



HIGH THROUGHPUT SCREENING FACILITY: CANCER BIOLOGY

Target Identification and Drug Discovery

VANDERBILT UNIVERSITY
MEDICAL CENTER

824 Roberson Research Building

VANDERBILT INSTITUTE of CHEMICAL BIOLOGY
Harnessing the Power of Chemistry to Improve Human Health

www.vanderbilt.edu/hts

HTS Cancer Biology



Cancer Biology HTS models:

- Protein-protein interaction
- 3D tumor cultures
- Kinase/ATPase activity
- Yeast, zebrafish, other organisms
- Receptors, channels, transporters
- Mouse xenografts
- Tumor cell lines
- Tumor biopsies
- Cell-based expression

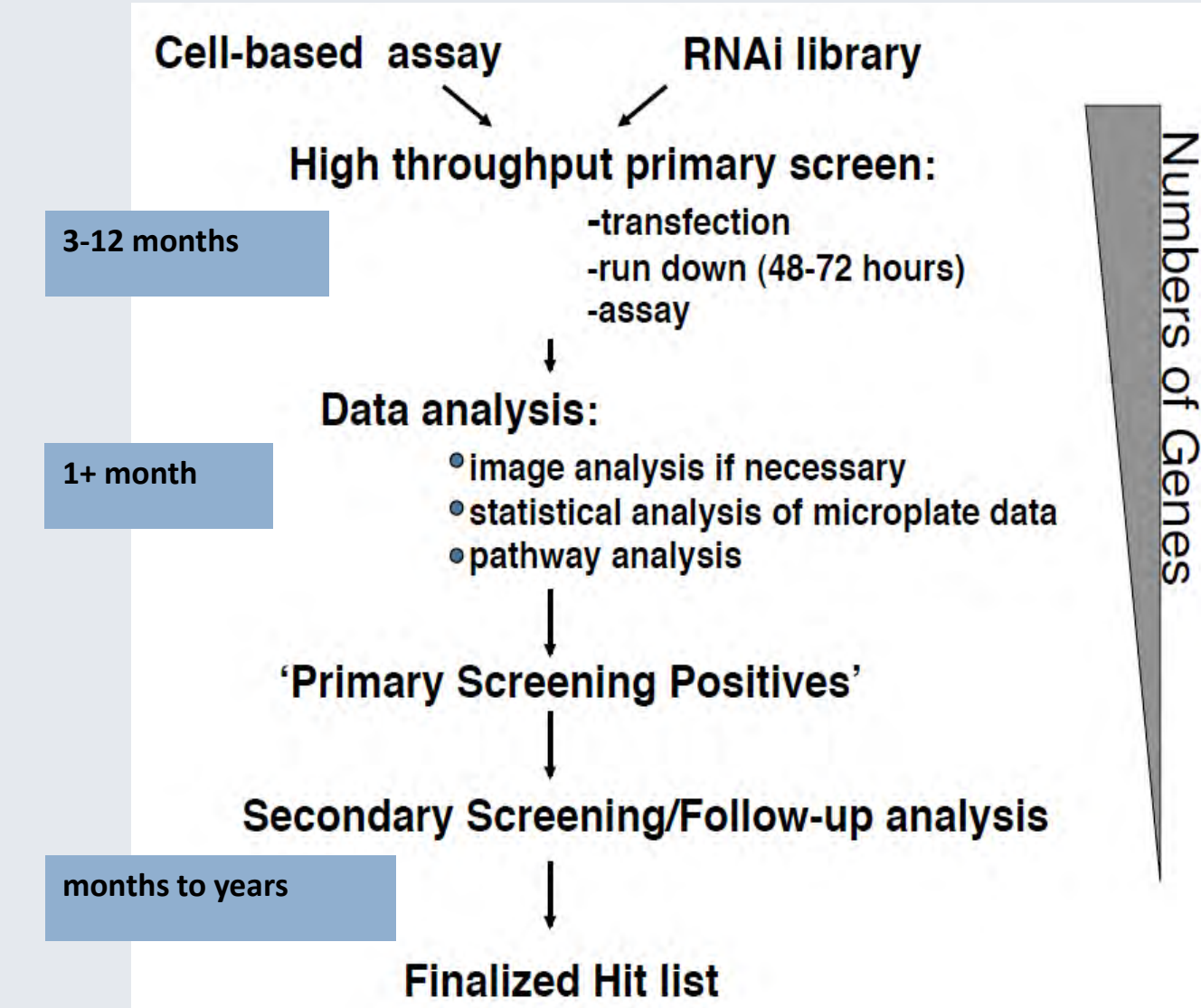
Target Identification: siRNA screening

NOW AVAILABLE at the Vanderbilt HTS Facility!

Dharmacon Human siRNA Libraries				Druggable Genome	
Library	Type	No. of genes	No. of oligos or pools	No. of library plates	Format
Kinase-pooled	siGENOME	714	720	9	96-well
Kinase-Individual	siGENOME	714	2,880	36	96-well
