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Multiparametric analysis of cell health status using flow cytometry

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ABSTRACT

Cellular responses to drug treatment can be varied and heterogeneous. Often, a multiparametric approach is necessary to identify cellular pathways that are responding or affected. To maximize the number of parameters analysed per sample, we aimed to combine several functional sensors into one assay. In addition, we set out to streamline the workflow focusing on live cell responses. To this end, we designed a flow cytometry-based detection system centered on a single incubation condition for sensors that have minimal spectral overlap. Analysing Jurkat cells treated with camptothecin, a topoisomerase inhibitor that triggers cellular apoptosis, we were able to simultaneously detect the loss of cells in G2/M phase using the stoichiometric DNA dye, Hoechst 33342; an increase in apoptotic cells with a sensor for activated caspase 3/7 (CellEvent Caspase-3/7 Green); the loss of mitochondrial membrane potential with tetramethylrhodamine, methyl ester (TMRM); and an increase in dead cells as well as cells in late apoptotic stage, using a SYTOX viability dye. We also tested another four-sensor combination that focused on detecting cellular stress. Upon prolonged camptothecin induction, we were able to detect an increase in mitochondrial stress with MitoSOX, a sensor that detects mitochondrial oxidative stress; an increase in cellular stress with a CellROX reagent specific for reactive oxygen species. There was also a loss in mitochondrial membrane potential as well as a greater loss of cellular viability. In summary, using a model of cellular response to drug treatment, we performed multiparametric cell status analysis with a combination of cellular detection reagents on a flow cytometer.

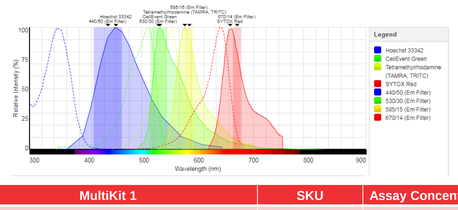
INTRODUCTION

Determination of cell status has often been limited to single parameter analysis. This approach limits the number of parameters examined, usually resulting in sequential testing using different reagents that target distinct biological readouts. Compounding the limited throughput of a single readout assay, single parameter analysis have the potential to miss out on other confounding biological effects. Flow cytometry leads itself to multiparametric analysis. In this study, we highlight the use of flow cytometric analysis in detecting four different cell status parameters in a single analytical run. One set of sensor will detect cell cycle state, induction of apoptosis, mitochondrial health and cell viability. The second set will determine the stress state of the cell by measuring mitochondrial stress, cellular stress, mitochondrial health and cellular viability. These sensors are marketed as single parameter reagents, but in this study, we show that four different sensors are compatible in live cell staining to demonstrate cellular health. Furthermore, we are able to identify hits from a compound library using a combination of these sensors on a flow cytometer.

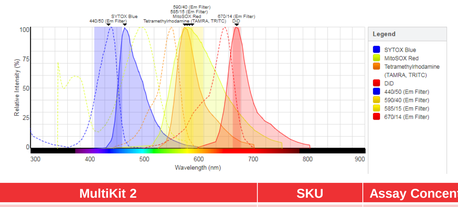
MATERIALS AND METHODS

Verification testing: Jurkat cells were resuspended at 1 x 10^6 cells/ml, and 1 ml of cells were stained with sensors in FACS tubes. A concentration range from 0.1 uM to 10 uM of camptothecin was used to stimulate the cells for 18 hours, in 37ºC with 5% CO_2. Individual sensors outlined in table 1 was added either individually or in combination. Sensors were incubated with the cells for 30 minutes at 37ºC with 5% CO_2. After that period, cellular responses were analyzed using an Attune NxT Acoustic Cytometer.

Treatment for compound library analysis: For this test,10,000 cells in 80 ul of RPMI 1640 + 10% fetal bovine serum were plated into each well of a 96 well plate. Compounds from one plate of the Tocriscreen Mini (Tocris, Cat No.2890) were used. The initial 10 mM stock was diluted to 100 uM. This allowed for a 1:10 addition of the compounds to 90 ul of cell to result in the final compound concentration of 10 uM. Incubation of the compounds with the cells were carried out in 37ºC with 5% CO_2 for 18 hours. After which, components of MultiKit 1 and MultiKit 2 were added to the final assay concentrations. Cells were returned to a cell culture incubator for 30 mins prior to flow cytometric analysis, using an Attune NxT Acoustic Cytometer with the attached Autosampler.



