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3D Microscope Imaging System based on the Transport of Intensity Equation

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Abstract. This study aims to develop and implement a low-cost, high-resolution three-dimensional biological sample imaging system based on the light intensity transmission equation (TIE) technology to obtain the three-dimensional phase information of biological samples in a non-destructive way. The system can provide non-staining, real-time three-dimensional reconstruction images through a common optical path design, combined with a standard LED light source, CMOS sensor and optical components. The goal of this study is to provide a simpler and lower-cost imaging technology for biology, medicine and other related fields, especially for cell imaging, pathological analysis and other aspects.

Keywords: TIE; microscopy; 3D imaging.

1. Introduction

With the increasing demand for three-dimensional (3D) cell imaging, an essential tool in life sciences, biology, and educational research, 3D Microscopy has become one of the most popular research topics nowadays [1, 2]. Unlike traditional two-dimensional imaging, 3D cell imaging provides researchers with a full morphological context, thereby improving the accuracy of cell structure analysis [3]. To overcome the limitations of 2D projection, in the 1950s, Marvin Minsky conceptualized the very first 3D microscope called confocal laser scanning microscopy (CLSM). However, the immaturity of this solution, such as requiring a complex laser system and scanning mechanism, makes this solution hard to apply and widespread. Later developments like two-photon excitation microscopy and stimulated emission depletion microscopy (STED) brought improvements in axial resolution while containing the expense of system complexity and cost. The most recent folution for 3D microscopy is label-free quantitative phase imaging (QPI) which offering 3D imagings by applying the Transport of Light Intensity Euation, specifically capturing the phase shift of any tested samples [4-7]. The Transport of Light Intensity Function, which also inspire my system was firstly rigorously analyzed by Teague in 1983, provides a powerful framework for reconstructing phase image by using focused image, a sharp image where light rays meet at the image plane, and defocused image, a blurry image where light rays miss the image plane, of a sample. Nevertheless, for all the 3D microscopy methods we mentioned, besides their tones of benefits such as: critical for studying complex phenomena, perfectly provides a more realistic and comprehensive understanding of cellular behavior and interactions, and making it an indispensable step in not only in modern biomedical investigation, but also an ideal way to improve the quality of science education system by using 3D image to label more clear pictures for students, the high cost of these microscopes based on theories we mentioned were unaffordable for many scientific researchers and most schools.

As students who previously took Biology and Physics, our study aims to develop and implement a low-cost, high-resolution three-dimensional biological sample imaging system based on the light intensity transmission equation (TIE) technology, thereby obtaining the three-dimensional phase information of biological samples in a non-destructive manner. This system can provide non-staining, real-time three-dimensional reconstruction images through a common optical path design, combined with a standard LED light source, CMOS sensor, and optical components. It is not for myself, but for millions of students who cannot afford a regular 3D microscope, yet it necessarily promotes an understanding of science. In this paper, we'll mainly disscuss the design of the TIE 3D micro-system

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and how does the TIE theory behind it functions well on our system to explain how we could convert 2D image into 3D in such a low-cost way, and lastly illustrate how we use the microlens array and human blood cells as sucessful examples to varify the correctness of the system and algorithm.

2. Basic Principles

$$\frac{\partial I(x,y,z)}{\partial z} = -\frac{1}{2\pi}\nabla\cdot\left(I(x,y,z)\nabla\phi(x,y,z)\right) \quad (1)$$

- I (x, y, z) am the light intensity distribution at position (x, y, z)
- Φ (x, y, z) is the phase information of light
- ∇ is a three-dimensional gradient operator, representing the partial derivative in space.

The Transport of Intensity Equation (TIE) is a mathematical model that describes how the intensity of light propagates along the optical axis. It provides a method for retrieving phase information from intensity variations without requiring interferometry or staining. The fundamental principle of TIE is based on the relationship between the axial intensity gradient and the phase of a wavefront.

