#P13030 - Novel Method for Prioritizing Hit Compounds using Multiple Parameters and Multiple Cell Types on the CellCard™ System

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Abstract

The CellCard™ System enables collection of multi-parametric data from ten cell types in a single microtiter well. Using this technology we screened a small library looking for activities that affect mitotic index and caspase 3 activation. The data set was analyzed both in the original feature space of mitotic index and caspase 3 measures in the cell types, and in the space of principal components. We used several multidimensional analytical tools to identify unique 'hit' compounds. Interestingly, the principal component approach allowed us to prioritize compounds based on their inferred mechanism of action and cell type selectivity. The combination of the CellCard™ System's ability to provide robust multi-parametric data across cell types within a single well and appropriate data analysis methods will enable scientists to identify and more rationally prioritize novel selective hit compounds early in the discovery process.

Introduction

The desire to obtain more biologically relevant data is expanding the use of cell-based assays in drug discovery. These assays are performed and analyzed in ever more sophisticated ways (e.g. high content screening) that allow the collection of multiparametric information about cells affected by the screened compounds. The CellCard system described here adds a new dimension to the data quality/quantity mix by simultaneously analyzing several cell types in the same microplate well. The system is based on the use of encoded carriers (CellCards®) that permit the reading and analysis of cellular responses, and at the same time allow decoding and the attribution of these responses to the appropriate cell line. CellCards are rectangular particles with an expandable color barcode and a transparent section upon which cells can be grown and imaged for cellular readout. Multiplexing cell lines allows assay controls and data normalization within each well, reducing well-to-well variability. It also allows the simultaneous interrogation of multiple targets and thus concurrent potency and selectivity screening. This may significantly reduce the time required to take a compound from primary screening into the clinic.

Figure 1. CellCards.

A. Drawing of a CellCard with two coding bands on each side and a recessed clear cell readout area in the middle

B. Composite image of a CellCard with cells

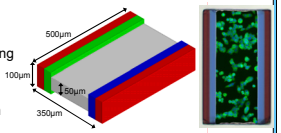
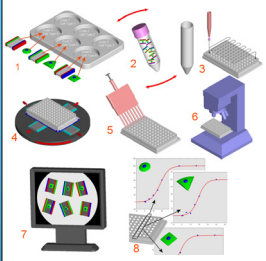
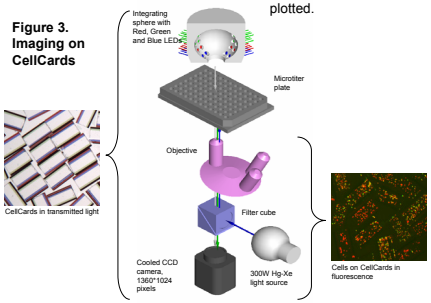


Figure 2. Procedure for performing assays on CellCards



1.The carriers are dispersed in 6-well plates, the cells are plated onto of the carriers and incubated overnight to allow cells to attach and spread;
2. The carriers with attached cells are combined and mixed;
3. Approximately 100 carriers are dispensed from the mixing tube into each well of a microtiter plate;
4. The carriers are dispersed to minimize overlaps;
5. The assay is performed as usual;
6. Images of each well are acquired in both brightfield and fluorescence mode;
7. Images are analyzed to decode the carriers and make assay-specific measurements;
8. Numerical data is analyzed and plotted.

Figure 3. Imaging on CellCards



Screen of the NCI structural diversity set

- 1840 compounds, 24 plates
- 24 hour incubation
- Combined Mitotic Index/Caspase-3 Cleavage assay
- Three fixed Intra-well controls: MI, C3, NEG
- Seven living cell types per well: A549, AdrRes, HCT116, HT29, MCF7, OVCAR3, SK-MEL28

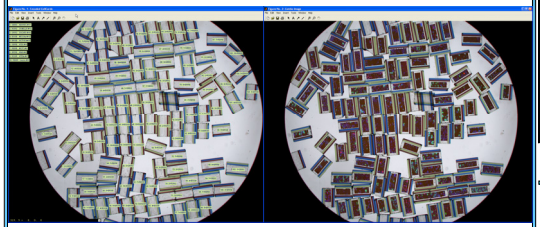


Figure 4. Recognition of CellCards.

