

PHASE DETECTION FOR OPTICAL MICROSCOPIC TOMOGRAPHY

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When investigating the tridimensional distribution of fluorescence markers, fast data acquisition is important for rapid biological processes. In the microscopic scale, several tomographic methods have been applied as the classic confocal scanning (CS) technique, where imaging different layers is attained by axially displacing the pinhole. In optical projection tomography (OPT) [1-3], three-dimensional information is computed from data sets acquired illuminating the sample for different directions. Also, optical coherence tomography[4] has been widely used but it is not able to operate in the incoherent fluorescence-light regime, except indirectly in the pump-probe scheme[5]. CS and OPT have the drawback that additional actions involving an extra consumption of time or an increase in setup complexity are needed for volume data acquisition respecting imaging a single two-dimensional section.

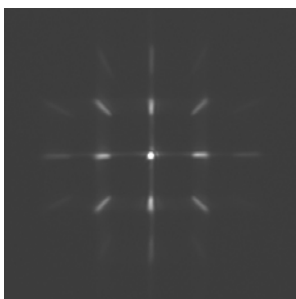


Figure 1: Example of a microlens image in the case of a simulated homogeneous emitter distribution

This presentation proposes the use of a new tomographic microscopy scheme for rapid tridimensional determination of incoherent emitters distributed in a volume. It is based on the combined use of a thin scanning beam to excite axially the sample and a detection camera coupled with a microlens array. The images acquired for each transversal point, contain phase –related with the emitters position– and concentration information that it is numerically recovered.

To test the acquisition system, experiments were carried out imaging a simulated fluorochrome distribution using an inverted microscope (Fig.1).

The characteristics and limitations of the system have been also investigated simulating the whole illumination and tomographic reconstruction process. For this purpose, between the different possibilities to generate a thin excitation beam in the focal plane of a microscope objective, an elongated energy distribution produced by an annular pupil was used.

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