Abstract

#P12024 - Quality Measures for Imaging-based Cellular Assays

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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.

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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:

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

Motivation for change:		*Effect:*
Throughput	→	lower resolution
Miniaturization	→	smaller areas, fewer cells
Better information	→	new algorithms

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:

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

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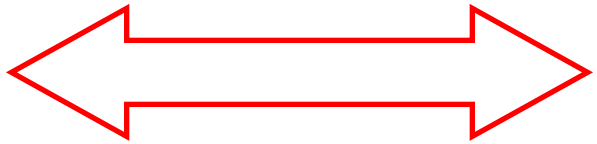
Quality assessment for cellular imaging assays now

Compare to visual assessment by a human



Use existing HTS quality measures

- *Very laborious*
- *Hard to quantify*
- *Subjective*

May not capture specific effects introduced by image analysis

Desired algorithm:

Sensitive to the variable of interest (e.g., concentration), but insensitive to all other variables (e.g., artifacts)

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Generalization of Z-factor

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

-inf 0 0.5 1

If the values of the assay for its 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 maps all positive values into a single value and all negative values into another single value.

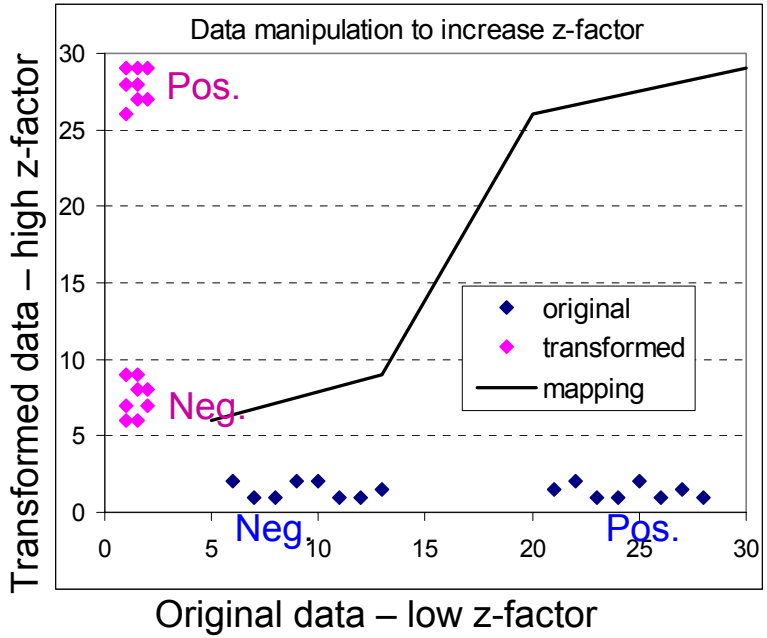
(2) $$V = 1 - 6\left(\frac{SD_{of\_fit}}{\left|M_{pos} - M_{neg}\right|}\right)$$

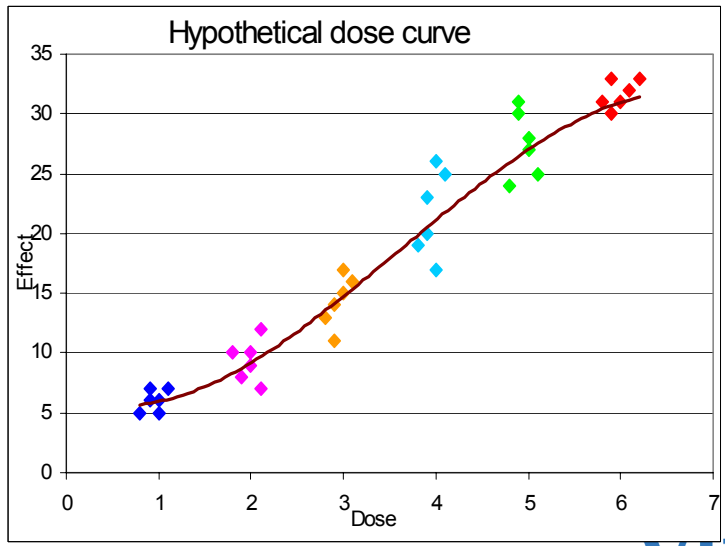
-inf 0 0.5 1

(3) $$SD_{of\_fit} = \sqrt{\frac{1}{n}\sum_{1}^{n}\left(f_{\text{model}} - f_{\text{experiment}}\right)^2}$$

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

(Alternative definition without a model)





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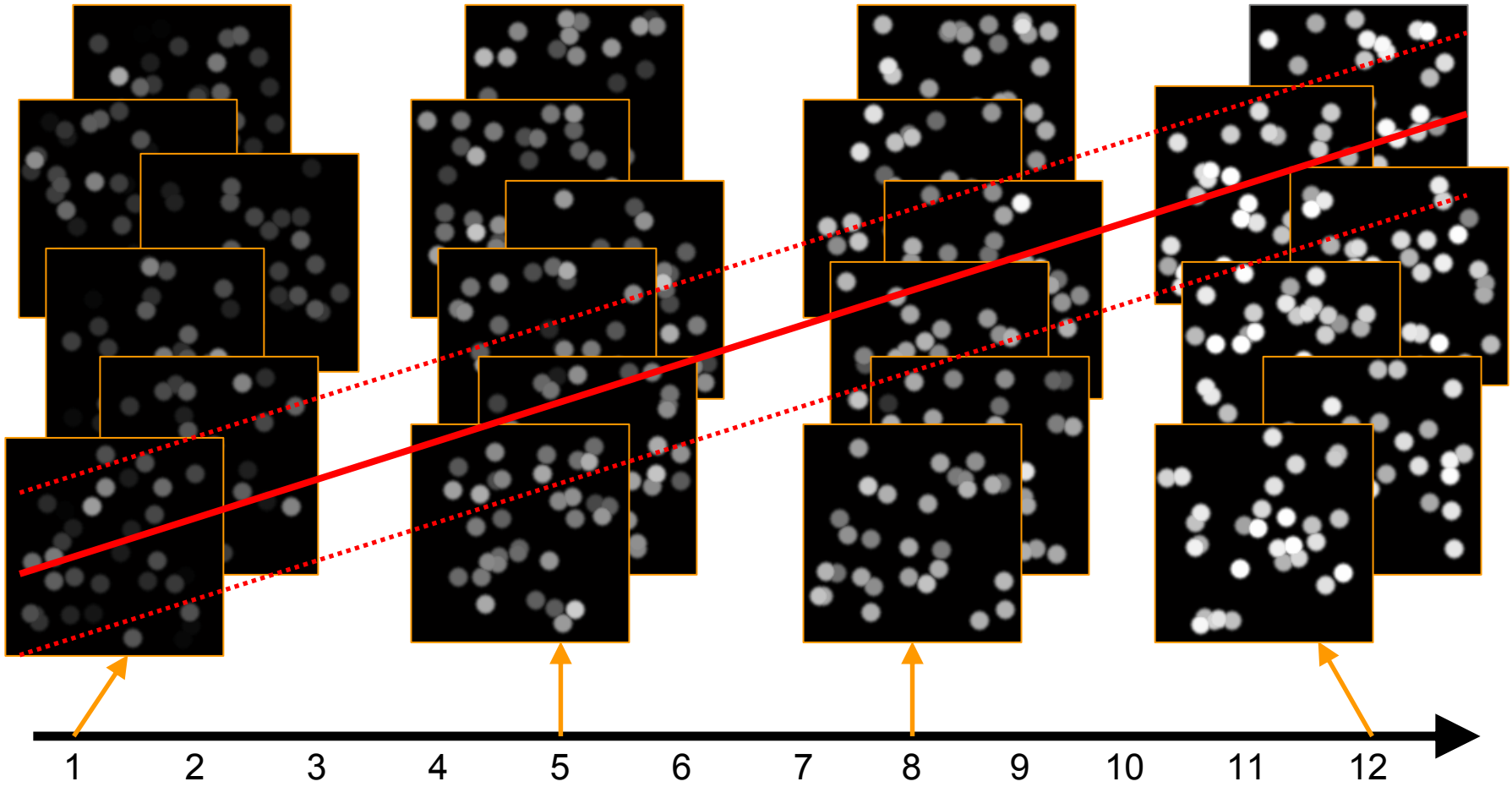
Monte Carlo simulation of dose-response and of two image-derived measures



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 increasing linearly with “dose”	$m_i = a + d*i$, i=1,…12; a=20, d=7
A number of replicas at each “dose”	15 replicas (images)

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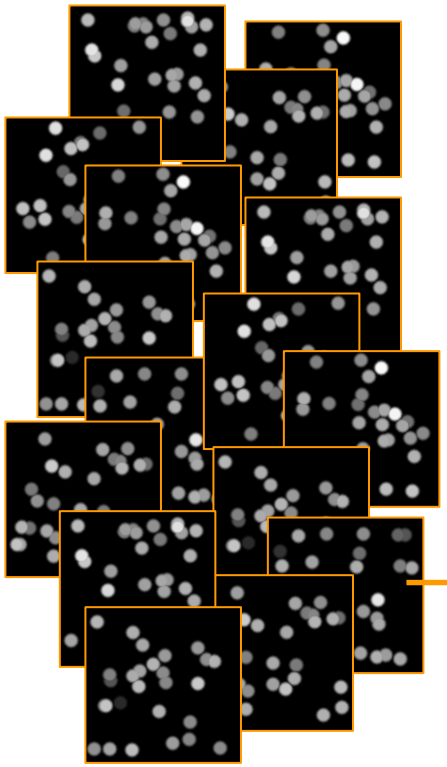
Calculations for Monte Carlo simulation



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)

These values are plotted and Z and V factors are calculated using formulas (1) and (4) .

