Validation of Neurite Outgrowth Measures in the IN Cell Analyzer 1000

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Abstract and Introduction

Fully automated quantification of neurite outgrowth is a difficult analysis task and requires validation. Comparison with manual scoring is the preferred method for validating automated quantification in most complex structural assays, including neurites.

We validated automated analyses of neurite outgrowth performed using the IN Cell Analyzer 1000 by comparison with manual scoring. On five parameters (total cell area and number, neurite length and number, neurite length per cell, and across six treatment conditions (retinoic acid), manual and machine scoring correlate highly (r > 0.95) and reflect the same biology. The machine took less than ½ hour to obtain data that required weeks of effort for two human scorers.

Methods

Cells

Neurite outgrowth

Neuro 2a cells plated for 8 hrs in 96 well plates

Retinoic acid treatment: 3 days

Figure 1. Cells treatment and labeling

Acquisition

Images were acquired at 1392 x 1040 pixels, 12 bit precision, 440 msec exposure. Total acquisition time (move/focus/acquire) was about 3 min for an entire 96 well plate. Both epifluorescence (4X and 10X objectives) and DIC (10X) optics were used.

Manual scoring

Two skilled technicians trained/neurites and cell bodies using MCID™ Elite image analysis software. A minimum length parameter was combined with visual evaluation to distinguish neurites from other structures.

Automated Scoring

The machine was given a set of scoring parameters (minimum and maximum neurite width, minimum neurite length, mean cell size per condition), defined prior to the analysis. The measurements required for the predefined process took about an hour to obtain.

Results With 10X Fluorescence

Scoring Reliability

Manual scoring was consistent. Both the inter-rater (same data, two raters) and intra-rater (same rater, data scored twice) reliability scores were > 0.98 (Pearson r). Machine resorces of the same data were identical.

• IN Cell Analyzer 1000 is validated with fluorescence imaging at a 10X objective power (toxic) high levels of measurement precision. IN Cell Analyzer 1000 is validated with fluorescence imaging at a 10X objective power (toxic) high levels of measurement precision.

• Automated system observed the same effects of treatment, and had the same measurement precision as human scorers.

• This poster was presented at the 8th Annual Conference of the Society of Biomolecular Screening, 08-10 September 2002. The images were created using ImageJ software.

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CONCLUSION

• IN Cell Analyzer 1000 is validated with fluorescence imaging at a 10X objective power.

• Performance remains acceptable at 4X.

• System also performs well at 10X with unstained specimens (DIC).

• Throughput of the automated system is orders of magnitude higher than with human scoring.

• Automated system observed the same effects of treatment, and had the same measurement precision as human scorers.

References