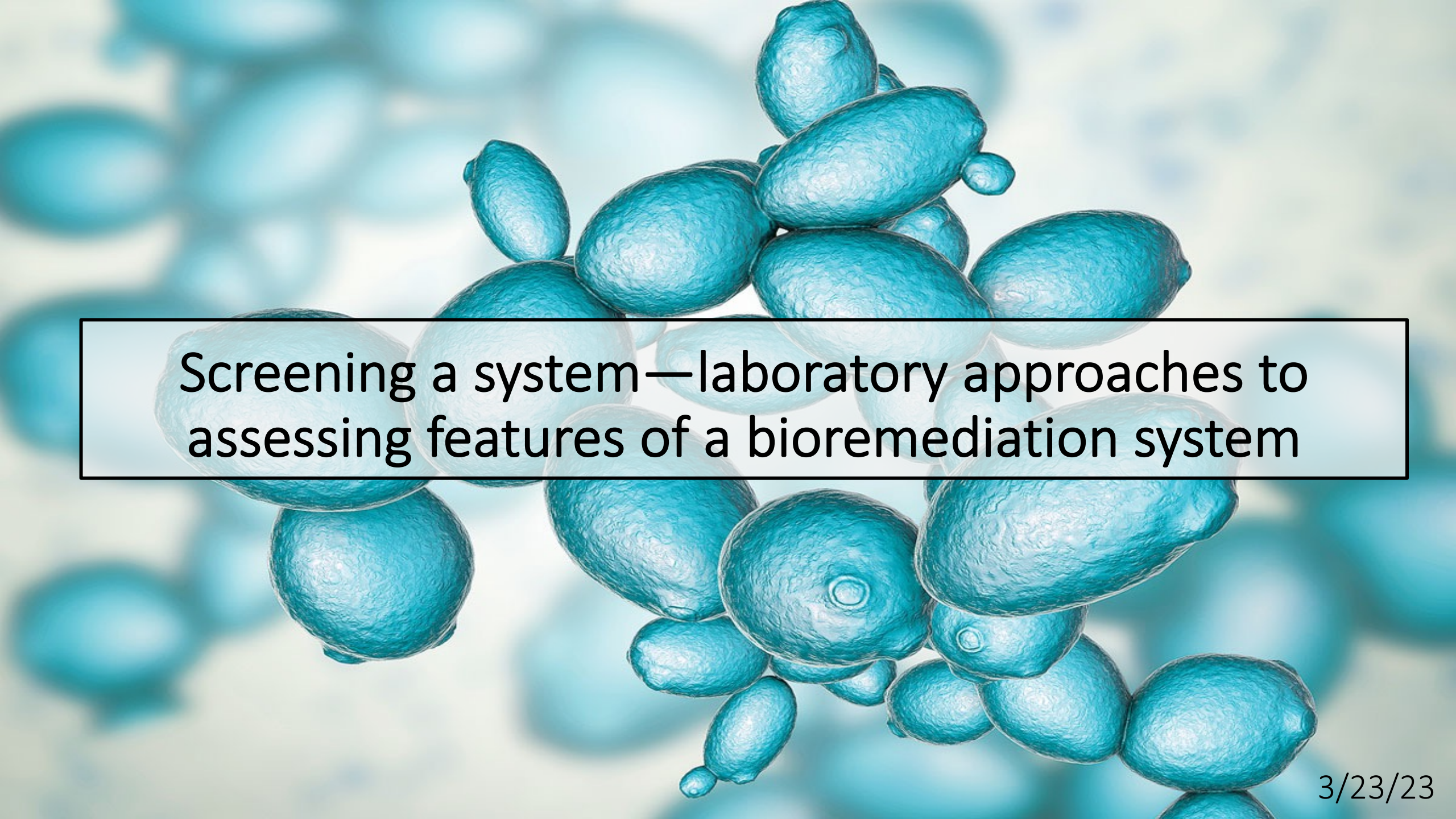


Screening a system—laboratory approaches to assessing features of a bioremediation system

3/23/23

Module Outline

- M2D1: Environmental heavy metal contamination
- M2D2: Model system – target selection and engineering approach
- M2D3: Model system – choosing a chassis host
- **M2D4**: Screening a system—assessing features of a bioremediation system
- M2D5: Analysis of elemental metals – laboratory and field approaches
- M2D6: Applying remediation strategies—advantages and pitfalls
- M2D7: Engineering a problem-specific bioremediation solution
- M2D8: Comm Lab

Intro

Design

Test

Apply

Review

Lecture overview

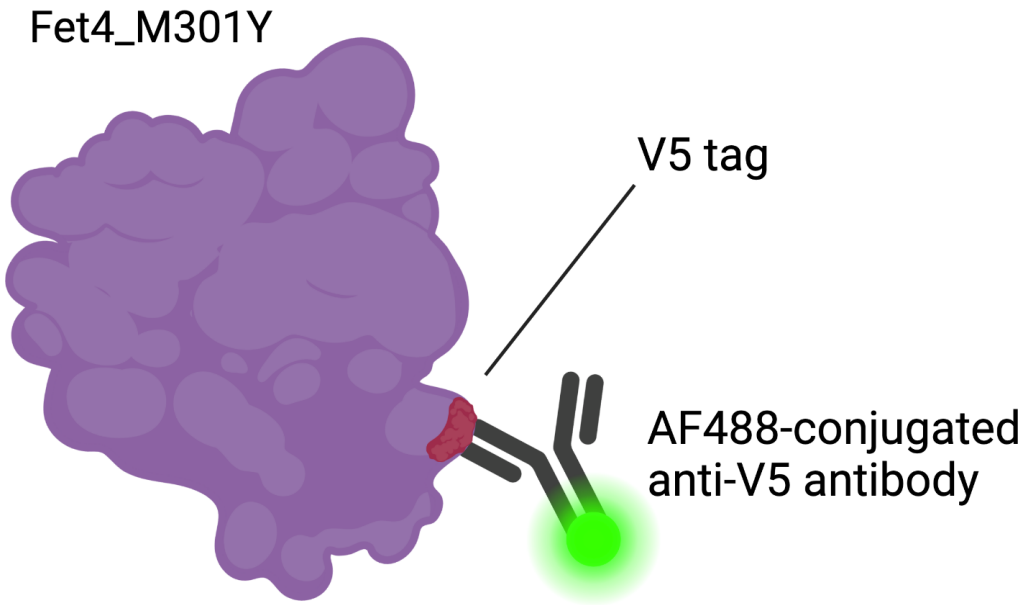
3 key parameters of our bioremediation system should be established

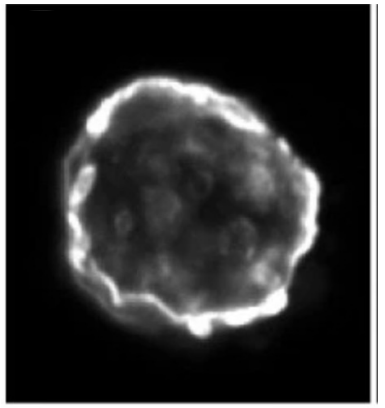
- Is our mutant protein expressed?
- Does our mutant transporter show any differential ability to take up metal?
- Does our cell tolerate metal exposure and uptake?