→ (ACI_j, PCT_j)

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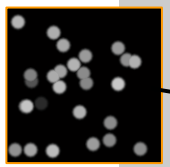
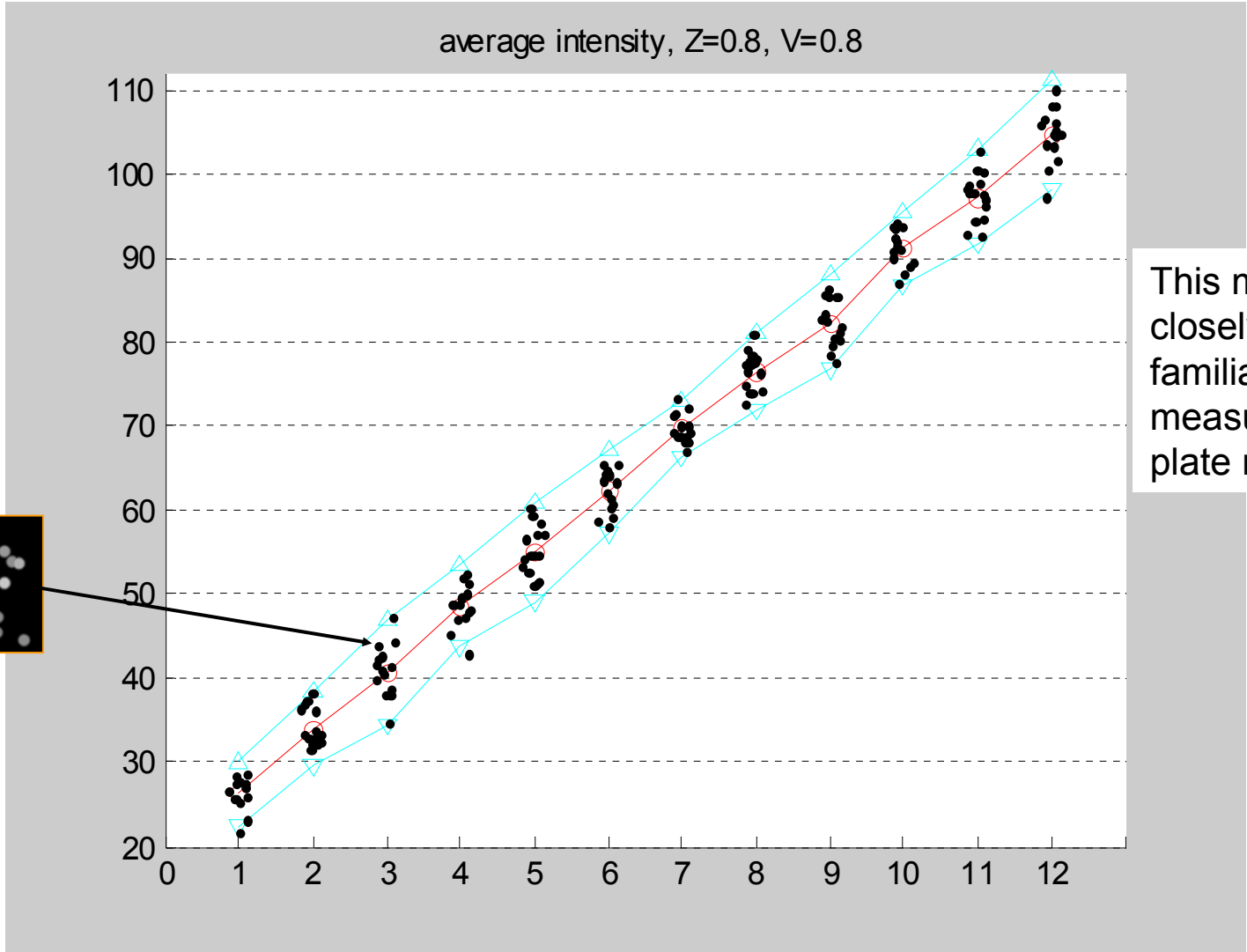
Simulation of “Average Intensity” measure





This measure most closely resembles familiar whole-well measurements of plate readers

Each black dot represents the population average of “average cell 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”.

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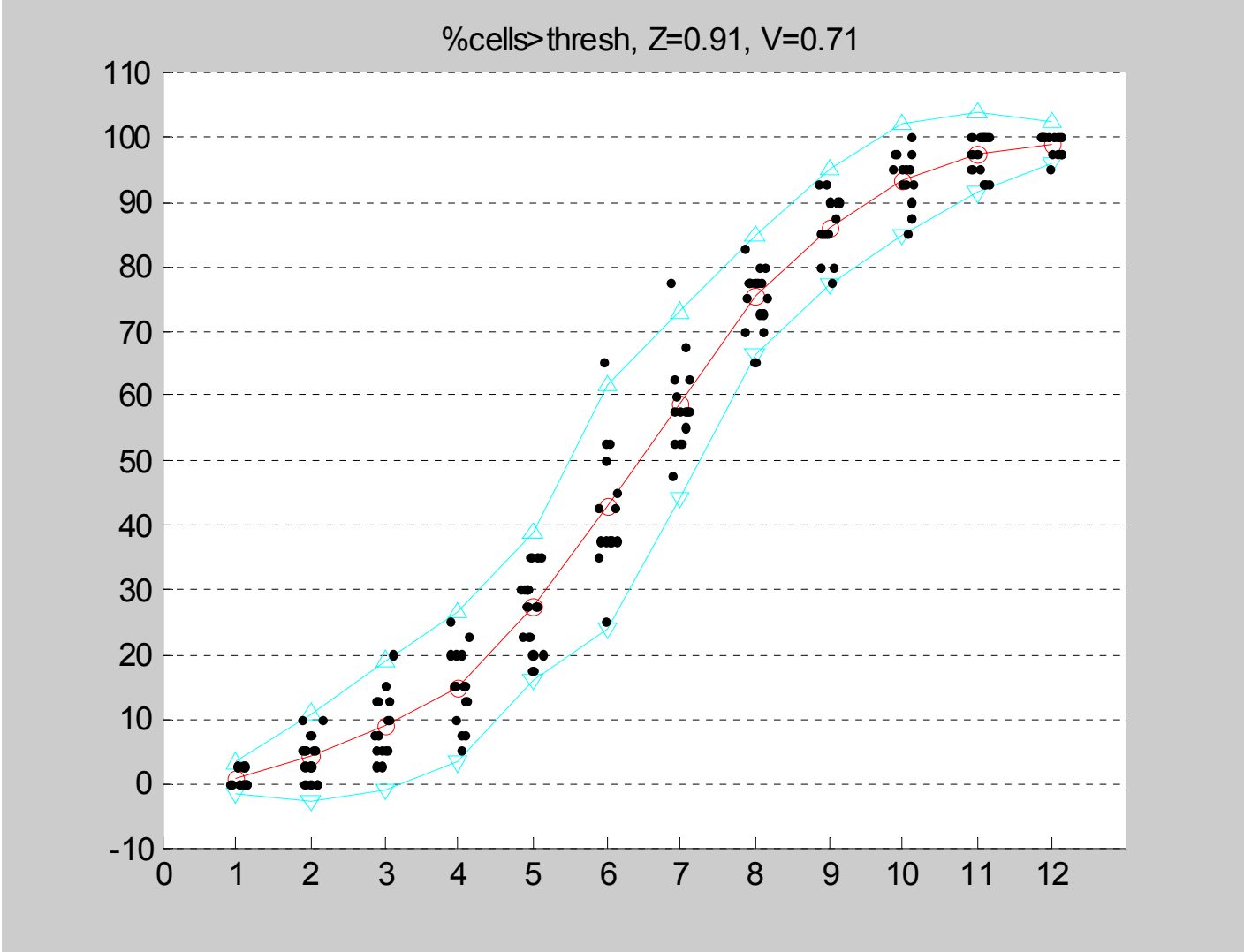
Simulation of “%Cells>Thresh” measure



Each black dot represents the “% 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”.

