Polarized Light Imaging in Life Sciences

Continuous Scanning Polarized Light Microscopy

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Polarized light imaging (PLI) is an imaging technique used to investigate the birefringent properties of samples. When examining biological samples under a microscope, the anisotropy in their interaction with light can arise from regular arrangements at either the molecular or macroscopic level. This information can be used to study the organization of fibers in connective tissues, neurons in grey and especially white matter of the brain, or to detect cancerous or abnormal tissues.

In the field of neurobiology, studying the spatial orientation and local density of fibers in the human brain at the level of individual neurons requires imaging thousands of brain tissue slices. Manual capture is impractical due to the extensive time it requires, as the number of images can reach hundreds of millions. To address this challenge, a high-speed mode for PLI has been developed based on the LMP1 microscopes. This mode significantly reduces the total acquisition time by capturing up to 18 frames per second through continuous image acquisition instead of automated sequential imaging.

This technology enables researchers to digitize a human brain using the PLI method on brain slices. By reducing the pure scanning time from 10 to 3 years with a single microscope, this advancement greatly expedites the process.

Fig. 1: First-generation PLI Microscope (2D imaging) without continuous scanning.

PLI – High-Quality Image Capture of Fine Structures

Polarized light microscopy (PLI) is a powerful technique used to analyze anisotropic materials, commonly found in minerals as well as biological tissues [1]. PLI is particularly suitable for research projects focused on studying the human connectome, an approach aimed at understanding the function of the human brain by deciphering the local and long-range interconnections between individual neurons [2]. To achieve this, thin histological slices of the brain are prepared and observed using a polarized light microscope. Compared to diffusion tensor imaging (DTI), a commonly used technique for imaging nerve fibers, PLI provides a resolution of nerve fiber tracings that is 1,000 times higher [3].

In PLI, the inherent or structural birefringence of a sample modifies the polarized light passing through it, allowing for the visualization of fine structures that are difficult to observe using conventional transmitted light microscopy [2]. By generating polarized light using a polarization filter, particularly circularly polarized light, the influence of the sample is directly related to the alignment of crystals or the arrangement of bundles of aligned submicroscopic fiber structures, making them indirectly visible [4]. However, acquiring a high-quality image of a large sample requires a substantial number of images, and using the PLI microscope (LMP) to scan an entire human brain would take approximately 10 years (Fig. 1). This limitation hampers comprehensive studies and emphasizes the need for further advancements in this field.

High-Speed Microscopy – Capturing a High Quantity of Images

High-speed microscopy is a technique that enables the rapid acquisition of large-area objects at a microscopic scale [5]. Industries such as semiconductors, electronics, and biology have a significant demand for efficient microscopic inspection methods, particularly when examining large surfaces. Traditional stop-and-go operations are being outpaced by the need for faster acquisition processes. In contrast, high-speed microscopy allows for continuous object movement during the recording process, resulting in a speed that can be up to 30 times faster than comparable stop-and-go methods.

Researchers are currently engaged in the digital mapping of the human brain, which involves imaging a high number of thin brain slices. Using a polarized light microscope without continuous scanning, this process would take over 10 years to create a 3D digital brain. However, high-speed microscopy, combined with automatic sample handling has enhanced the capabilities of the PLI microscope, enabling a significant acceleration of this process.

The Power of Both Worlds: Combining Quality and Quantity

The new PLI microscope, the LMP 3D (Fig. 4), has been developed by Taorad and the Fraunhofer Institute for Production Technology IPT to accelerate the image acquisition process. Instead of the conventional stop-and-go method, where the polarization filter pauses at each image capture, the microscope utilizes the continuous rotation of the polarization filter while capturing images. This adaptation allows for image acquisition at a rate of 18 frames per second. This brings a significant advantage: With the continuous scanning method, the total acquisition time is not directly proportional to the number of images.

