#P12024 - Quality Measures for Imaging-based Cellular Assays

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Abstract

Z-factor and related measures are useful in estimating assay variability in HTS caused by assay biology and by instrumentation. Imaging-based cellular assays introduce several new sources of variability: imaging resolution and other image acquisition parameters, size of the imaged area, image analysis algorithm and its parameters. The algorithms that derive assay measures from images may be complex and may saturate the values from the positive and negative states of the assay, thus artificially reducing variability. We propose a new quality measure, v-factor, which generalizes z-factor for a dose-dependent sequence of assay states. It gives a more realistic measure of the overall assay performance by accounting for intermediate points in the dose curve, which have higher variability due to effects of computation and of dispensing errors. The use of v-factor as a quality measure allows comparing algorithms and rationally determining imaging resolution and size requirements.

Introduction

In cellular imaging assays, the measure (or measures) used to characterize the assay is far removed from the signal registered by the camera. Different algorithms will produce different assay measures on the same image. This is especially acute for redistribution assays where the total intensity may not change and the assay result may depend more on the algorithm than on the raw image.

In high throughput drug screening it is common to evaluate the quality of assays by a statistical parameter that depends on the dynamic range and variability of the assay. Several such parameters have been introduced with z-factor being the most popular. For cell-based assays, z-factor above 0.5 is considered good. This type of measures proved to be very useful to capture and compare variability caused by assay biology and by instrumentation (e.g., pipetting). Cell assays based on imaging introduce several new variables: imaging resolution, size of the imaged area and the data extraction algorithm.

In addition to introducing new variables, cellular imaging assays may lead us to reconsider the quality measure itself. An assay measure derived from an image may be computationally very complex. It may contain operations that have the effect of saturating the values from the positive and negative states of the assay, thus artificially reducing variability. This may happen unintentionally and even without being realized. One way of dealing with this is the use in the quality measure of a dose-dependent sequence of assay states (dose-curve) with doses being close enough to each other, so that artificial manipulation would be impossible. We introduce such a measure - v-factor, which is the generalization of z-factor to the dose curve. The v-factor reverts to z-factor if there are only two dose points.

The v-factor is less susceptible to saturation artifacts caused by computation than z-value. There is also another subtle difference. Standard deviation in the middle of the dose-response curve is often larger than the standard deviation at the extremes even for non-imaging assays. This is because the maximal point on the curve is often determined at saturating concentration, and so any dispensing error has little effect on the response; the minimal point is usually zero concentration and it also avoids dispensing errors. In contrast, the effect of volume errors has its maximal effect in the middle of the dose-response curve. Taking the whole curve into account gives a more realistic measure of the assay data quality.

Variability in cellular imaging assays

Traditional sources of variability in screening:

- Assay biology,
- Equipment,
- Operator

Additional sources of variability in cell imaging:

- Resolution (magnification),
- Image size (number of cells)
- Data extraction algorithm

Methodology of the study:

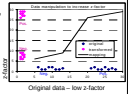
- Vary optical or interpolated magnification from 20X to 1X
- Subdivide images into fragments of decreasing size
- Compare different algorithms/measures
- Study **quality measure** as a function of magnification, size, and algorithm

Assay examples:

- Proliferation (Mitotic Index)
- Receptor Internalization (Transfluor)
- Nuclear Translocation

V-factor: generalization of Z-factor

(1) $$Z = 1 - 3\left(\frac{SD_{pos} + SD_{neg}}{\left|M_{pos} - M_{neg}\right|}\right)$$

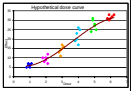


If the values of the assay for the positive and negative states do not overlap (and if they do it is not a very useful assay), the z-factor can be manipulated intentionally, by applying a mathematical transformation that moves all positive values into a single number and all negative values into another single value.