Druggable genome-pooled	siARRAY	7,304	7,502	97	96-well
*Whole-genome-pooled	siGENOME	32	~18,000	57	384-well
*Whole-genome-pooled	ON-TARGETplus	35	~18,000	57	384-well

- Formatted for 96 or 384-well plates
- Ready-to-transfect format
- Compatible with ImageExpress and all plate readers for high content or high throughput screening
- Can be used in conjunction with small molecule libraries

General siRNA screen workflow



Sterile-liquid handling screening system



Automated liquid handling and plate washer for cell plating, transfections/infections, media wash, drug addition, bulk dispensing, etc.

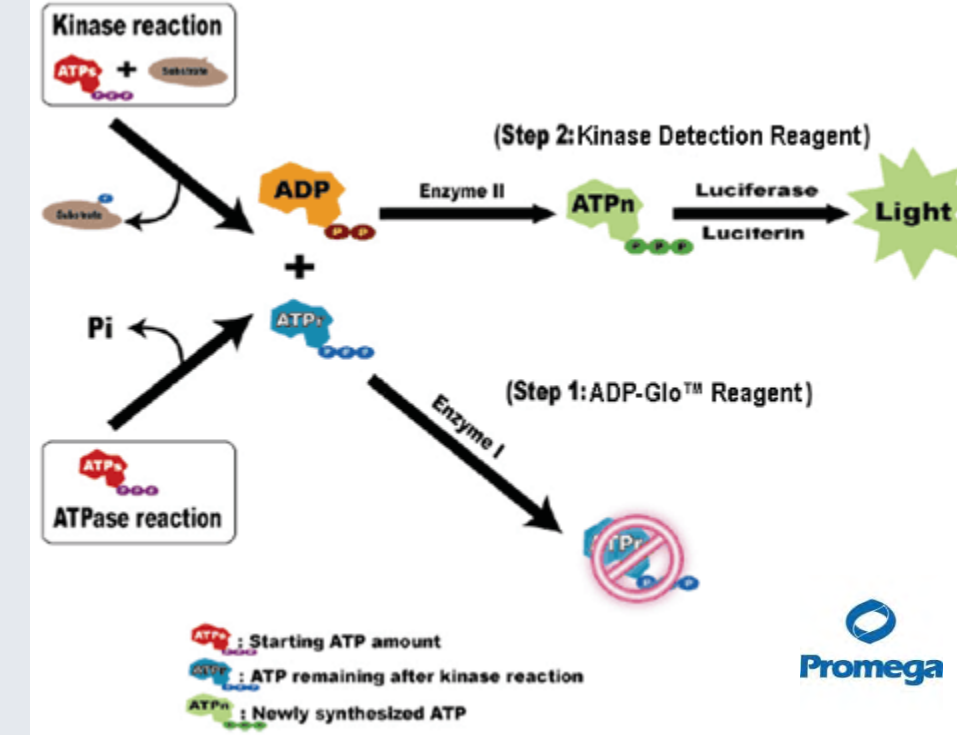
Kinase Activity & Protein-Protein Interaction

Assay Development and Screening for Cancer Targets

Targeting Kinases in Cancer

Deregulation of kinase activity has emerged as a major mechanism by which cancer cells evade normal cell growth and survival.

