Tissue Engineering Measurands

Carl G. Simon, Jr., Ph.D.

National Institute of Standards and Technology
United States Department of Commerce
Materials Measurement Laboratory • Biosystems & Biomaterials Division • Biomaterials Group

Tissue Engineering Measurands

- During the development lifecycle of a TEMP (tissue engineered medical product), many product properties are measured over many years along the way to a product release.
- Measurands for TEMPs are often complex, hard to measure and difficult to define.
- The selection of each measurand is motivated by the ultimate goal to establish that the product is safe and efficacious (product success)

Arora D, Babakhanova G, Simon Jr CG (2020) Tissue engineering measurands. *ACS Biomaterials Science & Engineering* 6, 5368-5376. https://doi.org/10.1021/acsbiomaterials.0c00475

Complexity of Tissue Engineered Constructs Makes Them Difficult to Assess and Describe

- Complexity can come from cells, which are
  - Responsive to their environment
  - Inhomogenous
  - Composed of thousands of different molecules
    - Composition can't be defined as with a small molecule drug

Challenging to identify a measurand to meaningfully describe such a complex system

Tissue Engineering Measurands Are Difficult to Select

- Desirable measurands have a bearing on product success, clinical efficacy
- A good place to start is the mechanism of action (MOA)
- Challenging to confirm the MOA
  - The only way to do this is a human clinical trial
  - Often not possible to test a hypothesis in human since the proper controls may not be ethical
    - Empty scaffold (no cells) could cause harm
    - Scaffold seeded with dead cells could cause harm
    - Immunosuppressive drugs may be required, which have harsh side effects
    - Sham surgery could be highly invasive

Challenging to identify a measurand which has a bearing on clinical efficacy or product success.

Miscommunication During Discussions of How to Characterize a Tissue Engineered Medical Product (TEMP)

What are we really measuring?

Cell Therapy Workshop, NIST, 2015

You can't call it viability since that isn't what you are measuring.

ASTM, Denver, 2019

Yes, but the platereader is detecting photons, not cell viability.

Cell Therapy Workshop, NIST, 2015

How do you validate that these markers are related to patient outcomes?

Phacilitate, Miami, 2020

We only want to measure properties that correlate with clinical efficacy.

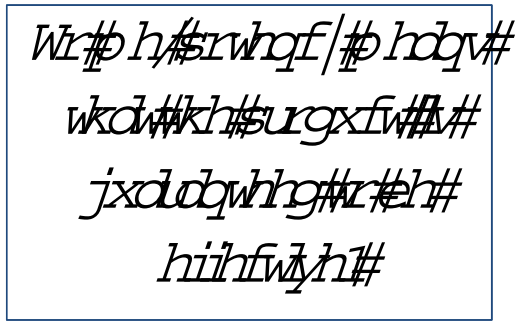
Scaffold Workshop, ARMI|BiofabUSA, 2018

Is it biologically relevant? If not, then why are we measuring it?

Cell Therapy Workshop, NIST, 2015

This may not have anything to do with curing the patient…

Scaffold Workshop, ARMI|BiofabUSA, 2018



Cell Therapy Workshop, NIST, 2015

Measurement

"the process that is undertaken to generate an estimate of the true value of a property of a material, along with an evaluation of the uncertainty associated with that estimate, which is intended for use in support of decision-making"

Possolo, A. NIST Technical Note 1900: Simple Guide for Evaluating and Expressing the Uncertainty of NIST Measurement Results. NIST, U.S. Department of Commerce, 2015.

quality, safety & efficacy

"measurement is typically done in the context of an experiment designed to make the property of interest accessible to a sensor in a measuring instrument"

Possolo, A.; Iyer, H.K.; Concepts and Tools for the Evaluation of Measurement Uncertainty. Review of Scientific Instruments 2017, 88, 011301.

"process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity"

Note: "presupposes a description of the quantity commensurate with the intended use of a measurement result"

VIM 3rd Edition: International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM), 3rd Edition. Joint Committee for Guides in Metrology (JCGM) 200:2012.