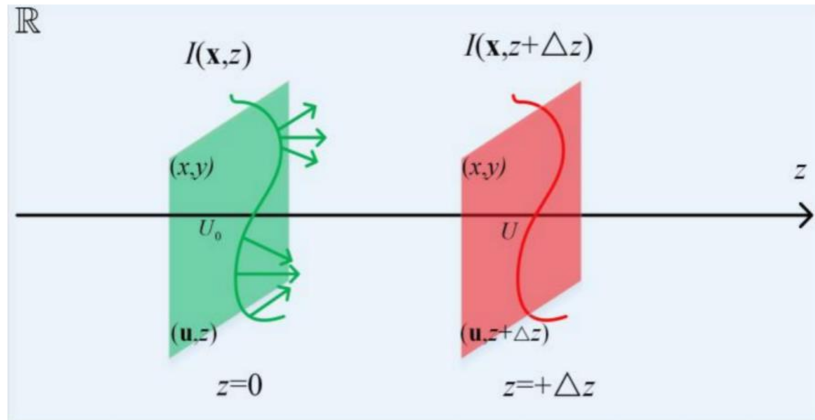

Figure 1. Schematic diagram of light intensity transmission equation

By measuring the changes in light intensity at different positions and combining it with the inversion of the TIE equation, researchers can obtain the three-dimensional phase information of the object and construct its three-dimensional structural model.

2.1. Assumptions

1. Assume that the scattering and absorption characteristics of biological samples are uniform and predictable within the measurement area.

2. Assume that the design of the optical system can ensure the accuracy of the measurement data and that the optical error is within an acceptable range.

3. Assume that the optical error can be minimized and the measurement accuracy can be improved through the design of the common optical path system.

2.2. Steps to Solve the TIE Equation

1. Capture a Focused Image using a CMOS sensor under LED illumination.

2. Capture Defocused Images. Two slightly defocused images are captured by shifting the sample or the imaging plane along the optical axis (z-direction).

3. Compute the Intensity Gradient. The TIE equation is solved using numerical techniques such as Fourier transform methods or finite difference methods.

$$\frac{\partial I(x,y,z)}{\partial z} = -\frac{\lambda}{2\pi} \nabla \cdot \left(I(x,y,z) * \nabla \phi(x,y,z) \right) \tag{2}$$

2.3. System design and construction

2.3.1. Overall light path system design.

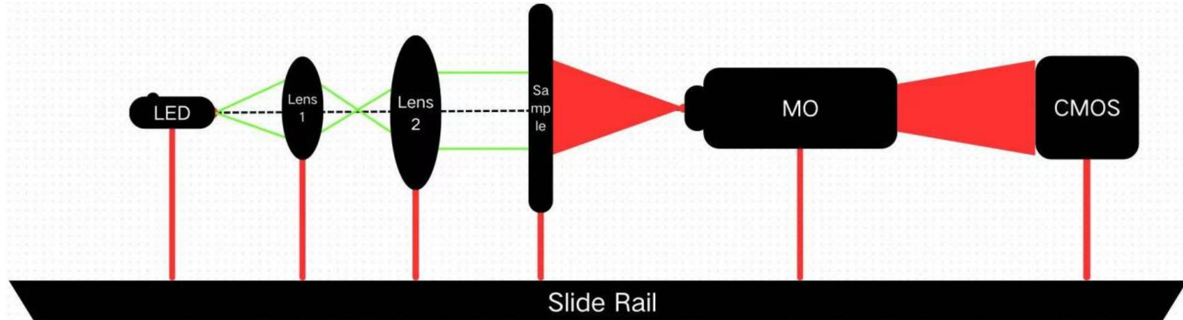

Figure 2. The light path system

The fixed LED light source emits light that passes through lens 1 and lens 2, which can expand and collimate the light source. The parallel light illuminates the sample and is magnified by the microscope objective lens, and finally recorded by the CMOS. In the actual experiment, after adjusting the distance between the microscope objective lens and the sample, a focused image is recorded by the CMOS, and then the positions of other devices are kept unchanged, and the sample is only slightly defocused to the left and right, and finally two defocused images are recorded by the CMOS.