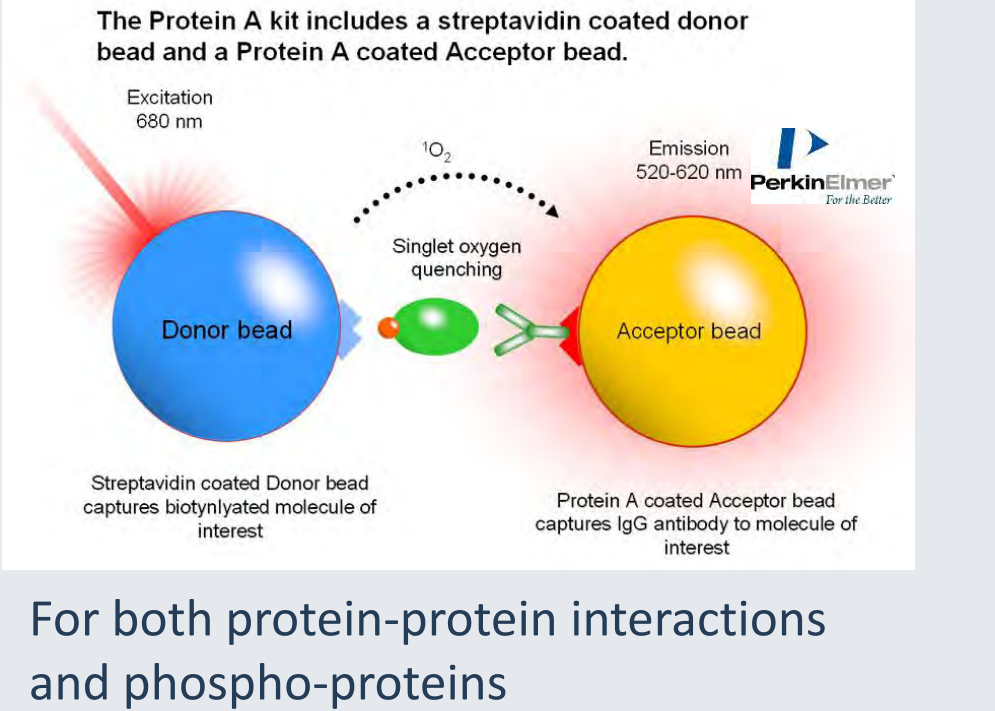
ADP-glo Assay for Kinase Activity



Assays in the Vanderbilt HTS Facility:

- Radioactive Technologies- scintillation proximity assays (SPA)
- Fluorescence- malachite green to measure inorganic phosphate
- Luminescence- enzyme-coupled assays (eg, ADP glo)
- Fluorescence Anisotropy (polarization)- small fluorescent peptide binding to large protein
- TR-FRET- Time-Resolved Forster Resonance Energy Transfer (eg, LANCE assay)
- Luminescent Oxygen Channeling- AlphaScreen and Surefire assays