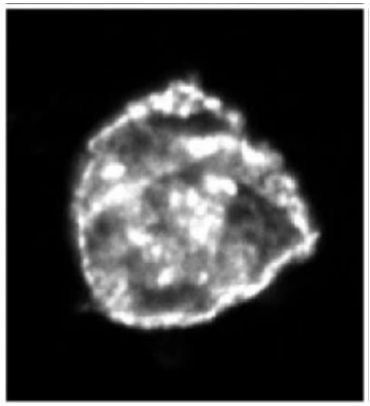
Protein expression

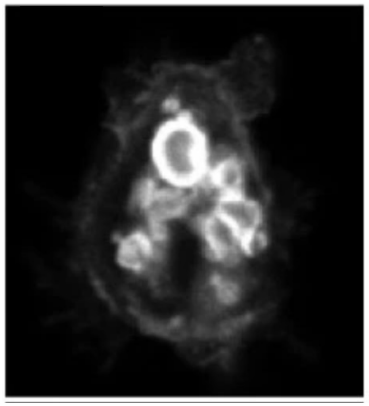
Fet4_mutant expression can be established with immunofluorescence

- Antibodies against protein or incorporated tag
- Confirms that mutant protein is translated and trafficked as expected
- Automated 96 well microscopy for adherent cells





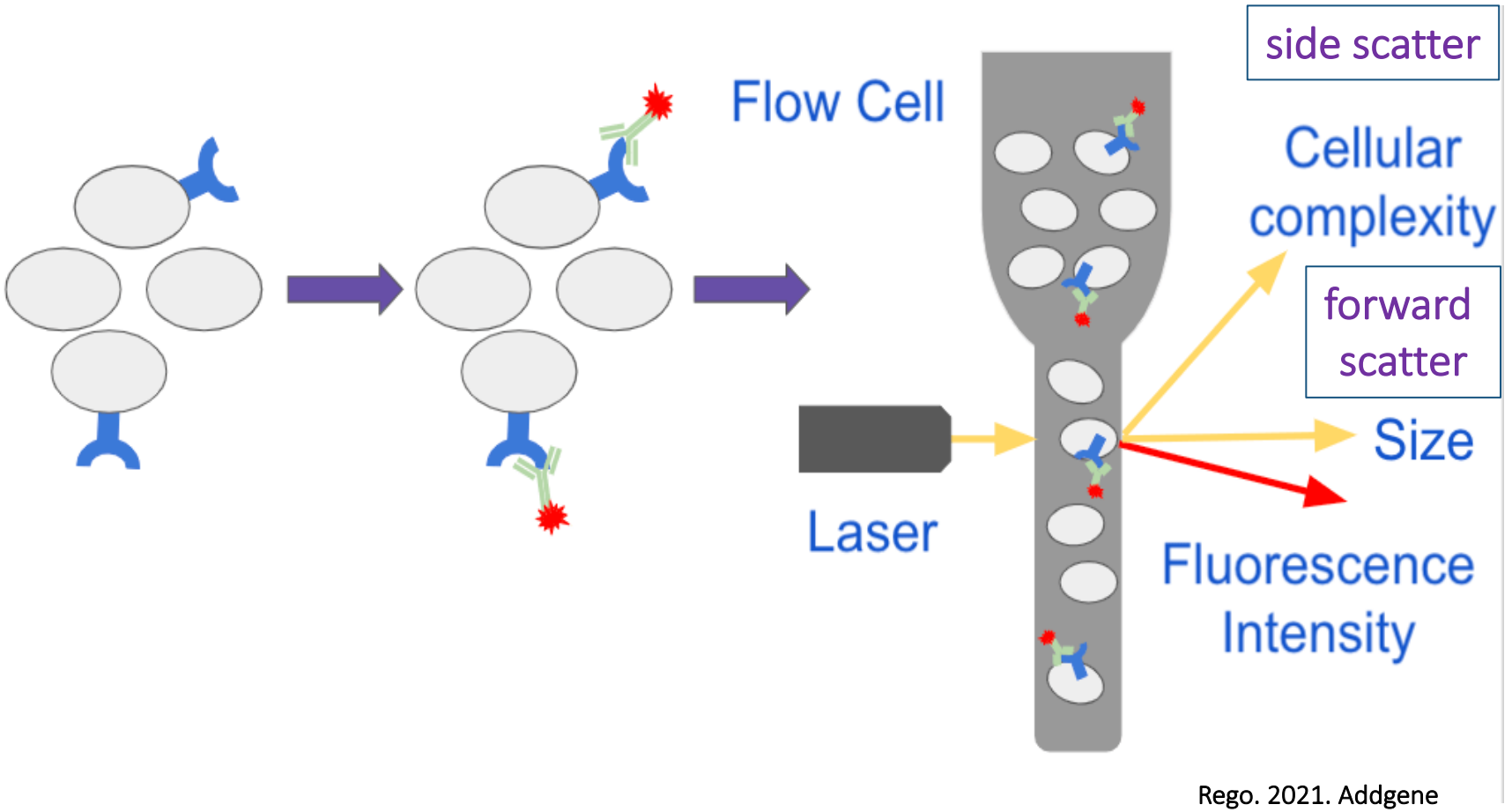




Flow cytometry can also be used to quantify protein expression

- Cells expressing mutant protein are labeled with fluorescent antibodies

- Labeled cells are passed in single file in front of a laser and visible light source
  - the scatter of the visible light provides information about cell size and granularity
  - fluorescent signal indicates protein labeling by antibody