Measurand

"the quantity or property intended to be measured"

- International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM), 3rd Edition. Joint Committee for Guides in Metrology (JCGM) 200:2012.
- Use of the word "intended" in this definition is intentional
  - It may be impossible to measure what you intend to measure
  - What is actually measured may not be what you intended to measure (due to experimental artifacts)

Nevertheless, the measurand is the property that you intend to measure, whether or not this is possible or achieved.

- *Measurand is personal*
- *No one can define it for you*
- *It is what you say it is*

Can make communication challenging, since we each have our own idea of what the measurand is & what it means

Measurand Chart:

Cell Viability in a Scaffold

Cell Viability in a Scaffold

- A TEMP is often composed of a three-dimensional template, called a scaffold, that has been seeded with cells
- The mechanism of action (MOA) for a scaffold-cell construct may be:
  - The cells build a new tissue in the patient through the processes of cell proliferation and deposition of extracellular matrix
  - The cells secreting factors that induce tissue regeneration or repair
- Since these MOAs may require live cells, a common and important measurand for a scaffold-cell construct is “cell viability in a scaffold”

Cell Viability

My Definition of a Viable Cell: "a cell that has 1) an intact membrane and the 2) capacity for metabolism, 3) motility, 4) proliferation and 5) reaction to stimuli" (Merriam-Webster's Dictionary, Encyclopedia Britannica, ASTM F2739)

Measuring Cell Viability: There are a ~100 different methods for assessing cell viability

- Each method has a unique measurand (they measure different things)
- Results from the various methods may not agree with one another, even when analyzing the same specimens

Case 1: Cell under a stress

High Dehydrogenase Activity => MTT => Live

Poor Membrane Integrity => Trypan blue => Dead

Case 2: Cell under a different stress

Low Dehydrogenase Activity => MTT => Dead

Good Membrane Integrity => Trypan blue => Live

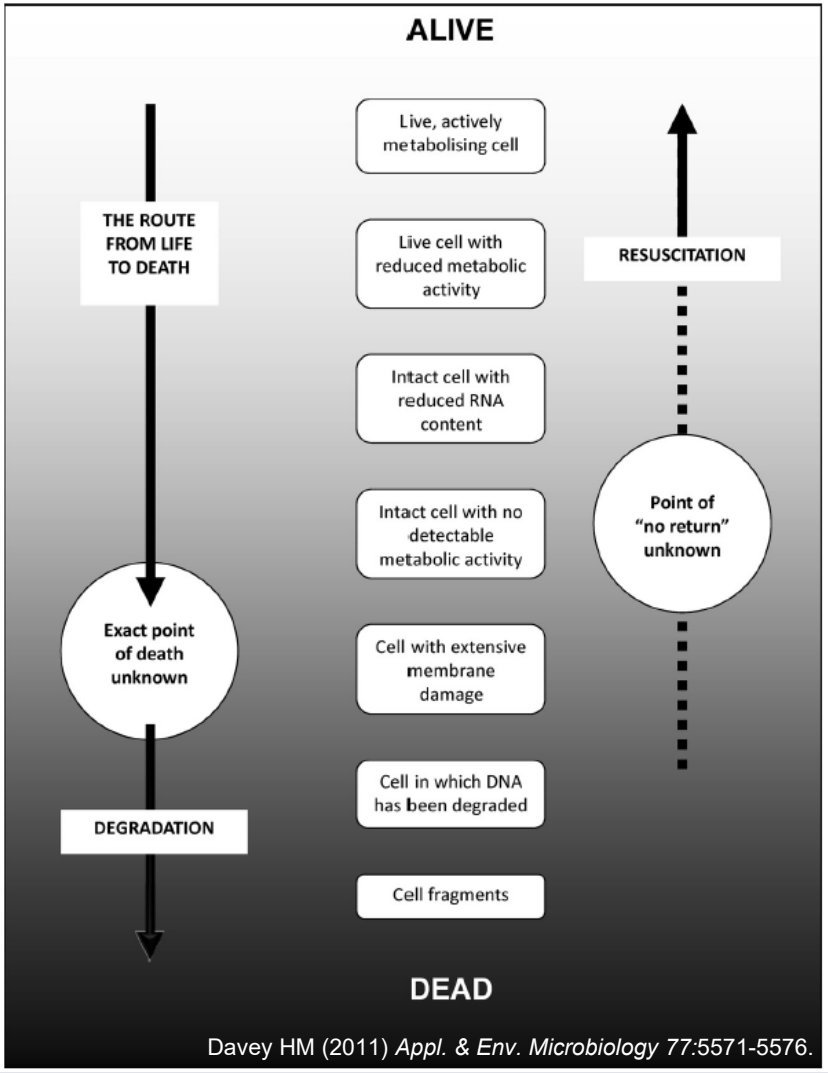
I assume this may be true for all measurement systems