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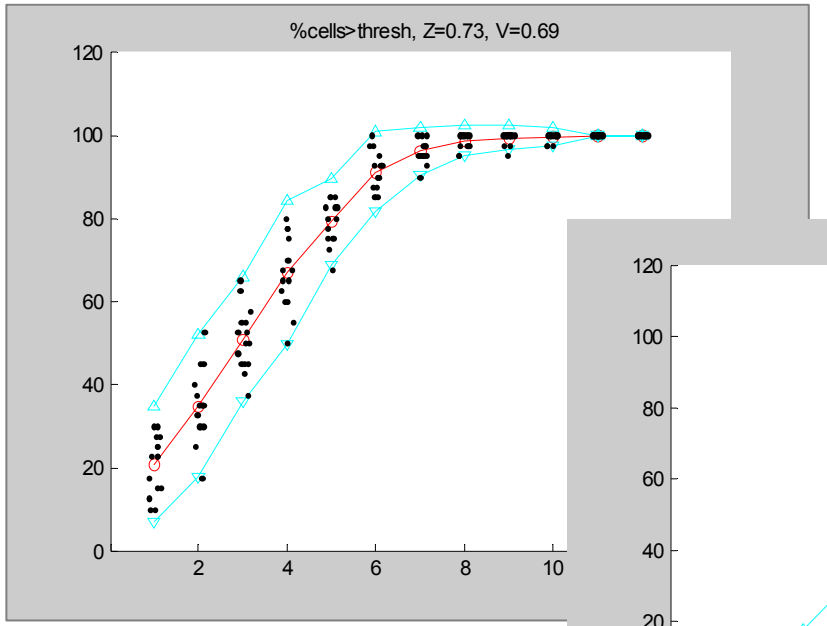
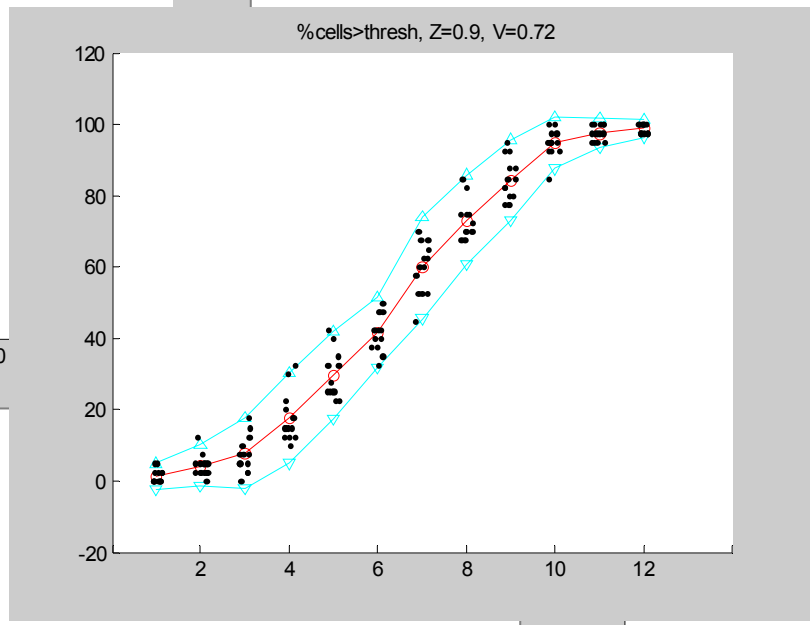
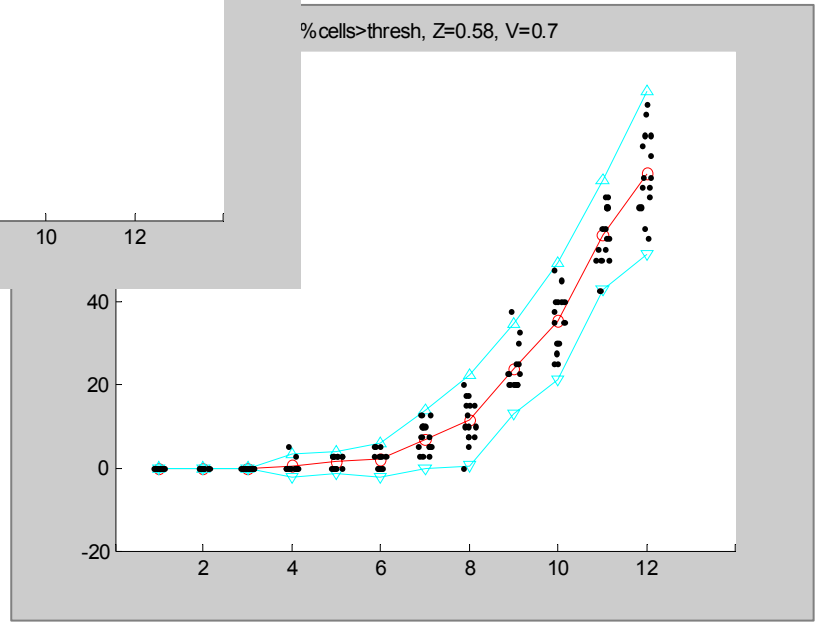
Simulation of “%Cells>Thresh” at different thresholds



Threshold = 40



Threshold = 65



Threshold = 95

Conclusion from simulations:

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

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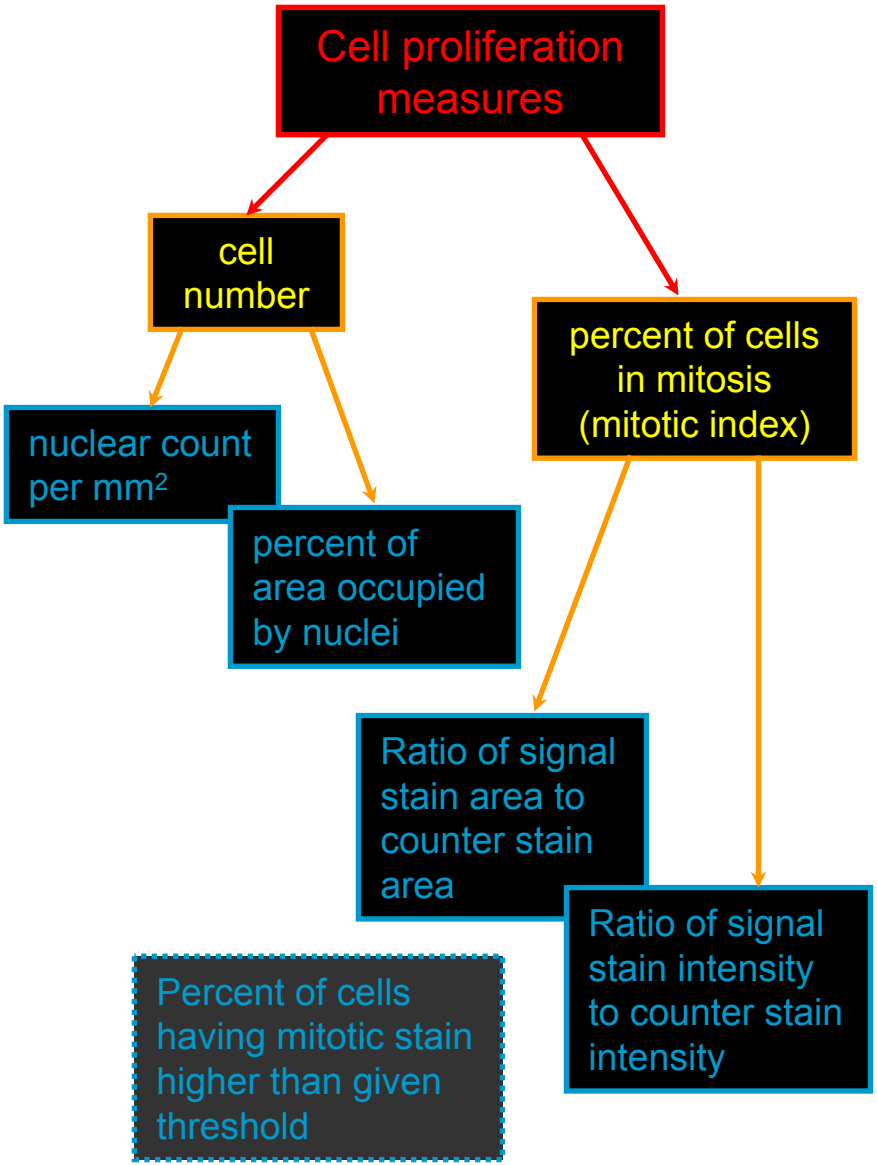
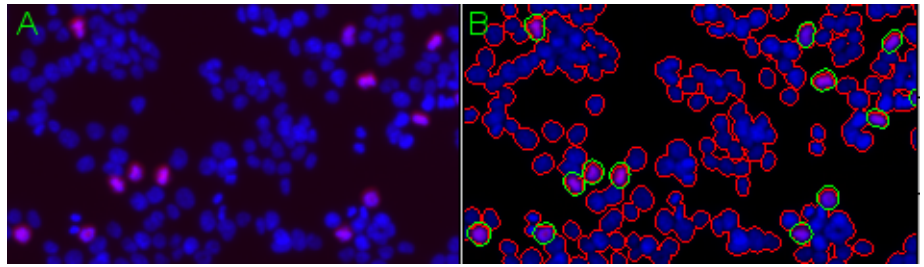
What you can do with a quality measure for an imaging assay

- Compare algorithms
- Estimate required scan areas (number of cells)
- Determine minimal imaging resolution

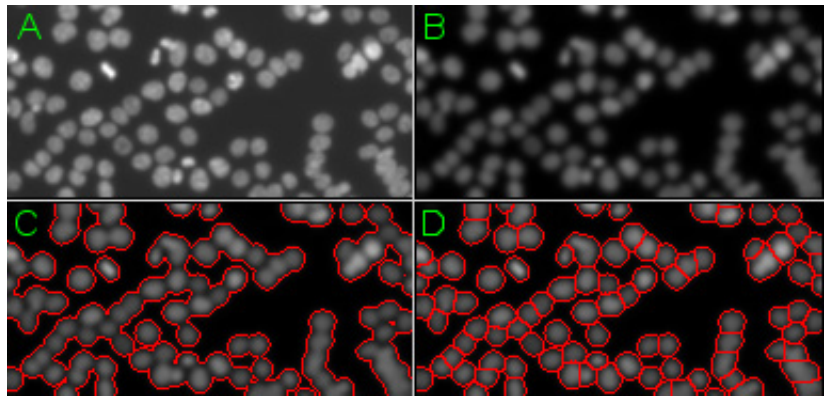
Measures of cell proliferation





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.