Previously, researchers had to limit the number of recordings in the classic stop-and-go mode to avoid excessively long recording times, which would have been problematic for the 3D approach of PLI technology. However, the continuous recording method fully exploits its potential when sampling more polarization and illumination angles. This is because the allowed exposure time per image decreases with an increasing number of polarization angles for a given polarizer rotational frequency (Fig. 3). Although significantly more data is produced, this only slightly increases the total acquisition time.

This innovation enables imaging specimens at intervals several times shorter than conventional PLI microscopy, resulting in significant time savings. To further optimize acquisition times, several additional developments have been realized. One is the incorporation of an autofocus system within the microscope. It utilizes a confocal chromatic distance sensor to scan the sample height and generate a height map for automated z-positioning, ensuring sharp images. The height map is used to track the objective lens height, optimizing image sharpness based on the specific tile.

Given the varied spatial extensions of brain slices, a sample may need to be divided into over 2,000 tiles due to the fixed field of view of the microscope optics and the selected tile overlap. Each tile requires data collection for multiple polarization angles, illumination angles – as oblique illuminations allow to resolve the inclinational ambiguity along z [2] –, and focal depths. As it is not necessary to capture the entire sample carriers due to the various size of the brain slices, an overview image of the entire specimen is taken using a second camera. This overview image is used to define the so-called region of interest (ROI) (Fig. 2). In one approach, a graphical user interface displays the overview image, and the ROI can be selected manually, or it is automatically defined using image analysis software.

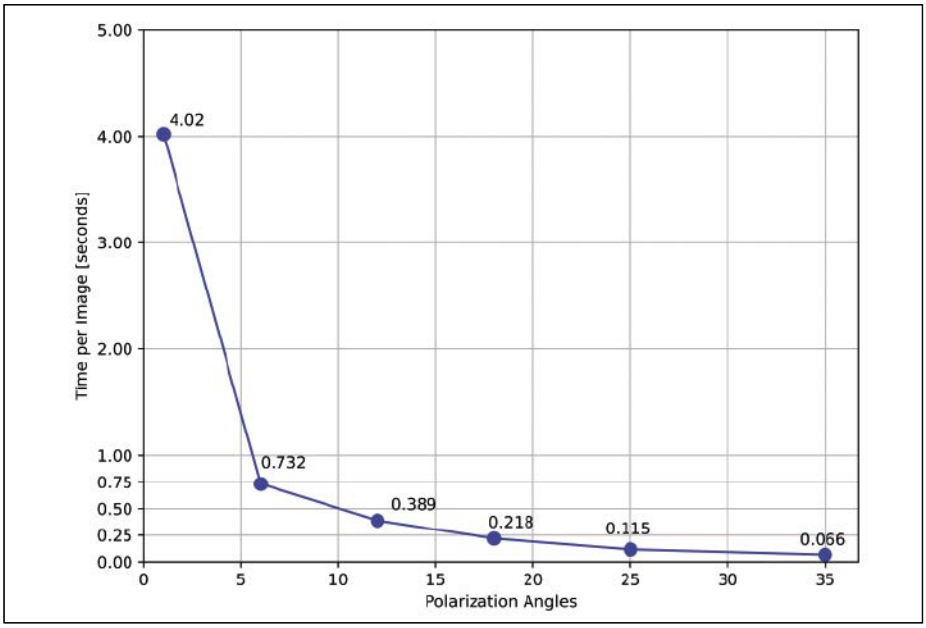

Fig. 2: Frame rate with respect to the number of polarization angles.