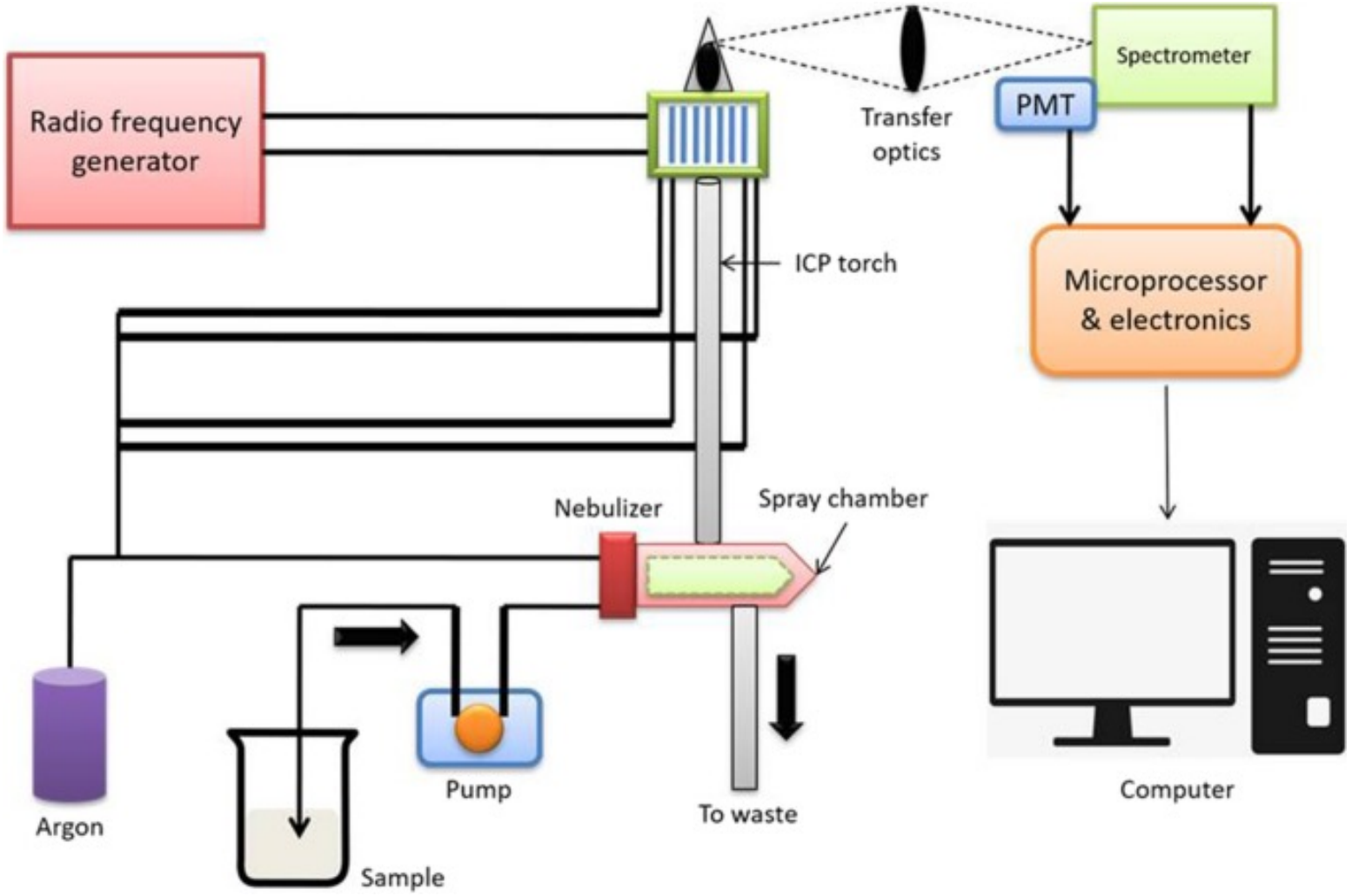


Rego. 2021. Addgene

Metal uptake

ICP-OES/MS can measure elemental concentration in a sample

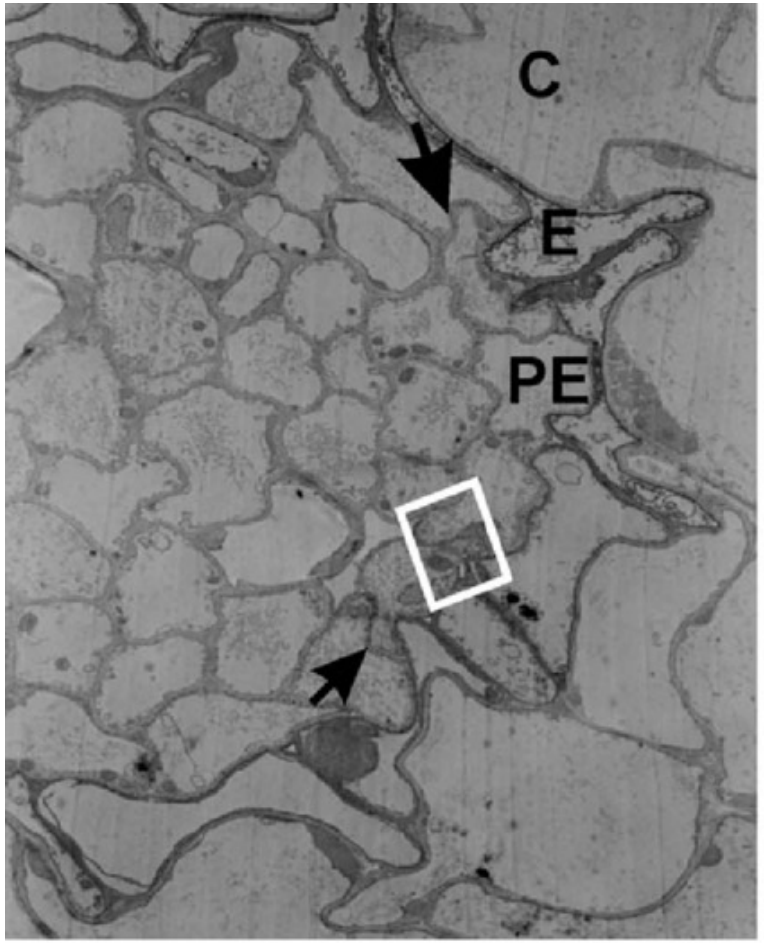
- Metal concentration can be quantified based on assessment of atomic properties
- Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)



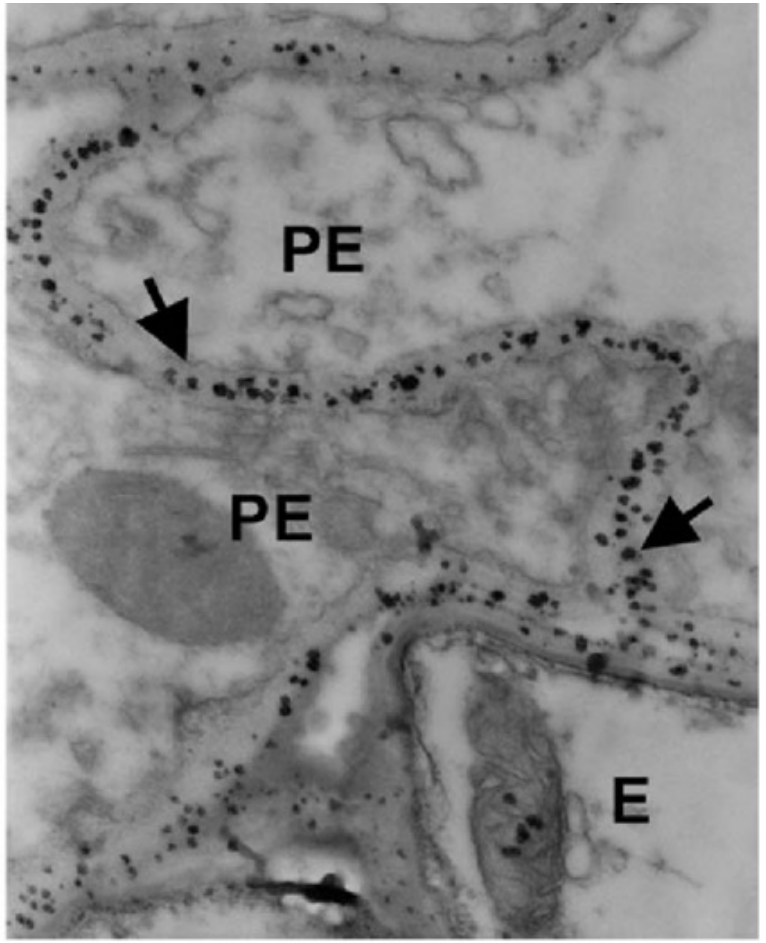
Electron microscopy can visualize metal collection in a cell