[that different measurements of an attribute may not agree]

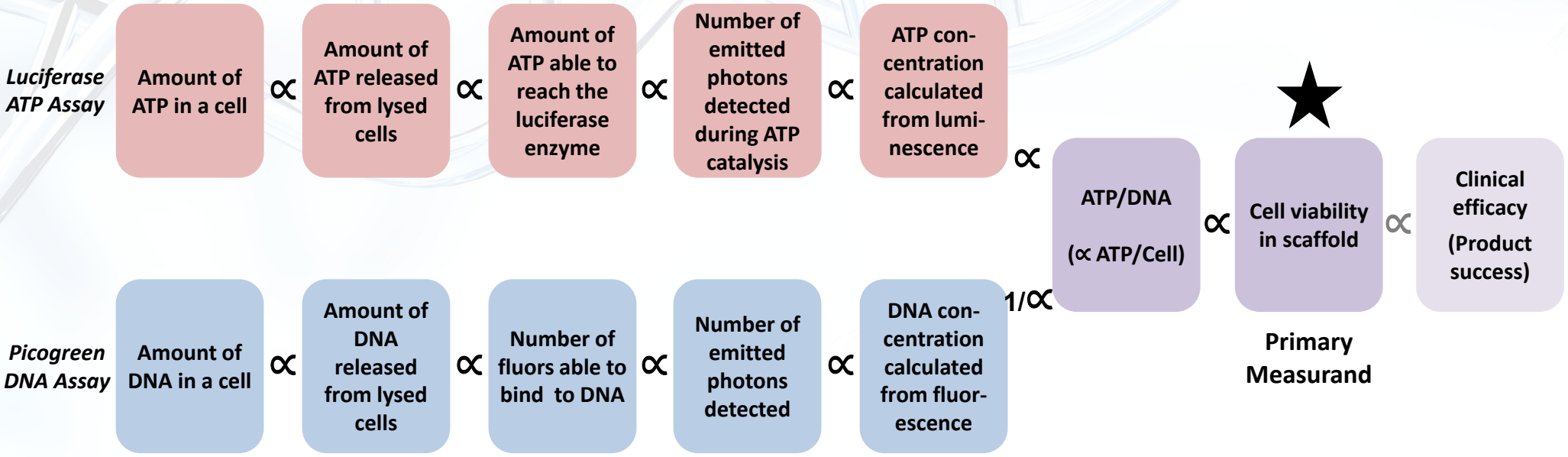
Cell Viability May Be a Continuum

The route from live to dead may contain multiple steps and could be a continuum.

- The order of the steps may change
- Not all of the steps may be observed



Measurand Chart: Cell Viability in a Scaffold (Every Box is a Measurand)



- Graphic to visualize the various measurands that may be present in a measurement
- A way to think about complex measurements to
  - Improve strategic thinking
  - Reveal the hidden assumptions

- Must make the allowance that there can be **multiple measurands per measurement**
- Similar to a process flow diagram
- Note that all possible measurands for this measurement are not present
- The relationships depicted by the chart are not absolute, but are meant to facilitate discussion

Arora D, Babakhanova G, Simon Jr CG (2020) Tissue engineering measurands. *ACS Biomaterials Science & Engineering* 6, 5368-5376. https://doi.org/10.1021/acsbiomaterials.0c00475

