Automated Kinetic Analysis in Individual Cell Motility Assays

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06 – LA – 3814 - ASCB

Introduction

Cell motility is a fundamental process central to embryonic development, immune response, wound healing, angiogenesis, tissue engineering and various disease processes, including cancer metastasis. The study of the mechanisms underlying cell motility is an important field in basic cell biology. Single cell motility assays allow scientists to put findings from a molecular and subcomponent level in the context of whole cell behavior, specifically movement. Despite the large and broad need for individual cell motility assays, and the improving performance and availability of automated live cell imaging systems, research efforts in individual cell motility analysis continue to be a tedious, largely manual and inexact process. This is due primarily to a lack of accurate and robust kinetic recognition tool, particularly for cells in phase contrast images.

We developed a kinetic recognition software to automatically recognize and track individual cells in time-lapse phase contrast images. We have implemented innovative kinetic cell morphology characterization measurements that extended the utility of the strong cell boundary recognition capability of the kinetic recognition software.

In this study we evaluate the efficacy of our methods in an experiment using Nocadozole, a drug known to depolymerize microtubules and inhibit cell motility. The results show that our methods correctly characterize the expected assay outcome. Furthermore, analysis of various measurements to score the assay show that the kinetic morphology measurements yield the best assay quality as evaluated using assay quality metrics such as the Z factor and assay signal to noise.

