

Making Use of Rejected Light - Improved Imaging with Multi-Channel Detection in Confocal and 4Pi Microscopy

Brynmor J. Davis, William C. Karl, Anna K. Swan, M. Selim Ünlü, and Bennett B. Goldberg
 Boston University, Department of Electrical and Computer Engineering, 8 Saint Mary's St., Boston, MA 02215, USA
 bryn@uiuc.edu

Abstract: Light usually discarded in a microscope can be collected in additional channels and used to reduce noise sensitivity. Optimal Fourier-domain processing is used to construct a single superior image from the multi-channel image set.

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1. Introduction

For many microscopy applications low signal level is a significant problem. This is particularly true in scanning fluorescence microscopy where only a limited number of photons are emitted by each fluorophore and the object must be scanned in three dimensions. Methods for improving the efficiency of both confocal and 4Pi systems have been proposed – additional detectors can collect light that would otherwise fall outside the confocal pinhole [1]; and the light exiting the destructive output of a 4Pi beamsplitter can be collected to form a second channel [2]. Here an appropriate image reconstruction algorithm for these systems is developed and the resulting performance quantified.

2. Methods and Results

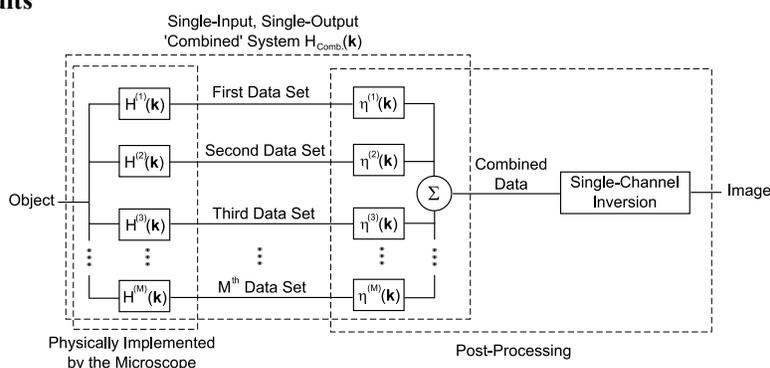


Fig. 1 - Systems diagram of a multi-channel microscope and the deconvolution algorithm. All systems are defined by their transfer function – $H^{(m)}(\mathbf{k})$ is the m^{th} channel's transfer function and $\eta^{(m)}(\mathbf{k})$ is the m^{th} processing filter.

The microscope and Fourier-domain post-processing algorithm form the system shown in Fig. 1. The processing filters are found by jointly minimizing the noise variance (a Poisson distribution assumed) at each spatial frequency. This results in the following definition, where * indicates the complex conjugate.

$$\eta^{(m)}(\mathbf{k}) = \frac{[H^{(m)}(\mathbf{k})]^*}{H^{(m)}(\mathbf{0})} \quad (1)$$

As shown in Fig. 1, this deconvolution process allows a 'combined transfer function' to be defined. This function indicates that at all spatial frequencies there is a higher SNR in the multi-channel instruments than in comparable single-channel systems. Simulations confirm that the imaging error is less in the multi-channel instruments at all signal levels. Quantitatively, the multi-channel systems have performances similar to single-channel instruments operating with up to 50% longer dwell times.

3. References

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