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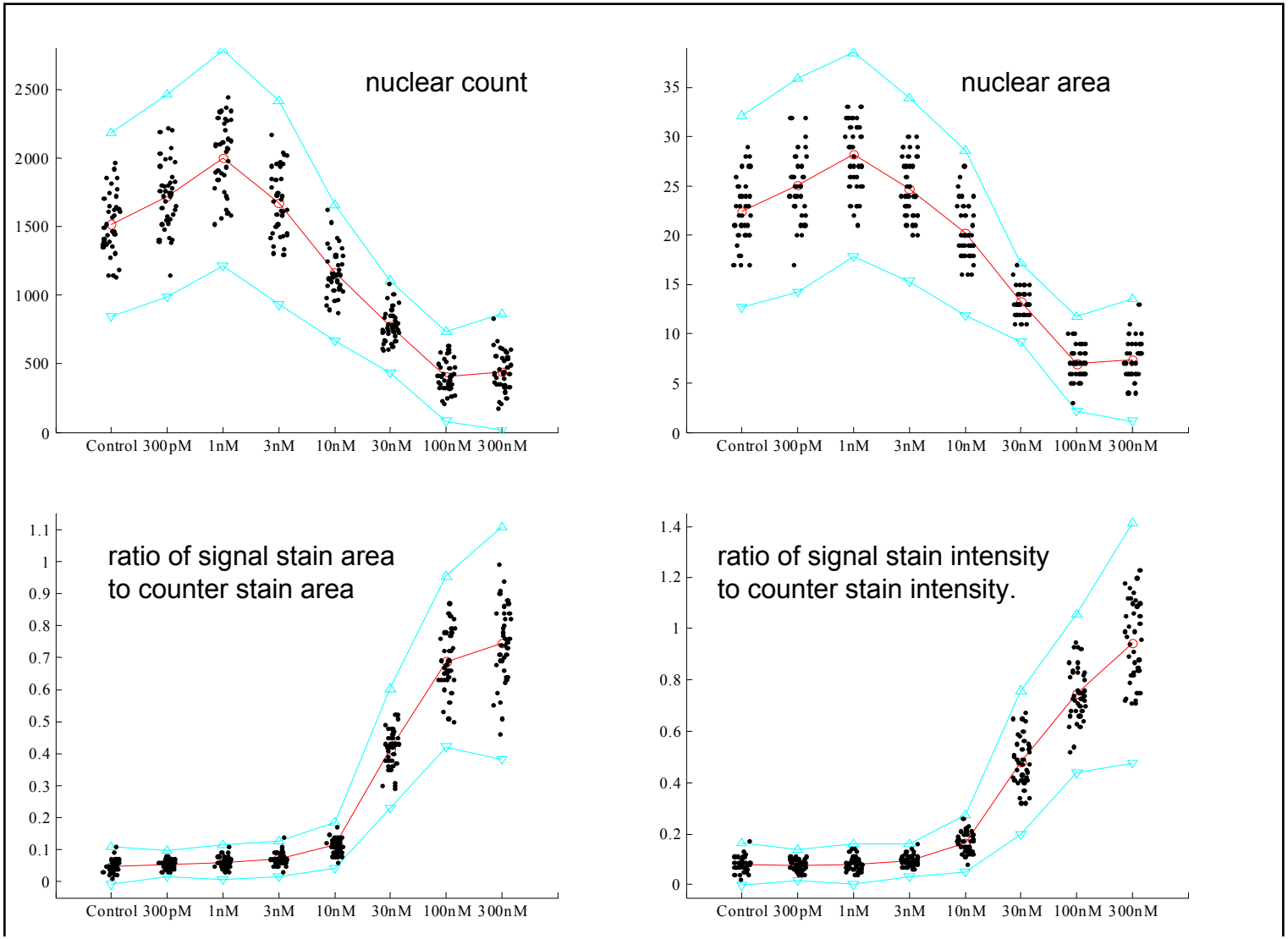
Dose curves for cell proliferation measures



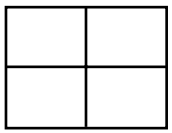
Response of HCT116 cells to Paclitaxel at different concentrations.
Dots are values from fragment images of 0.4mm^2 at 2X magnification.
Middle line - average, top and bottom lines - average +/- 3*SD.

