing light (sheet) microscopy techniques.

Technology

The device utilizes a microscope with a rotatable sample mounting, where a sample is rotated and simultaneously translated through the focal plane (Fig. 1A-B). This controlled movement forms a spiral imaging path that allows the sample to pass continuously through the focal plane at multiple angles, significantly reducing acquisition time. Typically, the spiral consists of 20 full rotations, images are acquired every 1° with high speed, e.g., at 60 frames per second (fps), and the total acquisition of 7200 images takes less than 2 min per specimen (Bassi et al., 2015). A bright-field or transmitted-light microscope captures digital images that are stored and processed to build a 3D model. This method's innovative approach minimizes motion blur and mechanical stress by maintaining a continuous motion path, which enhances the stability of live sample imaging.

Advantages

- Rapid tomogram creation reduces imaging time significantly (acquisition of 7200 images takes less than 2 min (Bassi et al., 2015)).
- Reduced amount of data during subsequent 3D reconstruction (improvement by a factor of 20 (Bassi et al., 2015)).
- Smooth spiral movement minimizes physical stress on live samples.
- High-resolution imaging suitable for translucent and non-fluorescent samples.
- Continuous movement path prevents motion artifacts in captured images.
- Capable of creating detailed 3D models without sample pretreatment.

Potential applications

- 3D imaging of biological specimens, especially fragile and live samples.
- Longitudinal studies of embryonic development in model organisms.
- Biomedical research requiring precise spatial resolution without fluorescence markers.
- Visualization of cellular and subcellular structures in transparent samples.
- Multimodal imaging in combination with ligh (sheet) fluorescence microscopy.
- High-throughput imaging in developmental biology and microbiology research.

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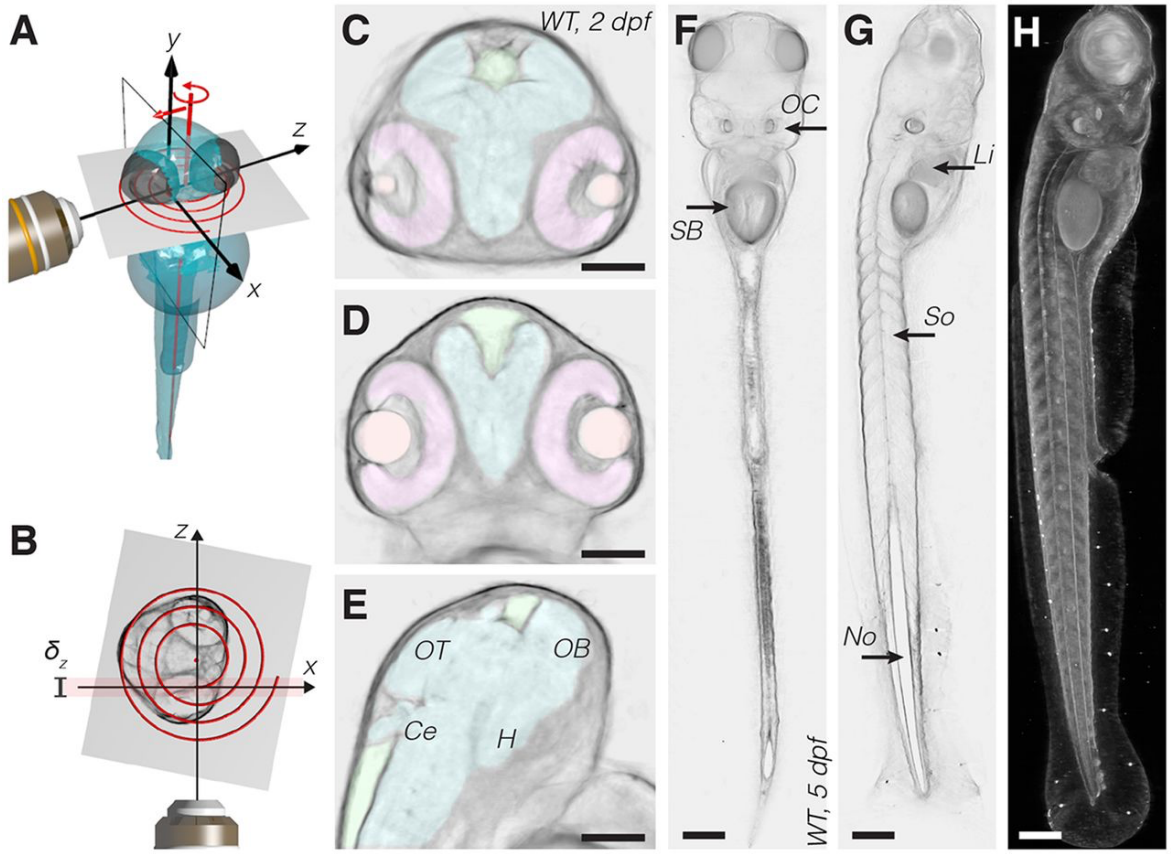

Figure 1: This figure illustrates the novel imaging technique for capturing high-resolution 3D images of live, transparent samples like zebrafish embryos. Panel (A) shows a schematic of the configuration, where the specimen is rotated smoothly through the focal plane while moving it along the detection axis, forming a spiral path. This movement allows multiple images from different angles to be captured without disturbing the sample. Panel (B) highlights how this spiral imaging creates a detailed, depth-resolved view. Panels (C) - (H) show reconstructed cross-sections of a zebrafish head and body, with clear views of organs such as retina (pink), eye lens (orange), brain ventricles (green), brain (cyan) (annotated brain domains: optic tectum (OT), hypothalamus (H), cerebellum (Ce) and olfactory bulb (OB)), swim bladder (SB), otic capsule (OC), liver (Li), somites (So), notochord (No). Scale bars: 100 μm. This technique provides a non-invasive way to visualize internal structures and follow development in live specimens over time (Bassi et al., 2015).

Patent Information

US10437038B2 (Application 20.02.2015)

Publications

Bassi et al., “Device and Method for creating an optical tomogram of a microscopic sample”, US 10,437,038B2, 8. October 2019

Bassi et al., Optical tomography complements light sheet microscopy for in toto imaging of zebrafish development. Development, 2015, 142. Jg., Nr. 5, S. 1016-1020.

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