AlphaScreen and Surefire Assays

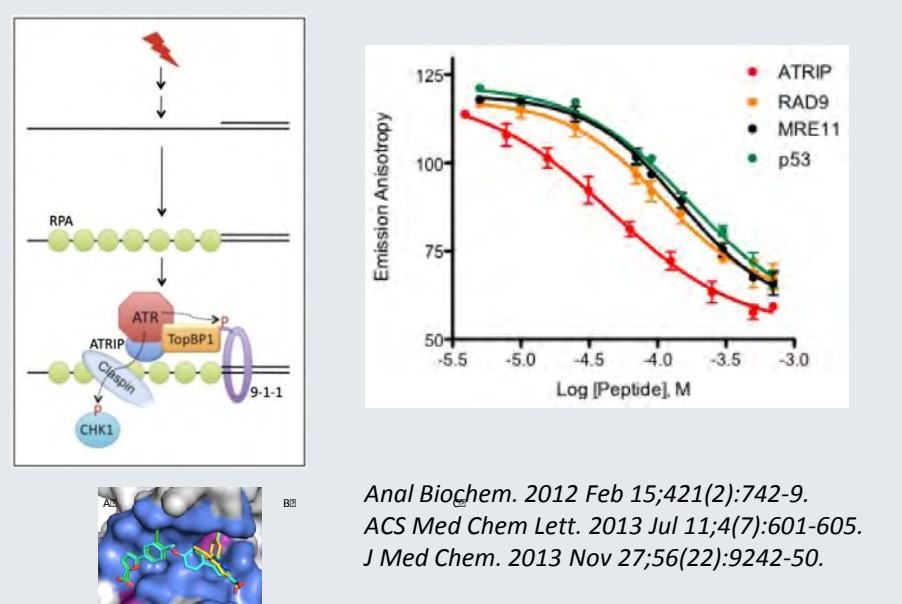


Targeting Protein-Protein Interactions in Cancer

Targeting of protein-protein interactions relevant to cancer is of fundamental importance and therapeutically significant. The tumor-promoting function of several aberrantly expressed proteins in the cancerous state is directly resultant of its ability to interact with a protein-binding partner, making them viable druggable targets.

RPA/ATRIP protein-protein interaction screen

- Developed fluorescent polarization assay for modulators of RPA/ATRIP protein-protein interaction
- Performed large screen with ~160,000 compounds (+16,000 fragment library)
- Utilized compound library, liquid handling, PE Envision plate reader
- Discovered small molecules that bind to RPA and interrupt binding partners on ssDNA



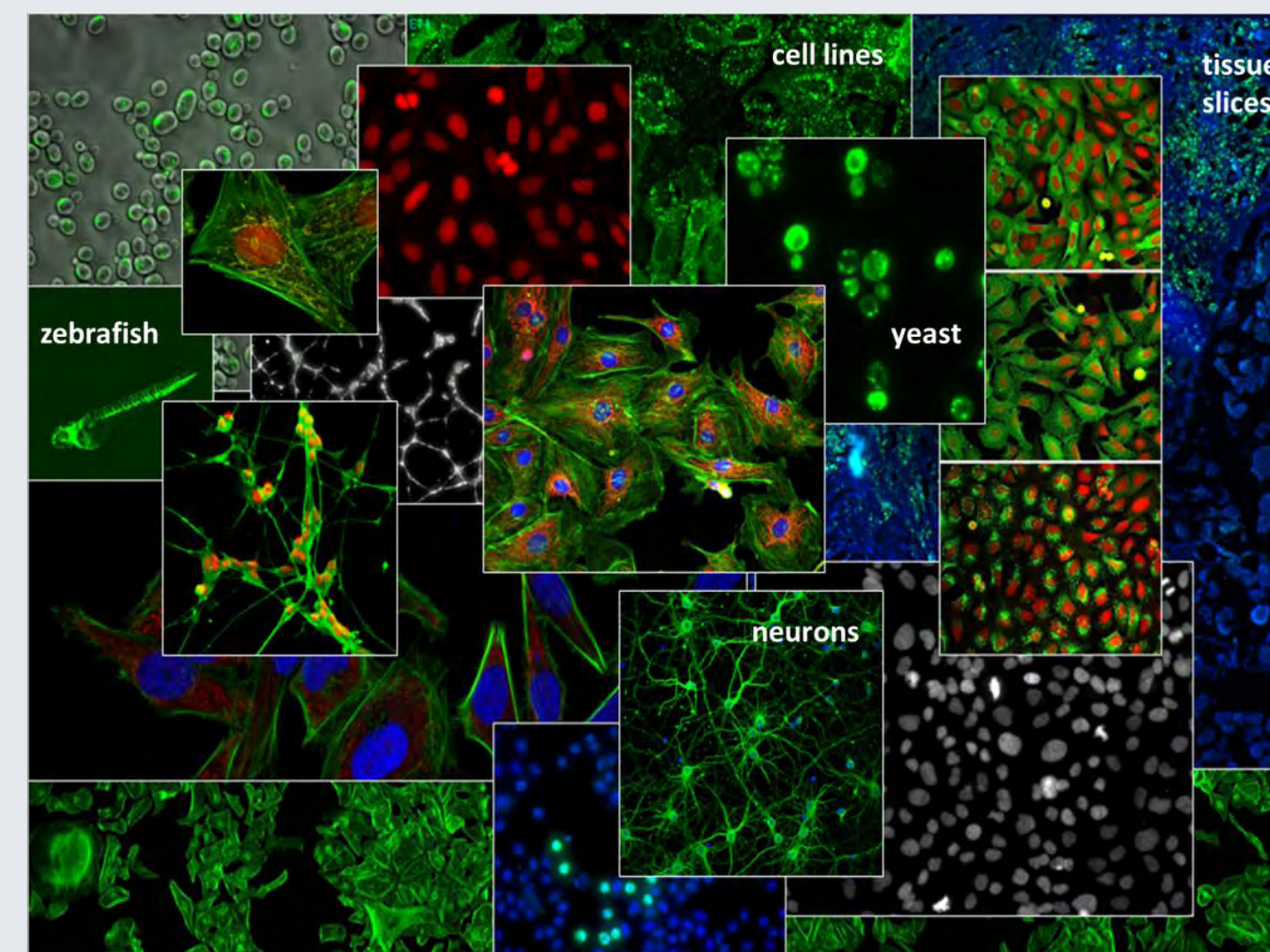
High Content Imaging- Automated Fluorescence Microscopy

