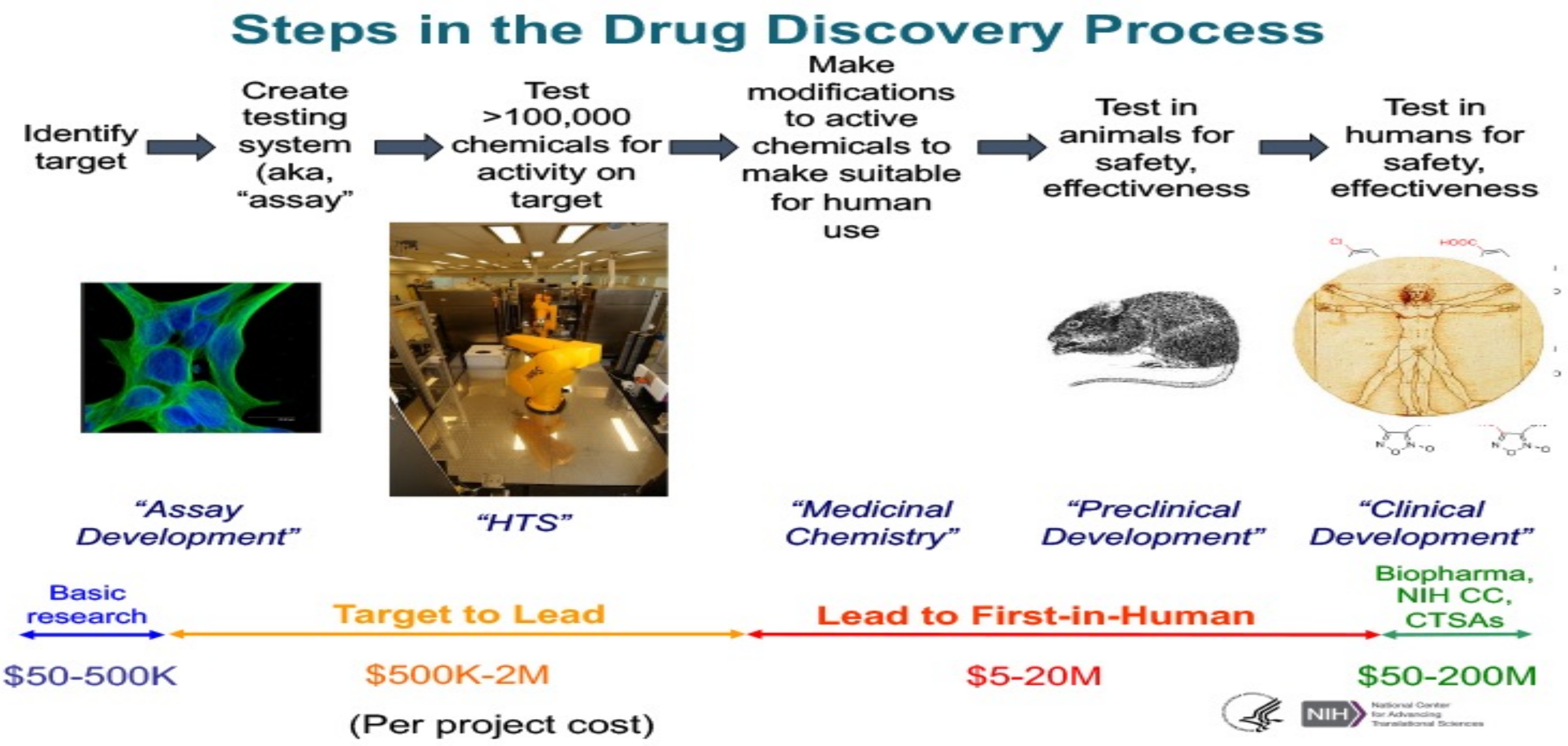
Small molecule discovery

Drug discovery steps

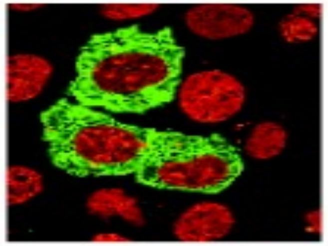


Screening assays

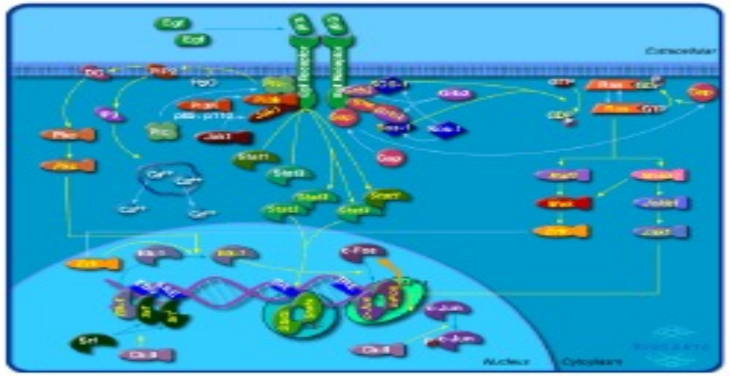
Range of Screening Assays

Extent of reductionism

Phenotype
(Image-based HCS, GFP, etc)

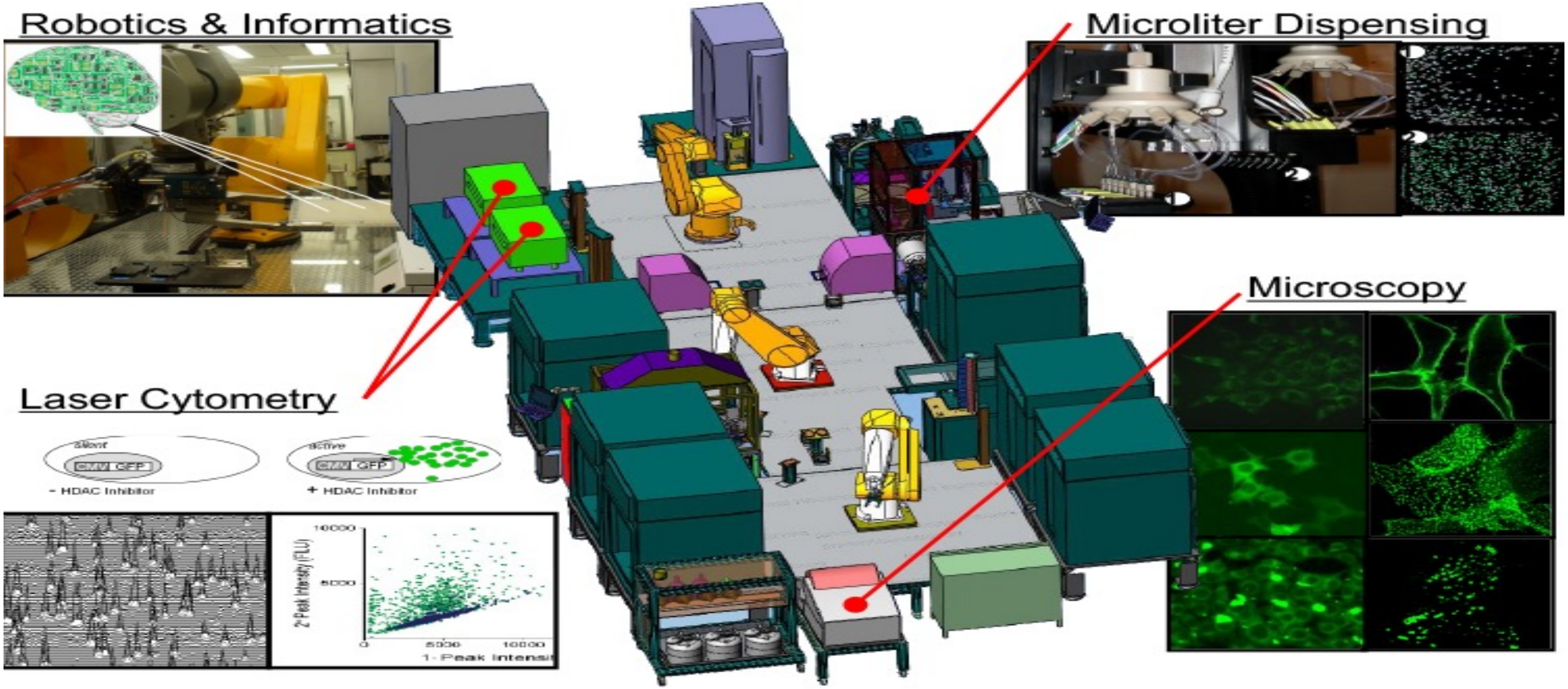


Pathway
(Reporters, e.g., luciferase, β-lactamase)

