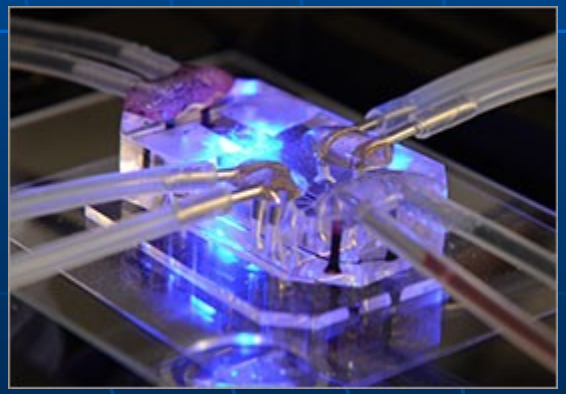
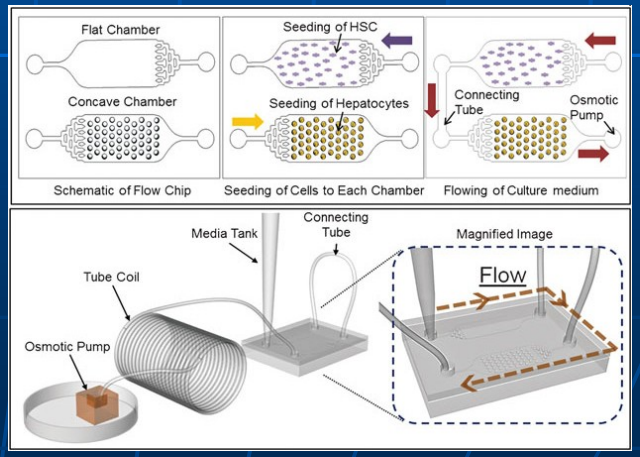
Validation of *in vitro* Devices: What Might That Look Like?

Validation of in vitro tools and models for preclinical drug discovery





Motivation for validation of new in vitro technologies

We want the correct answer…. an accurate comparison of in vitro results with clinical results

Requires data to support that claim.

Caveats:
What is the correct answer?
Biological variability of patients. (which patient is device representing?)
What entity(ies) should be measured that accurately relates assay output (measurand) and a clinical response.

- If assay data are noisy, it will be impossible to recognize correlations between measurand and clinical response.
- if we don't know the uncertainty or the sensitivity of our in vitro data, we may conclude that there is no correlation because we don't know that the assay is too insensitive to detect it.

Thought process for validation

What is (are) the desired function(s) of the device? (ex. stimulation of immune response; liver toxicity)

What will actually be measured (ex. Secretion of IL8; oxidation product of acetophenamin). (May require >1 measurand to evaluate function.)

How well does/do measurand(s) report function? [MARKER VALIDATION] How do you test that?

How well can you measure the measurand in your device?: reproducibility, accuracy, robustness, dynamic range, response function. Are the control experiments for reproducibility, robustness, etc. well documented? [ANALYTICAL VALIDATION]

On what basis have you designated limits for device performance, outside of which the results are considered invalid? [DEVICE VALIDATION]

Do the results predict clinical outcome [CLINICAL VALIDATION]

Development Stages and Validation

Basic science. Hypothesis re mechanisms

Refine Assays and measurements

Design/construct device and measurement stream

Validate analytical response of device

Comparative analysis to clinical data

Define limits of validated response

Accuracy
Precision
Ruggedness
Dynamic range
Response function
Specificity

Numerical limits for acceptable standard deviations, detection limit, response function, etc.

Accuracy
Precision
Ruggedness
Dynamic range
Response function
Specificity

Characteristic of Validated Assays

Accuracy: Orthogonal method
Precision: Repeatability (replicates); Reproducibility (different days, locations, technicians
Ruggedness: sensitivity to assay parameters
Dynamic range and Response function: Instrument benchmarking. +/- controls. Calibration curve. Limit of detection
Specificity: sensitivity to matrix effects/ impurities

Numerical limits for acceptable standard deviations, detection limit, response function, etc.

When we get a negative result from a sample, we want to have confidence that the assay accurately reports the characteristic of the sample and be assured that the result is not because of failure of the assay.

Validation entails understanding a device/assay sufficiently well that you know how it should perform every time it is run.