Quality of cell proliferation measures - 1

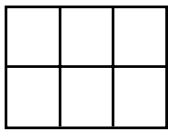
One camera frame at 10X: 1.4 mm²

0.70 mm²

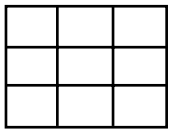
0.47 mm²



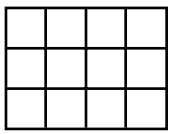
0.35 mm²



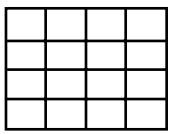
0.23 mm²



0.16 mm²

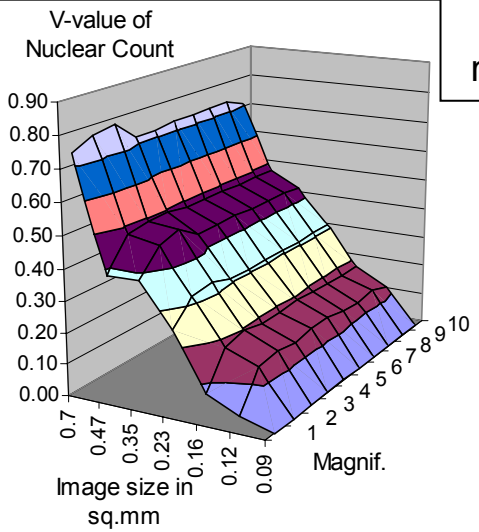


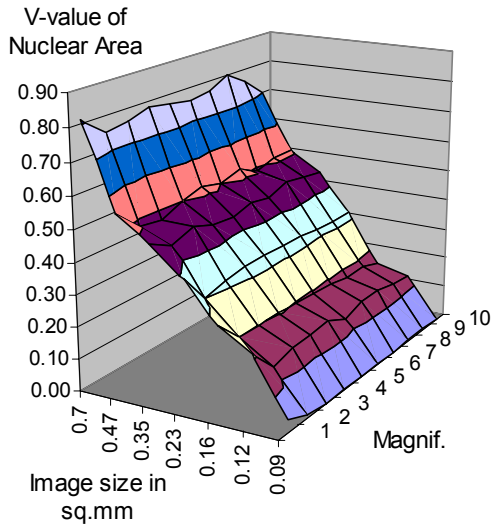
0.12 mm²



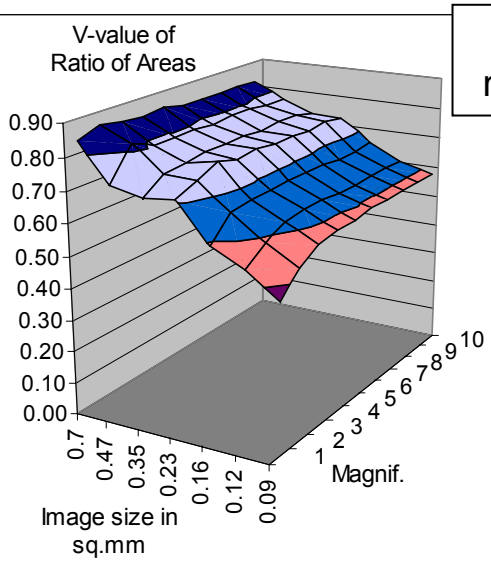
0.09 mm²

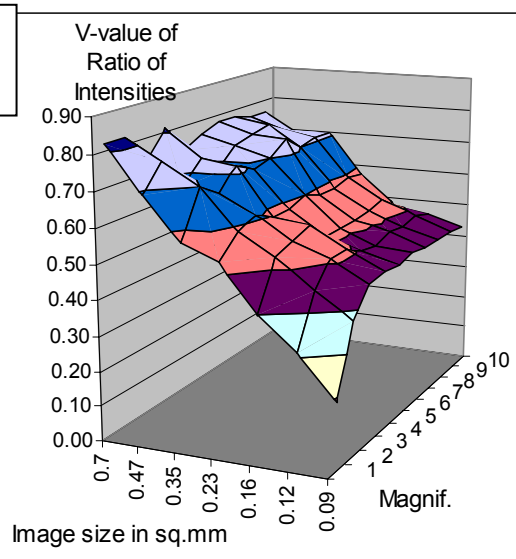
Absolute measures





Ratio measures





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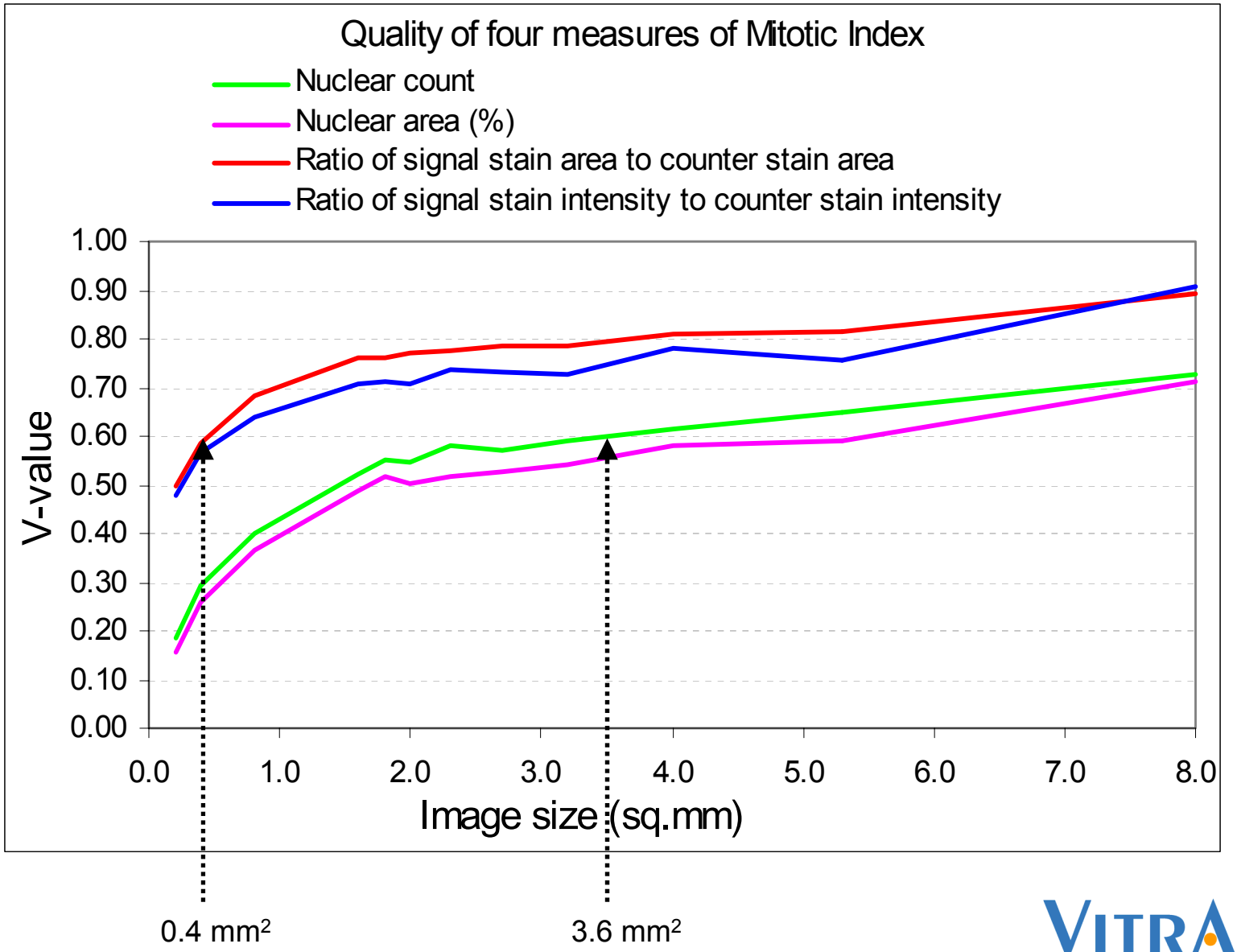
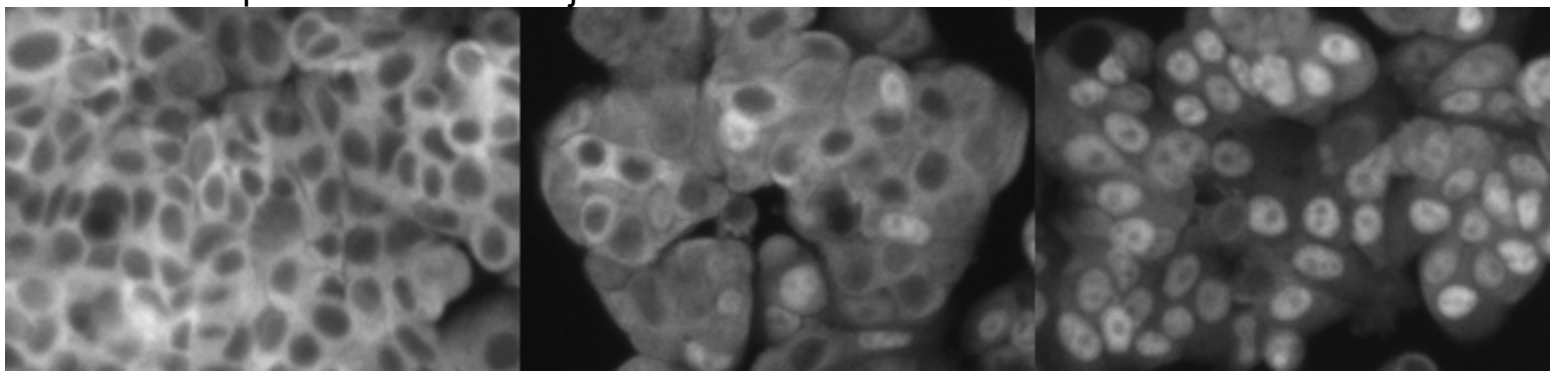
Quality of cell proliferation measures - 2

V-values of four measures of cell proliferation at magnification 2X as a function of image size.



Nuclear translocation assay

Translocation of transcription factor NFkB in MCF7 cells in response to TNFa.
FITC stain acquired with a 10X objective



Negative – bright staining in the cytoplasm

Intermediate

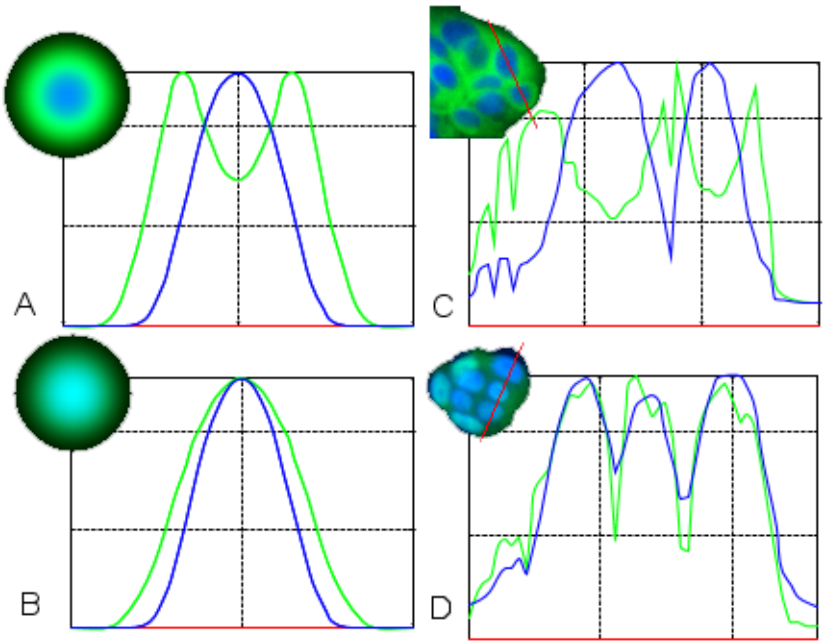
Positive – bright staining in the nucleus

Images and profiles through model and real cells.

A,B – model; C,D – real,

A,C – negative, B,D – positive.

Blue – counter stain, green – signal stain.



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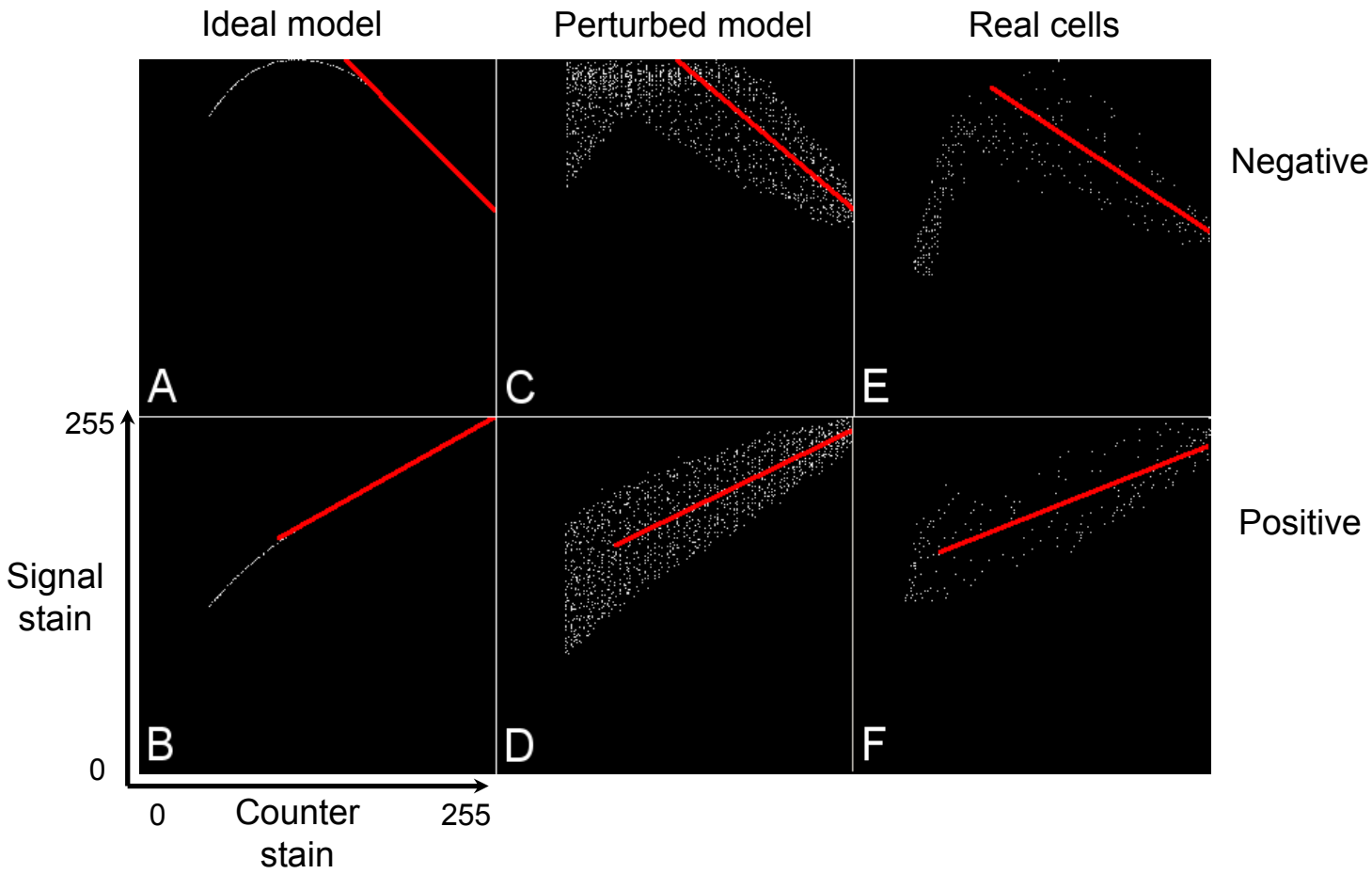
Model of signal and counter stain distribution in nuclear translocation assay



Translocation measure - slope of a straight-line segment approximating the right side of the cross-histogram