- Transmission Electron Microscopy (TEM)
  - send electron beam through a sample
  - generated image is shaded according to density

- TEM micrograph of root section from plant exposed to Cd for 14 days
  - Identified granules containing Cd in the cell wall of pericycle cells



2000x magnification

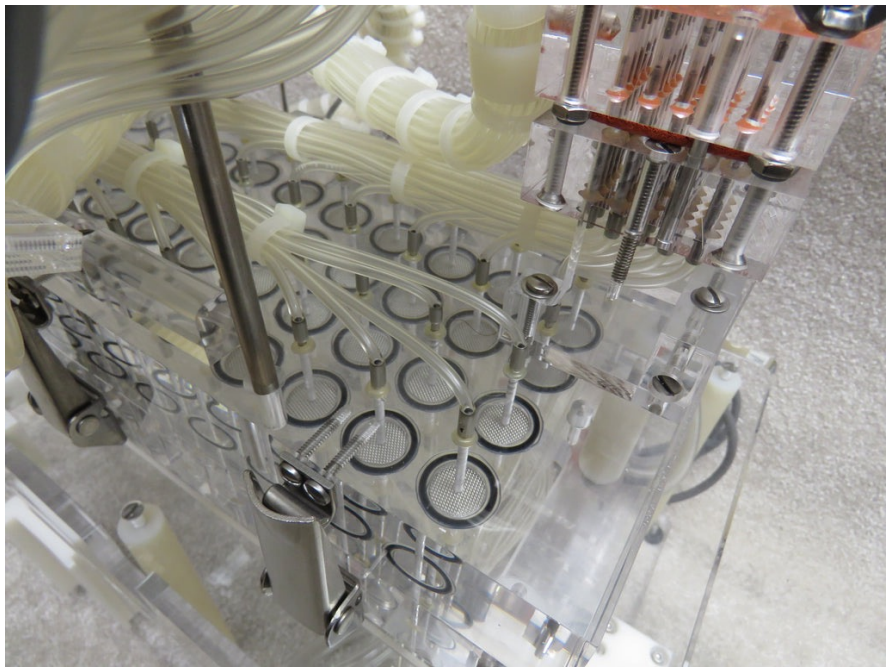


20,000x magnification

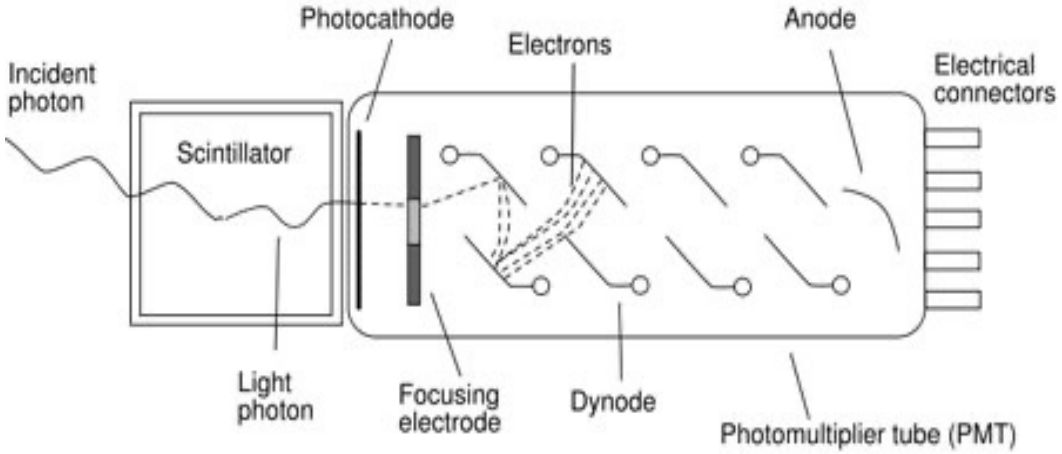
Wojcik, 2004

Radioactive metal isotopes can be utilized to monitor metal presence

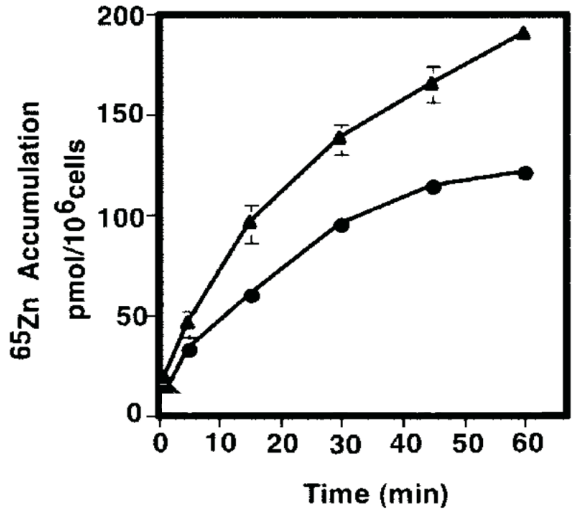
- Incubate cells with radioactive isotope of metal
  - Rapid wash to remove any radioactive material not bound to or accumulated within the cell
  - Use gamma scintillation counter to convert radioactive decay to light to an electrical pulse
  - High sensitivity