ImageXpress Micro XL



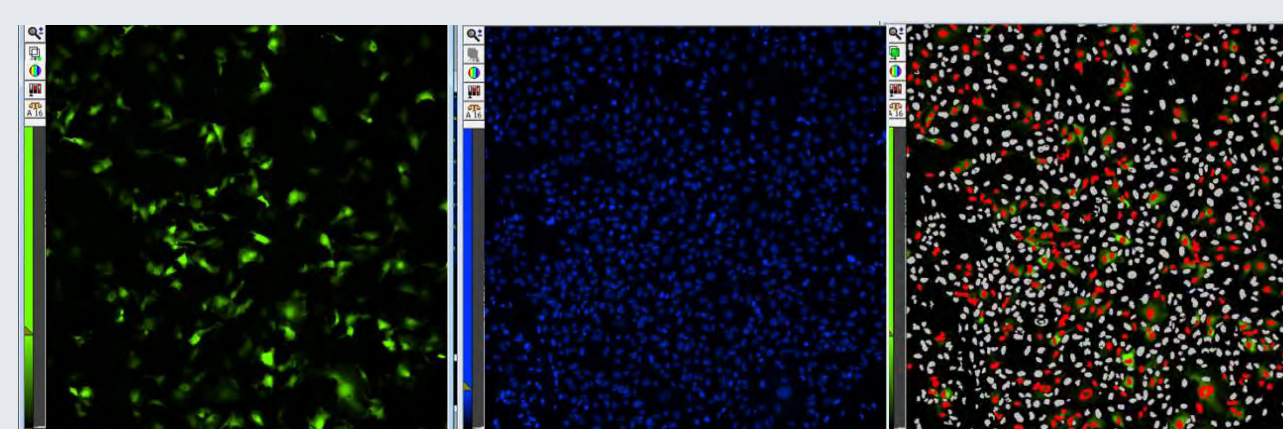
Features

- Sample format flexibility
- Wide-field CMOS camera
- 1x - 100x objectives
- Up to 5 fluorescent filters
- Transmitted light (phase contrast)
- Compatible for 6- to 1536-well plates and microscope slides
- High-speed laser and image-based autofocus
- Solid state light source
- Automation friendly



