Single-Cell Biology

Poh, et al., Single Cell Biol 2015, S1
DOI: 10.4172/2168-9431.S1-001

Letter to the Editor Open Access

Quantitative Image Analysis of Cellular Morphology Using Amnis® ImageStreamX Mark II Imaging Flow Cytometer: A Comparison against Conventional Methods

Stanley Poh[1], Evelyn Png[2] and Louis Tong[1-4]

[1]*Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[2]*Ocular Surface Research Group, Singapore Eye Research Institute, 11 Third Hospital Avenue, Singapore*
[3]*Singapore National Eye Center, 11 Third Hospital Avenue, Singapore*
[4]*Duke-NUS Graduate Medical School, Singapore*

Abstract

Chemotaxis, the directional cell migration guided by chemoattractant gradients, plays essential roles in many physiological processes, such as recruitment of neutrophils to sites of inflammation. Neutrophils detect chemoattractants by G protein-coupled receptors (GPCRs). Chemoattractant stimuli activate multiple signaling pathways to regulate directional migration of neutrophils. Recently, we identified a novel GPCR-mediated PLCβγ/PKCβ/PKD1 signaling axis that regulates cofilin activity through cofilin phosphatase slingshot 2 (SSH2) and remodels actin cytoskeleton during neutrophil chemotaxis. In the future, it will be important to understand how multiple signaling pathways are spatiotemporally regulated to precisely control the rapid remodeling of actin cytoskeleton in the leading front of chemotaxing neutrophils.

Keywords: Imaging; Flow; Cytometry; Fluorescence; Brightfield

To the Editor

Imaging of cell morphology is extremely important and useful in life sciences and biotechnology**.** Study of cellular morphology provides insights into physiological function of cells and pathological changes that may have occurred. One recent type of automated cellular imaging technology, the Amnis, combines the capabilities of microscopy and flow cytometry in a single platform and is able to perform cellular imaging and automatic calculation of quantitative cellular morphological indices [1-3]. The ability to screen and analyse large cell populations in a high-throughput manner is crucial in today's research [4].

To evaluate this imaging technology, we evaluated cellular morphology using Amnis® ImageStreamX Mark II imaging flow cytometer and compared this with experienced human assessment. Here, we validated 2 common imaging parameters, circularity (Figure 1A) and aspect ratio (Figure 1D). Circularity describes how ruffled or irregular the membranes of cells are while aspect ratio describes the overall shape of the cells in terms of how elongated cells are. 500 cells were obtained by pressing a commercially available membrane (Eyeprim, Opa Tech) on the conjunctiva of a human participant for 2-5 seconds. Membranes were then scratched; the resulting cells suspended and washed using a standard protocol. The Amnis imaging was performed as in the instruction manual provided by the manufacturer. In this study, we only evaluated images of the cells in the brightfield channel. 30 duplet cells, 17 poor quality cells and 2 cells which were a combination of both were excluded giving a study population of n=451.

Amnis software computed the two chosen imaging parameters automatically and those values were extracted for analysis. It computes circularity into continuous variables by measuring the radii of the cell from a centroid to the cell membrane in various sectors of the cell. It is a calculated index which was inversely related to the variance of these measured radii. A human assessor, who was blinded to the software's grading, graded the same set of cells into either high or low circularity by visualisation (Figure 1B). To achieve best possible prediction of circularity from automated circularity index, the Receiver Operating Characteristics (ROC) was used to detect the optimal threshold to dichotomise variables into high or low circularity grades (Figure 1C). At this threshold the machine scores achieved a sensitivity of 82.2% and specificity of 85.1% compared to the human assessor trained in visualisation of microscopic cell images (gold standard). Cross-tabulation was performed on these two sets of measurement, Table 1, and moderate concordance was obtained with Cohen's Kappa of 0.594 and Krippendorff's Alpha of 0.592.

The second parameter, aspect ratio, was calculated by Amnis software as the ratio of length of the cell's minor axis to its length along the major axis (Figure 1D). A human observer then assessed these cells in a similar fashion using ImageJ software along 2 perpendicular axes (Figure 1E). A Bland Altman plot was used to analyse this parameter where the difference between the automated and measured aspect ratio values was plotted against the mean of these 2 readings (Figure 1F). The mean of the differences was found to be -0.034 and 2 standard deviation of the differences ranged from -0.176 to 0.108. Hence, for most practical purposes, there was satisfactory agreement between the aspect ratio calculated by machine and the human assessment.

There are several caveats in the interpretation of our study. First, the automated assessment may have underperformed because we utilised a 'difficult' but real experimental setting of impression cytology where cell viability and morphology may have been disturbed to a greater extent than say in a peripheral blood sample. The presence of elongated or duplet-like cells may reduce the validity of automated measurements.