Recognized carriers are shown with class assignment. Overlapping carriers and those partially in the image are excluded.

Figure 5. Cellular readout on CellCards.

Measurement areas with composite fluorescence images are shown inscribed into each carrier. Blue color represents nuclear counterstain, green color represents mitotic stain, red color represents caspase 3 stain.

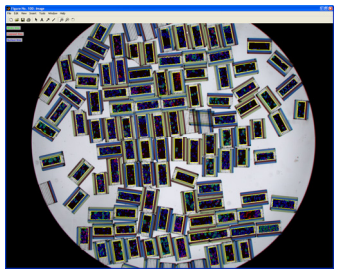
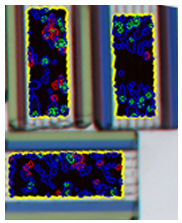


Figure 6. Cellular measurement on CellCards.

Measurement areas with composite fluorescence images and overlaid contours are shown inscribed into each carrier

Figure 7. Cell measures.



Measurement mask is shown by the yellow contour. AreaM is the area of this mask.
Blue contours outline areas where nuclear counterstain is brighter than the given threshold; they mark the presence of nuclei. AreaB is the area within blue contours.
Green contours outline areas where mitotic stain is brighter than the given threshold; they mark cells with high mitotic activity. AreaG is the area within green contours.
Red contours outline areas where caspase 3 stain is brighter than the given threshold; they mark cells with high caspase 3 activity. AreaR is the area within red contours.

Mitotic Index (MI) = AreaG/AreaM
Caspase 3 Index (CI) = AreaR/AreaM

(Alternatively these indices can be defined on the basis of average intensity or spot count)

Selection of compounds based on means and standard deviations of Mitotic Index and Caspase 3 Index among cell types

CellType	Compound ID	Plate ID	Well ID	MI	CI
A549	3036	3653	E11	2.08	4.72
SK28	3036	3653	E11	0.88	4.50
HCT116	3036	3653	E11	3.41	5.00
HT29	3036	3653	E11	1.35	12.26
MCF-7	3036	3653	E11	4.92	4.84
OVCAR-3	3036	3653	E11	7.01	25.27
ADRRES	3036	3653	E11	4.63	8.11
...					
A549	121384	3659	D04	1.64	0.98
ADRRES	121384	3659	D04	5.42	2.46
OVCAR-3	121384	3659	D04	8.16	8.45
HT29	121384	3659	D04	9.18	0.70
SK28	121384	3659	D04	3.11	1.97
MCF-7	121384	3659	D04	7.35	0.74
HCT116	121384	3659	D04	7.25	1.69
...					

1. Average of MI,
2. Standard deviation of MI (among cell types),
3. Average of CI,
4. Standard deviation of CI (among cell types)

In the absence of more specific information, we use standard deviation as a measure of selectivity of action of compounds on the cell types.

Figure 8. Fragment of the original table produced by the CellCard reader.

Figure 9. Distribution of compounds in the space of "activity" and "selectivity" of two assay parameters.

Goal – to find compounds with unique activity and selectivity profiles.