Brandel Cell Harvester



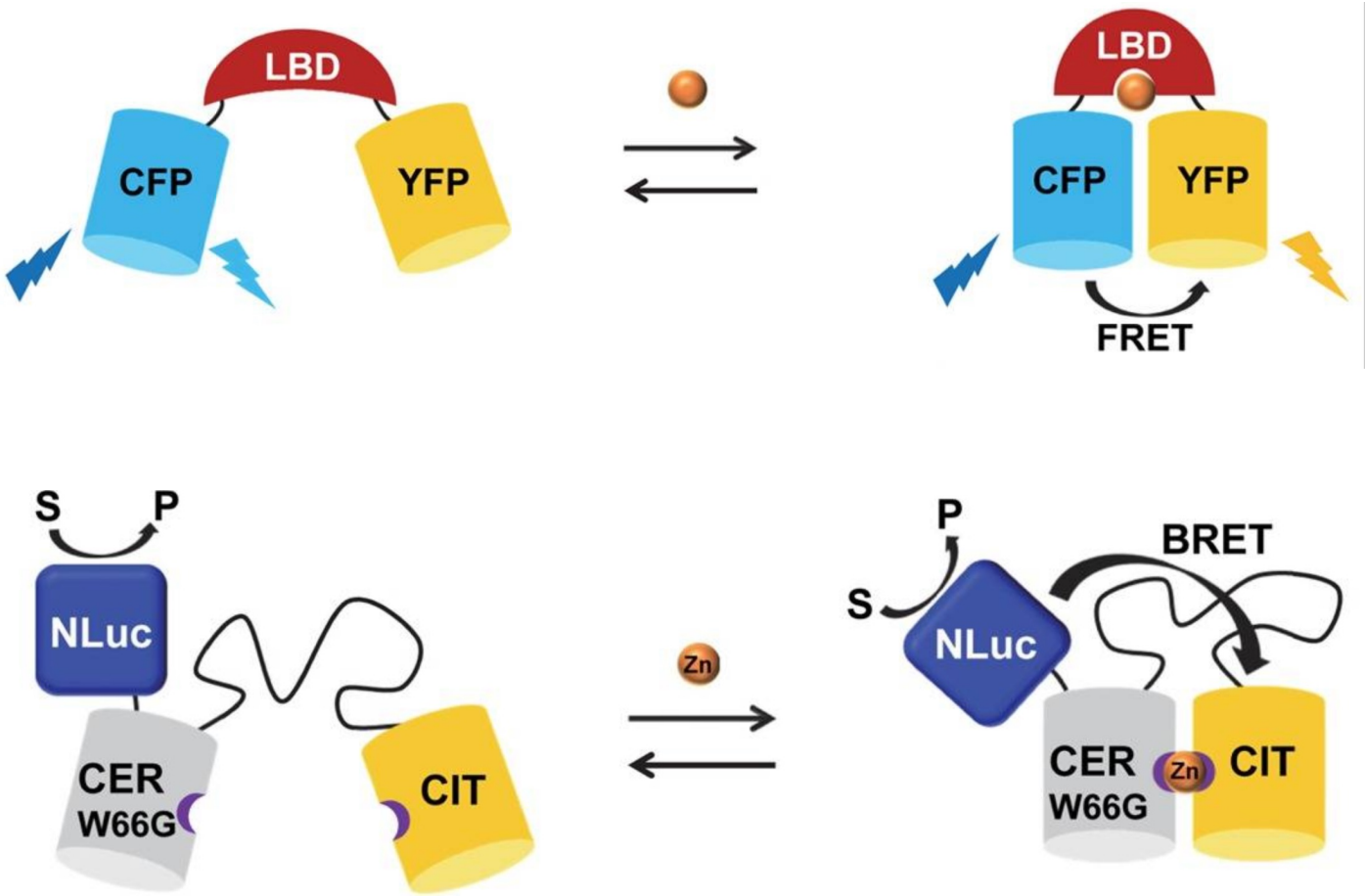
Stanford Scintillation Materials Group



Gaither, 2001

Engineered sensors can be used to detect metal in a cell

- Förster Resonance Energy Transfer (FRET)
  - Laser excited donor fluorophore
  - Acceptor fluorophore emits fluorescent signal
- Bioluminescence Resonance Energy Transfer (BRET)
  - Bioluminescent luciferase excites the acceptor flurophore
- Fluorescent probes
  - small enough to cross a membrane and bind to intracellular metal
  - not as sensitive as the sensors