Transformed data – high

Original data – low z-factor

(2) $$V = 1 - 6\left(\frac{SD_{pos} + SD_{neg}}{\left|M_{pos} - M_{neg}\right|}\right)$$

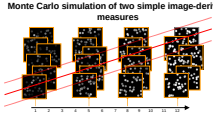


(3) $$SD_{M\_fit} = \sqrt{\frac{SS}{n}\left(J_{model}^{T} J_{experiment}\right)^{-1}}$$

(4) $$V = 1 - 6\left(\frac{Average\_SD}{\left|M_{pos} - M_{neg}\right|}\right)$$

(Alternative definition without a model)

Monte Carlo simulation of two simple image-derived measures



Circles ("cells") uniformly distributed in an image (40 "cells")

Intensities of circles normally distributed $N(m_i, s)$ (s = 17; intensity range 0-255)

Average intensities increase linearly with "dose" ($m_i = a + d^*i$, i=1,...12; a=20, d=7)

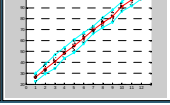
A number of replicas (images) at each "dose" (15 replicas)

At every "dose" point for each replica image two measures are calculated:

1. Population Average of average Cell Intensity (ACI)
2. % of Cells with intensity > Threshold (PCT)

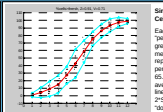
Z- and V- factors are calculated using formulas (1) and (4)

(ACI, PCT,)



Simulation of Average Intensity measure

Each black dot represents the population average of "cell" average intensity in one image, 15 replica images were generated per "dose" point. The red line represents averages of replicas. The cyan lines represent average +-2*SD of replicas within each "dose".

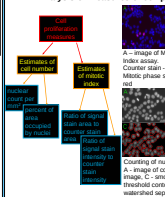


Simulation of "Percent of Cells > Threshold" measure

Each black dot represents the "percent of cells with intensity greater than threshold" measure in one image, 15 replica images were generated per "dose" point. Threshold = 65. The red line represents averages of replicas. The cyan lines represent average +- 2*SD of replicas within each "dose".

Conclusion from simulations: Even simple image-derived measures may behave differently from familiar whole-well measures

Analysis of measures of cell proliferation



A – image of Mitotic Index assay. Counter stain - blue. Mitotic phase stain - red

B – adaptive threshold contours. For the counter stain - red, for the signal stain - green.

Counting of nuclei:
A - image of counter stain, B - smoothed image, C - smoothed image with adaptive threshold contours, D – contours with watershed separation lines inside.

Dose curves for cell proliferation measures.

Response of HCT116 cells to Paclitaxel at different concentrations.

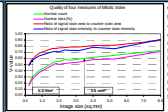
A: nuclear count, B: nuclear area, C: ratio of signal stain area to counter stain area, D: ratio of signal stain intensity to counter stain intensity.

Dots are values from fragment images of 0.4mm² at 2X magnification.

Middle line - average, top and bottom lines - average +/- 3*SD.

Quality of cell proliferation measures

V-factors of four measures of cell proliferation as a function of magnification and image size.

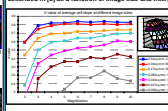


Quality of cell proliferation measures

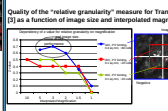
V-factors of four measures of cell proliferation at magnification 2X as a function of image size. The image size at which v-factors reach acceptable range for the ratiometric measures may be an order of magnitude smaller, than for the raw measures.

Analysis of other algorithms

Quality of the "slope" measure of the nuclear translocation algorithm described in [3] as a function of image size and interpolated magnification.



Quality of the "relative granularity" measure for Transfluor assay described in [3] as a function of image size and interpolated magnification.



Conclusion

- Imaging-based cellular assays have new computational properties compared to whole-well assays and their assessment calls for new quality measures.
- V-factor is less susceptible to computational artifacts than z-factor.
- V-factor is more sensitive to dispersing errors, which are larger in the middle of the dose curve.
- V-factor gives a more realistic measure of assay performance where it affects the derivative values (e.g., ED50) the most.
- V-factor can be used to compare different image analysis algorithms/measures.
- V-factor can be used to determine image resolution requirements.
- V-factor can be used to determine image size/cell number requirements.
- The high-resolution imaging community may benefit from a common library of normative assay images for comparing different algorithms.

References

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2. S. Murphy, S.J. Capper, S.M. Hancock, E. Adie, E.P. Roquemore, M. Price-Jones, S. Game and S. Swinburne "Is Z' Factor the Best Assessment for the Quality of Cellular Assays Delivering Higher Content?", *SBS poster 2003*

3. I. Ravkin, V. Ternov, A.D. Nelson, M.A. Zarowitz, M. Hoopes, Y. Varhovsky, G. Ascue, S. Goldburt, O. Beske, B. Bhagwat, H. Marciniak "Multiplexed high-throughput image cytometry using encoded carriers", *Proc. SPIE Vol. 5322, pp. 52-63, 2004 (Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues II; D.V. Nicolau, J. Enderlein, R.C. Leif, D.L. Farkas; Eds.)*