Computer-based recognition of drug-induced changes in the distribution of fluorescently labeled microtubules in NIH 3T3 cells

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Computer-based recognition of patterns in gene and protein sequences serves as the basis for the bioinformatics methods that underlie the fields of genomics and proteomics. Recent efforts have sought to extend the power of computer-based pattern recognition to the analysis of the subcellular distribution of fluorescently labeled proteins[1,2]. This approach will allow for quantitative comparisons between the distribution patterns of different proteins and/or the analysis of changes in protein distribution over time. This latter capability is particularly relevant to the study of the cellular response to toxic injury and the cellular basis of disease initiation and progression. The purpose of this study was to test the ability of computer-based pattern recognition software to distinguish between the patterns of fluorescently-labeled tubulin in NIH 3T3 cells responding to treatment with the microtubule altering drugs nocodazole and taxol.

NIH 3T3 fibroblast cells were cultured in DMEM plus 10% fetal bovine serum using conventional methods. For drug treatment cells were exposed to either 5 micromolar nocodazole or taxol for 1-3 hours at 37 degrees C. The fluorescent labeling of tubulin was accomplished using either a green fluorescent protein (GFP)-based method or immunofluorescent staining. For GFP labeling, cells expressing a stable form of GFP labeled alpha-tubulin[3] were obtained from Dr. Jonathan Jarvik (Carnegie Mellon University). For immunofluorescent labeling, cells were fixed and permeabilized in a mixture of formaldehyde and triton detergent and stained with a primary mouse monoclonal IgG antibody against alpha-tubulin followed by a goat anti-mouse IgG secondary antibody conjugated with fluorescein. Fluorescently labeled cells were viewed on an Olympus Fluoview 300 confocal microscope and digital images were collected. Digital files were then computer analyzed using the Support Vector Machine (SVM) method or the Statistical Image Experiment Comparator (SImEC). Both of these pattern recognition programs provide a numerical description of protein localization according to sets of features including Zernike moment features, Haralick texture features and features specifically derived for analysis of subcellular location (see http://murphylab.web.cmu.edu/services).

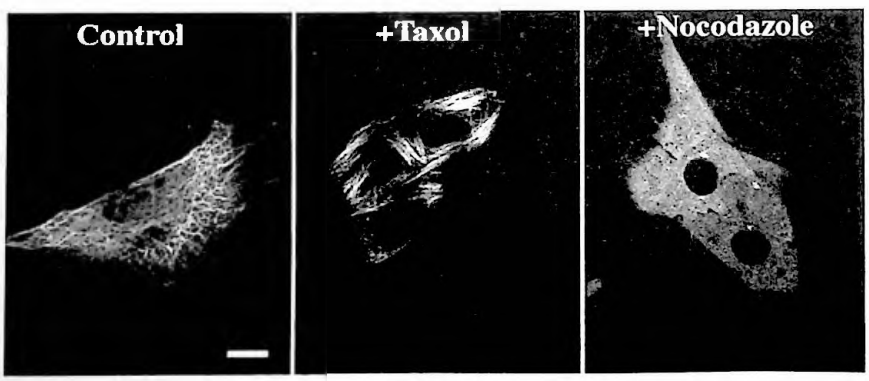

Figure 1: Confocal microscopic images of living NIH 3T3 cells expressing GFP-labeled tubulin. The control cell shows an extensive microtubule array, while the +taxol cells contain bundles, and the +nocodazole cells have lost their microtubules. These are examples of the images used for the computer-based comparative analysis. Bar = 10 microns.

Confocal microscopic imaging of either GFP expressing or immunofluorescently stained cells showed that control cells contained an extensive array of microtubules. This is in contrast with the drug treatments, with nocodazole leading to microtubule depolymerization and taxol leading to microtubule bundling. The matrix below provides a tabular depiction of the SVM results based on images of the GFP labeled cells.

	Computer Predicted Classification		
	Control	+Nocodazole	+Taxol
Actual Classification:			
Control	15	1	2
+Nocodazole	2	16	0
+Taxol	1	1	16

The matrix indicates that out of the 18 total control images (from 4 separate experiments), the SVM method erroneously classified only one as belonging to the +nocodazole group and 3 as belonging to the +taxol group. Two images were incorrectly categorized in both the +nocodazole and +taxol groups. The overall correct performance of the SVM method was 87% for the GFP method and 85% for the immunofluorescent staining method. The SImEC-based pair-wise comparisons (control versus +nocodazole; control versus +taxol; +nocodazole versus +taxol) for the GFP and immunofuorescence groups were all found to be statistically different using a Hotelling T2 test ($p = 0.05$). These results indicate that the computer-based pattern recognition software is a reliable means for the quantitative analysis of drug-induced changes in protein subcellular distributions. In addition they demonstrate that this approach could be used for the quantitative and high throughput analysis of toxin-induced subcellular reorganizations. This automated analysis of microscopic alterations has promise in the areas of tracking cell, tissue and whole animal exposure to toxic chemicals, in the high throughput analysis of drug effects, and in the diagnosis and progression staging of disease.

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