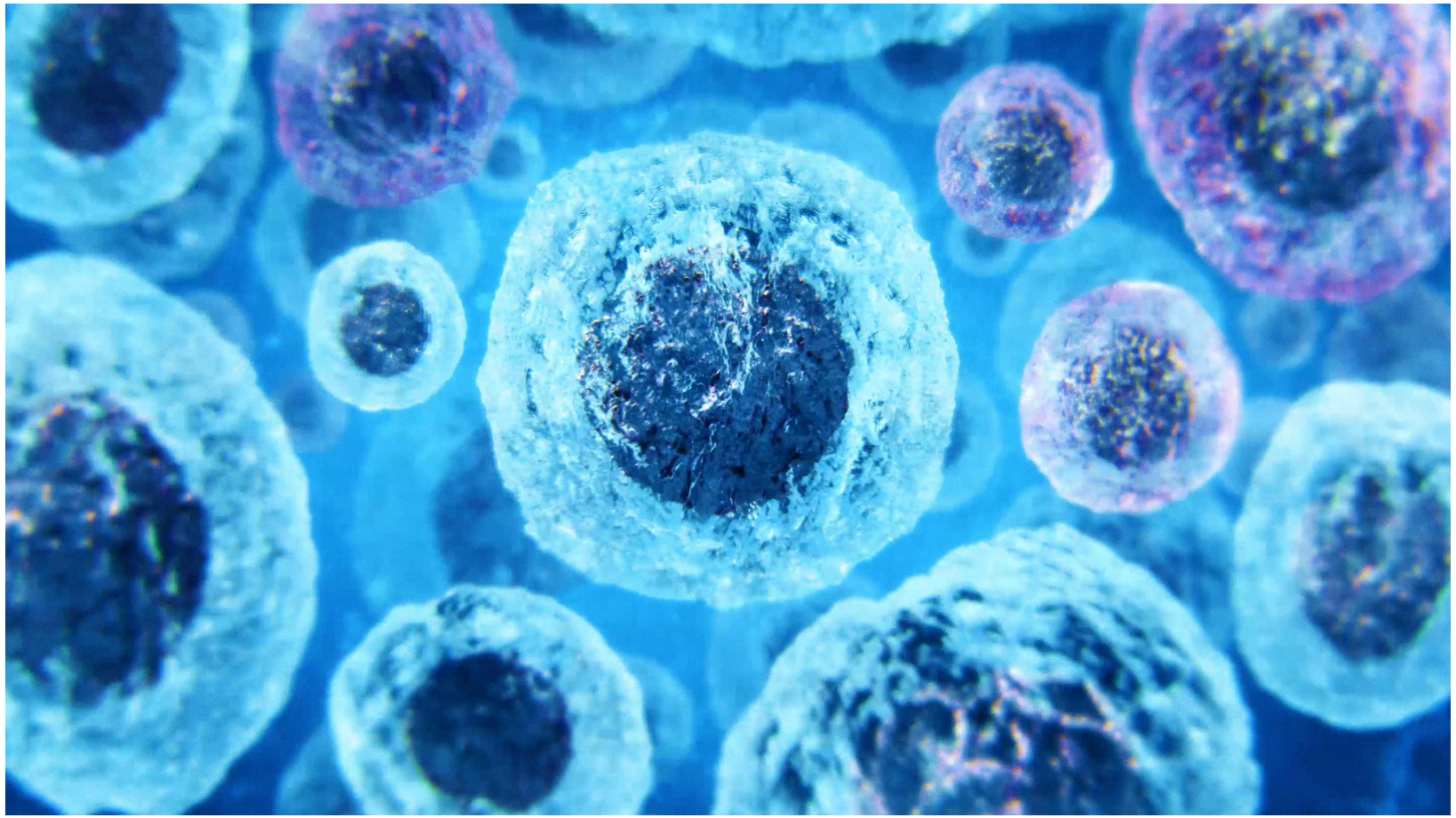


Aper. 2016

Cell tolerance of metal accumulation

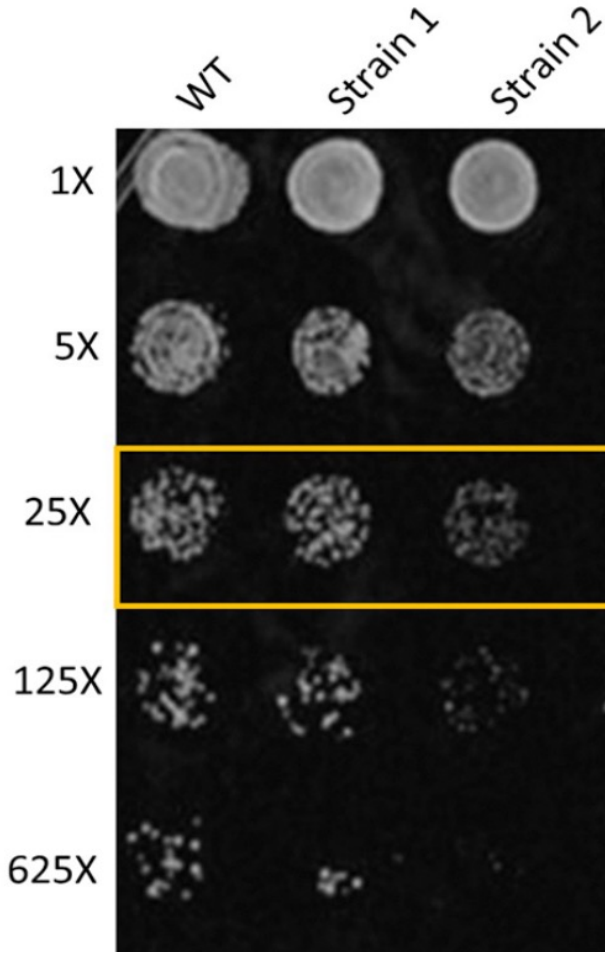
Different metrics can be used to assess cell viability

- Growth rate
- Membrane integrity
- Metabolic activity



Spotting assay can be used to count colonies and compare growth under different conditions

- Commonly used to evaluate bacterial or yeast growth and survival
- Measure the density of cells within a single spot of defined size
  - Count colonies
  - Densitometry
- Identify common dilution that will allow for most accurate comparison across groups



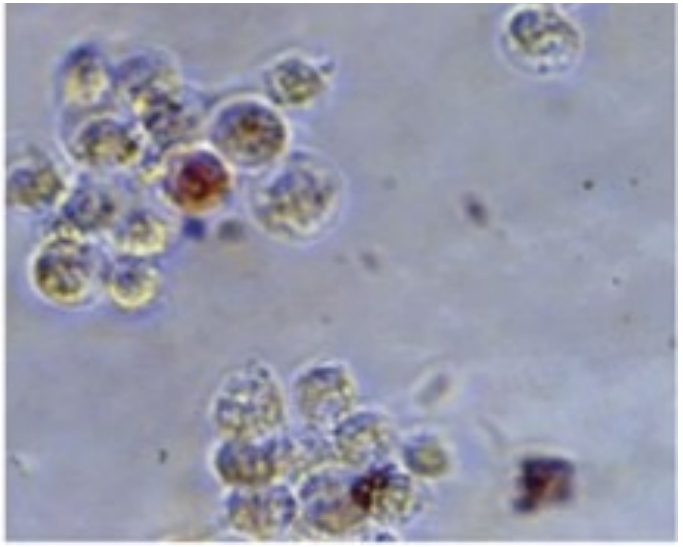
Petropavlovskiy. 2020

Vital dyes can be used to establish membrane integrity of cells

- Colormetric dyes

- Neutral red
  - weak cation dye
  - cross intact plasma membranes to concentrate in organelles like vacuoles and lysosomes
  - damage to those membranes prevents the incorporation of the dye because it can be washed out
  - Red= alive

- Evans blue
  - anionic dye
  - impermeable to membrane of living cells but able to accumulate in cells with compromised plasma membrane
  - Blue = dead
  - trypan blue is another example

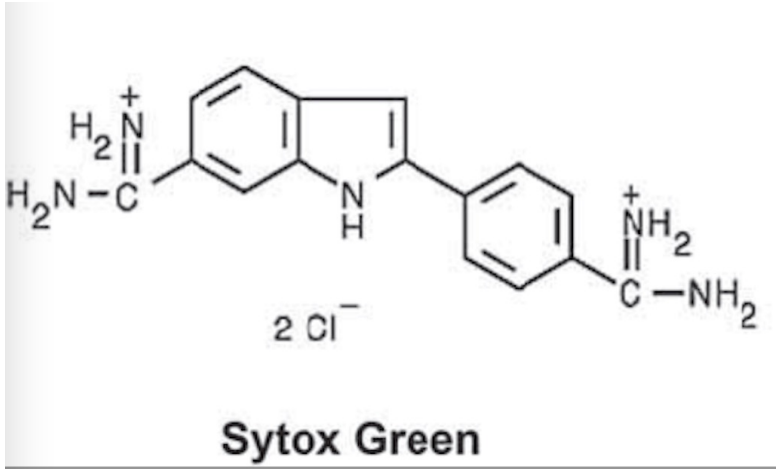


Neutral red

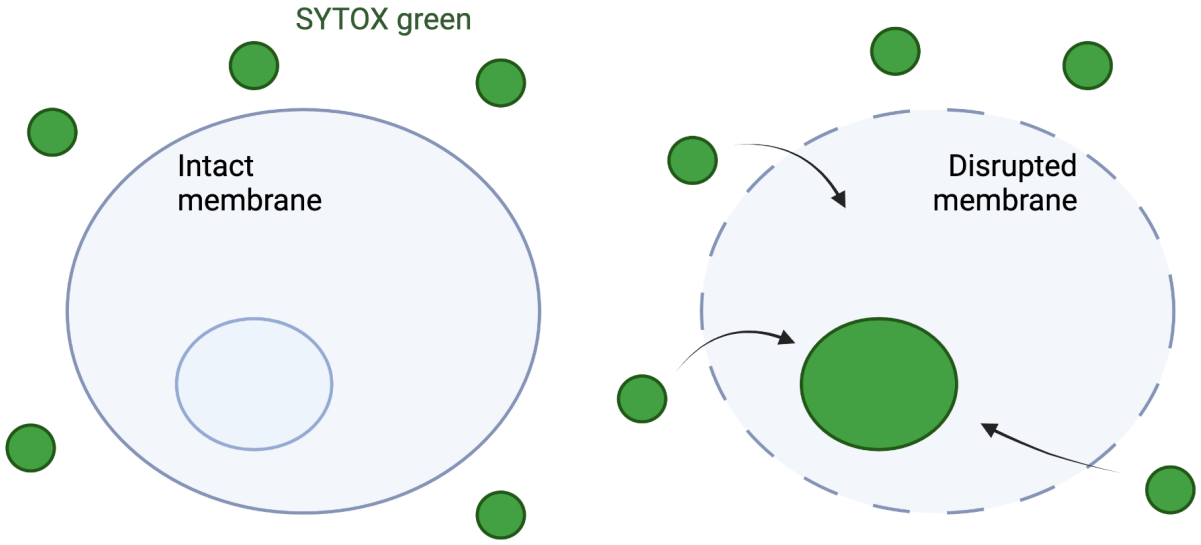
Evans blue dye

Nuclear fluorescent dyes can be used to indicate loss of membrane integrity

- SYTOX Green is a probe that cannot cross intact membranes
- One inside the nucleus, it binds to nucleic acids and fluoresces

