Using Multi-Element Detectors to Create Optimal Apertures in Confocal Microscopy

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Abstract— **A detection pinhole is used in confocal microscopy to reduce contributions from image planes outside of the focal plane. The size of this pinhole may be varied but the idea of a fixed circular aperture is ubiquitous. Here it is shown that an ideal detection aperture varies as a function of the spatial-frequency being imaged. A method for calculating such detection apertures is given, an example calculation is shown and a detector array is suggested as a means to approximate these varying detection apertures.**

I. Introduction

The detection pinhole is critical to the operation of a confocal microscope as it stops out-of-focus light reaching the detector. The size of this pinhole is often varied according to signal strength but few other variations are made. This paper considers what may be achieved if the aperture shape is different for each spatial frequency to be imaged. The variable shape is motivated by maximizing the signal-to-noise ratio across all of Fourier space. The optimal shape for each spatial frequency is found and the expected performance gain quantified.

The effect of a given physical aperture is fixed for all spatial frequencies, however a more flexible detector option is available. Recent advances in detector-array speed and sensitivity have made them suitable for applications such as confocal microscopy [1]. An array can be placed in the detection plane and the data collected from each detector-element decomposed using a Fourier transform. At each spatial frequency, the detector elements can be weighted to synthesize the desired aperture. The shape of this compound aperture is limited only by the size of the individual array elements. Using the results from previous work [2], it can be seen that even a fairly coarse detector array approximates the optimal apertures well.

II. Finding the Optimal Apertures

Each position in the detection plane can be surrounded by a small area and treated as an independent detector. The task will then be to find a complex weighting (which may vary with spatial frequency) for each position so that when all the position weightings are calculated, the aperture is given. For example, with a standard pinhole, all positions within the pinhole area would have a weighting of 1, as all the light incident on this area is summed to give the detected signal. All positions outside the detection pinhole would have a weighting of zero. The optimal weightings are found by satisfying an optimality criterion.

The optimal signal will be defined as that which has a maximum signal-to-noise ratio (SNR) at all values of the spatial frequency variable $\mathbf{k}$. This definition is well-matched to linear deconvolution methods, which can be represented as Fourier domain multiplications. Following such a deconvolution, this definition of optimality ensures that the power of the reconstruction's error-due-to-noise (measured by a mean-square-error or 2-norm metric) will be minimal. It should be noted that the other component of the reconstruction's error, the error-due-to-bias, is determined by the deconvolution method.

The optical transfer function for the detection area around position r will be denoted by $H_r(\mathbf{k})$; methods of calculating it are well-known [3]. The noise will be modeled as Poisson which means that the noise at a given discrete spatial frequency is zero mean, uncorrelated across $\mathbf{k}$ and has a variance proportional to $H_r(\mathbf{0})$. Minimizing the total noise level at $\mathbf{k}$, while keeping the signal strength constant gives the following weightings.

$$W_r(\mathbf{k}) \propto \frac{H_r^*(\mathbf{k})}{H_r(\mathbf{0})} \quad (1)$$

The spatial distribution of these weightings gives the optimal aperture for imaging spatial frequency $\mathbf{k}$. Note that the spatial position is denoted by r.

III. Example Calculation

The calculations of the previous section were performed for a fluorescence confocal system with linearly polarized excitation along the x axis and with an excitation wavelength of 488nm. The detection wavelength wavelength was 530nm and the numerical aperture of the system was 1.35. A demagnified detection area of 2μm by 2μm was considered. The calculated apertures at some illustrative spatial frequencies are shown in Fig. 1. The behavior of the apertures is intuitive — a smaller pinhole is used to image the fine detail of higher spatial frequencies while a larger pinhole is used to gather more signal at lower frequencies. Imaging lateral frequencies introduces directional structure in both the magnitude and phase of the aperture.