For all your imaging and quantitative analysis needs

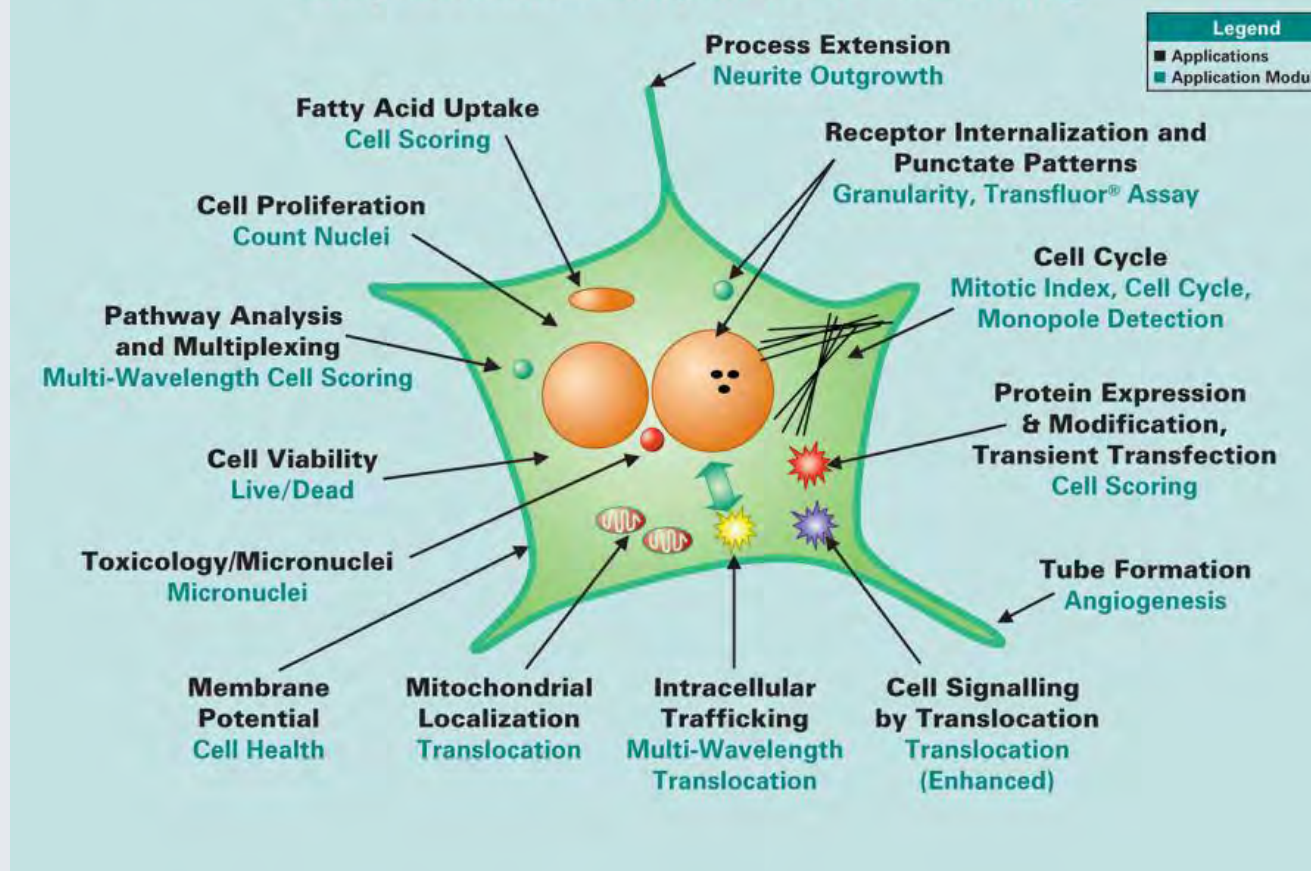
The Molecular Device's ImageXpress Micro XL is an automated microscope imager for high content screening (HCS). The MetaXpress and Powercore software uses common or custom-made application modules for fast image acquisition (multiday and time-lapse tracking) and full image analysis. The modules use size, intensities, and distances to analyze for cell scoring, counting nuclei, micronuclei, or foci, cell health, cell cycle, translocation, angiogenesis, mitotic index, proliferation, granularity, neurite outgrowth, viral/bacterial infection, and much more. This can be used to screen a variety of models including but not limited to, yeast, virus, bacteria, cells (live or fixed), tissue, TMAs, 3D culture models, and whole organisms (eg, zebrafish).



