DEVELOPMENT OF A DOUBLY WEIGHTED GERCHBERG-SAXTON ALGORITHM FOR USE IN MULTIBEAM IMAGING APPLICATIONS

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The development of multifocal techniques in laser scanning multiphoton microscopy has allowed for large improvements in the speed, whilst maintaining both high temporal resolution and signal-to-noise associated with laser scanning techniques. One technique, using a holographic pattern projected onto a spatial light modulator to generate the multibeam array, is being used much more frequently since it offers much greater flexibility in terms of photon efficiency and distribution as well as having complete programmability. The Gerchberg-Saxton algorithm 1 is the most common holographic generation algorithm and whilst not computationally intensive, suffers from non-uniformity issues associated with the generated beamlet pattern intensity.

We report on the development of a doubly weighted Gerchberg-Saxton algorithm (DWGS) to enable generation of uniform beamlet arrays with a spatial light modulator for use in multiphoton multifocal imaging applications. The algorithm incorporates the Weighted Gerchberg-Saxton (WGS) algorithm 2 as well as feedback of fluorescence signals from the sample measured with a Single Photon Avalanche Diode (SPAD) detector array. This technique compensates for issues associated with non-uniform illumination onto the spatial light modulator, the effects due to aberrations and the variability in gain between detectors within the SPAD array to generate a uniformly illuminated multiphoton fluorescence image. We demonstrate the use of the DWGS with a number of beamlet array patterns to image muscle fibres of a 5 day old fixed Zebrafish larvae.