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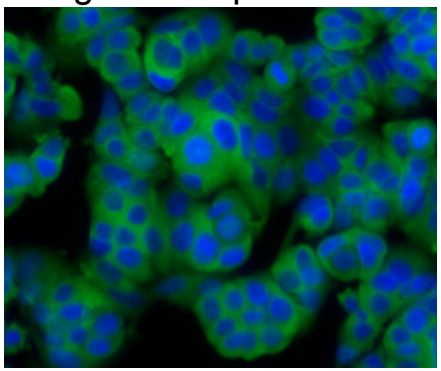
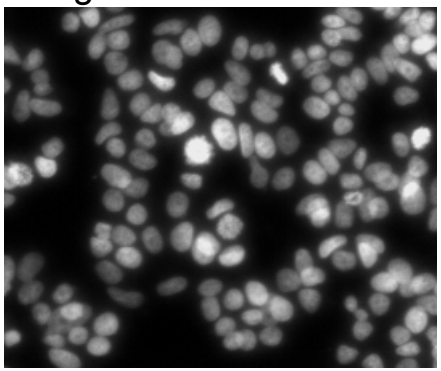
Cell-by-cell intensity normalization

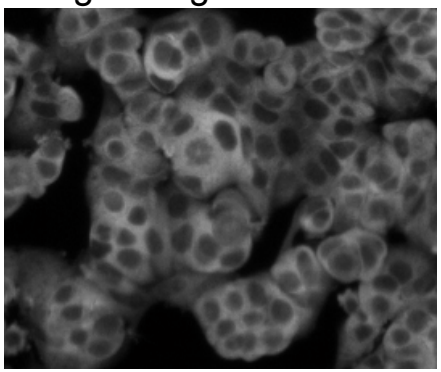
Original composite



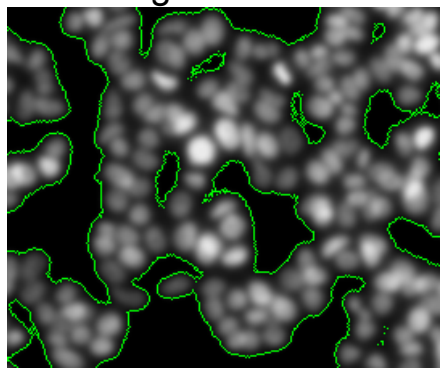
Original counter stain



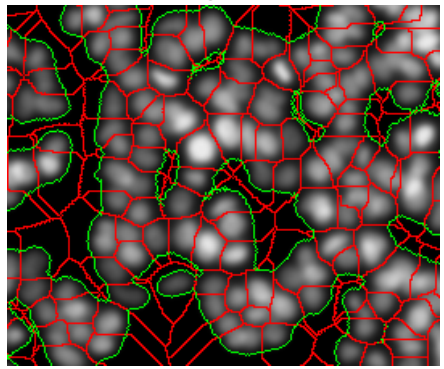
Original signal stain



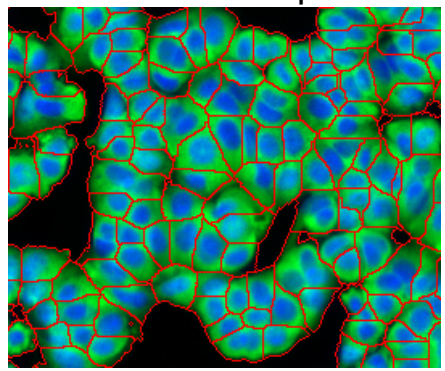
Adaptive contours separate areas of counter stain from the background



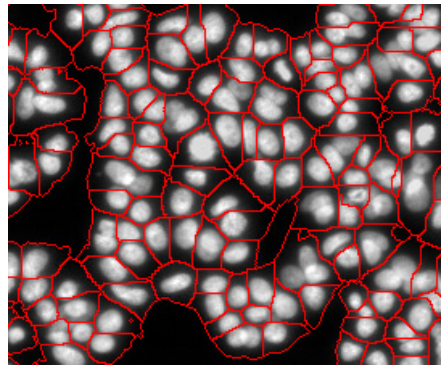
Cell separation lines are watershed of inverted smoothed counter stain above background