***Corresponding author:** Louis Tong, Singapore National Eye Center, 11 Third Hospital Avenue, Singapore 168751, Singapore, Tel: +65-62277255; Fax: +65-63224599; E-mail: Louis.tong.h.t@snec.com.sg

Received May 30, 2015; **Accepted** June 08, 2015; **Published** June 10, 2015

Citation: Poh S, Png E, Tong L (2015) Quantitative Image Analysis of Cellular Morphology Using Amnis® Imagestreamx Mark II Imaging Flow Cytometer: A Comparison against Conventional Methods. Single Cell Biol S1: 001. doi:10.4172/2168-9431.S1-001

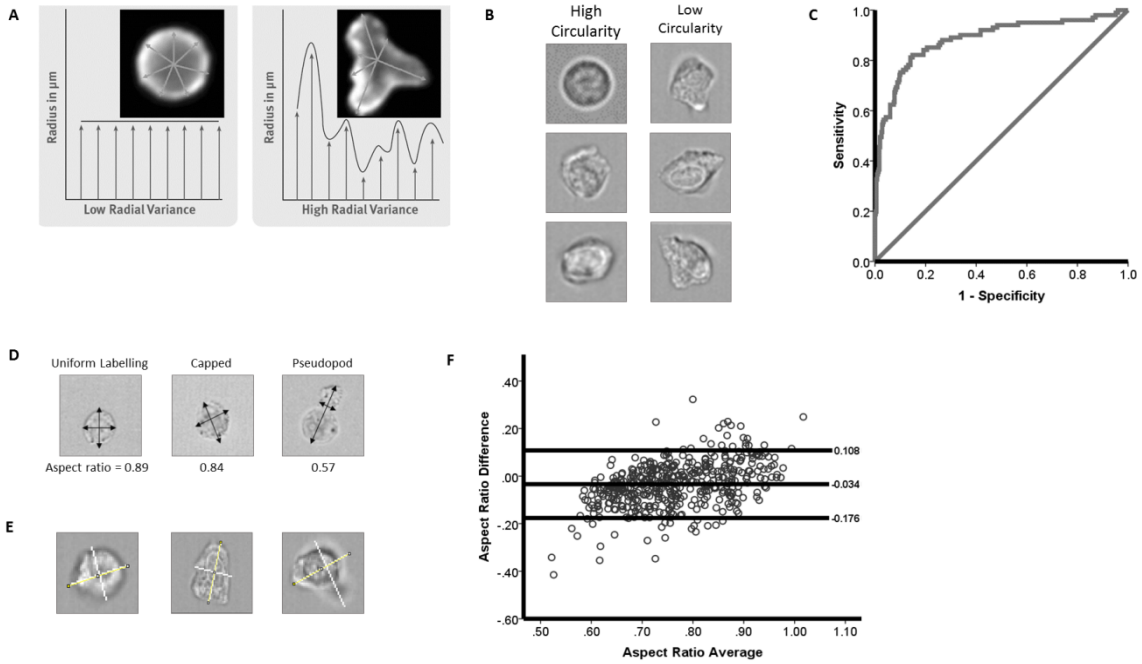

Figure 1: Comparison of automated cellular morphology analysis with human assessment. A specimen of 500 cells derived from conjunctival impression was imaged and two specific imaging parameters, circularity and aspect ratio, were computed automatically by the Amnis platform. (**A)** Circularity describes how ruffled or irregular the membranes of cells were (adapted from www.amnis.com '*Measuring Shape Change in Monocytes*'). This is calculated by measuring the radii of the cell from a centroid to the cell membrane in various sectors of the cell. The circularity is a calculated index which was inversely related to the variance of these measured radii. Examples of a cell with low radial variance hence high circularity score is shown on the left and the converse, on the right. (**B)** shows, on subjective assessment of the brightfield images by human grading, examples of cells with high (left column) and low (right column) circularity. (**C)** To achieve the best possible prediction of circularity (human assessment) from automated circularity index, the Receiver Operating Characteristics was analysed (jagged line: area under curve=0.884; 95% confidence interval=0.841 – 0.927). (**D)** The 'Aspect ratio' is a parameter that describes how elongated cells are (adapted from Poh CT et al. '*Quantitative Analysis of Pseudopod Formation with the ImageStream Cell Imaging System*'). This is computed by the Amnis system as the ratio of length of the cell's minor axis to its length along the major axis. (**E)** The same type of imaging analysis as **D** was performed by a human assessor using ImageJ along 2 perpendicular axes. (**F**) Bland Altman plot is shown, comparing the agreement between the automated 'aspect ratio' and the assessment by human measurement. The difference between the automated and measured values was plotted against the mean of these 2 readings.

We have already excluded such cells from the analysis, so this issue may not be so significant. Second, the assessment may show even greater agreement had we used immunofluorescent images from the Amnis, with staining of specific cell membrane proteins to delineate the outline of cells.

Further refinement of the automated algorithms in the image analysis may improve performance. We were unable to ascertain the number of radial measurements employed by the Amnis to ascertain circularity. Increasing the number of radii may increase the accuracy of this index.

In conclusion, the degree of concordance between machine and human evaluation for circularity is moderate and is high for aspect ratio. These two cellular indices may be applied to screening cell morphology in a high-throughput manner in experiments.

References

1. Basiji DA, Ortyn WE, Liang L, Venkatachalam V and Morrissey P (2007) Cellular image analysis and imaging by flow cytometry. Clin Lab Med 27(3): 653-670.
2. Henery S, George T, Hall B, Basiji D, Ortyn W, et al. (2008) Quantitative image based apoptotic index measurement using multispectral imaging flow cytometry: a comparison with standard photometric methods. Apoptosis 13(8): 1054-1063.
3. Ortyn WE, Hall BE, George TC, Frost K, Basiji DA, et al. (2006) Sensitivity measurement and compensation in spectral imaging. Cytometry A : the journal of the International Society for Analytical Cytology 69(8): 852-862.
4. Hui L (2013) Mass Spectrometry for Single Cell Imaging. Single Cell Biol 2: e118.

This article was originally published in a special issue, **Progress in Single Cell Technology** handled by Editor(s). Dr. Yusuf Tutar, Cumhuriyet University, NJ, Turkey