Cell Line	Organelle/Purpose	Integration Time (ms)	Laser Power (%)	Focal Height (µm) ^f
A549	DNA ^a	80	55	-4.4
	RNA (Nucleoli) ^b	60	50	-1.2
	ER (Endoplasmic reticulum) ^c	40	40	-2.4
	AGP (Actin cytoskeleton + Golgi + Plasma membrane) ^d	60	60	-4.4
	Mito ^e	20	15	-6.4
ARPE-19	DNA ^a	120	60	-4.8
	RNA (Nucleoli) ^b	20	45	-1.6
	ER (Endoplasmic reticulum) ^c	40	40	-4.0
	AGP (Actin cytoskeleton + Golgi + Plasma membrane) ^d	100	40	-4.8
	Mito ^e	120	80	-6.8
HepG2	DNA ^a	100	80	-2.0
	RNA (Nucleoli) ^b	40	30	-1.6
	ER (Endoplasmic reticulum) ^c	20	35	-2.4
	AGP (Actin cytoskeleton + Golgi + Plasma membrane) ^d	80	60	-2.4
	Mito ^e	80	60	-2.4
HTB-9	DNA ^a	100	60	-4.4
	RNA (Nucleoli) ^b	80	60	-2.8
	ER (Endoplasmic reticulum) ^c	120	70	-4.4
	AGP (Actin cytoskeleton + Golgi + Plasma membrane) ^d	80	60	-4.4
	Mito ^e	20	40	-6.8
MCF7	DNA ^a	80	40	-3.2
	RNA (Nucleoli) ^b	40	30	-1.6
	ER (Endoplasmic reticulum) ^c	80	35	-3.2
	AGP (Actin cytoskeleton + Golgi + Plasma membrane) ^d	60	40	-4.8
	Mito ^e	20	65	-6.4
U-2 OS	DNA ^a	80	50	-3.6
	RNA (Nucleoli) ^b	20	45	-0.4
	ER (Endoplasmic reticulum) ^c	20	50	-3.6
	AGP (Actin cytoskeleton + Golgi + Plasma membrane) ^d	120	50	-4.4
	Mito ^e	20	80	-6.8
^a Visualized with Hoechst-33342. Imaged using 405 nm laser with 435-480 nm emission filter. ^b Visualized with SYTO-14. Imaged using 488 nm laser with 500-550 nm emission filter. ^c Visualized with Alexa Fluor 488-conjugated concanavalin-A. Imaged using 488 nm laser with 500-550 nm emission				

Supplementary Table 1. List of channel-specific image acquisition settings for six human-derived cell lines.

^a Visualized with Hoechst-33342. Imaged using 405 nm laser with 435-480 nm emission filter. ^b Visualized with SYTO-14. Imaged using 488 nm laser with 500-550 nm emission filter. ^c Visualized with Alexa Fluor 488-conjugated concanavalin-A. Imaged using 488 nm laser with 500-550 nm emission filter. ^d Visualized with Alexa Fluor 568-conjugated phalloidin multiplexed with Alexa Fluor 555-conjugated wheat germ agglutinin. Imaged using 561 nm laser with 570-630 nm emission filter. ^e Visualized with Mitotracker Deep Red. Imaged with 640 nm laser with 650-760 nm emission filter. F focal height in reference to default values determined by the Opera Phenix HCS imaging system when using PerkinElmer CellCarrier-384 Ultra microplates.

Supplementary Figure 1

Qualitative comparison of chemical-induced morphological phenotypes across cell lines

- Supplementary Figure 1A. Ca-074-Me
- Supplementary Figure 1B. Etoposide

Supplementary Figure 1A. Ca-074-Me



Supplementary Figure 1B. Etoposide



Supplementary Figure 2

Potency Magnitude Plots and Accumulation Plots for Visualizing Phenotypic Effects of Test Chemicals

- Supplementary Figure 2A. Sorbitol
- Supplementary Figure 2B. Saccharin
- Supplementary Figure 2C. Amperozide
- Supplementary Figure 2D. Fluphenazine
- Supplementary Figure 2E. Tetrandrine
- Supplementary Figure 2F. Ca-074-Me
- Supplementary Figure 2G. Metoclopramide
- Supplementary Figure 2H. NPPD
- Supplementary Figure 2I. Fenbendazole
- Supplementary Figure 2J. Oxibendazole
- Supplementary Figure 2K. Taxol
- Supplementary Figure 2L. Rotenone
- Supplementary Figure 2M. Latrunculin B
- Supplementary Figure 2N. Etoposide
- Supplementary Figure 2O. Rapamycin