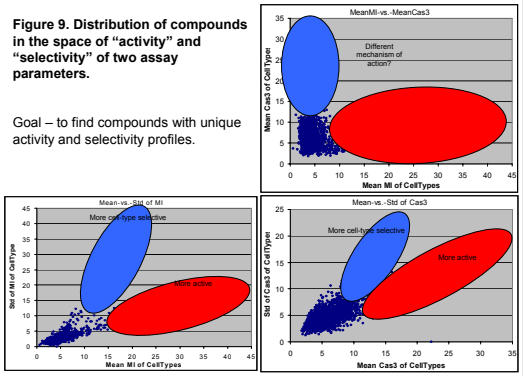
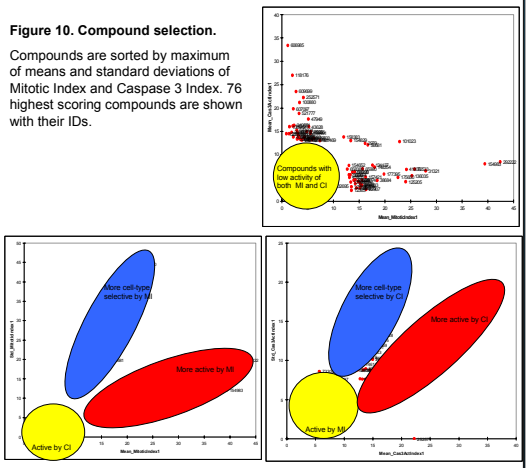


Figure 10. Compound selection.

Compounds are sorted by maximum of means and standard deviations of Mitotic Index and Caspase 3 Index. 76 highest scoring compounds are shown with their IDs.



Selection of compounds based on Principal Component Analysis of Mitotic Index and Caspase 3 Index in cell types

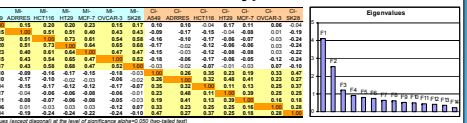
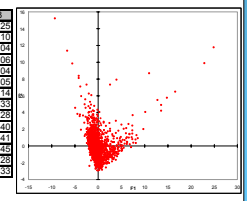


Figure 11. PCA.

Correlation matrix, eigenvalues and eigenvectors.

All compounds are shown in the space of the first two principal components.

Eigenvectors:	F1	F2	F3
MI-A549	0.11	0.22	0.25
MI-ADRRES	0.32	0.09	-0.10
MI-HCT116	0.40	0.13	-0.04
MI-HT29	0.40	0.17	-0.06
MI-MCF-7	0.36	0.13	-0.04
MI-OVCAR-3	0.37	0.09	0.05
MI-SK28	0.35	0.21	-0.14
CI-A549	-0.18	0.34	-0.33
CI-ADRRES	-0.13	0.41	0.28
CI-HCT116	-0.17	0.27	-0.40
CI-HT29	-0.11	0.38	0.41
CI-MCF-7	-0.11	0.33	0.45
CI-OVCAR-3	-0.08	0.34	-0.28
CI-SK28	-0.23	0.30	-0.33



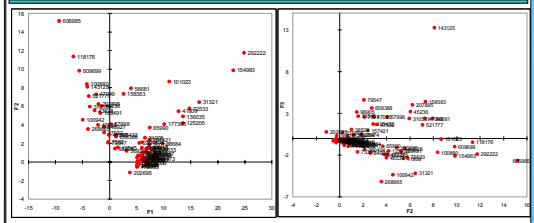


Figure 12. Compound selection. Compounds are sorted by maximum of absolute values of their principal components (all or selected components can be used). 76 highest scoring compounds are shown with their IDs.

Comparison of the methods

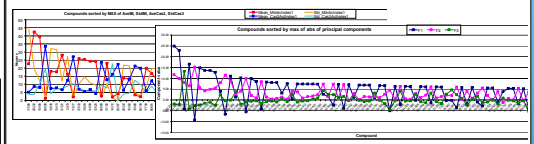


Figure 13. Lists of compounds selected by the two methods. Overlap = 62%.

Conclusion

Inclusion of cell-type selectivity and multiple assay parameters into compound prioritization schemes enables the identification of unique compounds that would have been overlooked using a single parameter, activity only approach. In theory, the incorporation of such novel approaches will lead to the identification of unique chemical scaffolds, pharmacophores, SAR, and intellectual property.

CellCard Product Suite