

## Image Analysis of the Mitotic Spindle

### The Mitotic Spindle

The *mitotic spindle* (see Figure 1) is a temporary and critical nuclear structure that serves as the machinery for chromosome segregation during cell division. The tubulin-based structure of the spindle is also the target of many anti-cancer drugs. Furthermore, there are many proteins that interact with the spindle in unknown ways. In this effort, automated image analysis tools were developed to quantitatively characterize the mitotic spindle under a variety of conditions including gene knockout and drug treatment. These image analysis tools will allow life science researchers to explore the roles of many proteins in cell cycle progression, spindle checkpoint, and/or chromosome segregation and, thereby, aid in the discovery of new drug targets.

### Technology

Five cell types were established for study, including wild-type, two different gene knockouts, and drug-treatment at two different concentrations. The cells were fixed and probed with fluorescent-labeled, anti-tubulin antibodies that attach to the spindles of metaphase cells. The spindles were then imaged via confocal microscopy, resulting in a three-dimensional (3D) stack of images for each spindle.

Image processing tools were developed to extract a set of quantitative features from each 3D spindle image stack. These features are based on the spindle's intrinsic coordinate system, which is referenced to the spindle center of mass and principal axes of inertia (see Figure 2). Statistical analysis of these features across all the image stacks in the database has indicated that

significant structural differences — that are too subtle to be quantified and/or perceived by a skilled human observer — can be elucidated between the positive control data (wild-type vs. drug-treated) as well as between the test data (wild-type vs. gene knockout).

### Specifications and Features

- Automated 3D image analysis tools in the MATLAB environment.
- Multiple features automatically computed in batch mode.
- Statistical comparison of data sets.
- Tools will enable large-scale studies of protein deficiencies on the mitotic spindle.

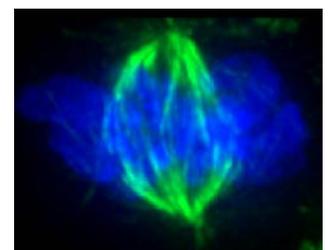


Figure 1. Chromosomes (blue) and the mitotic spindle (green) during cell division.

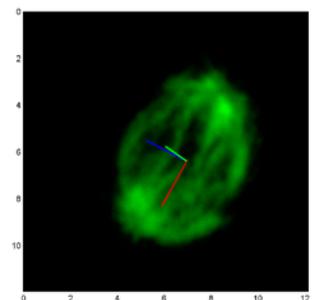


Figure 2. Spindle shown with principal axes of inertia and center of mass (intersection of axes) Note that this is a 2D rendering of a 3D volume. Scale is in microns.



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