FLUORESCENCE CORRELATION MICROSCOPY IN TISSUE: EFFECTIVE ELIMINATION OF ABERRATION BIAS USING ADAPTIVE OPTICS.

Joseph Gallagher[1,2], Jacques Derouard[1], Antoine Delon[1] and Irène Wang[1]

1. Univ. Grenoble Alpes, LIPHY, F-38000 Grenoble, France.
2. Alpao, Inovallée, 345 rue Lavoisier, Montbonnot Saint-Martin 38330, France.
Email: joseph.gallagher@univ-grenoble-alpes.fr

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Introduction

Fluorescence correlation spectroscopy (FCS) is a microscopy technique used to measure the concentration and dynamics of fluorescent molecules using optical microscopes. It is fast becoming a ubiquitous tool to measure protein dynamics in living organisms. More recently it is being applied as tool to measure mechanical pressure in tissues [3].

The sensitivity of the technique comes from its use of high numerical aperture immersion objectives, which generate well defined, diffraction limited PSFs with 3D volumes on the order of femtolitres. This permits the measurement of concentration and diffusion times of fluorescent species in subcellular structures. However, optical aberrations deform and enlarge this PSF which bias FCS measurements [1]. Even weak aberrations, such as those generated by single cells or cell monolayers, which would have a weak impact on imaging performance, can greatly perturb FCS measurements and render them almost useless [2]. Aberration correction using adaptive optics (AO) can be used to compensate for this, to have unbiased FCS measurements in situations previously thought prohibitive for the technique.

Results

In our lab we have implemented a custom confocal microscope for FCS measurements incorporating a deformable mirror (Alpao DM97-15, France) for AO. Paramount to the implementation of such a system is the understanding of the impact of aberrations on FCS measurements. We show that AO correction with a relatively small number of aberration modes is sufficient to remove aberration bias of FCS measurements. Our simulations and results of FCS measurements behind model aberrating samples and cell layers show the advantages and limitations of adaptive optics. These results bring us closer to our goal of reliable FCS measurements in spheroids, a tumour model.

References

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