Structured Illumination for *In-Vivo* Retinal Imaging

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Abstract: Structured illumination can potentially be used for superresolved axially-sectioned retinal images. A method for applying structured illumination to *in-vivo* retinal imaging is proposed.

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1. Introduction

In an imaging system, by illuminating the object with a pattern of sinusoidally varying irradiance, it is possible to enhance both lateral [1] and axial [2] resolution through wide-field (non-scanning) imaging. There has been considerable work in applying structured illumination to fluorescence micropscopy [1, 3] and to a lesser extent to reflectance microscopy [4]. There is some early work on proposing the application of structured illumination to retinal imaging [5–7] and fluorescence images of *ex-vivo* eyes [8].

In this work we focus on a modality that can be applied to *in-vivo* retinal imaging. As the retina is a three-dimensional structure consisting of distinct layers, a structured illumination system should be able to exhibit good axial and lateral resolution. The lateral resolution enhancements brought about by structured illumination could in future lead to superresolved imaging of the retina if combined with Adaptive Optics aberration correction.

Most of the literature on structured illumination focusses either on the axial sectioning properties of the technique, as proposed initially by Neil *et al.* [2], or on lateral superresolution following the method proposed by Gustaffson [1]. However the two methods are compatible with each other provided some trade-offs are made [1, 9]. Within the context of retinal imaging, both lateral and axial resolution are important.

Application of structured illumination to *in-vivo* retinal imaging differs from its counterpart in microscopy in another important way, namely the non-stationarity of the target being imaged due to eye movements. This factor is more problematic in structured illumination retinal imaging than other retinal imaging modalities because the relative phases of the sinusoidal gratings play an important role in the reconstruction of the final image [5, 7]. For this work we have chosen a protocol that acquires consecutive frames at video rate in which the relative phase of the sinusoidal pattern with respect to the retina changes in between frames due to eye movements. The relative phase is then calculated from the images using an algorithm that will be described in detail in a future publication.

2. Fizeau Fringe Projection in a Structured Illumination Ophthalmoscope

The axial sectioning properties of structured illumination imaging can be understood by observing the change that occurs to the frequency domain as the system is defocussed, as shown in Fig. 1. When an object is illuminated with a sinusoidal pattern, its frequency spectrum is convolved with the frequency spectrum of the sinusoidal grating resulting in copies of the information contained within the frequency spectrum shifted to the ± 1 order. If we now consider the case of incoherent imaging with an unaberrated system, we note that as the defocus increases, the modulation of the spatial frequencies decreases at different rates depending on the spatial frequencies considered, with the dc term and other low spatial frequencies showing no or very little change. The information contained within the shifted components (centred at $\pm\nu$, where ν is the spatial frequency of the illuminating sinusoidal pattern) does attenuate with defocus. Structured illumination algorithms [2, 5, 7] extract this information while discarding the information centred at the zero order, thus giving an image whose overall energy attenuates with defocus. The rate of attenuation with defocus is therefore a function of the rate of decrease of the Modulation Transfer Function (MTF) with defocus at any given frequency, with the largest rate of attenuation being achieved at a frequency of $\nu = \nu_0/2$ where ν_0 is the cut-off frequency of the transfer function [2, 4].

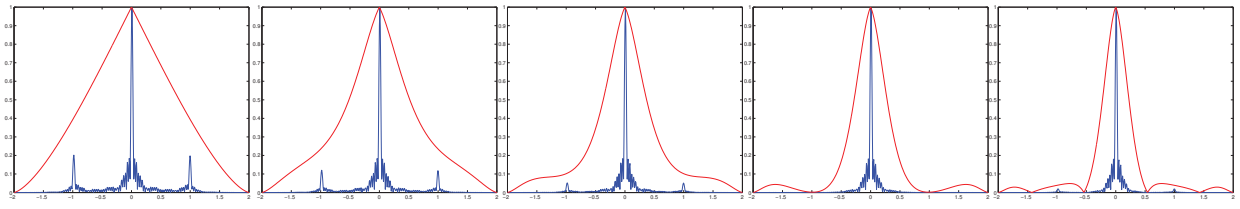

Fig. 1. Fourier transform of the image of a sinusoidally illuminated object (blue) superimposed on a plot of the Modulation Transfer Function of an unaberrated incoherent imaging system (red). Axes are modulation (y-axis) and spatial frequency (x-axis). Left most image is in focus. Defocus increases with each successive plot. The fourth image if for a defocus of u=8 ($u = 4knz\sin^2(\alpha/2)$ as defined in Refs [6, 7, 10]) and corresponds to the minimum reached in the integrated intensity curve (for constant modulation) shown in Fig. 2.