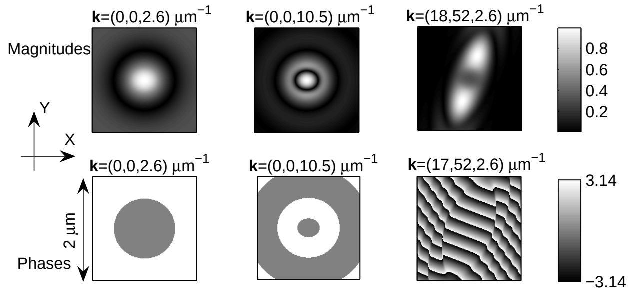

Fig. 1. Optimal apertures at illustrative spatial frequencies for the example system considered. Magnitudes are displayed on the top row and phases on the bottom. All detector areas are 2μm by 2μm in demagnified space.

The performance of the optimally varying detector was compared against three systems with the standard pinhole detection aperture. Pinhole diameters of 250nm, 500nm and 1000nm in demagnified units (approximately 0.5, 1 and 2 Airy units) were simulated. The optical transfer function (OTF) was calculated for each and compared to that of the optimized system. The results are shown in Fig. 2. Note that the OTFs have been normalized so that the noise level is the same in each system, which means the OTF with the higher value has the higher SNR. As expected, the optimized system outperforms each individual pinhole at all spatial frequencies.

IV. Realizing a Varying Aperture

To implement the optimized detection aperture described here, it is necessary to have a detector that can approximate the aperture shapes for all spatial frequencies simultaneously. An obvious choice for this is a detector array where the data from each detection element can be mathematically combined after data acquisition. Indeed, using a small detector array in place of a confocal pinhole has been suggested [4].

The case of a 5×5 square detector array with array-element side length 250nm has previously been examined by the authors [2]. The data collected by each region of this array is Fourier transformed, multiplied by a $\mathbf{k}$ dependent weighting and summed to give a noise optimized data set. The weighting is calculated using (1), where $H_r(\mathbf{k})$ is the OTF of the r^{th} of the 25 detection regions. This approximates the ideal apertures described in this paper. The ideal apertures are essentially those realized by a very finely pixelated detector array.

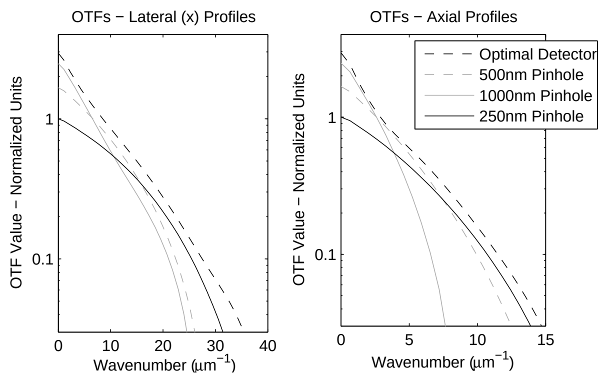

Fig. 2. Semi-logarithmic profiles of the normalized optical transfer function for the optimized system described and several single-pinhole systems with various pinhole sizes.

Despite the somewhat coarse subdivision of the 5×5 detection area, it performs at a reasonable level compared to the ideal system, at least at the spatial frequencies that are usable in a confocal microscope. As the strength of a confocal fluorescence signal is generally low [5], it is not usually possible to reconstruct the higher spatial frequencies that are weakly passed by the instrument. The performance gap between the ideal detection apertures and the 5×5 array increases as the spatial frequency increases. However, even at $\mathbf{k}$ values passed with only 2.5% of the ideal system's maximum SNR, the ideal system's SNR is still less than twice that of the 5×5 array. Thus a small detector array provides a reasonable approximation of the ideal apertures.

V. Conclusion

It has been shown that an ideal confocal microscope would have a detection aperture that varies with spatial frequency. Simulation results show that this variable aperture can be expected to out-perform any single pinhole shape. A variable aperture cannot be achieved with a physical aperture where the shape is fixed, but it can be approximated using a detector array. Post-acquisition processing is used to weight each detection element differently at each spatial frequency and thus achieve the effect of a spatial-frequency dependent aperture.

An important caveat to this result is that the noise properties of the different detection regimes may not be equal. It was assumed that the noise was Poisson limited and that the same proportion of incident photons were detected for all systems. In practice, the detection efficiency and noise properties will vary with detector type [6].

Acknowledgment

This work was partially sponsored by the NIH under grant NIH 5R01 EB00756 and by the NSF under grant NSF DBI 01388425.

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