2.3.2. Hardware selection.

Considering the imaging of biological samples, and based on the diffraction effect of the light source, interference should be avoided as much as possible. The low-power (5mw) and low-coherence LED light source was finally selected for imaging. The final application was the 525nm wavelength LED light source of Daheng Optoelectronics. Furthermore, in order to expand and collimate the light source and shorten the size of the overall optical path as much as possible, the final focal lengths of the two lenses were 15mm and 50mm respectively, and the distance between the two was exactly 65mm. At this time, the light source can achieve a magnification of 3.33 times. In terms of the selection of microscope objective lenses, more consideration is given to the measurement accuracy requirements of the actual measurement samples. Microscope objective lenses with different magnifications can be used. In this experiment, the magnification used is 20 times and the numerical aperture is 0.4. For the final CMOS selection, we chose the Daheng Optoelectronics HD-R630M model, with a single pixel size of 2.4 microns and an overall pixel size of 3072*2048.

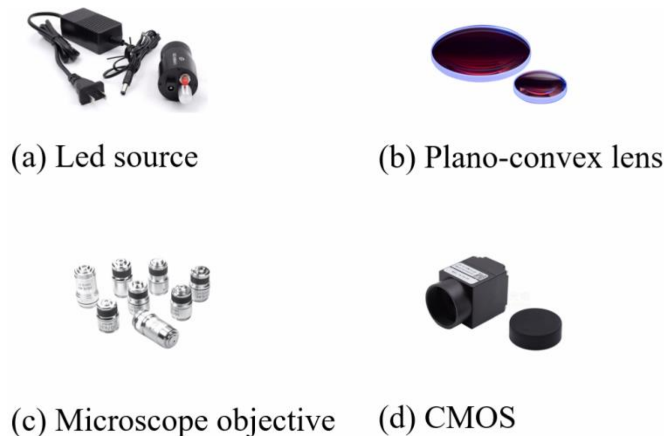

Figure 3. The hardware used in this system

2.3.3. Building.

During the overall construction process, in order to ensure that all devices are coaxial, we place all devices under the same horizontal rail for operation. First, install the light source and adjust the direction of the light source to ensure that the direction of the light source is parallel to the direction of the rail. Then adjust the distance between the two lenses to the sum of the focal lengths of the two lenses, that is, 65mm, and then adjust the height of the light source and the two lenses as a whole to ensure that the three are on the same horizontal line. A white board can be placed behind the lens. When the size of the image does not change with the forward and backward movement of the white board, a standard beam expansion collimated light source can be obtained. Then place the sample, the microscope objective lens and the CMOS at the same height, then fix the sample position, adjust the distance between the microscope objective lens and the sample, and observe whether the image recorded by the CMOS is focused on the computer through the system software. Keep adjusting the front and back distance of the microscope objective lens until focusing is achieved. Then, based on the position of the focusing distance, the sample can be slightly moved to the left and right and the specific distance can be recorded. At the same time, CMOS records the image. At this point, the optical path system is adjusted. The best actual optical path system is shown in Figure 4.

Figure 4. Actual optical system.

3. Experiment

3.1. Experimental methods and steps

The experimental pross can be divided into three steps: obtaining a focused image, obtaining a defocused image, and solving the phase based on the light intensity transmission equation. Each step is crucial. The following is a detailed experimental design.

3.1.1. Obtaining a focused image.

Purpose: Obtain a sample image under ideal focusing conditions to provide reference data for the defocused image.

(1). Turn on the LED light source to ensure that it can illuminate the biological sample and adjust the LED light intensity to achieve the optimal brightness.

(2). Use the focusing lens (lens 1 and lens 2) in the optical system to focus the light source on the target position of the sample.

(3). Use a high-resolution CMOS sensor to capture a focused image of the sample. Ensure that the details of the image are clear and the structure of the sample is clearly visible under optical magnification.

(4). Record the focused image and save it as the data basis for subsequent processing.

The focused image must be clear and correct to serve as the benchmark data for phase inversion. If the quality of the focused image is poor, it may affect the accuracy of subsequent data.