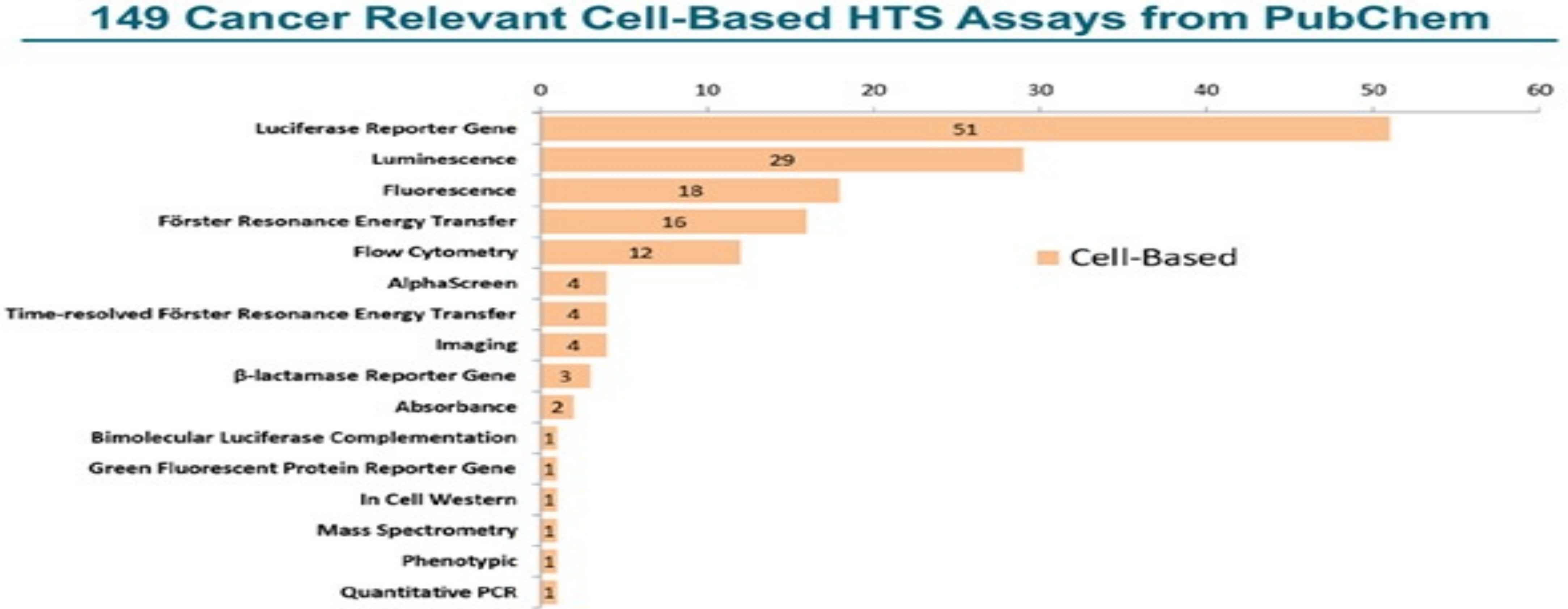


Protein
(Enzyme readouts, interactions, etc)

Robotics


Robotics & Informatics
Microliter Dispensing
Microscopy
Laser Cytometry
CMV GFP
- HDAC Inhibitor
+ HDAC Inhibitor
10000
5000
0
2° Peak Intensity (FLU)
1- Peak Intensi
National Center for Advancing Translational Sciences

HTS assays



Coussens, N. P., Braisted, J. C., Peryea, T., Sittampalam, S. G., Simeonov, A. and Hall, M. D.
Small Molecule Screens: A Gateway to Cancer Therapeutic Agents with Case Studies of FDA-Approved Drugs *Pharmacological Reviews*, October 2017, 69 (4) 479-496

Assay expense

Important Considerations for Choosing an Assay

- **Assay expense**
  - Cost per well
  - Disposal cost(s)

Instrumentation

Important Considerations for Choosing an Assay

- Assay expense
  - Cost per well
  - Disposal cost(s)
- **Available instrumentation**
  - **Select the best possible assays based on the available instrumentation**

Throughput

Important Considerations for Choosing an Assay

- Assay expense
  - Cost per well
  - Disposal cost(s)
- Available instrumentation
  - Select the best possible assays based on the available instrumentation
- **Assay throughput**
  - **Miniaturization reduces the cost per well**

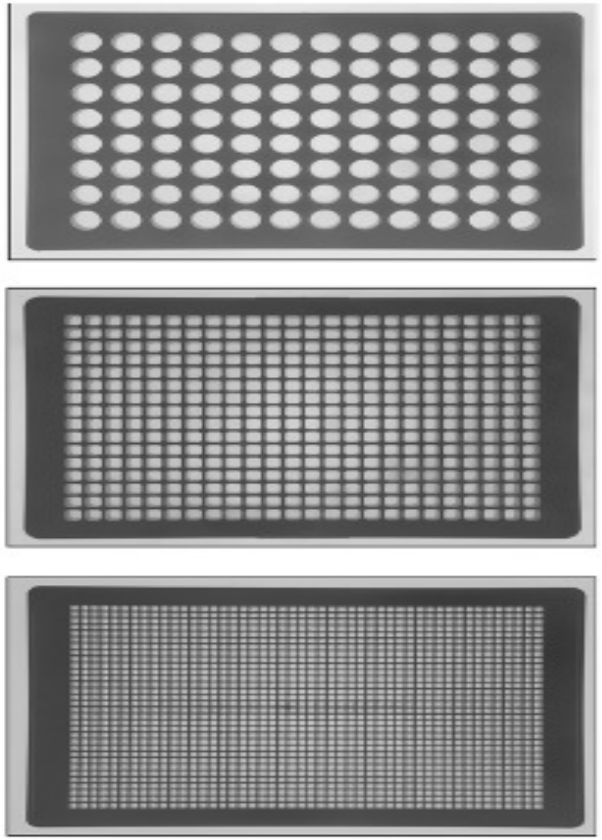
Miniaturization

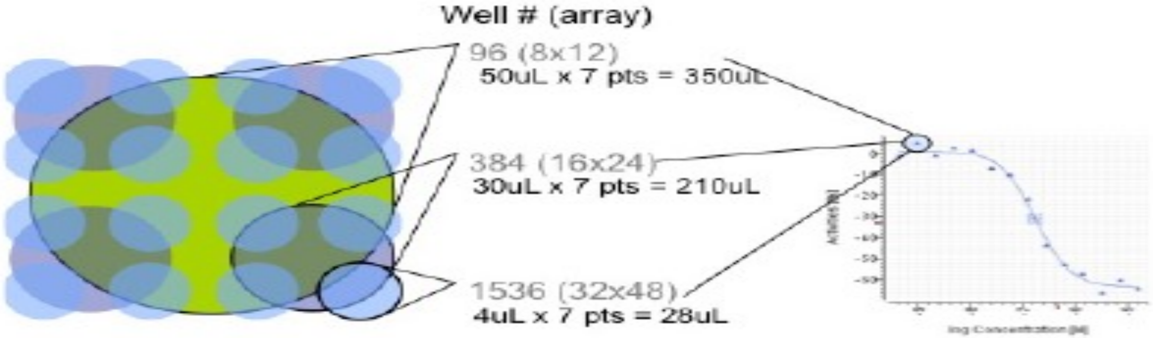
Assay Miniaturization Saves Time and Reagents

96-well
~50,000 cells/well

384-well
~5,000 cells/well

1536-well
~500 cells/well





	96	384	1536
Plates per 100,000 compounds:	1,042	261	66
Assay volume (uL):	50-200	30-50	2-8
Adherent cell seeding density:	~10,000	~2,000	~500

Horman, Shane R. "Complex High-Content Phenotypic Screening." Special Topics in Drug Discovery. InTech, 2016.

Multiplex

Important Considerations for Choosing an Assay

- Assay expense
  - Cost per well
  - Disposal cost(s)
- Available instrumentation
  - Select the best possible assays based on the available instrumentation
- Assay throughput
  - Miniaturization reduces the cost per well
- **Ability to multiplex**
  - **Can the response be measured by a single parameter; is multiparametric output possible?**
  - **Increased data per sample**
  - **Can guide hit slection by differentiating selectivity among related targets**
  - **Can distinguish pathway inhibition from cytotoxicity in a cell-based assay**

Reagents

Important Considerations for Choosing an Assay

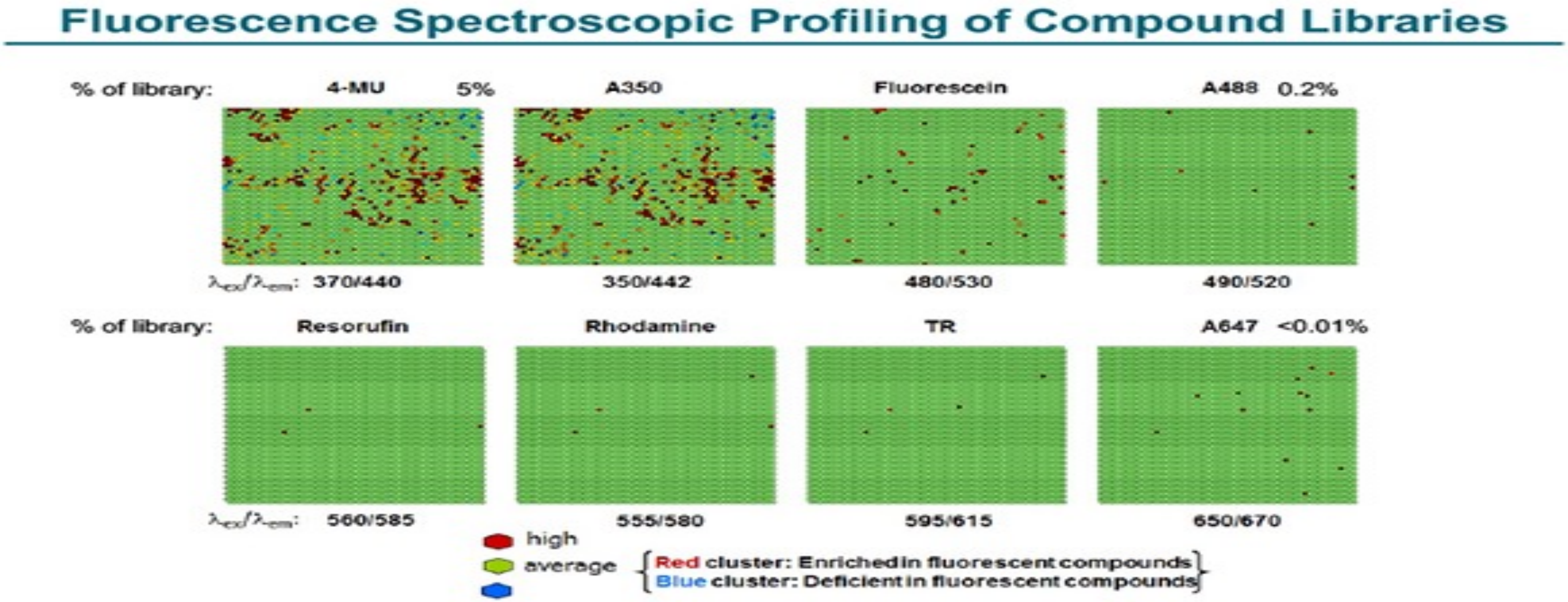
- Assay expense
  - Cost per well
  - Disposal cost(s)
- Available instrumentation
  - Select the best possible assays based on the available instrumentation
- Assay throughput
  - Miniaturization reduces the cost per well
- Ability to multiplex
  - Can the response be measured by a single parameter; is multiparametric output possible?
  - Increased data per sample
  - Can guide hit slection by differentiating selectivity among related targets
  - Can distinguish pathway inhibition from cytotoxicity in a cell-based assay
- **Reagents**
  - **Stablility for hours is important**
  - **Consistency is critical (ideally obtain a large quantity from a single lot)**
  - **All reagents need to be validated (cell lines, antibodies, enzymatic purity, etc.)**