- Spatial distribution of targets in cells
- Individual cell and organelle morphology
- Combined multiple measurements per cell
- Multiple populations of cells isolating multiple phenotypes

Analysis & Screening of Cancer Targets and Mechanisms

Application Modules for Hundreds of Assays



Analysis & Screening of Cancer Targets and Mechanisms

- Angiogenesis/endothelial tube formation
- Bi- and multi-nucleated cell detection
- Biomarker analysis
- Budding yeast screening
- Cell counting
- Cell cycle analysis
- Cell migration
- Cell pathway analysis
- Cell proliferation
- Cell signaling by translocation
- Cell viability
- Channel and transporter uptake
- Clustering target molecules
- Cytotoxicity and apoptosis
- Fatty acid uptake, adipogenesis
- Kinase activation
- Micronuclei and genotoxicity analysis
- Mitochondrial and nuclear localization
- Mitosis/cell division
- Monopolar spindle detection
- Neurite outgrowth/ process extension
- Pathway analysis and multiplexing
- Protein expression/immunofluorescence
- Protein movement
- Protein phosphorylation
- Quantifying cellular punctate staining
- Ratiometric intracellular [Ca2+]
- Receptor internalization
- Receptor recycling
- Stem cells differentiation
- Studying intracellular structures
- Transfection efficiencies

Examples of High Content Imaging:

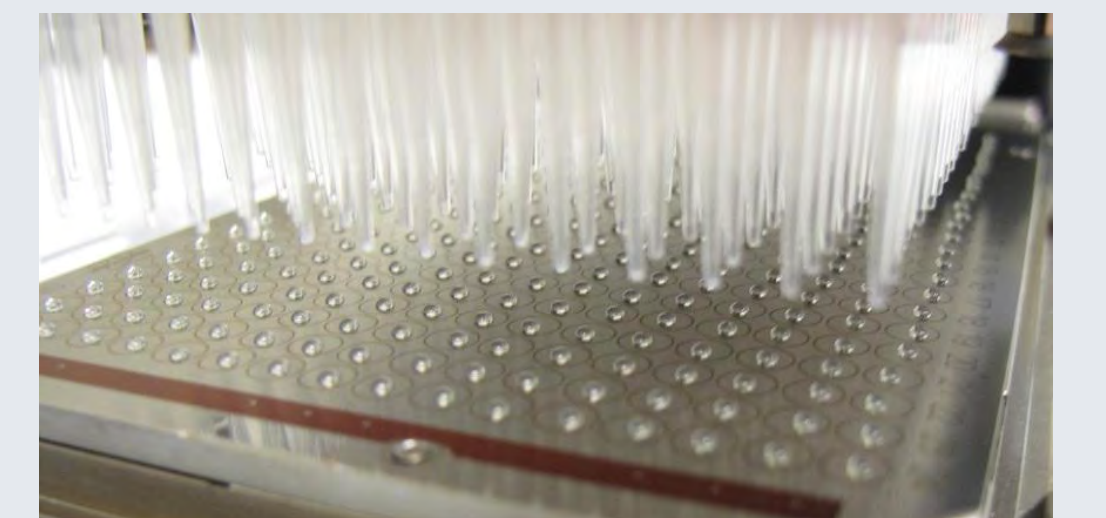
HTS Compound Library Screening

Pilot and Targeted Libraries

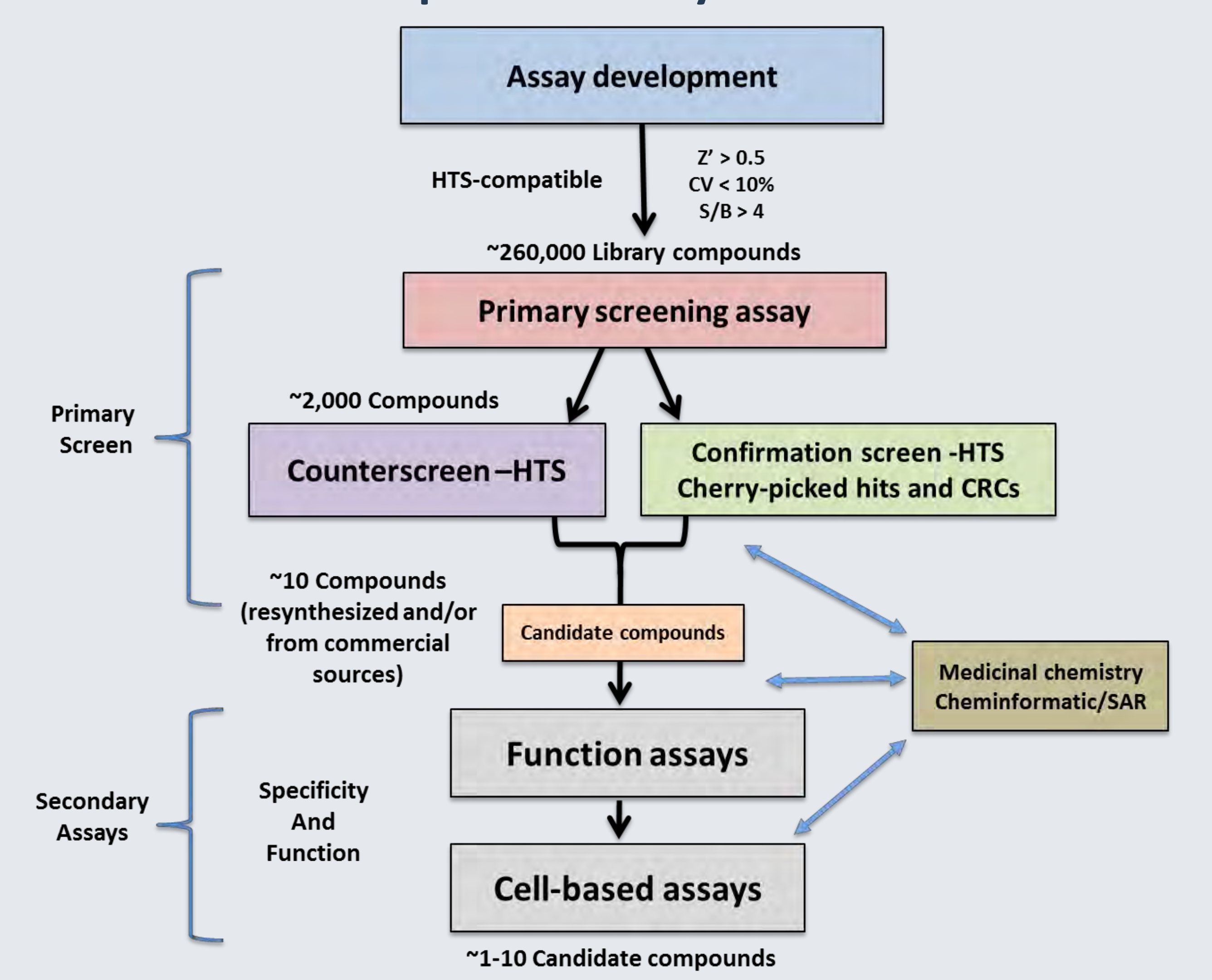
Collection	Description	#cmpds
Spectrum	Known bioactive cmpds	2,000
NIH Clinical I and II	cmpds with a history of human clinical trial use	730
Ion Channel	Ion channel targeted library	6,000
Kinase Inhibitor	3 sources: GSK, Roche, Enzo	665
Marnett Collection	NSAID derivatives	212
Fesik Fragment Library	Diverse collection of fragment molecules from 8 vendors	16,000
Cayman Lipid Library	Broad variety of bioactive lipids	1,000
Epigenetics Collection	Small molecule modulators with biological activity for use in epigenetic studies	51

Diverse Discovery

Collection	#compounds
VICB	160,000
Optimized Set	100,000



General compound library screen workflow



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