3.1.2. Obtain defocused images.

Purpose: By obtaining defocused images, further phase information is provided, providing more light intensity data for phase solution.

(1). By adjusting the position of the focusing lens, ensure that the focusing light path deviates slightly from the sample surface, and obtain two defocused images at different focal lengths. The position of each defocused image is set as front defocus and back defocus.

(2). Keep the position of the light source and CMOS sensor fixed, and only adjust the focusing lens to obtain sample images at different focal lengths.

(3). Record and save the front defocused and back defocused images. By changing the focal length, information of different depth layers is obtained to provide light intensity data for subsequent calculations.

The light intensity change of the front and back defocused images must be significant and can reflect the sample structure at different focal lengths. This is the key data for phase inversion.

3.1.3. Phase solution based on the light intensity transmission equation.

(1). Data input:

Input the focused image, the front defocused image and the back defocused image into the computing system. Obtain the gradient and divergence by calculating the light intensity change between the focused image and the defocused image.

(2). Calculate the gradient and divergence:

Calculate the gradient and divergence of each image, and obtain the gradient information of each point according to the relationship between light intensity and phase.

(3). Phase inversion:

The light intensity data is processed by Fourier transform and inverse Fourier transform to invert the phase distribution and obtain the three-dimensional structure map. The precision and accuracy of phase inversion are the key to determine the quality of three-dimensional reconstruction, so noise and error must be strictly controlled during the calculation process.

3.2. Microlens array measurement

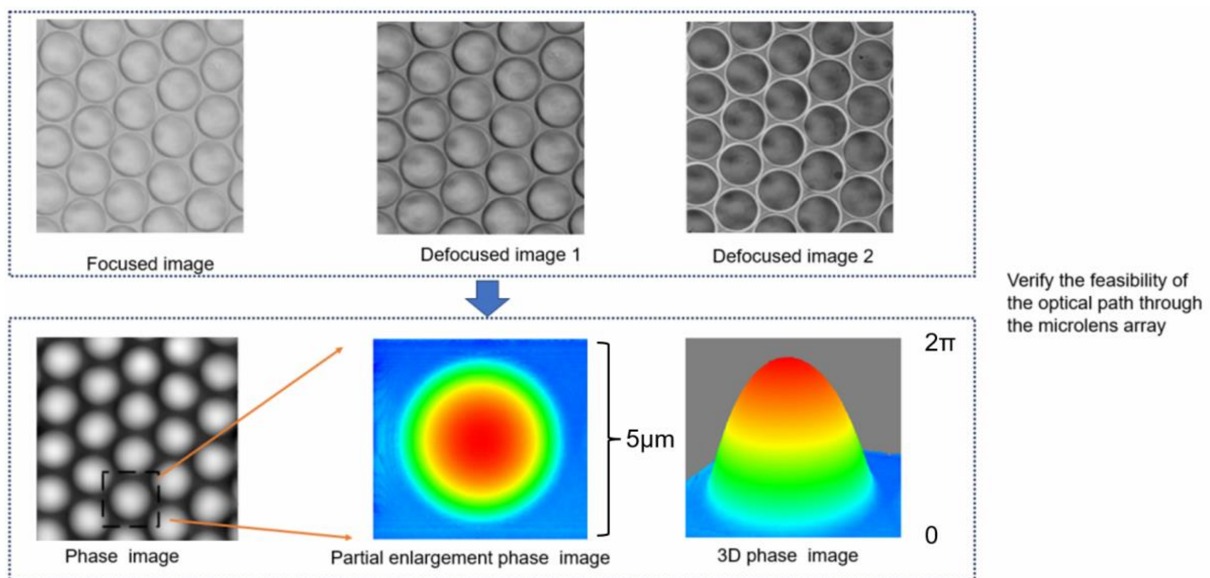

Figure 5. Microlens array measurement

The length of the single microlens array used in our measurements is 5 microns. Measuring the length of the microlens array verifies the system's lateral resolution. Considering that the system's axial height measurement only provides a phase representation, and 3D reconstruction requires a complete solution for phase unwrapping, which is beyond current capabilities, only the overall 3D outline of the object can be qualitatively measured in the axial direction, represented by phase.