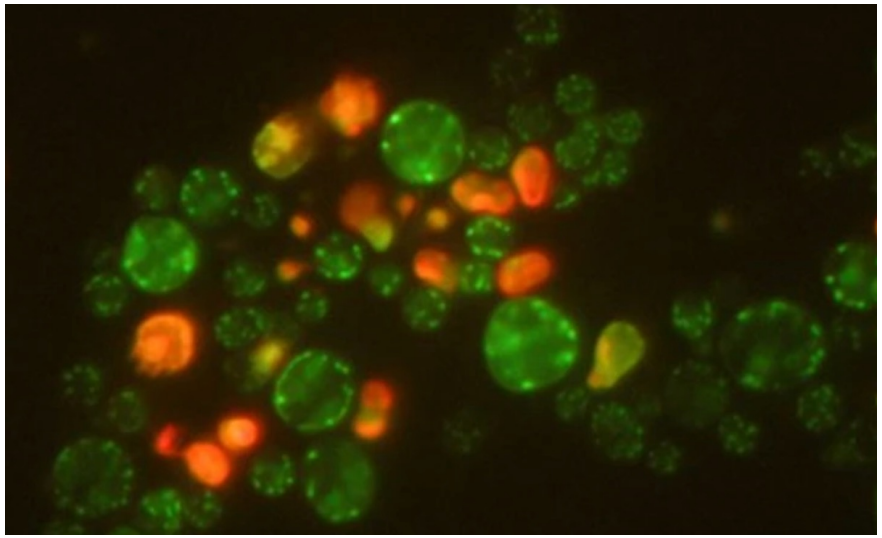


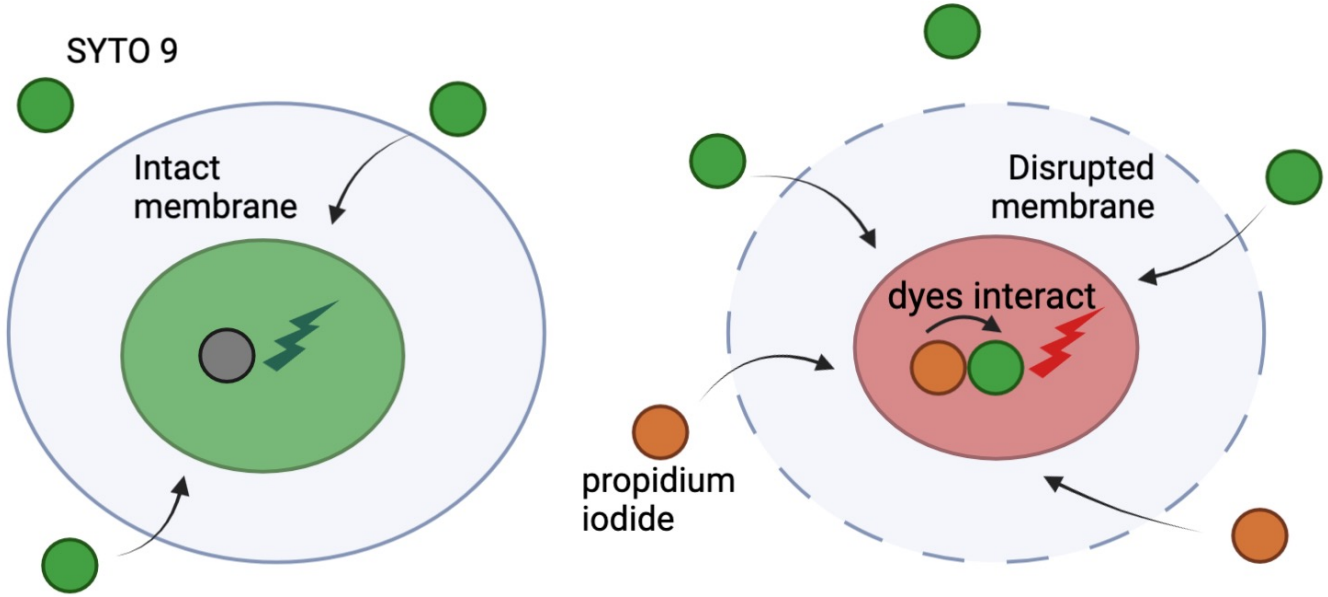
Sytox Green



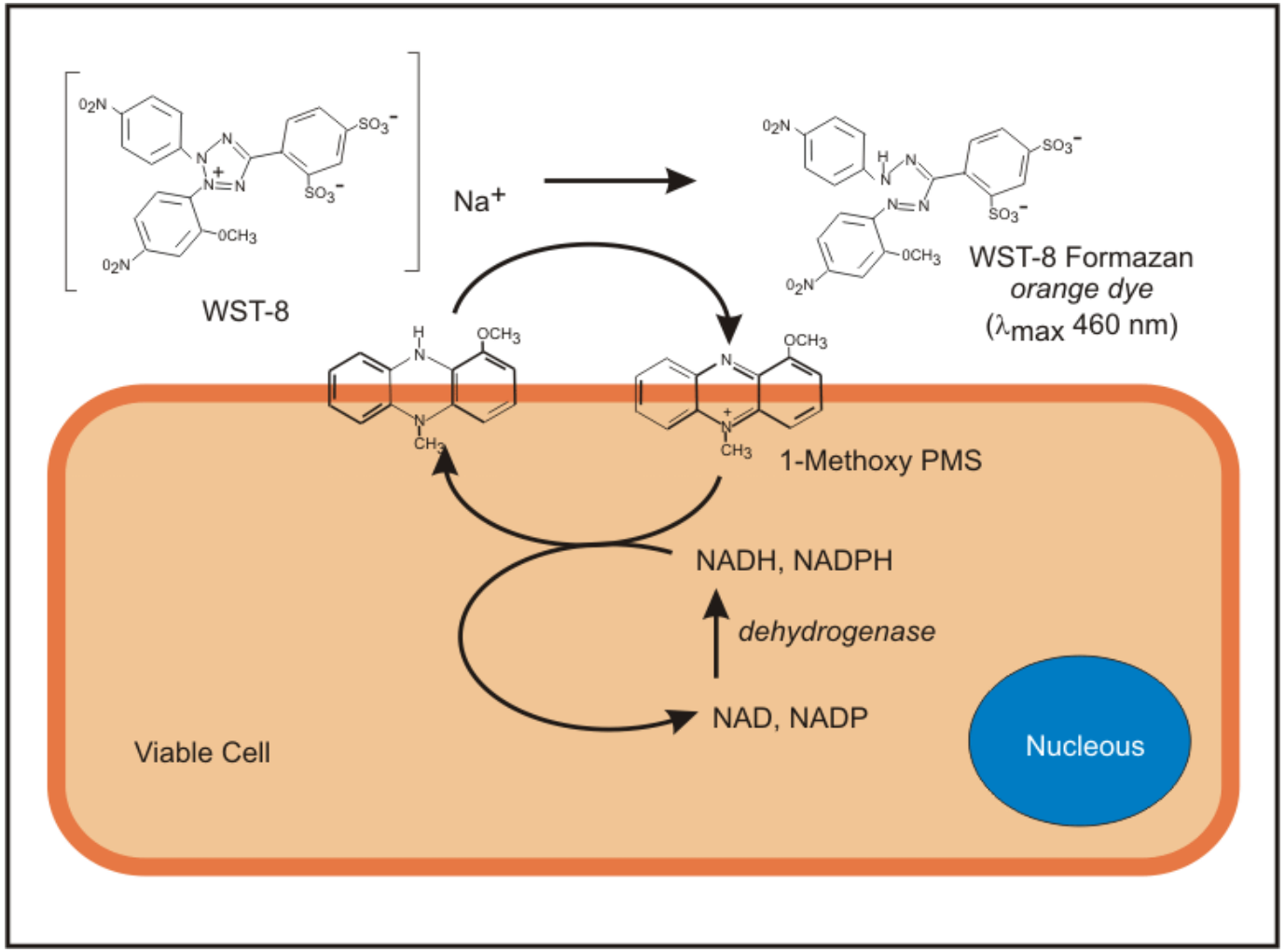
LIVE/DEAD assays can simultaneously mark both states of cells within a population