Supplementary Figure 2A. Sorbitol







Supplementary Figure 2B. Saccharin



Supplementary Figure 2C. Amperozide

• DNA

AGP

Mito

Intensity

s

С

R Radial

Р Profile

Nuclei Ring ٠

RNA

ER

T Texture

Axial А

Symmetry





Shape / Position

DNA

RNA

AGP

Mito

T Texture

Intensity

M Morphology

C Comptactness

Cell / Cytoplasm

S Symmetry

A Axial

Nuclei

Membrane

R Radial

Ρ Profile

٠ Ring

Supplementary Figure 2D. Fluphenazine

۲

• DNA

AGP

Mito

s

С

R Radial

Ρ Profile

•

Nuclei ٠ Ring

RNA

Intensity

Symmetry

T Texture

Axial А







Supplementary Figure 2E. Tetrandrine

۲

• DNA

AGP

Mito

s

С

А Axial

R Radial

Ρ Profile

•

Nuclei ٠ Ring

RNA

Intensity Т

T Texture







Supplementary Figure 2F. Ca-074-Me

۲

• DNA

۲ AGP

Mito

Т Intensity

s

С

R Radial

Ρ Profile

•

Nuclei ٠ Ring

RNA

ER

T Texture

Axial А





Supplementary Figure 2G. Metoclopramide

۲

DNA

AGP

Mito

I Intensity

T Texture

s

С

A Axial

R

P Profile

Ring

RNA

M Morphology

Symmetry

Radial

Nuclei

Membrane

Comptactness

Cell / Cytoplasm

ER

Shape / Position





Supplementary Figure 2H. NPPD

۲

DNA

AGP

Mito

s

С

R

P Profile

•

Nuclei

Ring

RNA

I Intensity

M Morphology

Symmetry

Comptactness

Cell / Cytoplasm

T Texture

A Axial

Radial

Membrane

ER

Shape / Position





Supplementary Figure 21. Fenbendazole

DNA

RNA

ER

AGP

Mito

Intensity

Symmetry

Axial

Radial

Profile

Nuclei

Ring







Supplementary Figure 2J. Oxibendazole

DNA

RNA

ER

AGP

Mito

Intensity

Symmetry

Axial

Radial

Profile

Nuclei

Ring

Comptactness







Supplementary Figure 2K. Taxol

Shape / Position

DNA

RNA

ER

AGP

Mito

Intensity

Symmetry

Axial

Radial

Profile

Nuclei

Ring

Comptactness

Cell / Cytoplasm







Supplementary Figure 2L. Rotenone

۲

• DNA

۲ AGP

Mito

s

С

R Radial

Ρ Profile

•

Nuclei ٠ Ring

RNA

Intensity

T Texture

Axial А

ER





Shape / Position

DNA

RNA

AGP

Mito

T Texture

A Axial

Nuclei

Membrane

S Symmetry

Radial

Intensity

Morphology

C Comptactness

Cell / Cytoplasm

• ER

М

R

Ρ Profile

•

٠ Ring

Supplementary Figure 2M. Latrunculin B

۲

• DNA

AGP

Mito

s

С

А Axial

R Radial

•

Nuclei

٠ Ring

RNA

Intensity

T Texture

Profile Ρ







Supplementary Figure 2N. Etoposide

DNA

RNA

ER

AGP

Mito

Axial

Radial

Profile

Nuclei

Ring

Intensity





DNA

RNA

AGP

Mito

Intensity

Radial

Profile

Nuclei

Ring

Cell / Cytoplasm

Morphology

• ER

L

•

Supplementary Figure 2O. Rapamycin

۲

• DNA

AGP

Mito

1 Intensity

s

С

А Axial

R Radial

Р Profile

•

Nuclei ٠ Ring Membrane

RNA

ER

T Texture

M Morphology

Symmetry







Supplementary Figure 3

Plots of Cell Count and Cell Viability (Well level values normalized to DMSO Control)

- Supplementary Figure 3A. Sorbitol
- Supplementary Figure 3B. Saccharin
- Supplementary Figure 3C. Amperozide
- Supplementary Figure 3D. Fluphenazine
- Supplementary Figure 3E. Tetrandrine
- Supplementary Figure 3F. Ca-074-Me
- Supplementary Figure 3G. Metoclopramide
- Supplementary Figure 3H. NPPD
- Supplementary Figure 3I. Fenbendazole
- Supplementary Figure 3J. Oxibendazole
- Supplementary Figure 3K. Taxol
- Supplementary Figure 3L. Rotenone
- Supplementary Figure 3M. Latrunculin B
- Supplementary Figure 3N. Etoposide
- Supplementary Figure 3O. Rapamycin
- Supplementary Figure 3P. Berberine Chloride

Supplementary Figure 3A. Sorbitol



Supplementary Figure 3B. Saccharin



Supplementary Figure 3C. Amperozide



Supplementary Figure 3D. Fluphenazine



Supplementary Figure 3E. Tetrandrine



Supplementary Figure 3F. Ca-074-Me



Supplementary Figure 3G. Metoclopramide



Supplementary Figure 3H. NPPD



Supplementary Figure 3I. Fenbendazole



Supplementary Figure 3J. Oxibendazole



Supplementary Figure 3K. Taxol



Supplementary Figure 3L. Rotenone



Supplementary Figure 3M. Latrunculin B



Supplementary Figure 3N. Etoposide



Supplementary Figure 3O. Rapamycin



Supplementary Figure 3P. Berberine Chloride