Materials and Methods

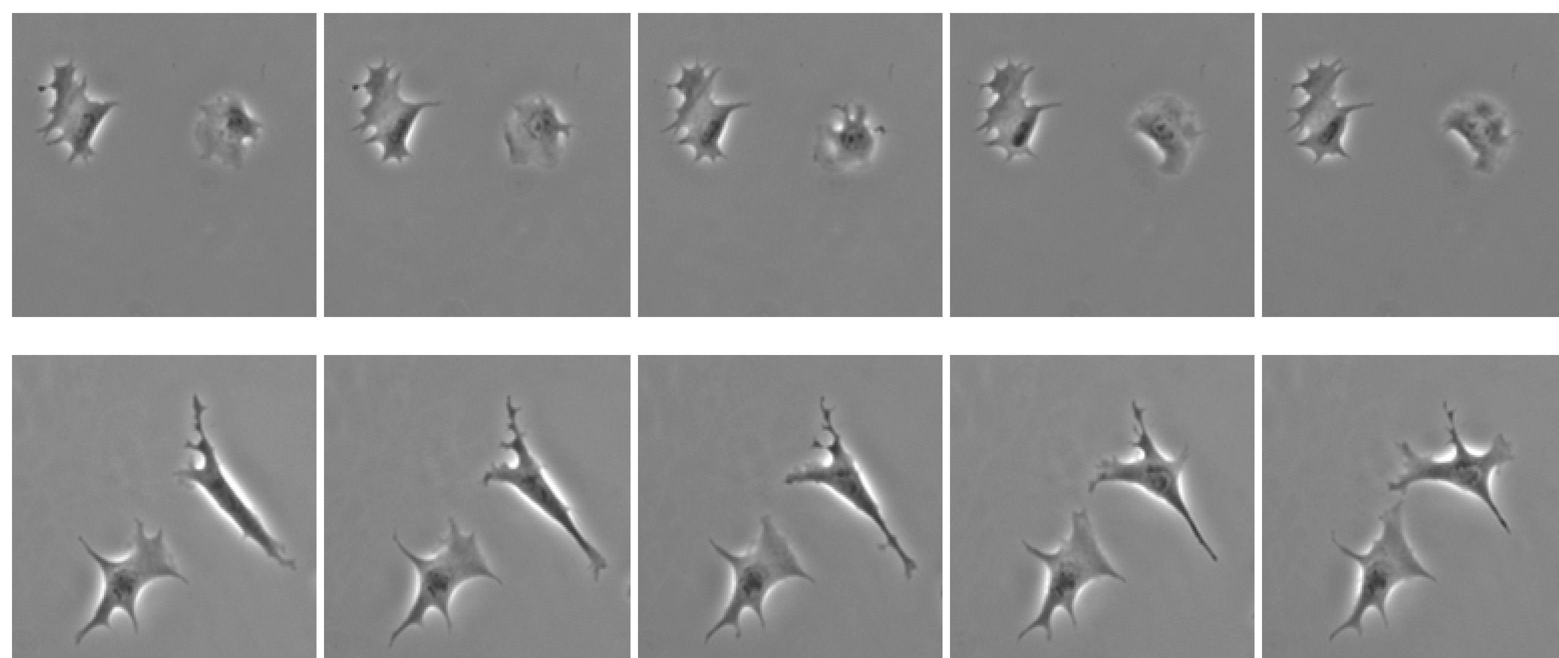

Figure 1. Real images of cell movement under Nocadozole treatment & washout after treatment were used in the study. Study set consist of (i) three sets of phase contrast movies subjected to 0.5 micromolar dosage of Nocadozole and washed out after the pre-treatment, and (ii) three sets of phase contrast movies subjected to 0.125 micromolar dosage and washed out after the pre-treatment. The above representative image frames show 0.5 micromolar dosage in the upper row, and washed out in the lower row, with a time interval of 10 minutes. It can be intuitively seen in the images that that the cells undergoing different drug treatment exhibit differing cell shapes. The higher dosage cells appear to be more compact and rounded in contrast to the wash out cells which have a stretched or extended morphology.

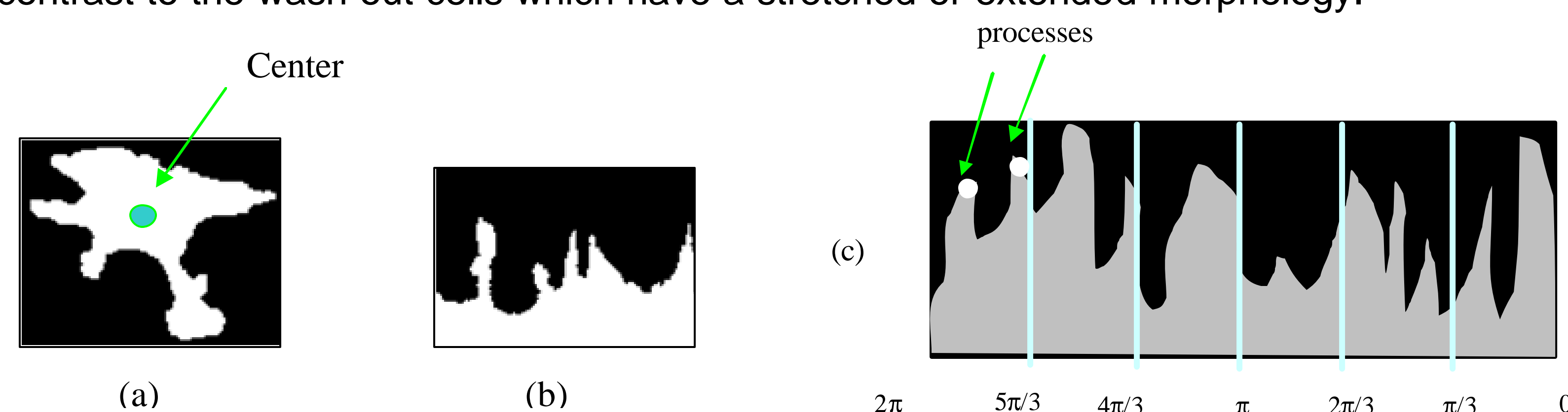

Figure 2. Polar domain morphological profiling measurement. Many shape measurements to quantifying kinetic morphology can be made using a polar coordinate transformation; (a) shows a cell region mask and its center; (b) shows the polar coordinate transformation of (a); (c) illustration of the polar domain morphological profiling measurements.