In the case of a coherent imging system, this will no longer be the case and therefore there is no axial sectioning in a coherent system, as demostrated by Karadaglić analytically [4]. In microscopy, the structured illumination is achieved either coherently (fringe projection) in the case of fluorescence imaging where the incoherence is delivered by the independance of the fluorophores, or by imaging a sinusoidal grid onto the target (grid projection) [3, 4]. The former is therefore not suitable for non-fluorescent imaging in the eye, and the double pass involved in the latter results in a severely reduced modulation in the acquired images, particularly for the spatial frequencies required and in the presence of aberrations. We have therefore proposed a Fizeau projection system using a Michelson arrangement in which an extended low-coherence light source, such as an LED, is used to give suitable spatial incoherence. As the fringes are formed directly by interference at the retina, the fringes are only imaged in single pass. This method of illumination also has the advantage (as does the grid projection technique used in microscopy) that the modulation of the fringes decreases with defocus, reducing the contribution of these planes further, as shown in Ref. [7] and discussed below.

3. Simulation of a Structured Illumination Ophthalmoscope

The image formation process of the Structured Illumination Ophthalmoscope (SIO) has been simulated in MATLAB (The Mathworks, Inc) by multiplying the appropriate sinusoidal pattern onto a plane of a 3D object and calculating the image of that plane by using Fourier optics methods. The effect of various parameters on the axial sectioning performance were investigated; these include the choice of spatial frequency of the illuminating sinusoidal pattern, the number of frames required to generate a sectioned image, the effect of change of modulation of the fringes with defocus and the effect of aberrations [7]. Here we highlight the effect of the change in modulation with defocus brought about by the localization of the Fizeau fringes and hence the reduced fringe visibility at planes away from the plane of localisation. The change in modulation was measured in our system by placing a CCD camera at the retinal plane and translating it axially, recording the fringes at different axial planes and extracting their modulation in MATLAB by finding the value of the first order peaks in the Fourier domain. These modulations were then input into the simulation to compare the axial sectioning with the case when the fringe contrast remains constant with defocus. Figure 2 shows the integrated intensity plots (total irradiance in an image plane as a function of axial position [10]) for both cases showing that the variable fringe contrast resulting from the Fizeau fringe projection method gives a slightly improved axial resolution (defined by the full width at half maximum of the plot) and apodization of the side lobes when compared to the output simulated for the conventional fringe projection illumination using coherent light.

4. Conclusion

Future work on this project includes the collection of *in-vivo* retinal images using the SIO prototype being developed and quantification of the axial and lateral resolving powers. The axial sectioning capabilities of structured illumination and confocal microscopy have been shown to be almost identical in the ideal cases [4, 7]. However, in the case of confocal microscopy applied to retinal imaging (the confocal Scanning Laser Ophthalmoscope) compromises on pinhole size are often required affecting the axial sectioning. In structured illumination there is no rejection of light as in the confocal microscope case and hence these trade-offs are not required. The optimal frequency might not be practically possible to use in all circumstances in structured illumination, but the axial sectioning performance is fairly robust to

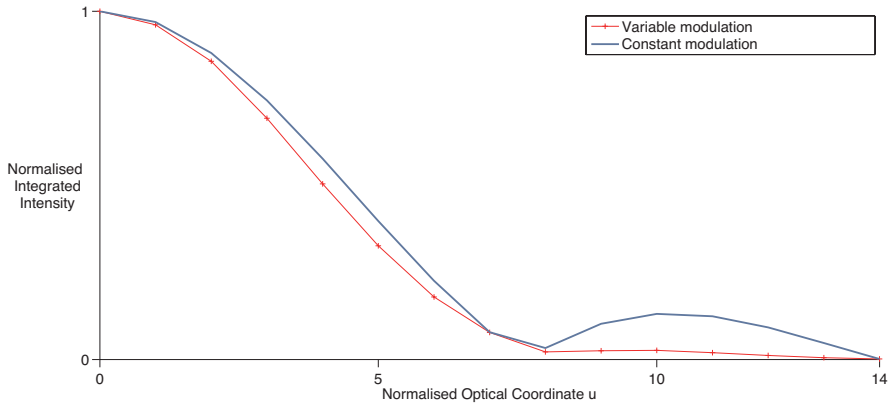

Fig. 2. Normalised integrated intensity against normalised axial optical coordinate u (defined in Fig. 1) representing defocus for the case of constant modulation with defocus, as in the case of coherent fringe projection illumination, and the modulation present in our current implementation of the Fizeau fringe projection technique which decreases with increasing defocus.

moderate deviations from this ideal frequency [7]. In terms of lateral resolution, structured illumination can surpass the diffraction limit [1]. The combined axial and lateral resolution performance of structured illumination therefore make it an ideal candidate for delivering superresolved, axially sectioned retinal images.

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