Measurement Model
&
Multiple Measurands

GUM Is a Model for Expressing [Output Quantities] in Terms of [Input Quantities]

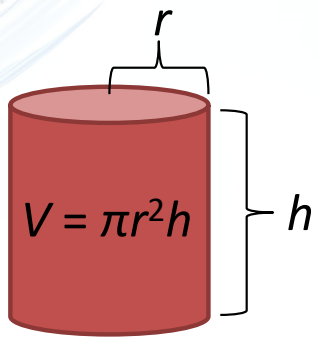
GUM: JCGM, 2008.

1) Express mathematically the relationship between the measurand Y and the input quantities X_i on which Y depends: $Y = f(X_1, X_2, ..., X_N)$.

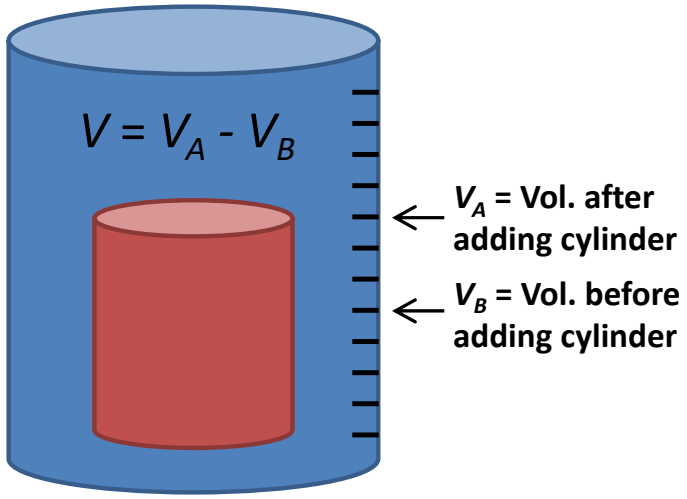
Possolo, A. NIST Technical Note 1900, 2012.

(1) **Measurand & Measurement Model.** Define the *measurand* (property intended to be measured, §2), and formulate the *measurement model* (§4) that relates the value of the measurand (output) to the values of inputs (quantitative or qualitative) that determine or influence its value.

Multiple Measurands: Volume of the Cylinder

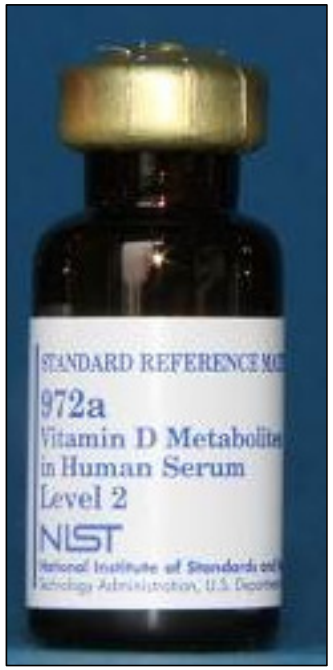


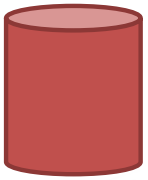
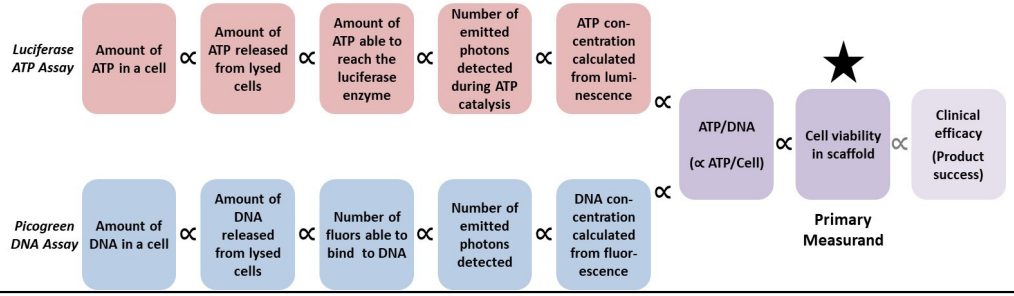
- Two different systems for measuring cylinder volume are shown
  1) Measure radius & height with a ruler
  2) Measure volume of water in a graduated cylinder before & after immersion of the cylinder
- In both cases, you have two different input values that are used to determine the measurand
- However, the primary measurand is the same: "Volume of the cylinder"