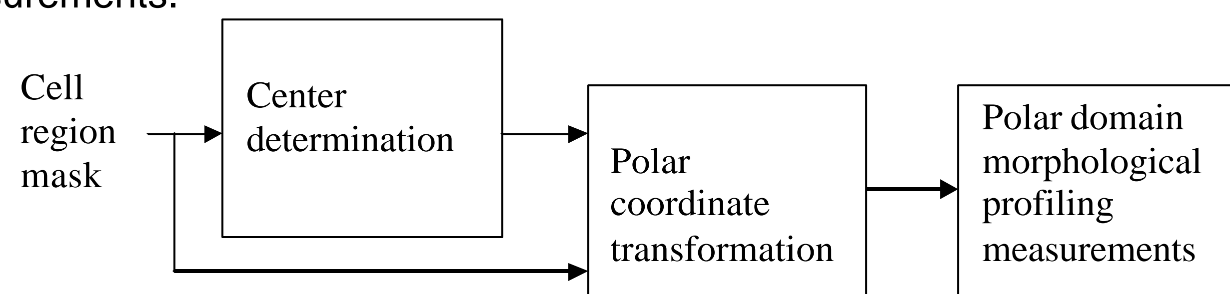

Figure 3. The processing flow for the cell morphological profiling measurements Many features could be derived in the polar domain including the maximum radius, average radius (normalized by the maximum radius), standard deviation of radius, the number of processes (peaks), the average process radius, the standard deviation of process radius. The above measurements can be calculated for the whole range (0 to 2p) or for each of the 6 ranges: 0-p/3, p/3-2p/3, 2p/3- p, p-4p/3, 4p/3-5p/3, 5p/3-2p (see Figure 2 (c)).

Results

	Z Factor Scores		S:N Scores	
	0.125 Noc	0.5 Noc	0.125 Noc	0.5 Noc
Velocity	-7.8551	-1.2418	0.4568	1.8185
Average Speed	-37.2368	0.3603	0.1016	6.6281
Acceleration	-4.0772	-0.5863	0.8070	2.6427
Minor / Major Axis Ratio	0.1542	0.4702	4.7773	7.6394
Filling Ratio	0.1499	0.4551	4.8314	7.5185

Table 1. Kinetic morphology measurements yield best assay quality. Each cell in the table shows the assay quality score Z-factor or S:N comparing the test condition (0.125 or 0.5 micromolar Nocadozole) to the control (Nocadozole washout) for the automated analysis.

The Z factor and S:N are defined as $Z = 1 - \frac{(3\sigma_s + 3\sigma_c)}{|\mu_s - \mu_c|}$ and $S:N = \frac{|\mu_s - \mu_c|}{\sqrt{\sigma_s^2 + \sigma_c^2}}$ where σ_s and σ_c are standard deviations of the treated sample and control populations, and μ_s and μ_c are their means. Reasonable assay quality scores for Z-factor and S:N are greater than 0 and 3.0 respectively, and excellent quality scores are greater than 0.4 and 6.0 respectively.

These results confirm that our methods produce the expected assay outcome. For each measurement, its assay quality score improves as you move from the lower to higher drug concentration. This is what we would expect for the Nocadozole assay as the mean of the higher dosage would be further away from control; thereby rendering a better quality score. Furthermore, the novel kinetic morphology measurements (minor/major axis ratio, filling ratio) of this study have better quality scores as compared to common motility metrics (velocity, average speed, acceleration).