Interference

Important Considerations for Choosing an Assay

- Assay expense
  - Cost per well
  - Disposal cost(s)
- Available instrumentation
  - Select the best possible assays based on the available instrumentation
- Assay throughput
  - Miniaturization reduces the cost per well
- Ability to multiplex
  - Can the response be measured by a single parameter; is multiparametric output possible?
  - Increased data per sample
  - Can guide hit slection by differentiating selectivity among related targets
  - Can distinguish pathway inhibition from cytotoxicity in a cell-based assay
- Reagents
  - Stablility for hours is important
  - Consistency is critical (ideally obtain a large quantity from a single lot)
  - All reagents need to be validated (cell lines, antibodies, enzymatic purity, etc.)
- **Potential for assay interference**
  - **Fluorescent compounds can interfere with fluorescent readouts**
  - **Colored compounds might interfere with luminescence**

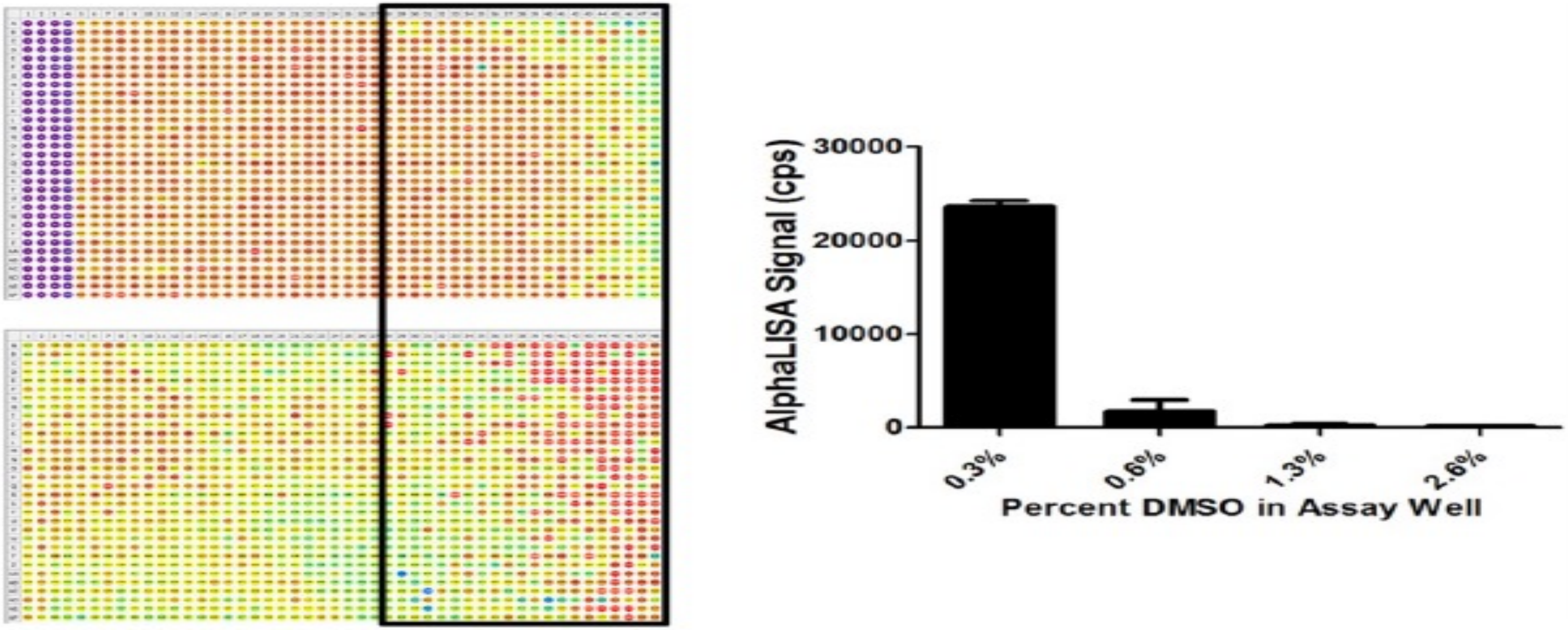
Spectroscopic profiling



Simeonov, A., Jadhav, A., Thomas, C.J., Wang, Y., Huang, R., Southall, N.T., Shinn, P., Smith, J., Austin, C.P., Auld, D.S. and Inglese, J., 2008. **Fluorescence spectroscopic profiling of compound libraries.** *Journal of Medicinal Chemistry*, 51(8), 2363-2371.

Tolerance to DMSO

Determination of Assay Tolerance to DMSO/Vehicle is Important



Yasgar A., Jadhav A., Simeonov A., Coussens N.P., **AlphaScreen-Based Assays: Ultra-High-Throughput Screening for Small-Molecule Inhibitors of Challenging Enzymes and Protein-Protein Interactions**. *Methods Mol Biol*. 2016;1439:77-98.

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Homogenous format

Important Considerations for Choosing an Assay

- **Homogenous assay format is preferred for screening**
  - Add reagents, mix and measure (no solution removal or wash steps)
  - Automation friendly
  - Reduces variability
  - Decreases hands-on time
  - Improves reproducibility

Time required

Important Considerations for Choosing an Assay

- Homogenous assay format is preferred for screening
  - Add reagents, mix and measure (no solution removal or wash steps)
  - Automation friendly
  - Reduces variability
  - Decreases hands-on time
  - Improves reproducibility
- **Time required for assay**
  - Off-line reagent preparation
  - Is temperature equilibration required
  - Actual assay time
  - Kinetic versus end point read
  - Time required for data analysis and record keeping

Signal stability

Important Considerations for Choosing an Assay

- Homogenous assay format is preferred for screening
  - Add reagents, mix and measure (no solution removal or wash steps)
  - Automation friendly
  - Reduces variability
  - Decreases hands-on time
  - Improves reproducibility
- Time required for assay
  - Off-line reagent preparation
  - Is temperature equilibration required
  - Actual assay time
  - Kinetic versus end point read
  - Time required for data analysis and record keeping
- **Signal stability**
  - **Does the response occur rapidly or within a few minutes or hours?**
  - **Longer signal stability allows for flexibility in automated systems**
  - **Longer signal stability minimizes differences among plates within a stack**

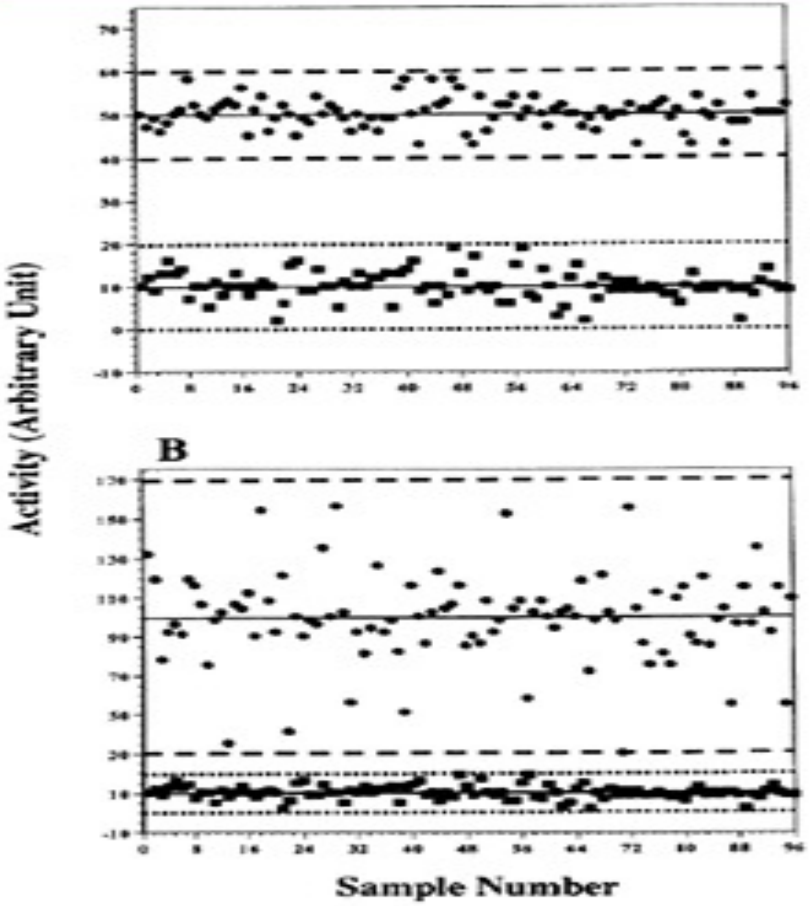
Sensitivity

Important Considerations for Choosing an Assay

- Homogenous assay format is preferred for screening
  - Add reagents, mix and measure (no solution removal or wash steps)
  - Automation friendly
  - Reduces variability
  - Decreases hands-on time
  - Improves reproducibility
- Time required for assay
  - Off-line reagent preparation
  - Is temperature equilibration required
  - Actual assay time
  - Kinetic versus end point read
  - Time required for data analysis and record keeping
- Signal stability
  - Does the response occur rapidly or within a few minutes or hours?
  - Longer signal stability allows for flexibility in automated systems
  - Longer signal stability minimizes differences among stacks of plates
- **Assay Sensitivity**
  - **Choice of readouts is important**
    - **Colorimetric<fluorescent<luminescent**

Evaluating assay

Evaluating Assay Suitability for Screening



S/N = 12

S/B = 5

z = 0.5

S/N = 27

S/B = 10

z = 0.1

$$S/N = \frac{\text{mean signal} - \text{mean background}}{\text{standard deviation of background}}$$

