Determining the optimal deconvolution method for images of fluorescently-labeled microtubules in T cells

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**Introduction:**

It is necessary to gather and analyze experimental data to create or refine a computational model of a biological system. Microscopy has long been a useful technique in biology, and it becomes even more useful with the application of algorithms designed to enhance images. Many scientists use confocal microscopy, but with the right analysis, wide-field light microscopy can supply just as much information.

All microscope images contain some non-random blurring as a result of light passing through the lenses. The pattern of the blur has been well studied, and is referred to as the Point-Spread Function (PSF). The PSF is an oblong “football” of light, with concentric rings of light around it, which get larger as you get farther away from the actual source of light. It has an hourglass shape when viewed in the x-z or y-z plane (Wallace, et al). An experimental PSF can be obtained by viewing the patterns of light produced by very small fluorescent beads. Because the PSF is known, deconvolution algorithms have been developed to reassign the blurred light back into the pixel of the image that it really came from. This can drastically improve the quality of the image, but it can also leave artifacts and noise, or break up parts of the image that are actually continuous. This has caused particular difficulty in analyzing images of filaments in biological systems (Wallace, et al).

Microtubules play an important role in the response of killer T cells to infectious cells. The microtubules have been shown to re-arrange themselves so that the aster from which they radiate faces the target cell (or, in some experiments, a surface coated with an antigen). Along
with this motion, the T cell effector vesicles move to that side of the cell to release their
cytotoxic molecules. It is still unknown exactly how the microtubule aster moves within the cell,
and what drives this motion. There is speculation, but the observations and theories often
conflict with each other. With more information from images of the cells, computational models
of the microtubule behavior could be developed. If the movement of the microtubules could be
modeled, this information could be used to determine the forces that are driving the
microtubules. T cells are very small, and therefore the images are hard to acquire and analyze.
This fact, coupled with the difficulty in using deconvolution algorithms to study images of
microtubules, demands a study of the best way to use the available algorithms.

Methods:

The images to be analyzed are 3-dimensional images of T cells on a thin glass surface
coated with antigen. Fluorescently-labeled tubulin subunits have been inserted into the cell so
that the microtubules will be visible. Images have been previously obtained of live cells over the
course of time, as well as fixed cells. The fixed cell images are better suited for study through
deconvolution. They have a higher resolution, and smaller steps in the direction of the z-axis.
Experimental PSF images have also been obtained using the same microscope.

The Matlab software by MathWorks, including the Image Processing Toolbox, will be
used to perform deconvolution on the images. This software contains several common
algorithms. All of the algorithms have strengths and weaknesses. For example, Wiener
deconvolution, an inverse filter method, is more likely to amplify noise in the image. The Lucy-
Richardson deconvolution is an iterative algorithm that cuts down on noise. The choice of
algorithm will be explored, as well as the optimal parameters, to determine how best to process
images of these cells.
**Expected Results:**

The goal of this research is to determine the most effective method for deconvolving images of microtubules in T cells, a problem that has not been solved yet. The deconvolved images obtained will be evidence of the success of the procedure. The deconvolution of the images will allow the microtubule length and bending to be measured computationally. Ideally, this method will also be useful for images of live T cells, so that the microtubule motion can be measured. By measuring the motion of microtubules in T cells, conclusions can be drawn about the forces driving the movement of the microtubule aster, allowing for the creation of computational models of T cell activity. These models, when verified experimentally, would provide information on this topic that observation alone never would.

**References:**