MultiKit 1	SKU	Assay Concentration
Hoechst 33342	H21492	10 ug/ml
CellEvent Caspase-3/7 Green Flow Cytometry Assay Kit	C10427	500 nM
MitoProbe TMRM Assay Kit for Flow Cytometry	M20036	20 nM
SYTOX Red Dead Cell Stain, for 633 or 653 nm excitation	S34859	5 nM



MultiKit 2	SKU	Assay Concentration
SYTOX Blue Dead Cell Stain, for flow cytometry	S35857	1 uM
MitoSOX Red Mitochondiral Superoxide Indicator, for live-cell imaging	M36008	1 uM
MitoProbe TMRM Assay Kit for Flow Cytometry	M20036	20 nM
CellROX Deep Red Flow Cytometry Assay Kit	C10491	500 nM

RESULTS

Figure 1. Individual cell status sensors can be used in combination

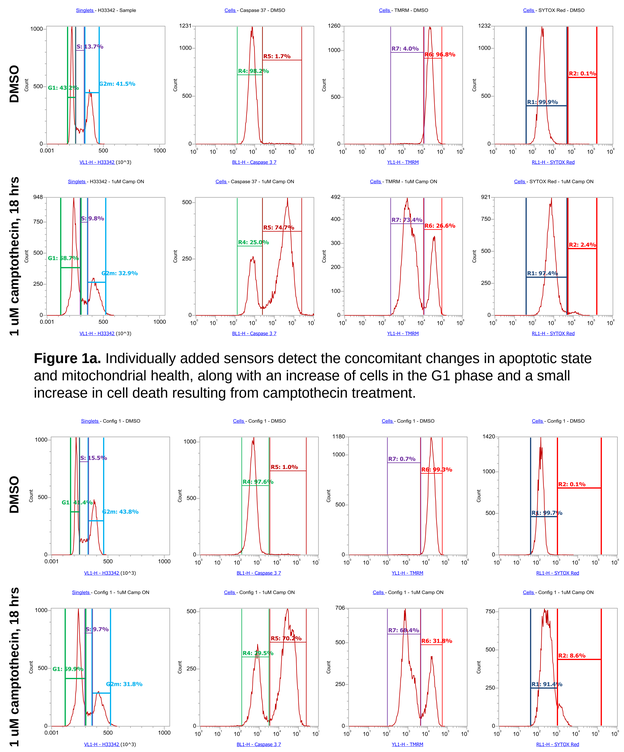


Figure 1a. Individually added sensors detect the concomitant changes in apoptotic state and mitochondrial health, along with an increase of cells in the G1 phase and a small increase in cell death resulting from camptothecin treatment.

Figure 1b. Sensors added in combination detected camptothecin-related effects similar to Figure 1a. Apoptotic state (70% vs 75%), mitochondrial health (68% vs 73%), increase of cells in the G1 phase (60% vs 59%) and the increase in cell death (8.6% vs 2.4%). Detection was performed without fluorescence compensation.

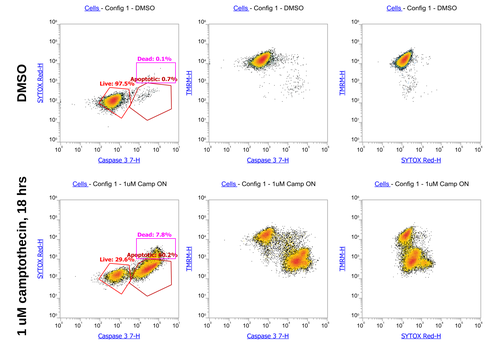


Figure 1c. Multiparameter analysis of Jurkat cells treated with 1 uM camptothecin for 18 hours show cells undergoing apoptosis with some that are dead/late apoptotic (Left Plots). Cell that undergo apoptotic changes have a reduce mitochondrial function as is evident in the loss of TMRM staining (Middle Plots). Loss of TMRM staining and gain of SYTOX staining is consistent with what is expected for dead/late apoptotic cells (Right Plots).