Multiple Measurands: NIST SRM 972a "Vitamin D Metabolites in Frozen Human Serum"

- Frozen serum with certified values of 25-hydroxyvitamin D_2 & 25-hydroxyvitamin D_3
- The sum of D_2 and D_3 is generally considered a reliable indicator of "vitamin D status" (primary measurand the physician desires to assess health)



Measurand	Function	Source
Y	General model for measurands: $Y = f(X_1, X_2, \ldots, XN)$ 	GUM, 2008
Volume of a Cylinder	$V = \pi r^2 h$ 	Geometric relationship
Vitamin D Metabolites in Frozen Human Serum	[25-hydroxyvitamin D_2] + [25-hydroxyvitamin D_3] 	NIST SRM 972
Cell Viability in a Scaffold		This talk

Definitional Uncertainty

Definitional Uncertainty

True value:

- The “true value (of a quantity)” is the “value consistent with the definition of a given particular quantity” (GUM, 2008)
- True value “is a value that would be obtained by a perfect measurement” (GUM, 2008)
- “true values are by nature indeterminate” (GUM, 2008)

Definitional uncertainty:

- The “component of measurement uncertainty resulting from the finite amount of detail in the definition of a measurand” (VIM, 2012)
- “a measurand cannot be completely described without an infinite amount of information” (GUM, 2008)

Consequences

- The true value of a measurand is what you say it is (true value is dependent on how you define the true value)
- Accuracy does not exist (not in an absolute sense)
- Discussing product characterization will be challenging if you don’t agree on a common definition for the measurand

Definitional Uncertainty: Mass of the Sun

Example from Possolo is the mass of the sun:

- Possolo, A. NIST Technical Note 1900, 2015.
- The mass of the sun rapidly changes from moment to moment
- Even at a given instant, the mass of the sun is dependent upon how the volume of the sun is defined

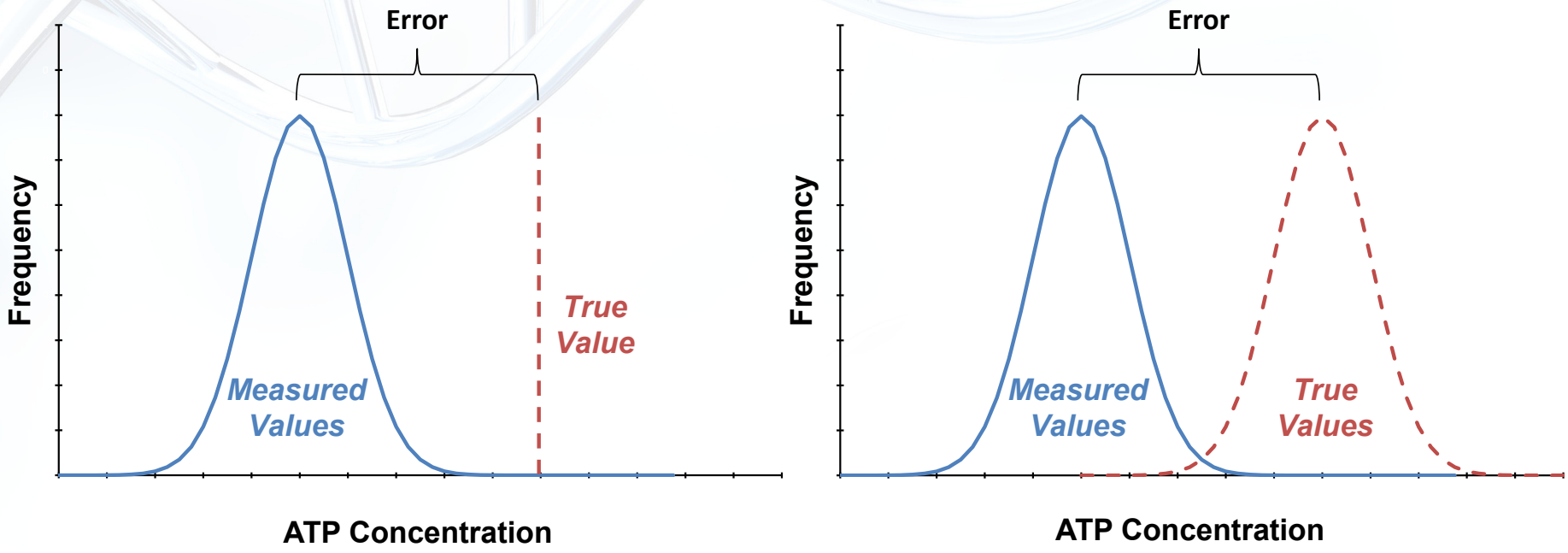
Definitional Uncertainty: Velocity of Sound