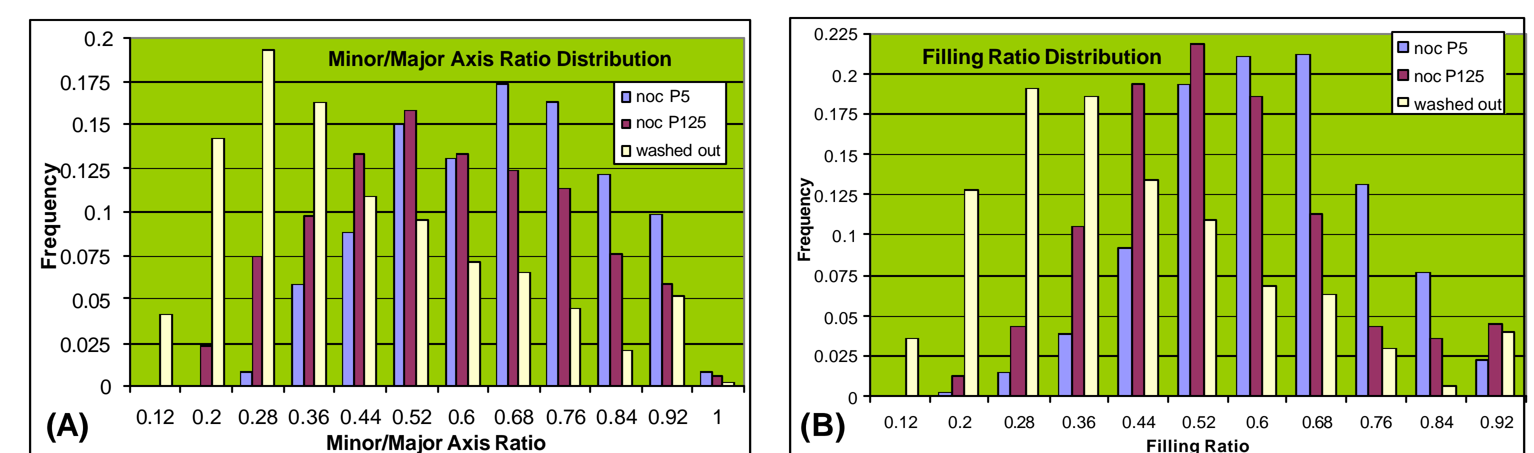

Figure 4. Kinetic morphology data support intuitive understanding of the phenotype

A) and B) provide the data to support the observation that the treated cells have a more compact phenotype than the washout cells. The distribution for the minor/major axis ratio is ordered from washout to increasing dosage in terms of a more rounded morphology, indicating that the control cells are less rounded (a ratio of 1 would be a perfect circle). The distribution of the populations for the filling ratio measurement tells a similar story.

	Z Factor Scores		S:N Scores	
	0.125 Noc	0.5 Noc	0.125 Noc	0.5 Noc
Velocity (manual)	-2.6798	-0.6016	1.1529	2.6075
Average Speed (manual)	-6.9574	-0.1752	0.5177	3.5913
Acceleration (manual)	-11.3156	-0.7554	0.3428	2.3738
Minor / Major Axis Ratio (auto)	0.1542	0.4702	4.7773	7.6394
Filling Ratio (auto)	0.1499	0.4551	4.8314	7.5185

Table 2. Automated kinetic morphology measurements compare well against manually determined motility measurements

To confirm that the improvement provided by the kinetic morphology measurements is not an artifact of our own automated analysis, in Table 2 we compare the assay quality of our automated morphology measurements with the motility measurements made from a tracking set produced manually. As you can see in the table, even when compared with manually derived motility measurements, the kinetic morphology measurements still provide superior assay quality.

Conclusion

We have developed and validated novel kinetic morphological measurements to characterize the cell motility assays. These results provide compelling evidence that the innovative kinetic morphology measurements can improve assay quality. Importantly this could provide researchers with more discrimination power to distinguish subtle differences in motility than is currently possible. In our next steps, we will continue to quantify the discrimination power of various motility metrics, including standard model parameters (coefficients of persistence and diffusion), and our own morphology based cell state classification based measurements. We will also come up with new kinetic morphology based motility models using our kinetic measurements.

This work was supported in part by grant number 1R43GM076780-01 from the National Institute of General Medical Sciences, KKG is a Department of Defense Breast Cancer Research Program Postdoctoral Fellow.