- SYTO 9 green-fluorescent nucleic acid stain
  - Can cross intact membranes and label all cells in a population
- Propidium iodide nucleic acid stain
  - Only enters cells with damaged membranes
  - Interacts with STYO 9 to produce a red fluorescence
- Cells with intact membranes stain fluorescent green
- Cells with damaged membranes stain fluorescent red





Reduction of tetrazolium salts creates color change proportional to metabolic activity

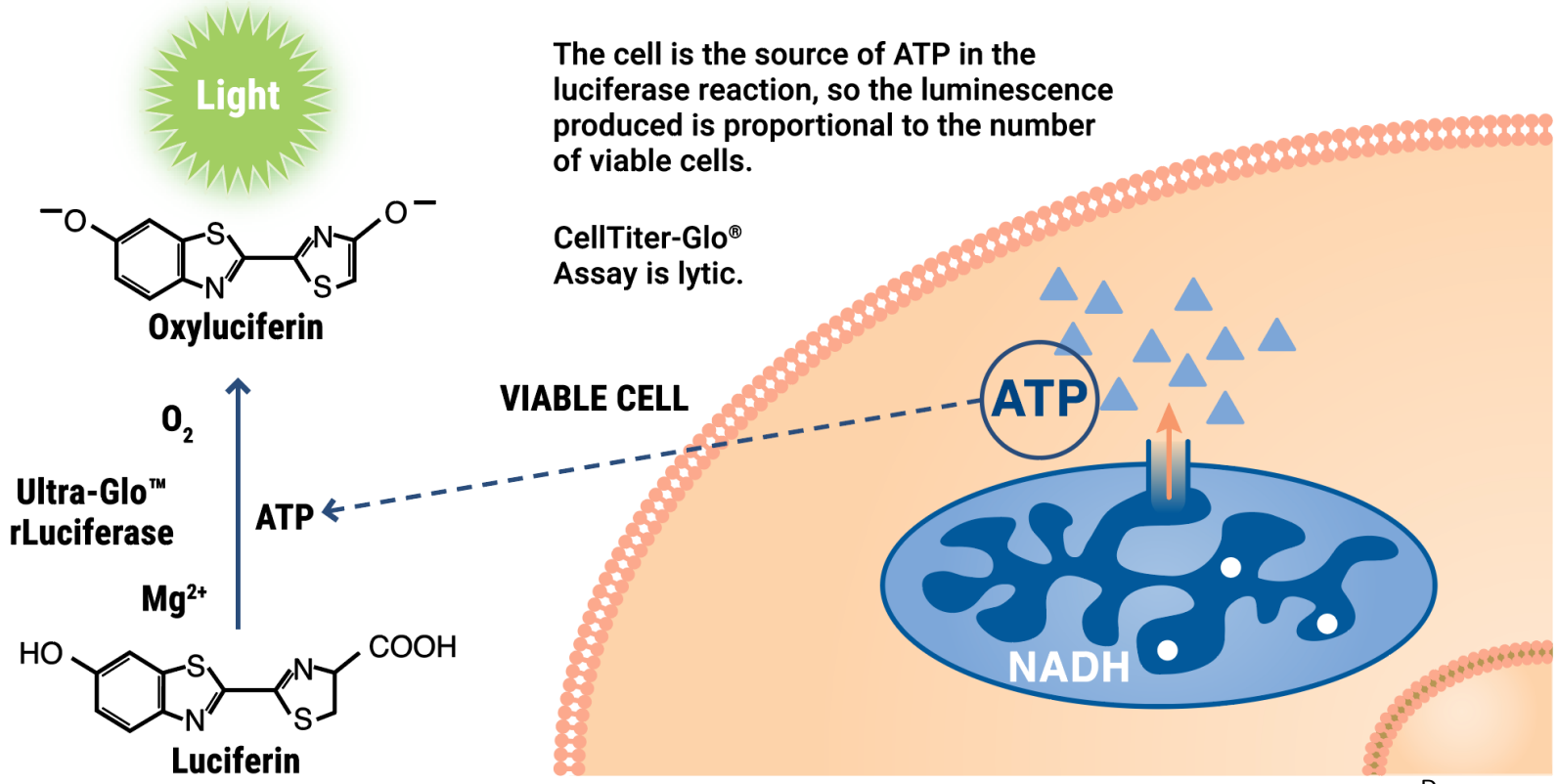


Held. 2009

- Tetrazolium salts can reduced into formazan as a by product of metabolic processes
- Color change is proportional to metabolic activity
- MTT = purple formazan precipitates
  - production kills cells
  - less sensitive
  - less expensive
- WST-8 = soluble orange formazan
  - cells survive assay
  - more sensitive
  - more expensive

ATP content can be used as a proxy for cellular physiology and metabolic activity

- Luciferase utilizes ATP to oxidize Luciferin to Oxyluciferin
  - Emits light as a result
- Luminescence is proportional to ATP and thus to cell metabolic activity
- Metabolic activity can change without resulting in cell death
  - In contrast to loss of membrane integrity



Promega

What are you doing in lab today?

- Experimental lull!
  - Biology moves slower than grand plans…
- Align sequencing to determine presence of mutations
- Prepare coverslips for the immunofluorescence experiment that begins after Spring break/ Journal article presentation week