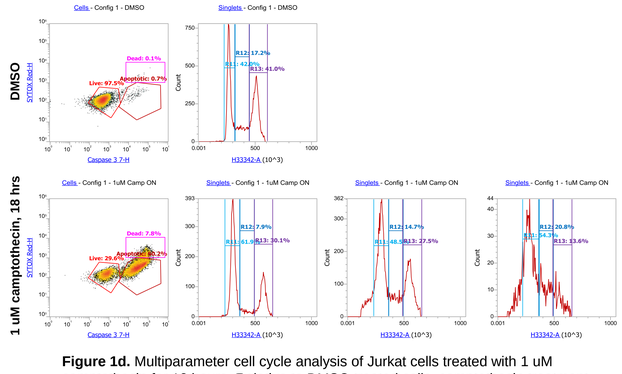


Figure 1d. Multiparameter cell cycle analysis of Jurkat cells treated with 1 uM camptothecin for 18 hours. Relative to DMSO treated cells, camptothecin treatment resulted in loss of cells in G2M phase (Bottom Histograms). The loss corresponded to the cellular states; treated live cells show a loss of S phase cells, with an increasing loss of G2M phase cells as the cells undergo apoptosis.

Figure 2. Individual cell status sensors to detect cellular stress

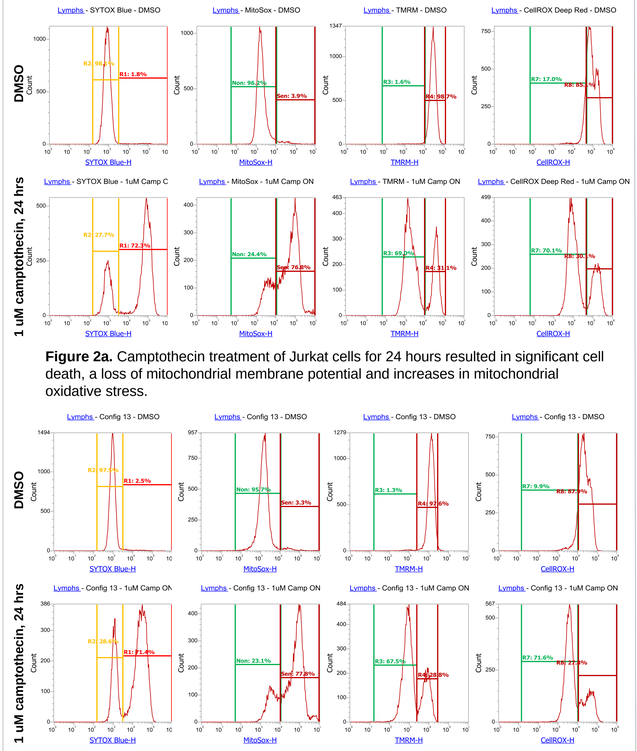


Figure 2a. Camptothecin treatment of Jurkat cells for 24 hours resulted in significant cell death, a loss of mitochondrial membrane potential and increases in mitochondrial oxidative stress.

Figure 2b. Using MultiKit 2, the effects of camptothecin on Jurkat cells were detected in a multiparametric analysis. Consistent with the results in Figure 2a, treated Jurkat cells showed an increase in cell death, a loss of mitochondrial membrane potential and increases in mitochondrial oxidative stress. Unlike MultiKit1, using MultiKit 2 required fluorescence compensation. Uncompensated data shown in lower, boxed row.

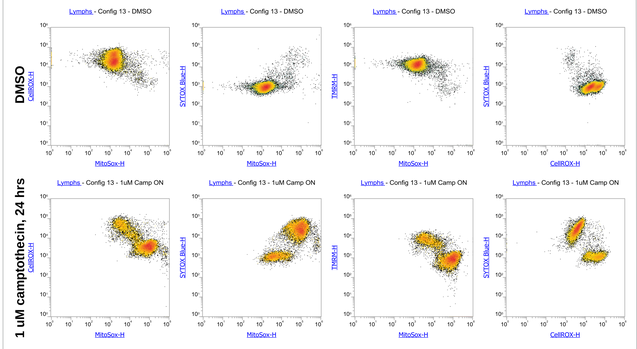


Figure 2c. Multiparameter analysis of Jurkat cells treated with 1 uM camptothecin for 24 hours. There was no induction of cellular stress by camptothecin treatment, however, mitochondrial stress was detected along with a loss in mitochondrial membrane potential.