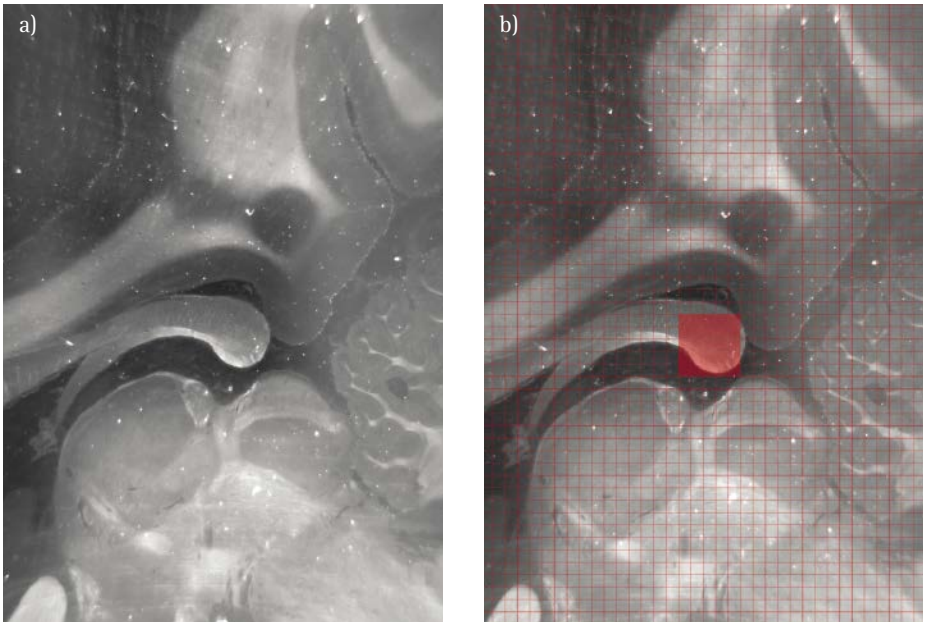

Fig. 3: Brain slice section (A) and its partition in tiles (B). Red tiles are selected for a microscope scan.