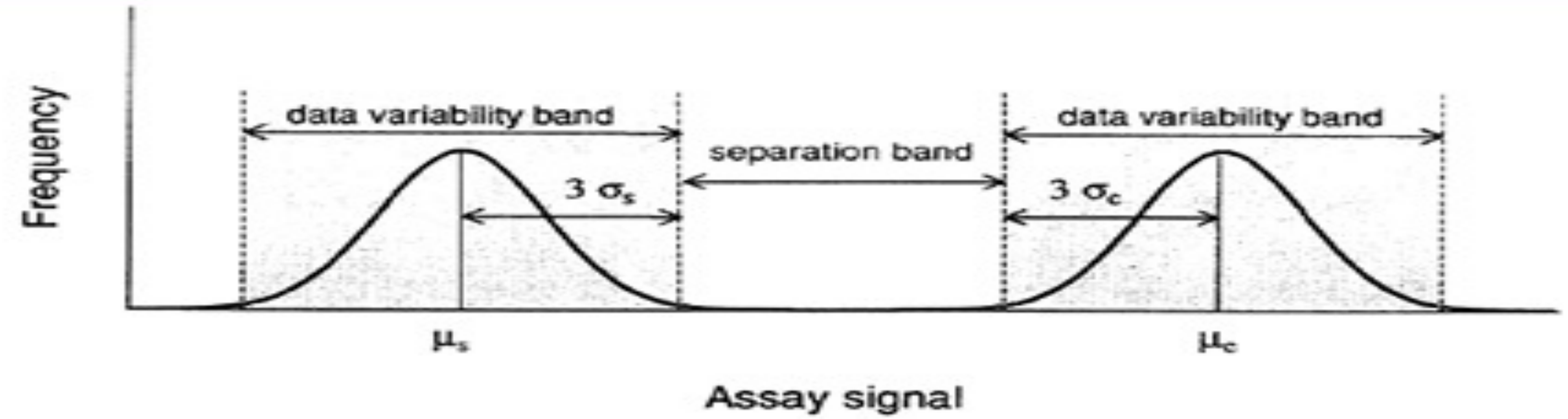
$$S/B = \frac{\text{mean signal}}{\text{mean background}}$$

$$Z = 1 - \frac{3SD \text{ of sample} + 3SD \text{ of control}}{|\text{mean of sample} - \text{mean of control}|}$$

A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Zhang JH, Chung TD, Oldenburg KR. J Biomol Screen. 1999;4(2):67-73.

Suitability

Evaluating Assay Suitability for Screening



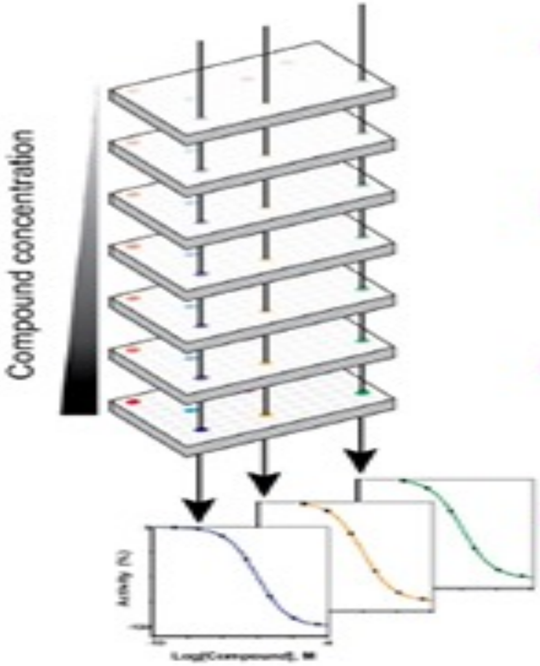
$$Z = 1 - \frac{3SD\ \text{of sample} + 3SD\ \text{of control}}{|\text{mean of sample} - \text{mean of control}|}*$$

Z-factor value	*Structure of assay*	*Related to screening*
1	SD = 0 (no variation), or the dynamic range → ∞	An ideal assay
$1 > Z \geq 0.5$	Separation band is large	An excellent assay
$0.5 > Z > 0$	Separation band is small	A double assay
0	No separation band, the sample signal variation and control signal variation bands touch	A "yes/no" type assay
<0	No separation band, the sample signal variation and control signal variation bands overlap	Screening essentially impossible

A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Zhang JH, Chung TD, Oldenburg KR. J Biomol Screen. 1999;4(2):67-73.

Quantitative HTS

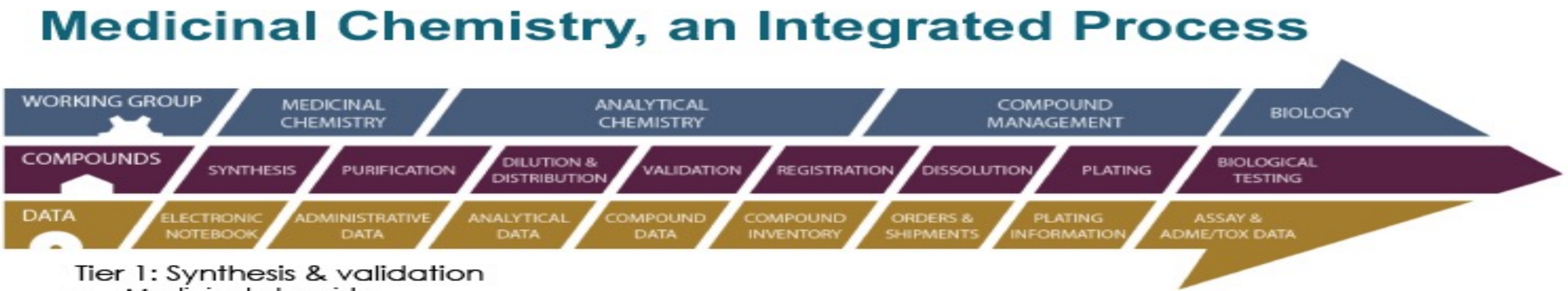
Improving the Process of Early Discovery: Quantitative High-Throughput Screening (qHTS)



- Conventional screening done at one concentration
  - Not appropriate for potency testing – "dose makes the poison"
- qHTS tests compounds assayed at multiple concentrations (range: 4 logs)
- Enabled by miniaturized assay volumes (2-8 µL per test) and informatics pipeline
- Generates *pharmacological actives* instead of statistical "hits"
  - Dramatically increases reliability
  - Dramatically reduces false positives and false negatives
- *To date, several hundred million datapoints from several hundred screens have been generated and deposited in the public domain.*

PNAS 103:11473

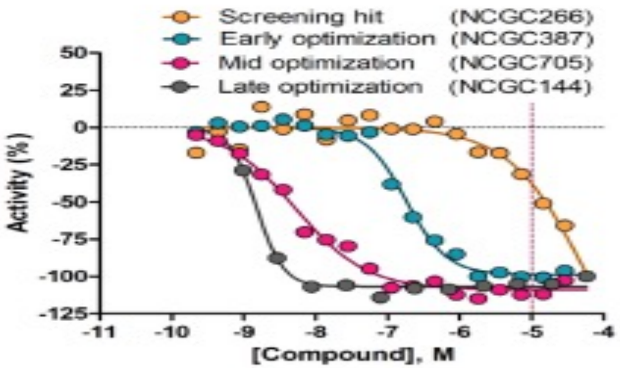
Medicinal chemistry

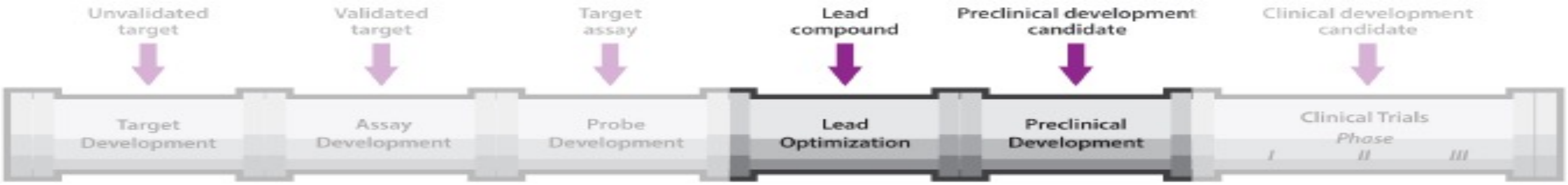


Tier 1: Synthesis & validation
- Medicinal chemistry
- Purification
- In vitro ADME