Figure 3. Screening with Tocriscreen Mini compound library

Position	Batch Specific Product Name	Brief Description	Position	Batch Specific Product Name	Brief Description
A01	NMDA	Selective NMDA agonist	E01	Spaglumic acid	Selective mGlu3 agonist
A02	(RS)-AMPA	Selective AMPA agonist	E02	N-Acetylglutyryl-D-glutamic acid	Potent convulsant
A03	cis-ACPD	Potent NMDA agonist. Also group II mGluR agonist	E03	MDL 73005EF hydrochloride	Potent and selective 5-HT1A partial agonist
A04	DNQX	Selective non-NMDA antagonist	E04	Ro 20-1724	PDE4 inhibitor
A05	L-Cysteinesulfinic acid	NMDA and mGlu agonist	E05	GBR 13069 dihydrochloride	Potent dopamine uptake inhibitor
A06	S-Sulfo-L-cysteine sodium salt	Group I agonist	E06	S-(-)-Atenolol	β1-antagonist. Active isomer of atenolol (Cat. No. 0387)
A07	ZAPA sulphate	Agonist at 'low affinity' GABAA receptor.	E07	β-CCB	Benzodiazepine inverse agonist, putative endogenous ligand
A08	L-Quisqualic acid	AMPA/group I mGlu agonist	E08	MDL 72832 hydrochloride	Potent 5-HT1A ligand
A09	Homoquinolinic acid	Selective, potent NMDA agonist	E09	YS-035 hydrochloride	Inhibits Kv outward/pacemaker current
A10	Kainic acid	Potent excitant and neurotoxin	E10	GBR 12909 dihydrochloride	Selective DA uptake inhibitor. Also σ ligand
B01	Kynurenic acid	Broad spectrum EAA antagonist	F01	Benzoquinonium dibromide	Nicotinic antagonist
B02	Guvacine hydrochloride	Specific GABA uptake inhibitor	F02	L-693,403 maleate	High affinity α ligand
B03	7-Chlorokynurenic acid	Potent competitive inhibitor of L-glutamate uptake	F03	SC-10	Protein kinase C activator
B04	Saclofen	Selective GABAB antagonist	F04	MY-5445	PDE5 inhibitor
B05	(R)-(+)-HA-966	NMDA partial agonist/antagonist, acts at glycine site	F05	Etazolate hydrochloride	PDE4 inhibitor
B06	Quinolinic acid	Endogenous NMDA agonist and transmitter candidate	F06	UK 14,304	α2 agonist
B07	Isoguvacine hydrochloride	Selective GABAA agonist	F07	Bromocriptine mesylate	Selective D2-like agonist
B08	2-Hydroxysaclofen	Selective GABAB antagonist	F08	ML 9 hydrochloride	Myosin light chain kinase inhibitor
B09	(S)-AMPA	Selective AMPA agonist. Active isomer of (RS)-AMPA	F09	SC-9	Protein kinase C activator
B10	(S)-(-)-HA-966	NMDA antagonist/partial agonist	F10	DPCPX	A1 selective antagonist
C01	5,7-Dichlorokynurenic acid	Potent NMDA antagonist, acts glycine site	G01	m-Chlorophenylbiguanide hydrochloride	Potent and specific 5-HT3 agonist
C02	(RS)-cTetrazol-5-ylglycine	Highly potent NMDA receptor agonist	G02	6-Chloromelatonin	Melatonin agonist
C03	(R)-3-Carboxy-4-hydroxyphenylglycine	Ionotropic glutamate receptor antagonist	G03	Carbetapentane citrate	High affinity σ1 ligand
C04	(RS)-3,5-DHPG	Selective group I mGlu agonist	G04	Chlormezanone	Skeletal muscle relaxant
C05	2-BFI hydrochloride	Potent, selective I2 ligand. Putative agonist	G05	5-Carboxamidotryptamine maleate	5-HT1 agonist. Also has high affinity for 5-ht5a and 5-HT7
C06	(RS)-4-Carboxy-3-hydroxyphenylglycine	Broad spectrum EAA ligand	G06	4-Chlorophenylguanidine hydrochloride	Urokinase inhibitor
C07	(S)-4-Carboxy-3-hydroxyphenylglycine	Group I antagonist/group II agonist	G07	Calpeptin	Calpain and cathepsin L inhibitor
C08	(S)-3-Carboxy-4-hydroxyphenylglycine	Group I antagonist/group II agonist	G08	(S)-(+)-Carbidopa	Aromatic L-amino acid decarboxylase inhibitor
C09	nor-Binaltorphimine dihydrochloride	Standard κ selective antagonist	G09	Clomipramine hydrochloride	5-HT re-uptake inhibitor
C10	1-Acetyl-4-methylpiperazine hydrochloride	Nicotinic agonist	G10	Cinanserin hydrochloride	Selective 5-HT2 antagonist
D01	4-Acetyl-1,1-dimethylpiperazinium iodide	Nicotinic agonist	H01	Capsazepine	Vanilloid receptor antagonist. Also activator of ENaC/δ
D02	(±)-(1,2-Diphenylethyl)piperidine maleate	NMDA antagonist, acts ion-channel site	H02	Dihydroergocristine mesylate	Partial α agonist. Non-selective
D03	A-7 hydrochloride	Calmodulin antagonist	H03	Flurofamide	Urease inhibitor
D04	Arecaidine propargyl ester tosylate	Muscarinic agonist	H04	4-DAMP	Muscarinic M3 antagonist
D05	3-Methyl-GABA	Activator of GABA amino-transferase	H05	Alendatin	Aldose reductase inhibitor
D06	N-Acetyltryptamine	Melatonin partial agonist (MT1/MT2). Also MT3 antagonist	H06	Nitrocaramiphen hydrochloride	Muscarinic antagonist, M1 > M2
D07	NBQX	Potent AMPA antagonist.	H07	Dihydroergotamine mesylate	Partial α agonist. Non-selective
D08	Arecaidine but-2-ynyl ester tosylate	Muscarinic agonist	H08	Dilazep dihydrochloride	Adenosine uptake inhibitor
D09	Nateglinide	Kir6 (KATP) blocker; displays high affinity for SUR1/Kir6.2 channels	H09	OR-486	Catechol-O-methyl transferase inhibitor
D10	(RS)-Atenolol	β1 antagonist	H10	1,3-Dipropyl-8-phenylxanthine	A1 selective antagonist