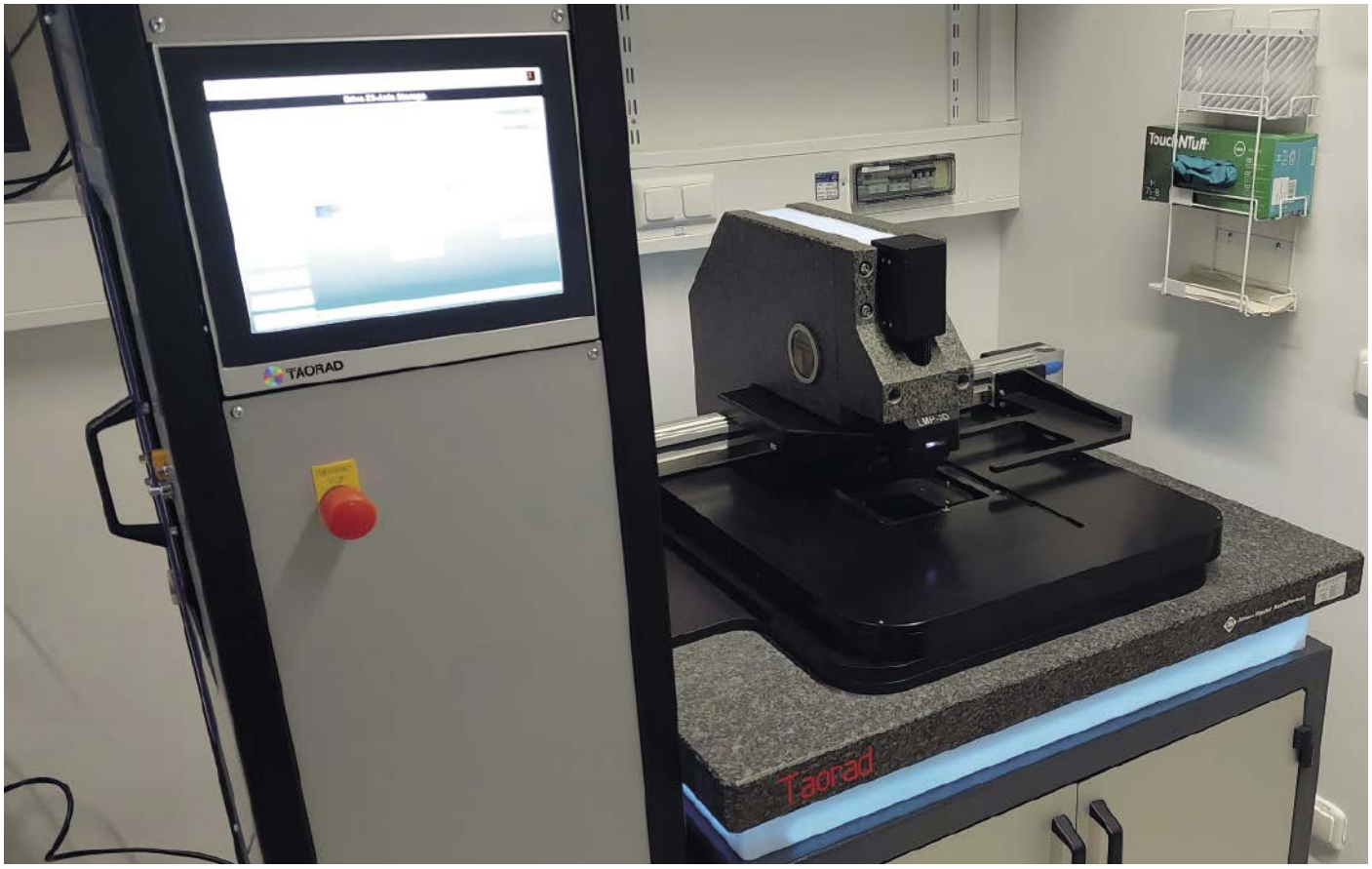

Fig. 4: The LMP-3D automated 3D-PLI microscope from Taorad.

Even after excluding unwanted tiles, imaging a whole brain can generate over 1.7 PB of image data, depending on chosen settings, emphasizing the importance of fast acquisition time. The continuous scanning PLI technology achieves an impressive speed of approximately 66 ms per image with 5° polarization steps.

A standard scan process, including 15° polarization angle steps (12 different angles in total in a 180° segment) from 5 illumination angles and one focus position, takes 24 s per tile, accounting for the time required to switch between illumination angles. Imaging an entire brain slice with 2,000 tiles takes around 13 h for a total of 360,000 images. Increasing the number of polarization angles to 36 per segment (5° angle step size) only increases the total time negligibly, due to the fast rotation of the polarization filter.

An additional enhancement made to the PLI microscope is the implementation of automated loading and measurement of specimens. Cataloged samples, including the overview image and ROI definition, can now be loaded onto the microscope stage using a linear axis. This automated process eliminates the need for human interaction during measurement. The continuous measurement capability enables more efficient and uninterrupted digitization of the human brain. By utilizing three adapted PLI microscopes to analyze samples simultaneously, 24 h a day, the imaging time can be reduced to less than one year.

Upon capturing all the necessary images, researchers can process the data and calculate a 3D model of the human brain. Artificial intelligence techniques can be employed to establish spatial context between the images. It is important to note that the PLI technology is not limited to brain slices and can be applied to other organic or inorganic structures as long as there are a sufficient number of samples available to generate a suitable model.

Summary

This advanced microscopy technology combines Polarized Light Imaging (PLI) with high-speed imaging to enable the rapid digitization of complex biological tissues, particularly the brain. It achieves this by quickly acquiring and visualizing fine structures. The system utilizes a continuously moving polarization filter, allowing it to capture up to 18 frames per second. Additionally, it can be easily automated, saving both time and personnel costs, and facilitating parallelization.

Some notable features of this technology include precise autofocus, automated oblique illumination, ROI detection, and loading for continuous operation (24/7). These capabilities contribute to reducing the time required for whole brain digitization from 10 to 3 years. Furthermore, by operating three microscopes in parallel, it is possible to potentially achieve this process within one year.

Overall, this technology offers significant advancements in the efficient and accelerated digitization of complex biological tissues, revolutionizing the study and understanding of structures such as the brain.

This ground-breaking microscopy technique has the potential to unlock, for the first time, the intricate secrets of how the human brain functions, ushering in a new era of unprecedented knowledge and understanding.

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