692 Automated Motion Detection and Quantitation of Cell Components: Application to Dynamic Populations of Microtubules in Living Cells

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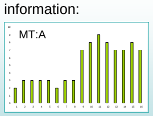
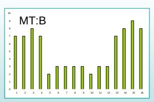
ABSTRACT

Live cell imaging technologies have advanced enormously over the past decade. However, in most cases, analysis remains a painstaking, tedious and largely manual task, limiting the quantity and quality of resulting data. Here, we describe (i) the development of computational methods that detects and quantifies changes in the location of objects of interest in living cells and (ii) the application of this software to the analysis of populations of GFP-labeled microtubules (MTs). More specifically, we developed image enhancement techniques to automatically track and quantify the growing and shortening behavior of populations of MTs in living cells. Our new method improves dramatically upon the currently available means to analyze MT behavior. Through automated detection of all visible MTs in a fluorescence image stack in a visible cellular region (>100 MTs), each stack produces considerably more usable data in considerably less time while removing possible unintentional operator bias. Additionally, our technique preserves intact, ordered event histories of MT populations, possibly elucidating novel MT population behaviors which cannot be described by single event frequencies and average rates calculated from parsed data sets. Finally, global analysis of MT populations could reveal regional, behavioral specificities and MT population inter-relations, possibly integral to specialized processes such as cell division and neuronal outgrowth. Taken together, this highly exportable technique improves our ability to address existing questions while making it possible to use statistics to ask novel questions regarding the behavior of MT populations (and other objects of interest) that have not been approachable previously.

LIMITATIONS OF MANUAL ANALYSIS

- Limited computational assistance
- Approximated measurements such as cutoffs to define attenuation event
- Possible user bias in MT selection and tracking
- Limited sample sizes
- Tremendous time and effort requirements per study
- Events are treated independently resulting in possible loss of information:





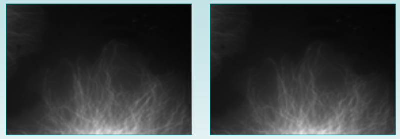
Same (growth) events for MT:A and MT:B occurring at different frames, same event rate (average growth = 5) but different behavior characteristics.

IMPROVEMENT SUMMARY

- All manual limitations reduced or eliminated
- Computation of statistics beyond traditional events
  - Broader set of descriptive statistics based on vastly larger data points
  - Streamlined data flow into large databases
- Introduction of behavior patterns
  - Events are not parsed before analysis, event context is preserved
  - Similarity measures based on behavior
  - Searching a database for similar behavior possible
- Standardization of analysis across MT research labs

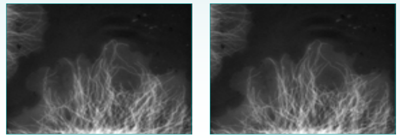
IMAGE ENHANCEMENT

I.



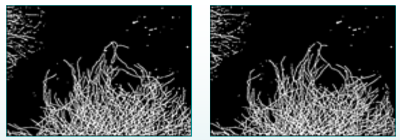
Original frames at time 1 (left) and time 2 (right).

II.



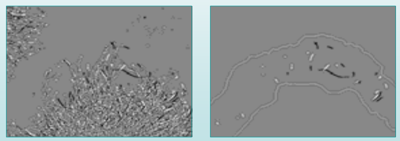
Preprocessed frames at time 1 (left) and time 2 (right).

III.



Binarized frames at time 1 (left) and time 2 (right).

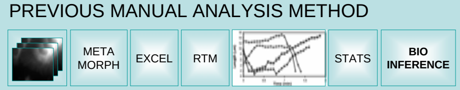
IV.



Difference frame showing MT activity (left), filtered events in activity region (right).
White = growth, black = shortening.