EXAMPLE The velocity of sound in dry air of composition (mole fraction) $N_2 = 0{,}780\ 8$, $O_2 = 0{,}209\ 5$, $Ar = 0{,}009\ 35$, and $CO_2 = 0{,}000\ 35$ at the temperature $T = 273{,}15$ K and pressure $p = 101\ 325$ Pa.

GUM, JCGM

- The air composition, the temperature and the pressure cannot be defined with infinite precision, which introduces uncertainty into the definition of the measurand

Definitional Uncertainty



Charles Ehrlich. Terminological aspects of the Guide to the Expression of Uncertainty in Measurement (GUM). Metrologia 51 (2014) S145–S154.

Cell Viability May Have Large Definitional Uncertainty: ATP/DNA

Defining what constitutes a cell:

- Cell numbers in a culture rapidly change as the cells divide
- Cell cultures contain many cell fragments that contain DNA. Are these considered cells?
- How dead would a cell have to be to no longer be considered a cell?
    - If the membrane degrades and the cytoplasm has dissipated but there is still a nucleus-like structure that contains DNA, would this count as a cell?

Defining ATP/cell:

- Cell preparations are typically inhomogenous & ATP/cell may vary between subpopulations
- There may be differences in ATP/cell within the human population
    - For an autologous indication that uses patient-derived cells, then each patient's construct will have a different true value for the ATP/cell in the scaffold

Cell Viability May Have Large Definitional Uncertainty: ATP/DNA

Defining ATP concentration:

- As with the mass of the sun, the ATP levels in cells may rapidly fluctuate
  - ATP is continuously dephosphorylated to release energy to support cell functions
  - ATP is continuously synthesized as the energy from food sources is used to drive the rephosphorylation of AMP & ADP
- There may be a challenge with defining what constitutes a molecule of ATP
  - Some fraction of ATP molecules will be complexed with enzymes, some by covalent bonds, as they are metabolized
  - Mitochondria, where most of the ATP is synthesized, comprise up to a quarter of the cell volume, which means that a significant fraction of ATP molecules may be complexed

"Ligase catalysis mechanism involves the lysine residue of the enzyme being covalently linked to the adenylyl group from ATP and form an intermediate"

Defining the Measurand

Measurand Should Be Defined Both Philosophically & Practically

Measurand = “the property intended to be measured”

- Philosophical measurand
  - Establishes a goal for the measurement
  - Can be abstract, impractical or impossible to measure
  - Should not mention a specific measurement system
- Practical measurand
  - Mentions the measurement system
  - Required for a coherent discussion of the value of a particular measurement
- Need to have both for a complex measurand such as “cell viability in a scaffold”