Special Components Requiring Preclinical Validation

Cells/ Characteristics
- Source and identity
- Reproducibility of source
- Conditions of handling/
- stability over time

Validate Cells:
Cell Line ID
Gene/ Protein expression
Growth rate
Morphology

Function/activity
- Response to drug
- Response to control
- Is measurement an accurate indicator of response

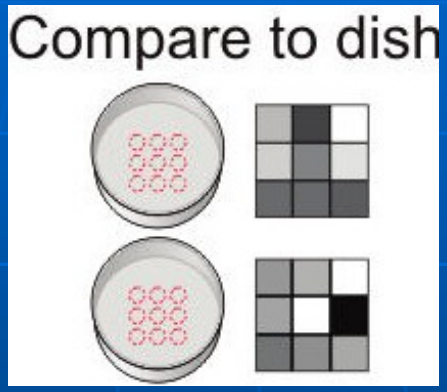
Validate that response that is being measured to drug and control is relevent to MOA

Device
- Materials
- Interaction with cells/reagents
- Reproducibility

?????

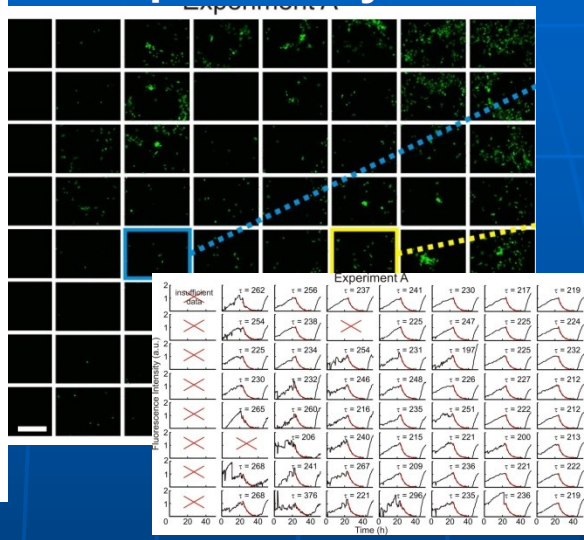
Reproducibility/Robustness in Microfluidics

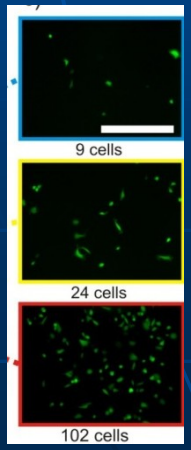
Accuracy



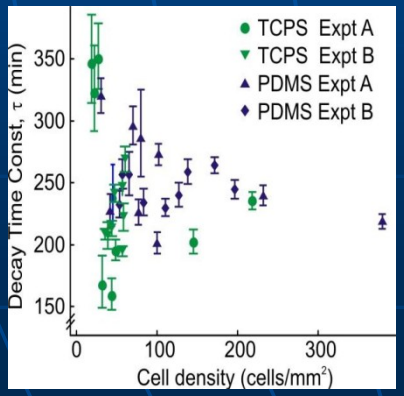
Accuracy
Precision
  Repeatability
  Reproducibility
Ruggedness
Dynamic range
Specificity