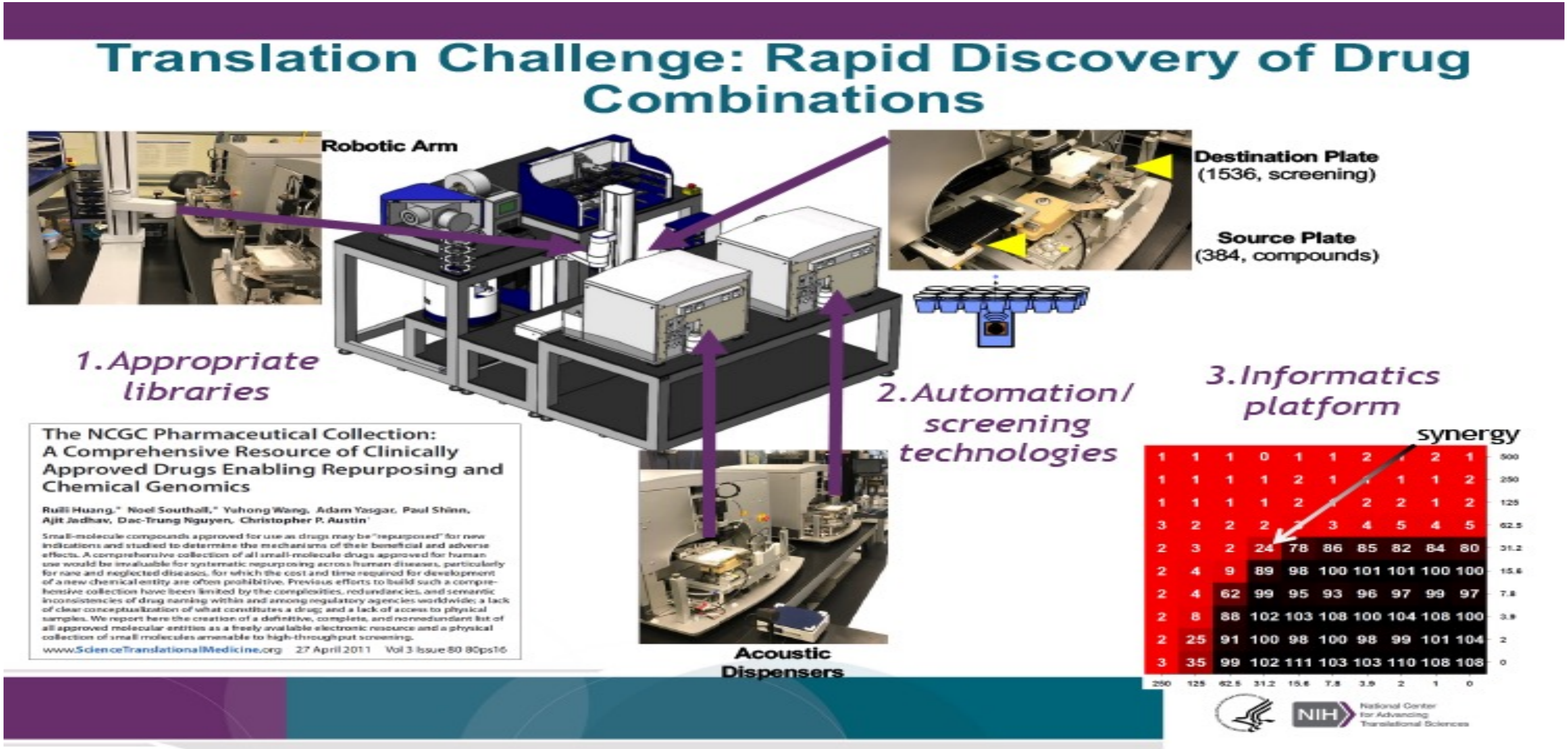
Tier 2: Compound profile expansion
- Met ID/CYP studies
- In vitro toxicology

Tier 3: Advanced Preclinical studies
- Formulation
- Scale-up
- In vivo PK/PD
- Preclinical toxicology

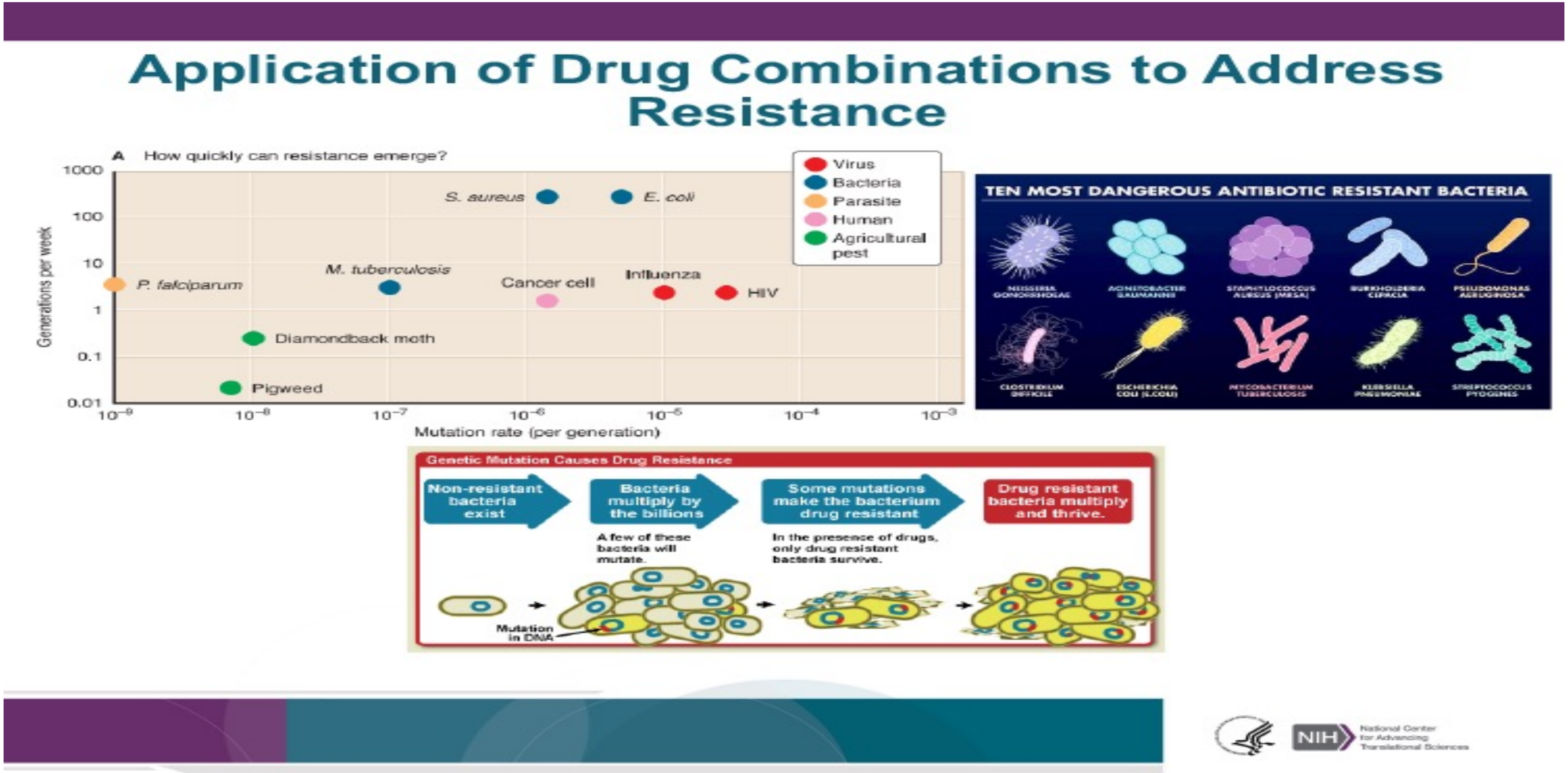




Drug combinations



Resistance



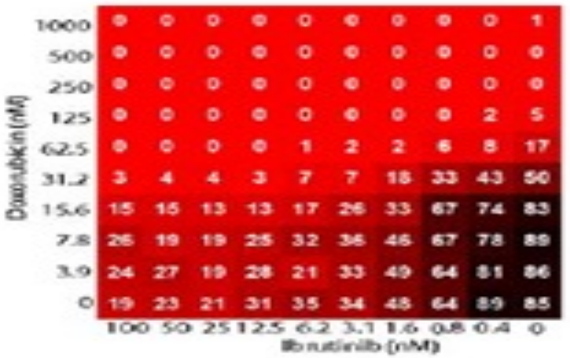
Application of Drug Combinations to Address Resistance
A How quickly can resistance emerge?
Generations per week
1000
100
10
1
0.1
0.01
S. aureus
E. coli
M. tuberculosis
P. falciparum
Cancer cell
Influenza
HIV
Diamondback moth
Pigweed
Virus
Bacteria
Parasite
Human
Agricultural pest
10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵ 10⁻⁴ 10⁻³
Mutation rate (per generation)
TEN MOST DANGEROUS ANTIBIOTIC RESISTANT BACTERIA
Genetic Mutation Causes Drug Resistance
Non-resistant bacteria exist
Bacteria multiply by the billions
Some mutations make the bacterium drug resistant
Drug resistant bacteria multiply and thrive.
A few of these bacteria will mutate.
In the presence of drugs, only drug resistant bacteria survive.
Mutation in DNA
NIH

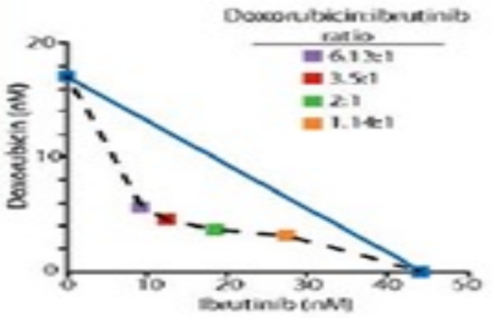
Dissemination

Dissemination of technology: combination screening to overcome drug resistance in cancer cells

- Applied to ABC subtype of Diffuse Large B-Cell Lymphoma (ABC-DLBCL)
- Ibrutinib is a BTK inhibitor that has activity against ABC DLBCL
- Lead investigators: Craig Thomas (NCATS) and Louis Staudt (NCI)
- Study evaluated 459 drugs *in combination* with Ibrutinib
  - » 6 x 6 concentration-response "matrix blocks", validation in 10 x 10 concentration-response matrix blocks
- DNA-damaging agents identified as synergizing with Ibrutinib in killing ABC DLBCL cell lines
- **Dissemination:**
  - » Protocols
  - » Source code for dispense

High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell–like diffuse large B-cell lymphoma cells