3.3. Human red blood cells

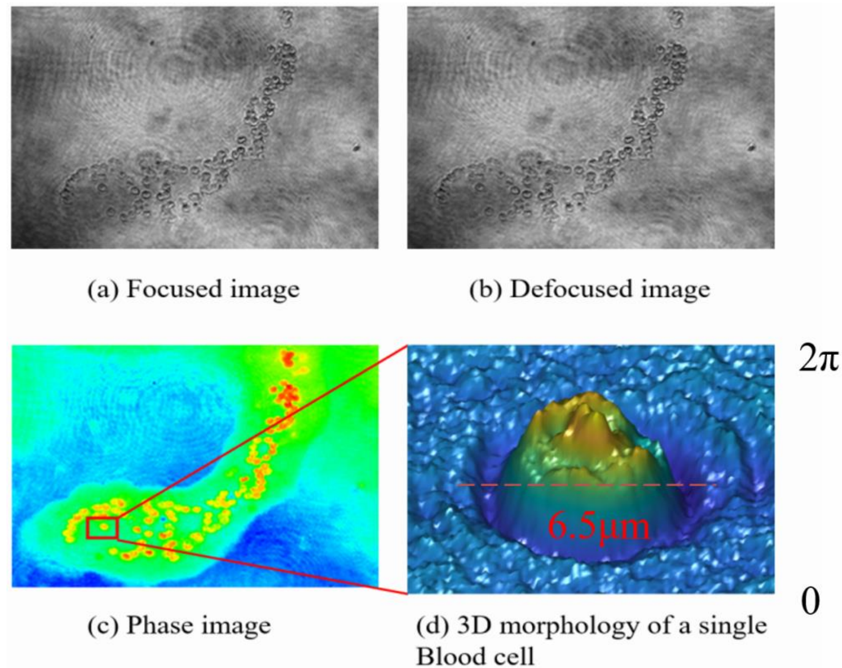

Figure 6. Human red blood cells measurement

Through the TIE equation, the 3D overall morphology of human blood cells can be observed to obtain more dimensional information. By measuring human red blood cells, it can be measured that the diameter of red blood cells is about 6.5 microns, and the three-dimensional morphology of red blood cells can be qualitatively measured as a whole. The next step is to obtain the three-dimensional morphological characteristics through an accurate phase unwrapping algorithm.

4. Conclusion

In this paper, we'll mainly disscuss the design of the TIE 3D micro-system and how does the TIE theory behind it functions well on our system to explain how we could convert 2D image into 3D in such a low-cost way, and lastly illustrate how we use the microlens array and human blood cells as sucessful examples to varify the correctness of the system and algorithm.

Next, we compare our designed system with ordinary optical microscopes and other three-dimensional imaging technology microscope systems. The transport-of-intensity equation (TIE) method offers significant advantages over conventional 2D microscopy and advanced 3D imaging systems by combining cost-effectiveness with functional 3D imaging capabilities. Unlike traditional brightfield microscopy that only provides qualitative 2D information, TIE enables quantitative phase reconstruction and 3D morphological analysis through computational processing of defocused intensity images, while maintaining the simplicity and affordability of standard microscope hardware. Compared to expensive 3D techniques like confocal or structured illumination microscopy that require complex optical systems, laser scanning, or fluorescent labeling, the TIE approach achieves micrometer-level axial resolution without specialized components, phototoxic illumination, or extensive sample preparation. This single-shot or limited focal-stack method provides rapid 3D reconstruction suitable for live-cell imaging, bridging the gap between the accessibility of conventional microscopy and the volumetric imaging capabilities of premium systems, making it

particularly valuable for clinical diagnostics and longitudinal biological studies where cost, speed, and cell viability are critical considerations. Typically, the hardware for a 3D microscopy system is expensive. For example, a confocal 3D microscopy system can cost over $80,000 USD. However, the overall hardware cost of the system proposed in this paper is kept under $1,000 USD, significantly reducing costs.

Figure 7. Confocal 3D microscope system

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