Repeatability



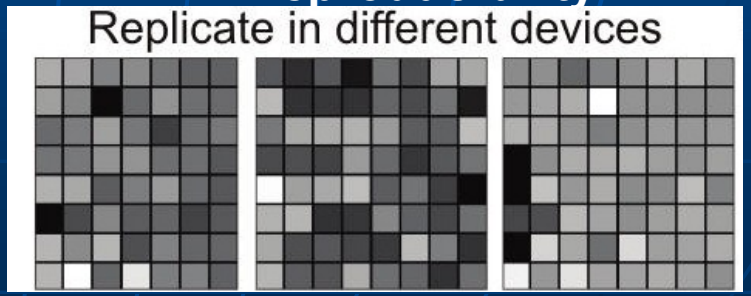


Ruggedness



Differences in cell number, tubing, lamp, flow rate, position in chamber

Reproducibility

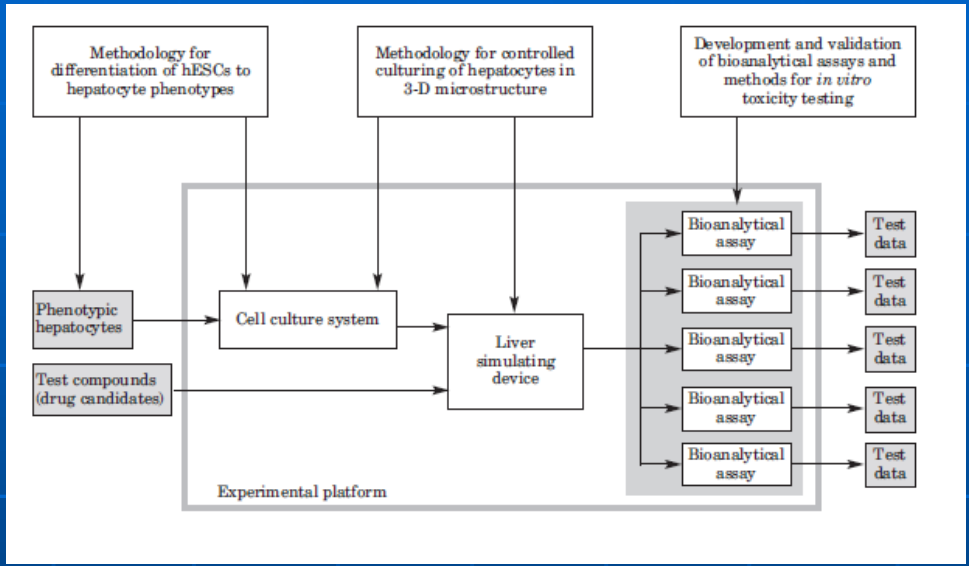


Cooksey et al Anal Chem 2011

Challenge of Working with Living Reagents

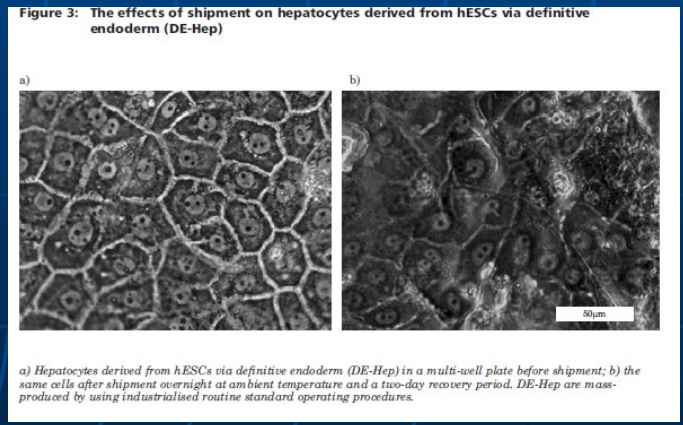
Robustness

- Source of living materials
- Transient nature of the stability of living components
- Manufacturing consistency



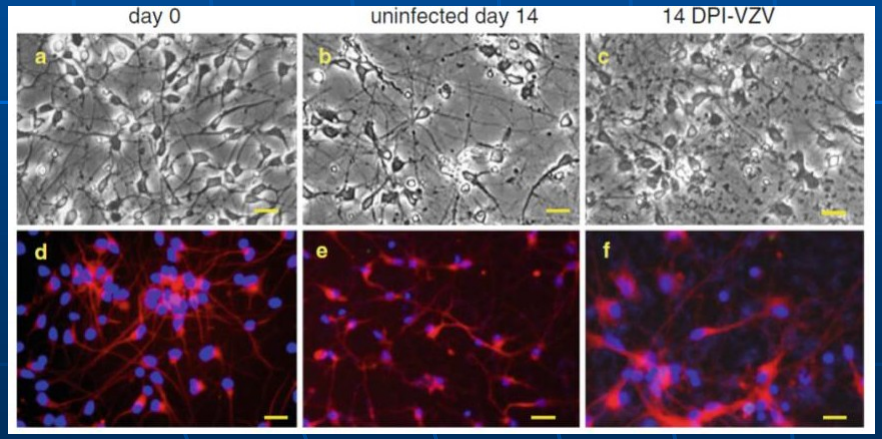
http://www.frame.org.uk/dynamic_files/39mandenius.pdf