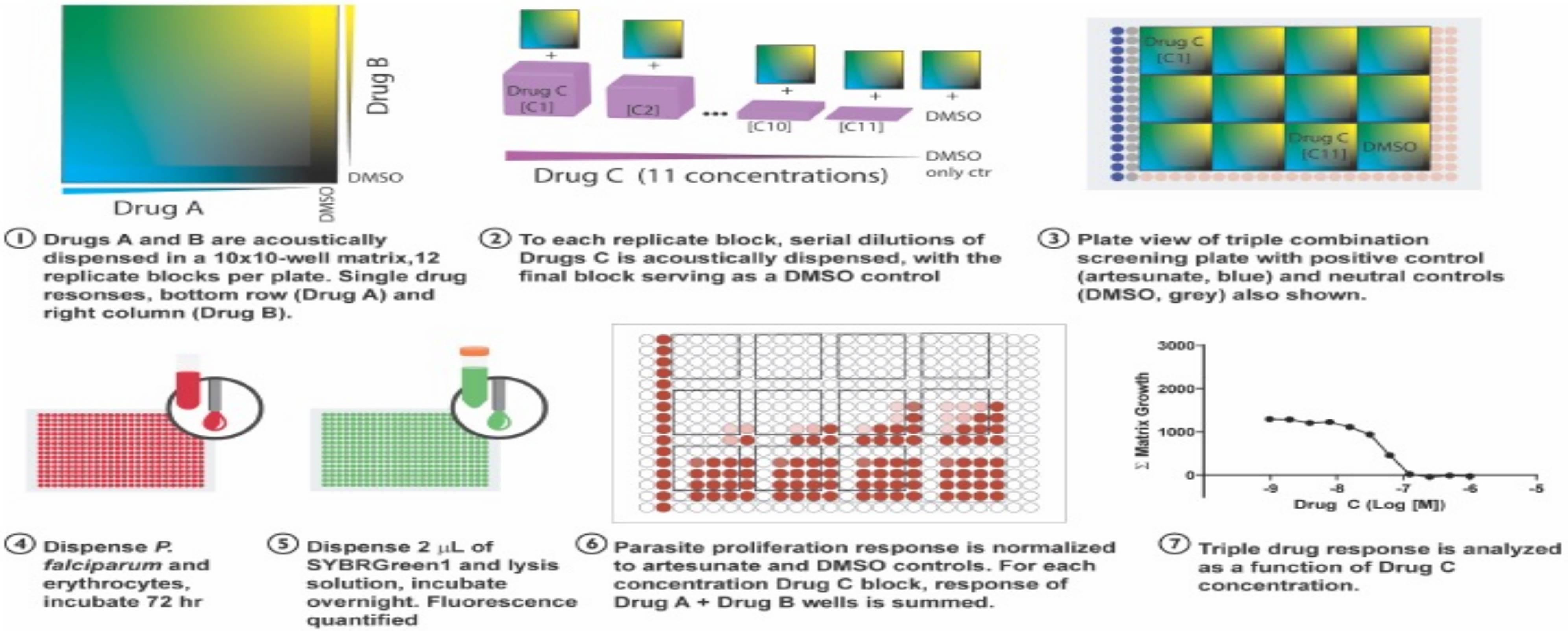


PNAS 111, 2349-2354

Triple drug combination

Example: triple drug combination screening to tackle resistance against artemisinin-based combination therapies in malaria

ACS Pharmacol. Transl. Sci. 2020, https://dx.doi.org/10.1021/acsptsci.0c00110?ref=pdf



VIPOR combination

ClinicalTrials.gov

Venetoclax, Ibrutinib, Prednisone, Obinutuzumab, and Revlimid (VIPOR) in Relapsed/Refractory B-cell Lymphoma

ViPOR Regimen Is Safe, Shows Impressive Activity in Relapsed/Refractory DLBCL

https://www.onclive.com/view/vipor-regimen-is-safe-shows-impressive-activity-in-relapsed-refractory-dlbcl

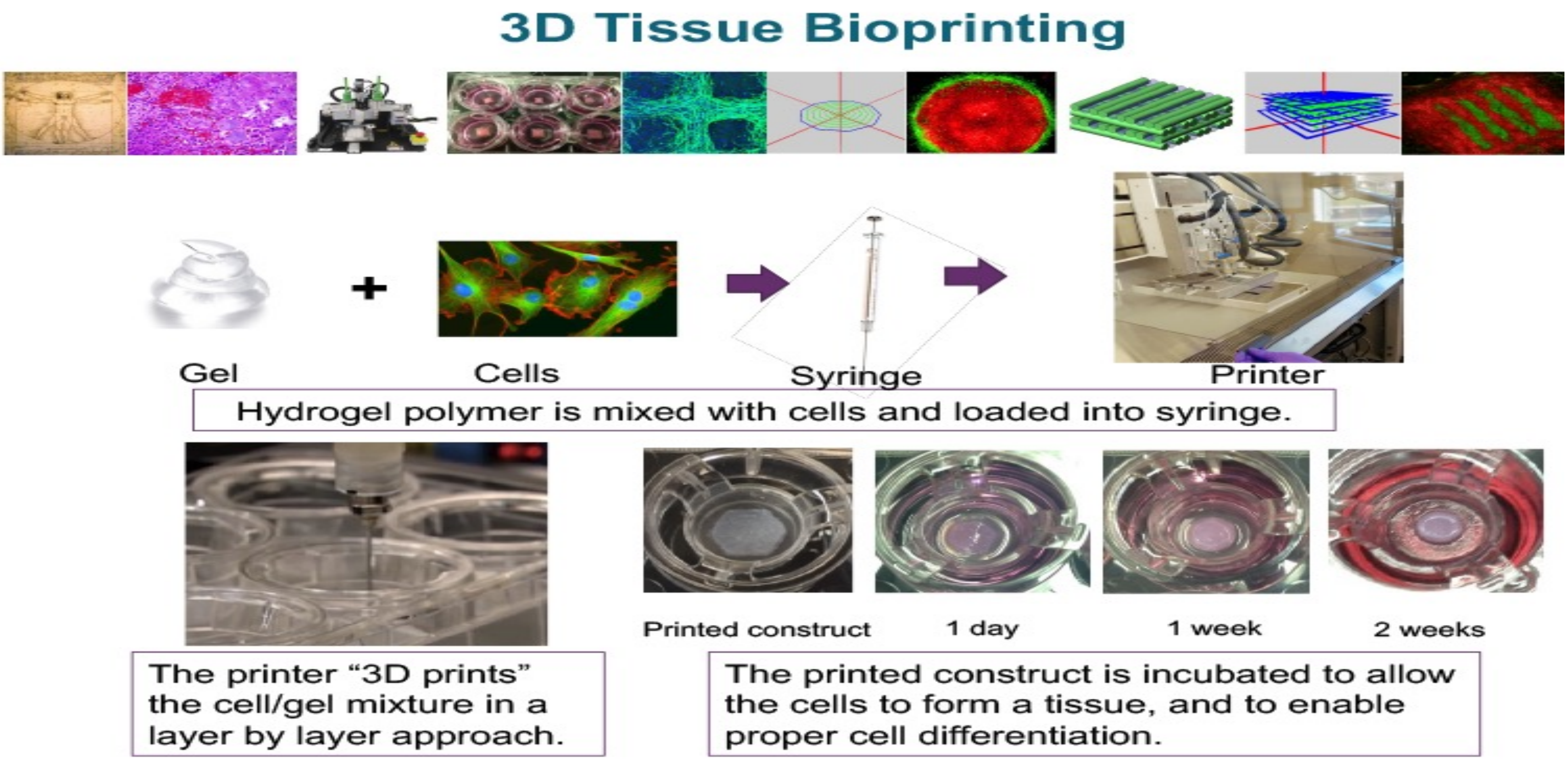
ViPOR Regimen Signals Benefit in Patients With Mantle Cell Lymphoma

https://www.targetedonc.com/view/vipor-regimen-signals-benefit-in-patients-with-mantle-cell-lymphoma

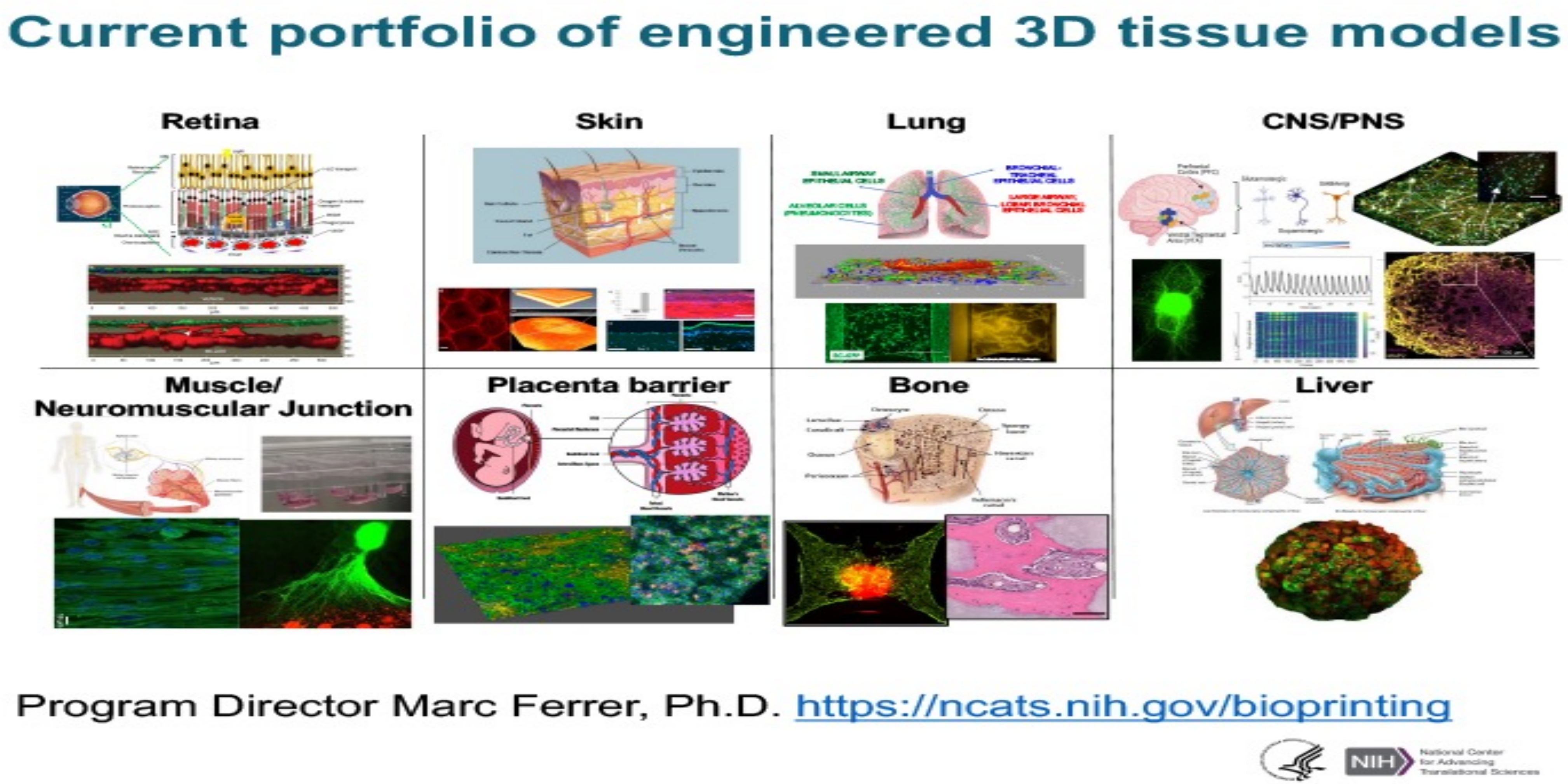
3D models


Increasing the predictivity of *in vitro* assays: a continuum of 3D models of healthy and diseased tissues
2D
Spheroids
Organoids
Printed Tissues
Organ-on-a-chip
Physiological complexity
HTS compatibility
NIH
National Center for Advancing Translational Sciences