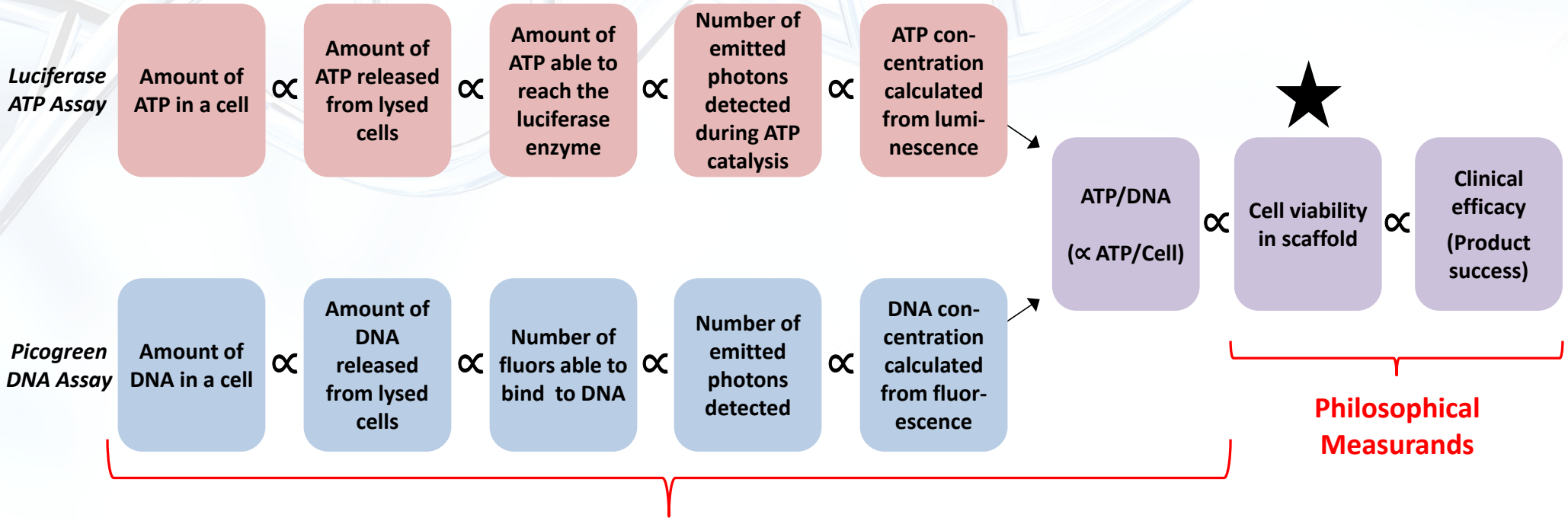
Measurand Should Be Defined Both Philosophically & Practically

Without reference to a measurement

In terms of the measurement

Philosophical Measurand (does not mention measurement system)	**Practical Measurand** (includes measurement system)
Mass of a penny	Position of weights on balance when pointer is horizontal on a triple beam balance
Cell viability	[Number of non-blue cells] divided by [total number of cells] as observed on a brightfield microscope following trypan blue staining

Cell Viability in a Scaffold Measurand Chart



Yes, but the platereader is detecting photons, not cell viability.

Cell Therapy Workshop, NIST, 2015

We only want to measure properties that correlate with clinical efficacy.