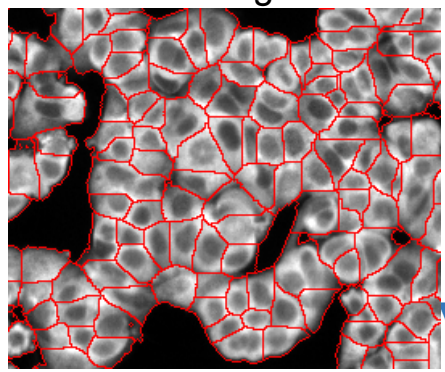
Normalized composite



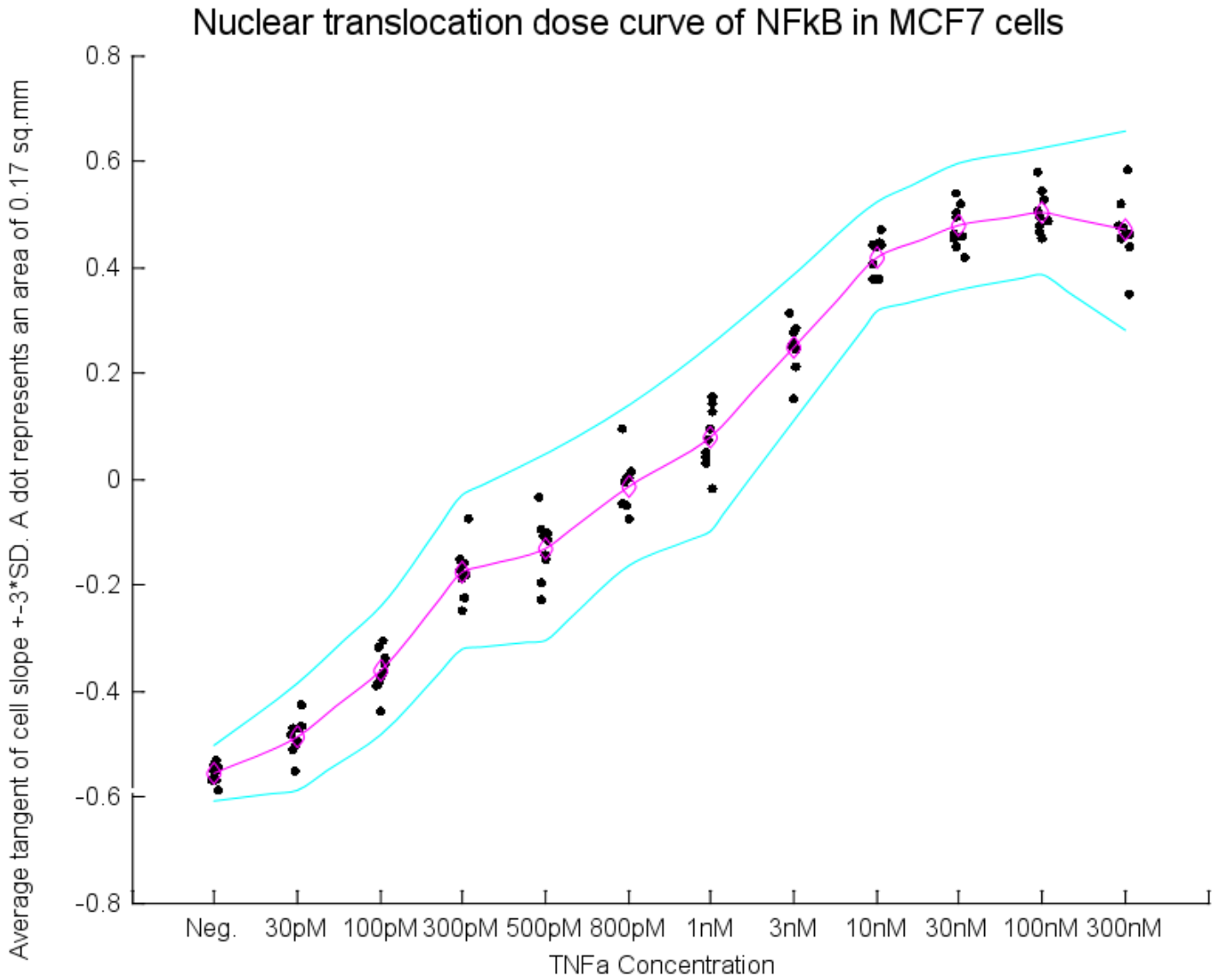
Normalized counter stain



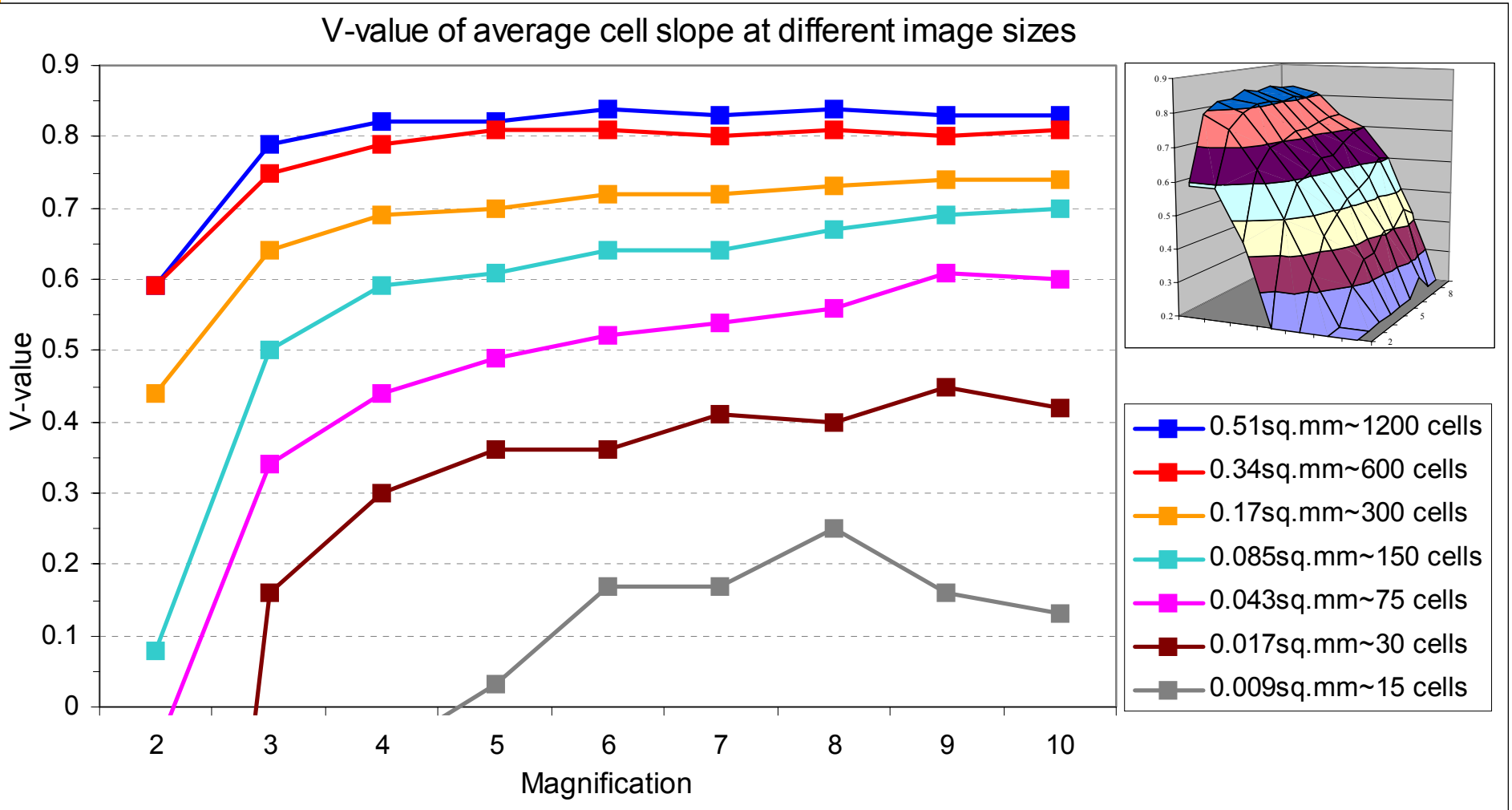
Normalized signal stain

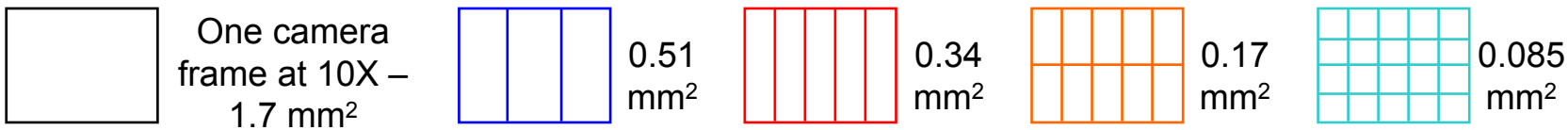


Nuclear translocation dose curve



Quality measures for nuclear translocation assay





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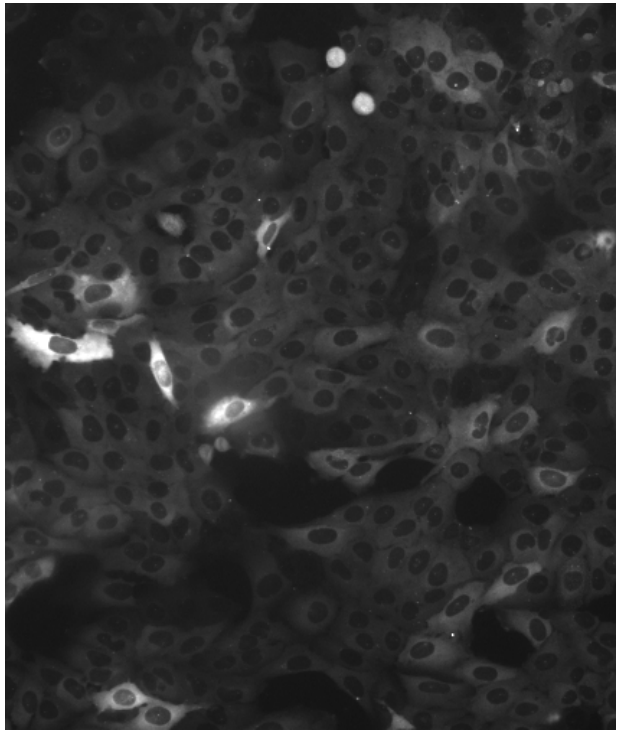
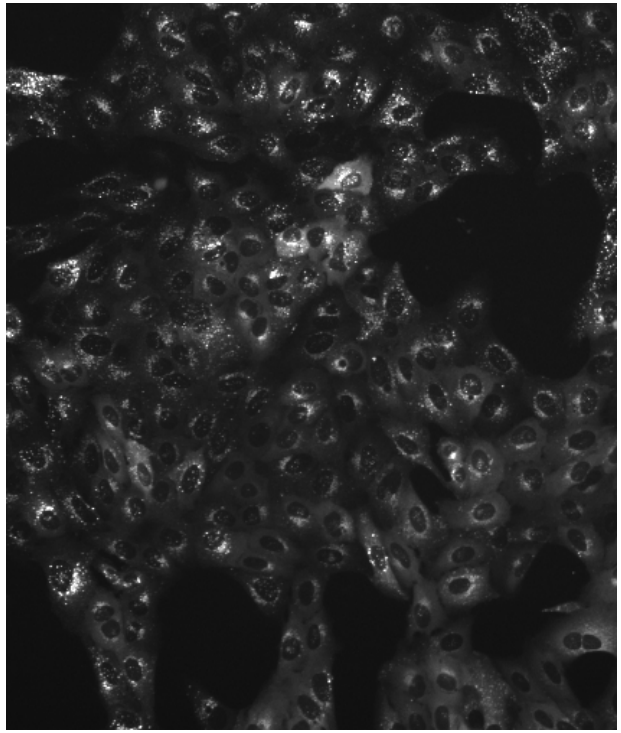
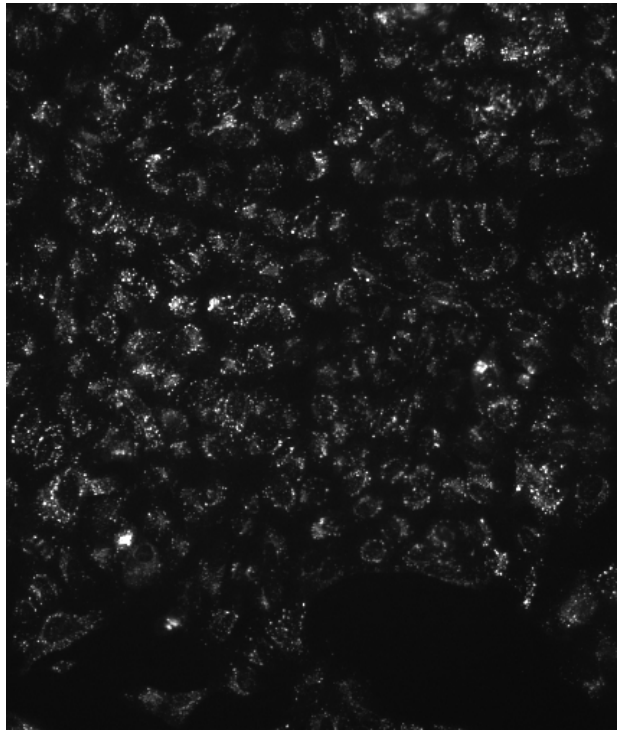
Receptor internalization (Transfluor) assay



Negative



Intermediate



Positive

Activity of G-protein coupled receptors (GPCR) is assessed by analyzing subcellular localization of GFP fused to β-arrestin. Receptor internalization causes staining to change from diffuse to granular. Images taken with 10X objective and 2*2 binning.

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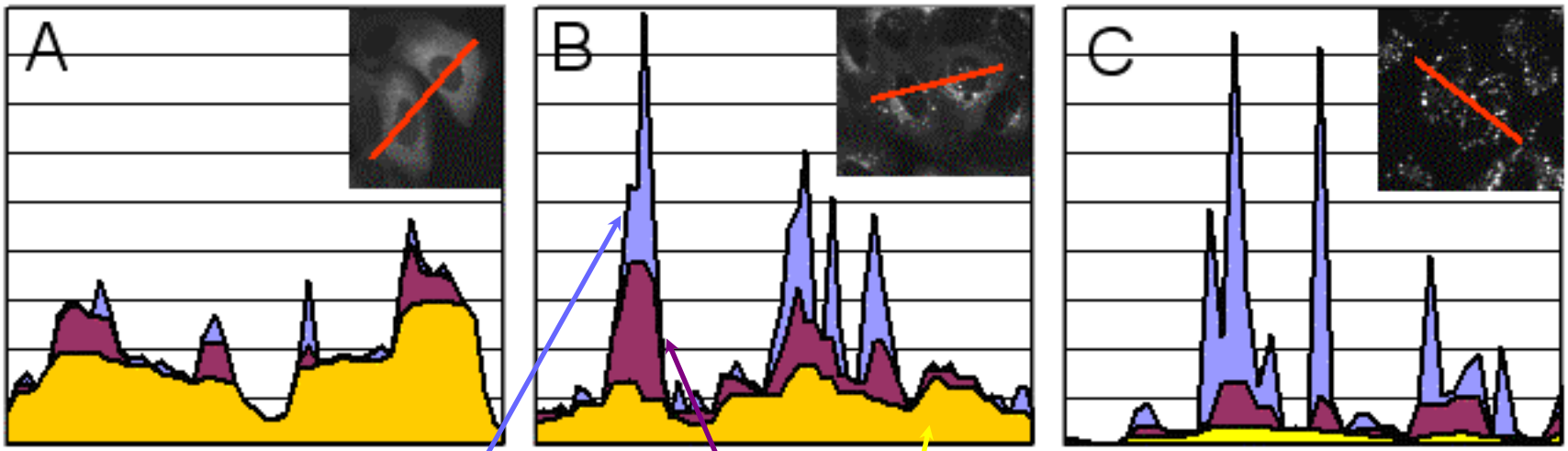
Analysis of granularity in Transfluor assay