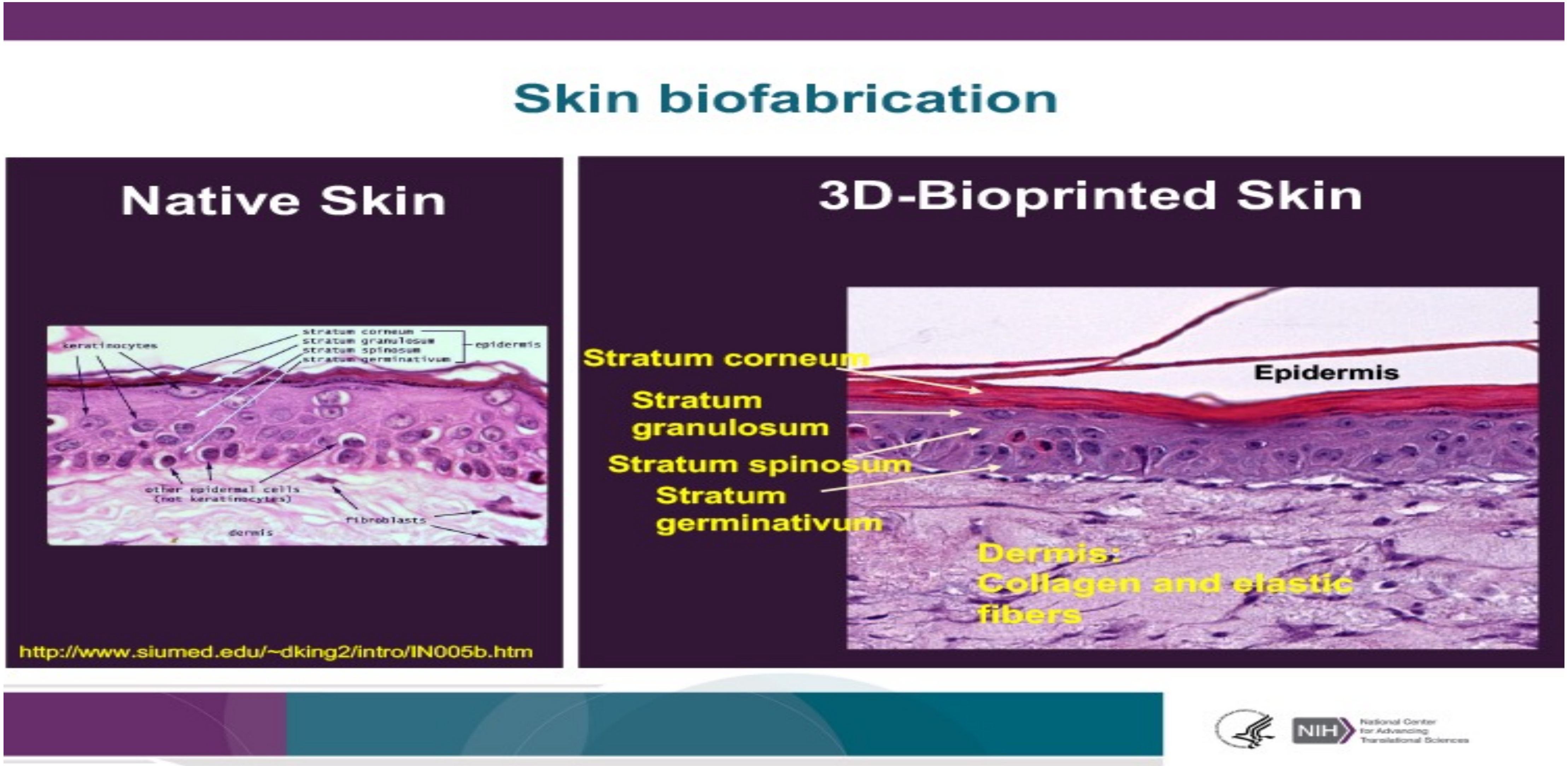
Tissue bioprinting



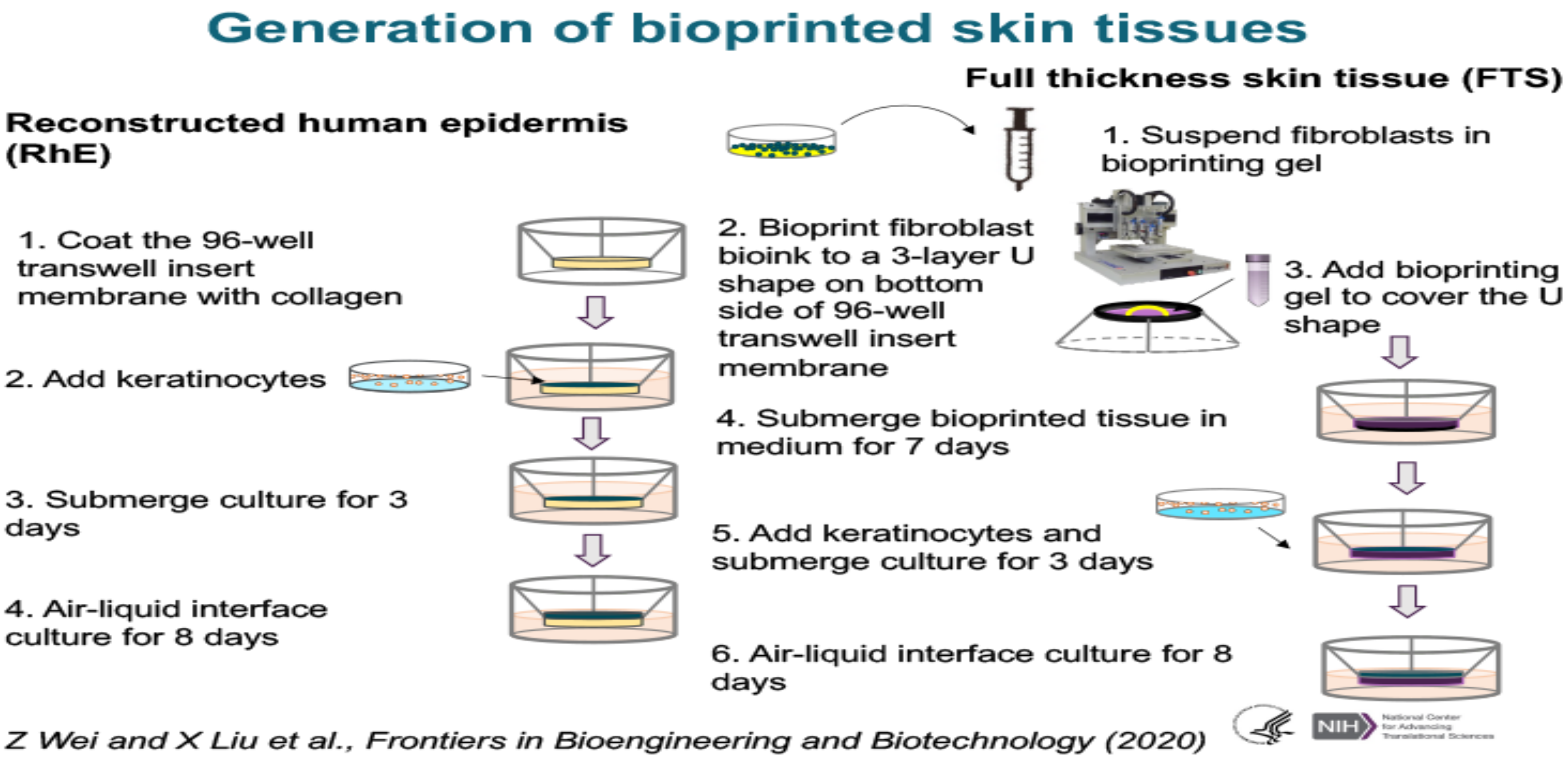
3D tissue models



Skin biofabrication



Skin tissue generation

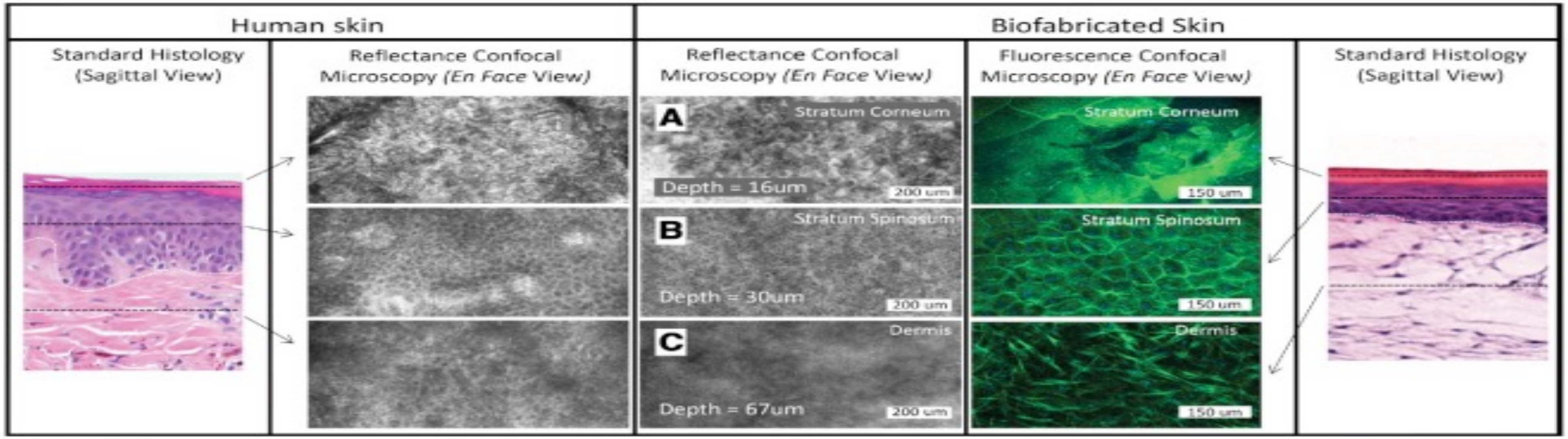


Tissue model

www.oncotarget.com Oncotarget, 2020, Vol. 11, (No. 27), pp: 2587-2596

Research Paper

A 3D biofabricated cutaneous squamous cell carcinoma tissue model with multi-channel confocal microscopy imaging biomarkers to quantify antitumor effects of chemotherapeutics in tissue