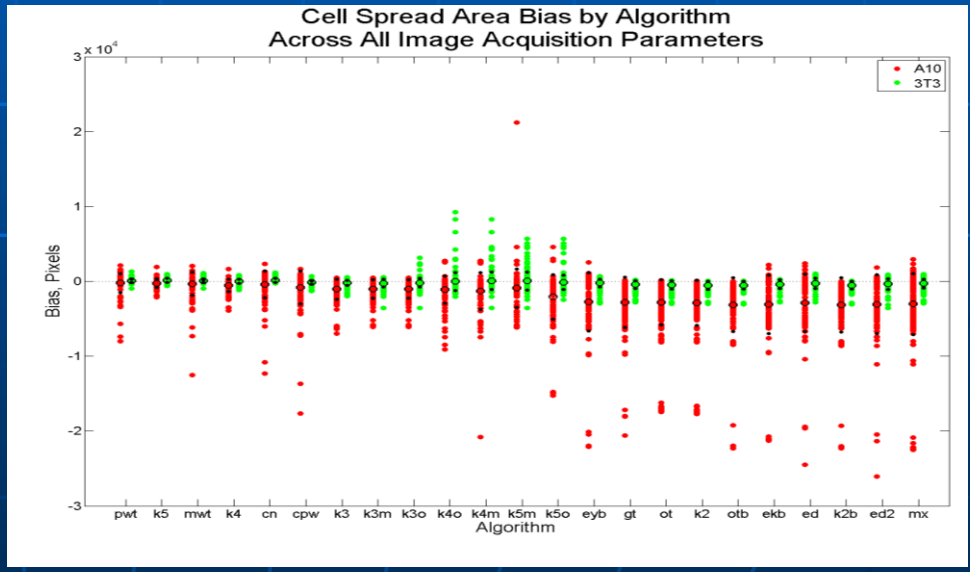
Validation will be an ongoing and interative process



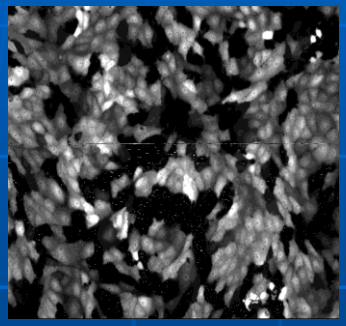
Evaluating the Accuracy of Analytical Software

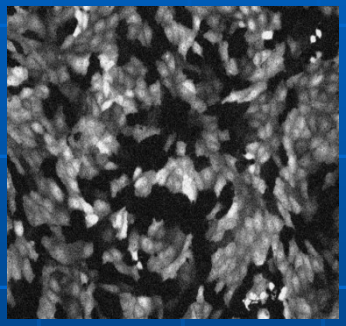
- Are image data analyzed in a quantitative fashion?
- How have the features been determined to be important?
- How were the algorithms tested for accuracy?
- Are the algorithms locked down?



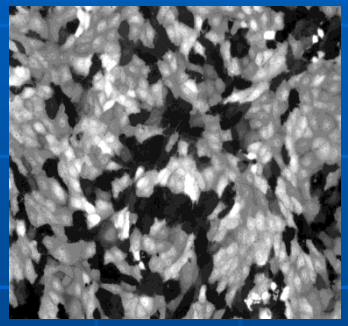


Evaluating the Accuracy of Instrumentation

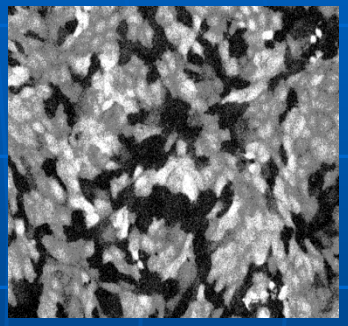




Poor signal-to-noise



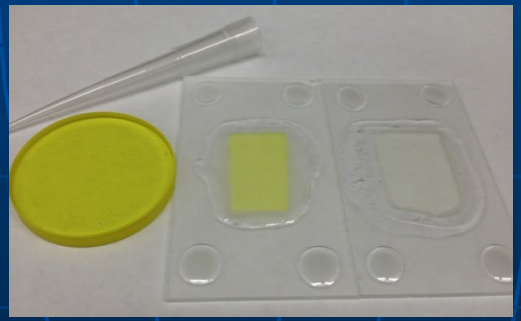
Non-linear response



Non-linear response +Poor signal to noise

Uranyl glass and Schott 475 GG:

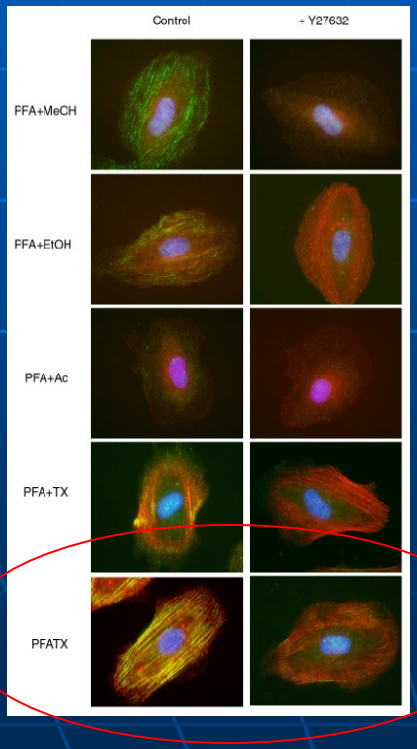
- Optical quality glass is homogenously fluorescent
- Robust and photostable
- Can be prepared as microscope coverslips

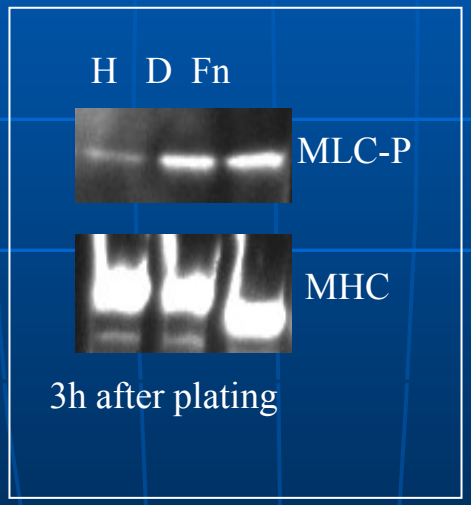


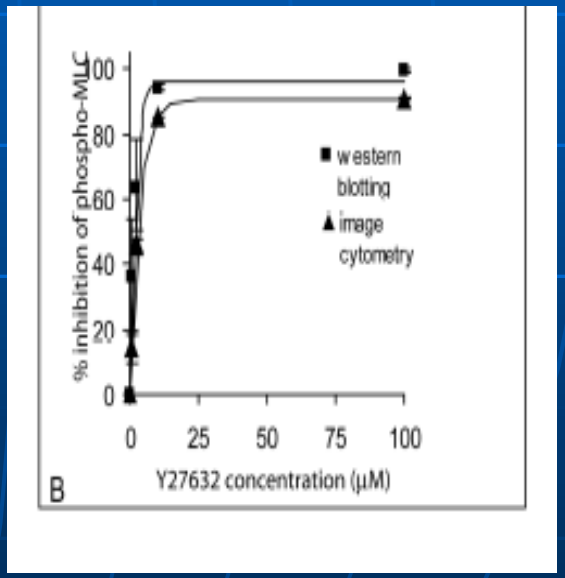
1) Saturation determination
2) Intensity calibration
3) Lower Limit of Detection (LLD)
4) Linear Dynamic Range

Other Examples of Validation

Validate protocols by comparing response with changes in protocol, and with ortholgonal method of analysis.







Bhadriraju et al, BMC Cell Biol 2007

How can a community go forward?

Validation takes at least as much effort as discovery.

Gathering sufficient knowledge (eg, reproducibility, control samples, reference materials) will require cooperation between labs.

Interlaboratory comparisons will require shared protocols, terminology, best practices.

Specific areas for consensus?

- Specification of device performance
- Validation of analytical response
- Validation of data handling/ analysis protocols
- Reduction of bias
- Sufficient sampling
- Source/ validation of activity of cells
- Cells representative of patient population
- Appropriate measurands for clinical response (MOA)

QUESTIONS?