Negative Intermediate Positive



Brightness profiles through cells:

in the original image (blue),

in the image opened by structuring element of size 1 (red)

in the image opened by structuring element of size 4 (yellow)

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Granular spectrum, relative granularity

Granular spectrum

$$G(n) = V(\gamma_{n-1}(X)) - V(\gamma_n(X))$$

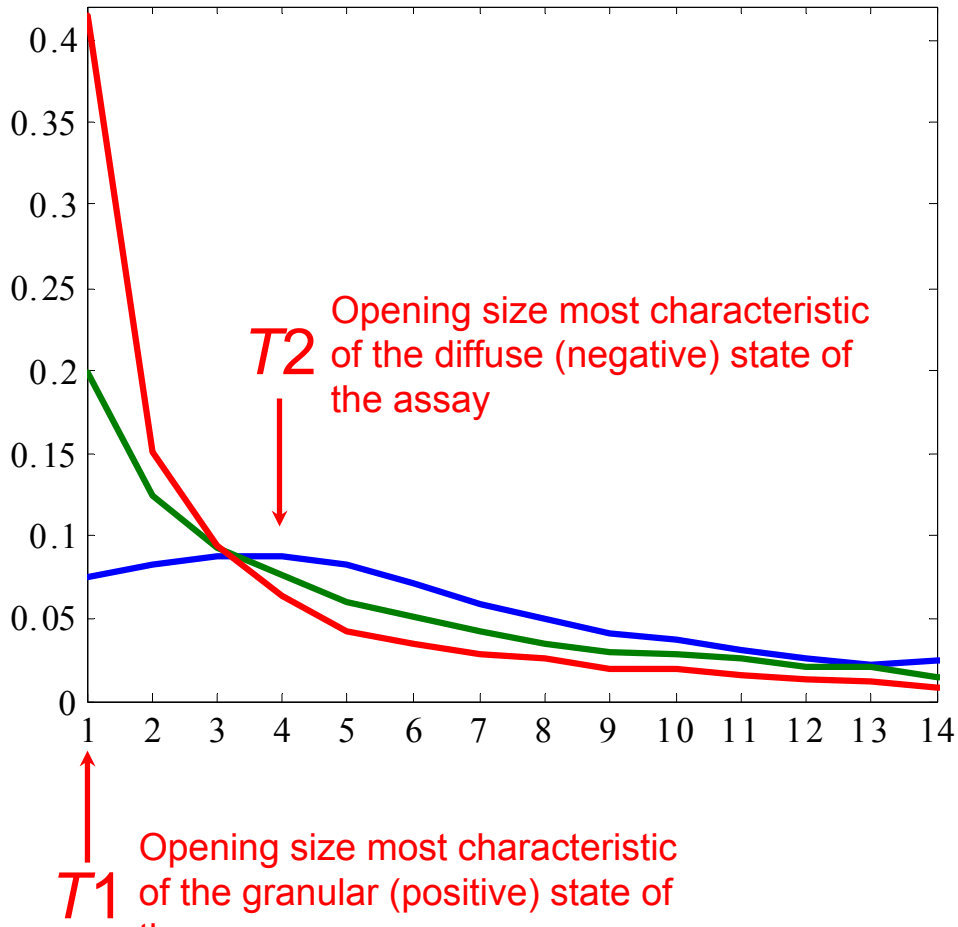
X - image, n - opening size

$\gamma_n(X)$ - n - th opening of image X

$V(X)$ - image volume (sum of pixel values)

Relative Granularity

$$RG = G(T1) / G(T2)$$



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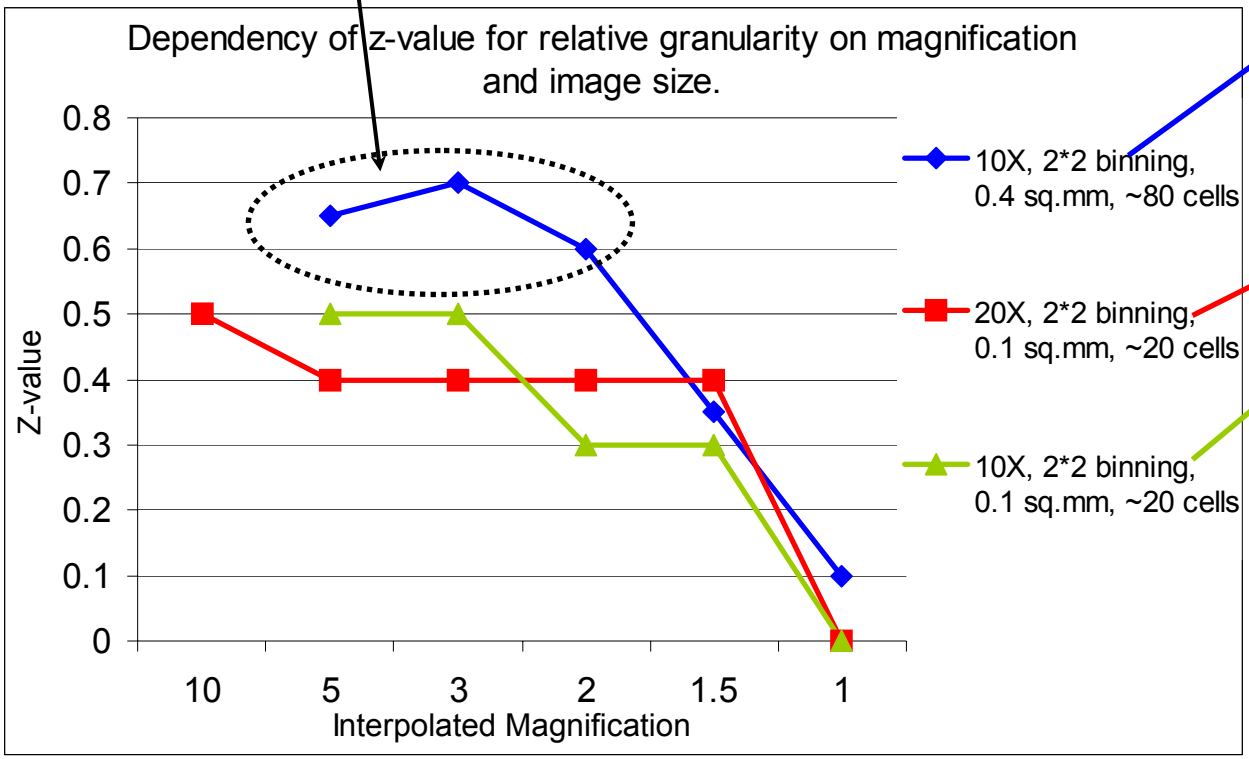
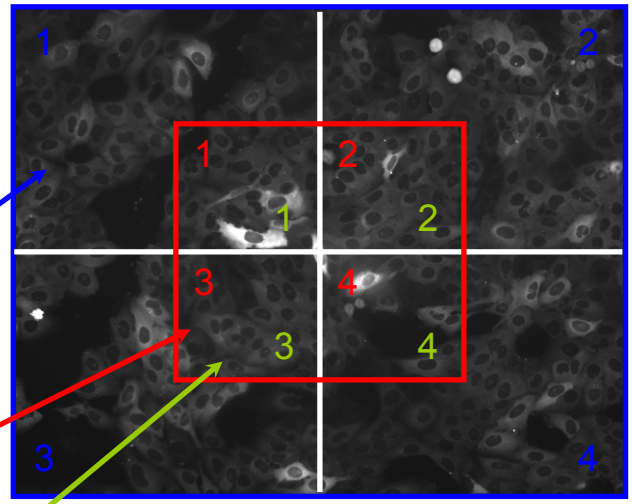
Quality measures for relative granularity

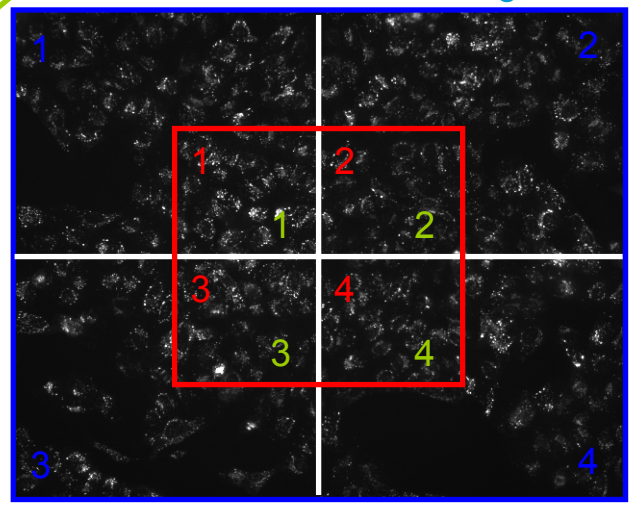
Good assay performance



Images used in calculation of z-value



Negative



Positive

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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 dispensing 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 HCS community may benefit from a common library of normative assay images for comparing different algorithms.

References

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