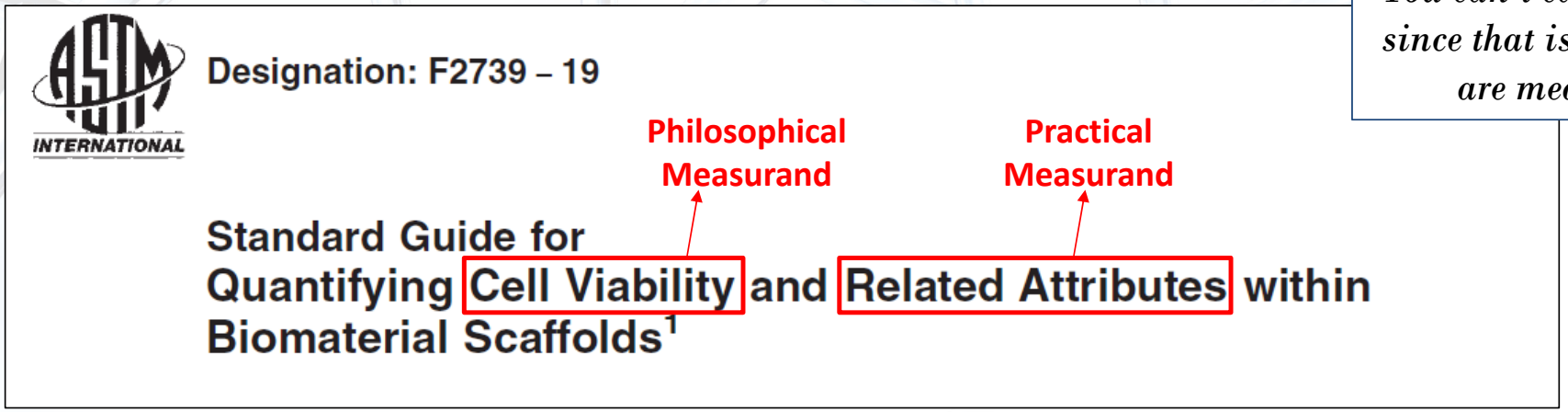
Scaffold Workshop, ARMI|BiofabUSA, 2018

You can't call it viability since that isn't what you are measuring.

ASTM, Denver, 2019

You can't call it viability since that isn't what you are measuring.

ASTM, Denver, 2019



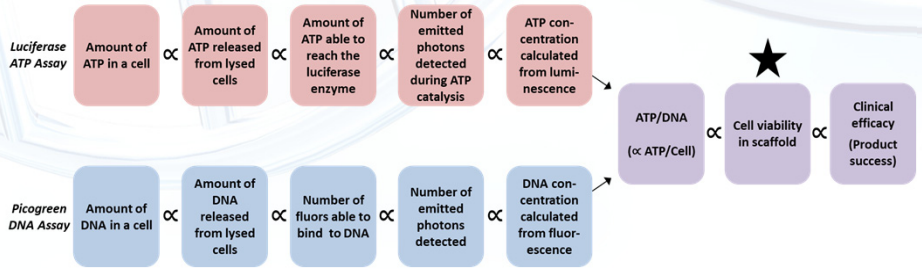
- An example where we mixed the philosophical & practical measurands
  - "viability" is the philosophical measurand (agnostic to the measurement system)
  - "related attributes" was meant to address the practical measurands (trypan blue, MTT, ATP, etc.)

Closing

Measurand Chart

- Graphical rendering of the relationship between measurands to make the information more accessible & easily consumed
- Formally map the chain of measurands & their relationships in a measurement system
- Reveal unrecognized assumptions
- Improve communication & strategic thinking
- Enhance interpretation of measurement results (what are we really measuring?)
- Help assess the value of measuring a given attribute
- Should not serve as an absolute; should be used to facilitate discussion

Summary



- Tissue engineering measurements can have many measurands
- Different test methods do not measure the same thing (different measurands)
- Tissue engineering measurands have large definitional uncertainty (inability to precisely define the true value)
- Coherent discussion requires that you define the measurand both philosophically & practically
  - Philosophically: without reference to a measurement system
  - Practically: in terms of the measurement system

Awareness of these issues enables strategic thinking when deciding 1) which product attributes should be characterized and 2) how they should be measured

Acknowledgements

NIST

Deepika Arora
Greta Babakhanova
Dave Duewer
John Elliott
Charles Ehrlich
Sheng Lin-Gibson
Will Guthrie
Anne Plant
Antonio Possolo
Marc Salit
Sumona Sarkar
Robert Waters

VIM, JCGM
GUM, JCGM
NIST Technical Note 1900 (GUM for NIST)
Malcolm Moos (FDA)
Richard McFarland (FDA / BioFabUSA)
Becky Robinson-Zeigler (FDA / BioFabUSA)

Thank you!

Arora D, Babakhanova G, Simon Jr CG (2020) Tissue engineering measurands. *ACS Biomaterials Science & Engineering* 6, 5368-5376. https://doi.org/10.1021/acsbiomaterials.0c00475