METHOD COMPARISON

PREVIOUS MANUAL ANALYSIS METHOD

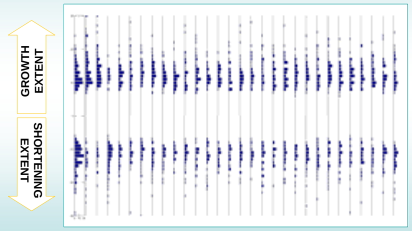


AUTOMATED ANALYSIS METHOD

IMAGE ENHANCEMENT ▷ EVENT QUANTITATION ▷ STATISTICAL MODELS

MT event verification vs manual method (%)				
	clear	noisy	broken	total
W Gaps	95 (21/22)	56 (18/32)	16 (1/6)	66 (40/60)
WO Gaps	100 (22/22)	81 (26/32)	16 (1/6)	81 (49/60)

GROWTH AND SHORTENING EVENTS IN A VIDEO



(1-30) EVENT COUNTS THROUGH VIDEO FRAMES

BEHAVIOR CHARACTERISTICS FOR TAU ISOFORMS

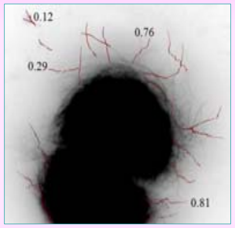
Modulation of MT dynamics by tau in living cells: implications for development and neurodegeneration. (Study by J. Bunker et al.)

	UNIN	3R	4R
WT	UNIN BUFF *3GST	0283R 65x3R 3R13	*035T 1004R 65x4R
Mutation		103RGV 103RRW	4RGV 4RDK 4RPL 4RSN 4RRW

CORRECT BEHAVIOR CLASSIFICATION (%)		
	(122) ALL VIDEOS	(32) GOOD VIDEOS
3 classes (random = 33.3%)	68.8	72.6
14 classes (random = 7.14%)	49.3	64.7

Estimated statistical models capture behavior differences.

MICROTUBULE POPULATIONS



Numbers describe the probability of individual MTs belonging to a particular class, based on behavior characteristics. It is possible to examine behavior patterns of MT populations.

TEST STUDY

Does βIII Tubulin Confer Taxol© Resistance?
(Study by K. Kamath et al.)

Experimental Outline:

- CHO cells injected with rhodamine tubulin for observation by fluorescence microscopy and dynamics tracking
- Subset was transfected with an inducible transgene, coding for bIII-Tubulin
- Cells with and without the induced bIII-Tubulin transgene were treated with paclitaxel and dynamics were recorded

Test Groups

I. βI : Induced, No Paclitaxel
II βIII: Induced, No Paclitaxel
III. βI: Induced,150nm Paclitaxel
IV. βIII: Induced,150nm Paclitaxel
V. βIII: Uninduced (tet) + 150nm Paclitaxel

Manual Findings

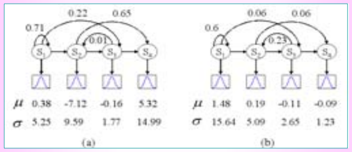
(III)(V) ⟷ (I)(II)(IV)
Dynamicity

Automated method performance:

Classification Performance on Test Cases	
Behavioral difference between (IV) and (V)	92.21% correct
Behavioral difference between (I,II,IV) and (III,V)	81.25% correct

NEW BEHAVIOR STATISTICS

Probabilistic models estimating statistics of a cellular process (microtubule dynamics) from observation sequences.



Model (a) estimates average dynamics between (-7.12, 5.32) pixels, as shortening and growth rates respectively, while model (b) estimates average dynamics between (-0.11, 1.48). Much less dynamic behavior in (III,IV).

First state of (a) has a probability (0.71) to stay in the same state: small growth events are likely to occur in longer periods than other events. (Not all probabilities shown.)

FUTURE APPLICATIONS

Tracking:
Our method could be applied to other dynamic cellular processes such as protein trafficking and sorting, cell differentiation and chromosome segregation.

Statistical modeling:
Drug-candidate or dose-range studies: Revealing similarity among congeners or concentration-dependent mechanisms (e.g. taxanes).
Protein mutation analyses: Evaluating individual and possible combinatorial affects of FTDP-17 mutations of tau upon MT dynamics, axonal transport.