Figure 4. Responses from compound library analysis

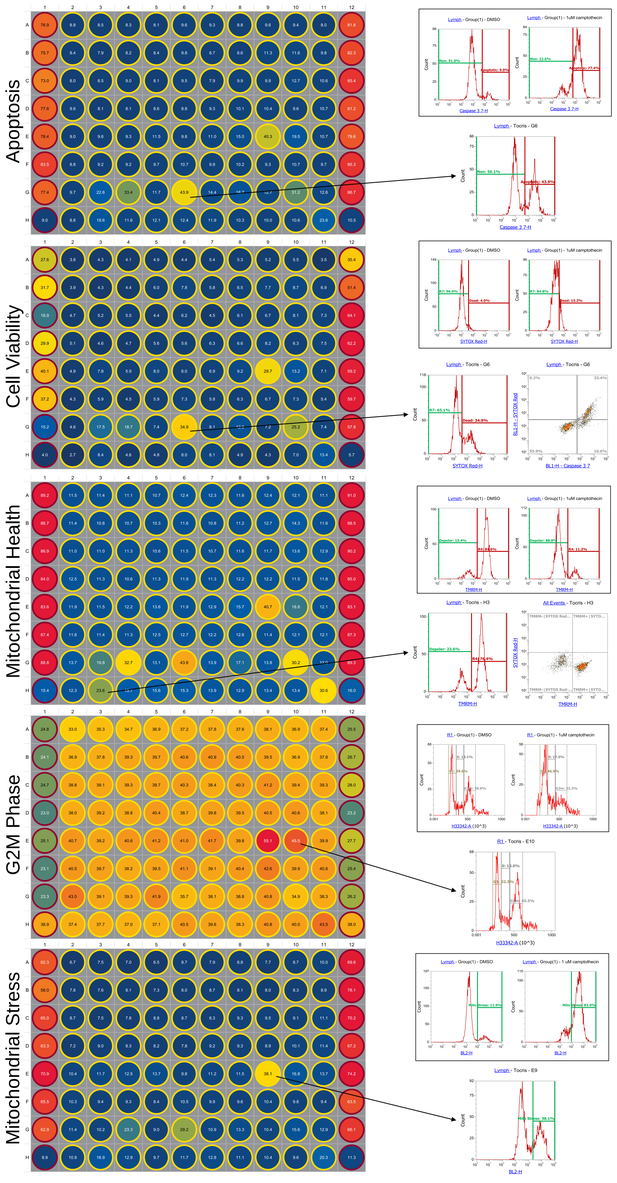


Figure 4. Multiparametric readouts from Jurkat cells in response to incubation with compounds from the Tocriscreen library. Control readouts are shown in boxed histograms. Heat map shows potential hits, and specific responses from those wells are highlighted (arrows).

CONCLUSIONS

- Single parameter detection reagents can be used in combination to create multiparameter kits
- Spectrally distinct reagents allows for experimental setup without employing fluorescence compensation
- Multiparameter kits can be tailored to determining cellular responses in drug compound library analyses.

REFERENCES

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