

Phase Detection for Quantitative Optical Microscopy

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Purpose

This presentation describes the use of two different wavefront sensors as key elements of optical systems able to provide quantitative information of microscopic samples. In the first case, a microlens array is used for fluorescent tomographic microscopy. In the second, the pyramid sensor is adapted for operation in a quantitative non-interferometric phase microscope.

Methods

When investigating the tridimensional distribution of fluorescence markers, fast data acquisition is important for rapid biological processes. On a microscopic scale, several tomographic methods have been applied such as classic confocal scanning (CS), whereby different layers are imaged by axially displacing the pinhole. In optical projection tomography (OPT)[1], three-dimensional information is computed from data sets acquired by illuminating the sample from different directions. Also, optical coherence tomography[2] has been widely used, but it cannot operate in the incoherent fluorescence-light regime. CS and OPT have the drawback that additional optomechanical operations involving extra of time or an increase in setup complexity are needed for volume data acquisition respecting imaging a single two-dimensional section.

As an advantageous alternative to the above mentioned techniques, we present a new scanning fluorescence microscopy method[3], which directly obtains tomographic information of fluorochrome concentration. Fluorescence emission is achieved by scanning the sample with a thinly extended excitation beam. Simultaneously, the generated light is analyzed with a Shack–Hartmann(SH) sensor placed after a microscope objective. The SH images contains information concerning the fluorochrome concentration and location in the sample. These images provide full volume information in a single scanning frame.

In the case of unstained microscopic biological media, a common problem in optical microscopy is the transparency of the samples, which means that their complete characterization is only possible through the detection of spatial variations in the refractive index imprinted in the phase. Several classical techniques (e.g. Zernike or DIC microscopes) transform phase variation into intensity, thus providing only partial information. More recently, interferometric quantitative techniques based on the Mach-Zender (digital holography)[4] have been used, with the drawback of their reliance on the use of temporal coherent light and the complexity of reference arms, not to mention the inherent mechanical stability required.

For this specific microscopy modality, we propose the use of a static pyramid wavefront sensor[5] for the direct phase detection of biological samples. Several potential advantages can be highlighted: the system can be adapted to conventional microscopes, it uses incoherent light, the gain is adjustable and a high and controllable sampling rate can be obtained.

Results

In the case of the proposed tomographic fluorescence method, an optical system was built to generate the excitation beam using an axicon lens. The microlens array was placed in a plane conjugated with the microscope objective exit pupil. As an example, Fig. 1 (a) shows the image obtained with a sample consisting of two fluorochrome layers spaced by $57\ \mu\text{m}$ excited by a $4\ \mu\text{m}$ thick beam.

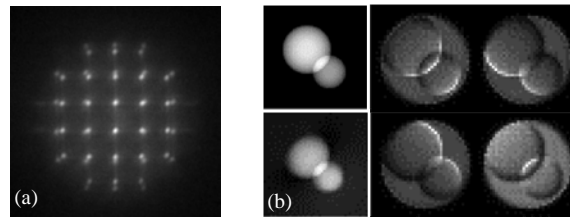


Fig. 1 (a). Microlens image of a sample consisting of a double fluorescent layer; (b). Top left: original phase object generated by two microspheres. Bottom left: reconstructed phase map. On the right: intermediate images generated by the refractive pyramid.

To test the proposed phase microscopy method, a modified inverted microscope was built. After the microscope objective, a refractive pyramid is placed generating four intensity distributions on a CCD camera (see Fig.1 (b)) which serves to reconstruct the phase map.

Conclusions

We have shown that wave aberration measurement techniques developed for astronomy can be applied for sample characterization in some modalities of optical microscopy in which phase detection is involved.

References

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