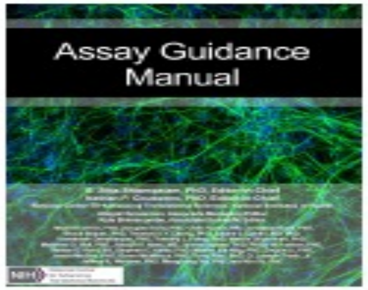


Collaboration between NCATS (Marc Ferrer) and Rockefeller University (Daniel Gareau)

Information

Book

Sharing internal know-how: Assay Guidance Manual (47 chapters/ 1,338 printed pages)



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In Vitro Biochemical Assays	10 Chapters
In Vitro Cell Based Assays	19 Chapters
In Vivo Assay Guidelines	2 Chapters
Assay Artifacts and Interferences	4 Chapters
Assay Validation, Operations and Quality Control	5 Chapters
Assay Technologies	2 Chapters
Instrumentation	2 Chapters
Pharmacokinetics and Drug Metabolism	1 Chapter
Glossary of Quantitative Biology Terms	1 Chapter

Website: https://ncats.nih.gov/expertise/preclinical/agm

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Facebook: www.facebook.com/assayguide

LinkedIn

Linkedin: www.linkedin.com/groups/7437344

https://ncats.nih.gov/agm-video

August 7th Videos

1. Austin, CP: **Welcome to the Assay Guidance Manual (AGM) Workshop**
2. Coussens, NP: **Strategies for Assay Selection & Robust Biochemical Assays**
3. Riss, T: **Treating Cells as Reagents to Design Reproducible Screening Assays**
4. Trask, OJ: **Assay Development Considerations for High Content Imaging**
5. Auld, DS: **Studies in Mechanisms and Methods in Assay Interferences**
6. Dahlin, JL: **Assay Interference by Chemical Reactivity**
7. Chung, TDY: **Basic Assay Statistics, Data Analysis & Rules of Thumb**
8. Devanarayan, V: **Reproducibility & Differentiability of Potency Results**
9. Sittampalam, GS: **Avoiding Artifacts & Interferences in Assay Operations**

March 26-27th Videos

1. Austin, CP: **Welcome to the Assay Guidance Manual (AGM) Workshop**
2. Coussens, NP: **Robust Assays Define Success in Preclinical Research**
3. Lal-Nag, M: **Target Identification & Validation in Translational Discovery**
4. Foley, TL: **Development & Validation of Cell-Based and Biochemical Assays**
5. Riss, T: **Treating Cells as Reagents to Design Reproducible Screening Assays**
6. Trask, OJ: **Assay Development for HCS & Best Practices for 3D HCS**
7. Roth, KD: **Mass Spectrometry for Drug Screening and Lead Optimization**
8. Dahlin, JL: **Bioassay Interference by Aggregation and Chemical Reactivity**
9. Patnaik, S: **Lead Selection and Optimization by Medicinal Chemistry**
10. Xia, M: ***In Vitro* Toxicological Testing Using a qHTS Platform**
11. Xu, X: ***In Vitro* Assessment of ADME Properties of Lead Compounds**
12. Kahl, SD: **Statistical Design of Experiments for Assay Development**
13. Guha, R: **Pharos Application to Target Evaluation and Drug Discovery**
14. Weidner, JR: **Assay Operations: Keeping Assays Robust and Reproducible**

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Assay guidance manual

Assay Guidance Manual Training Workshops

- Online Training Modules
- Upcoming Workshops
- Past Workshops

NCATS offers a variety of Assay Guidance Manual (AGM) training workshops throughout the year designed to share best practices and advice on robust assay design, development and implementation for researchers involved in the drug discovery process.

Online Training Modules

NCATS offers an online AGM training workshop in addition to the in-person AGM workshops held throughout the year. The online training workshop also features experts sharing best practices and expert advice on assay design, development and implementation. **View the video modules.**

NIH National Center for Advancing Translational Sciences

Assay Guidance Workshop on 3D Tissue Models for Antiviral Drug Development

Tue Jun 7, 11:00 AM - Wed Jun 8, 5:15 PM (EDT)

Zoom

Add to calendar ▾ Share ▾

THIS EVENT HAS ENDED

Video of the workshop is available at the links below:

Assay Guidance Workshop on 3D Tissue Models for Antiviral Drug Development (Day 1) (June 7, 2022)

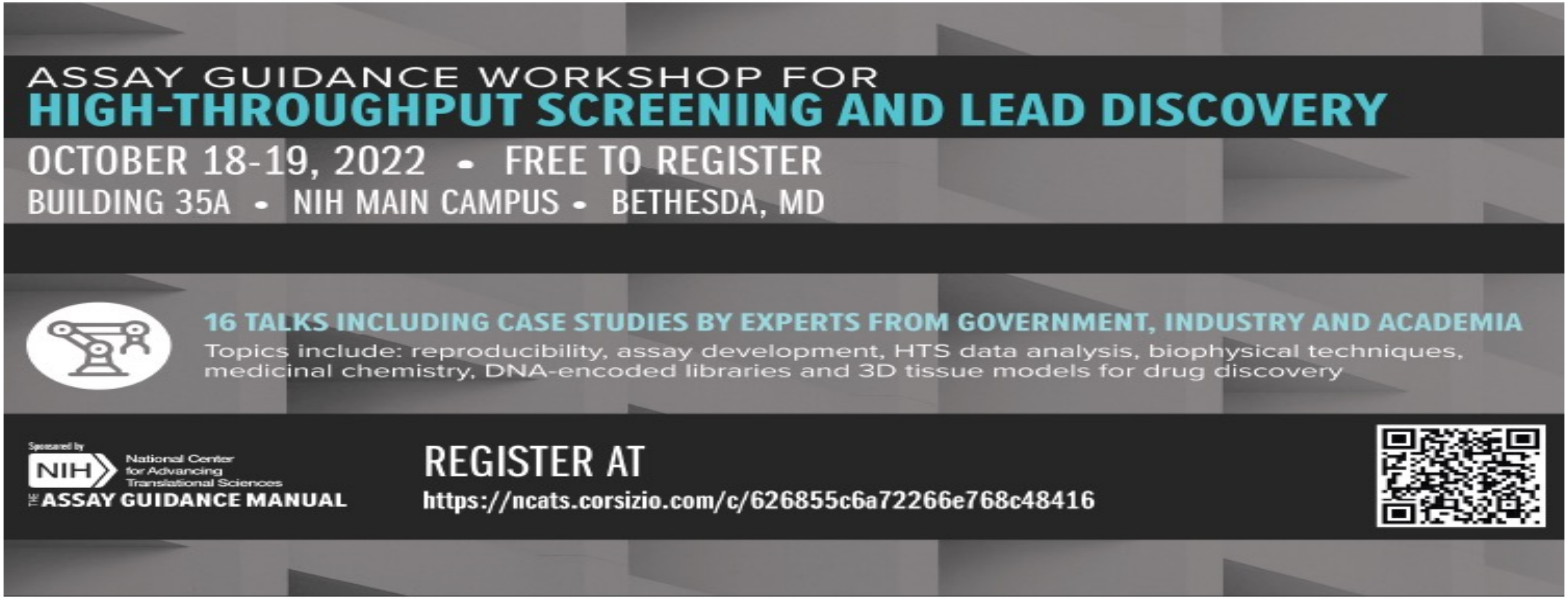
Assay Guidance Workshop on 3D Tissue Models for Antiviral Drug Development (Day 2) (June 8, 2022)

About the Workshop

The National Center for Advancing Translational Sciences (NCATS) Assay Guidance Manual (AGM) program is hosting a two-day workshop that will cover a broad range of critical concepts, including practical approaches and best practices, for developing standardized 3D cellular assays with the hope of helping the community to successfully develop therapeutics for future pandemic threats. This workshop is jointly organized by NCATS, the National Institute of Allergy and Infectious Diseases (NIAID) and the Bill & Melinda Gates Foundation. The overall goal of this workshop is to help scientists establish robust, reproducible, scalable, consistent, advanced 3D tissue models to study pandemic threat viruses.

https://ncats.nih.gov/expertise/preclinical/agm/training

WORKSHOP


ASSAY GUIDANCE WORKSHOP FOR
HIGH-THROUGHPUT SCREENING AND LEAD DISCOVERY
OCTOBER 18-19, 2022 • FREE TO REGISTER
BUILDING 35A • NIH MAIN CAMPUS • BETHESDA, MD
16 TALKS INCLUDING CASE STUDIES BY EXPERTS FROM GOVERNMENT, INDUSTRY AND ACADEMIA
Topics include: reproducibility, assay development, HTS data analysis, biophysical techniques, medicinal chemistry, DNA-encoded libraries and 3D tissue models for drug discovery
Sponsored by
NIH National Center for Advancing Translational Sciences
THE ASSAY GUIDANCE MANUAL
REGISTER AT
https://ncats.corsizio.com/c/626855c6a72266e768c48416

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