BioTek Synergy H1 Microplate Reader for Absorbance Measurements

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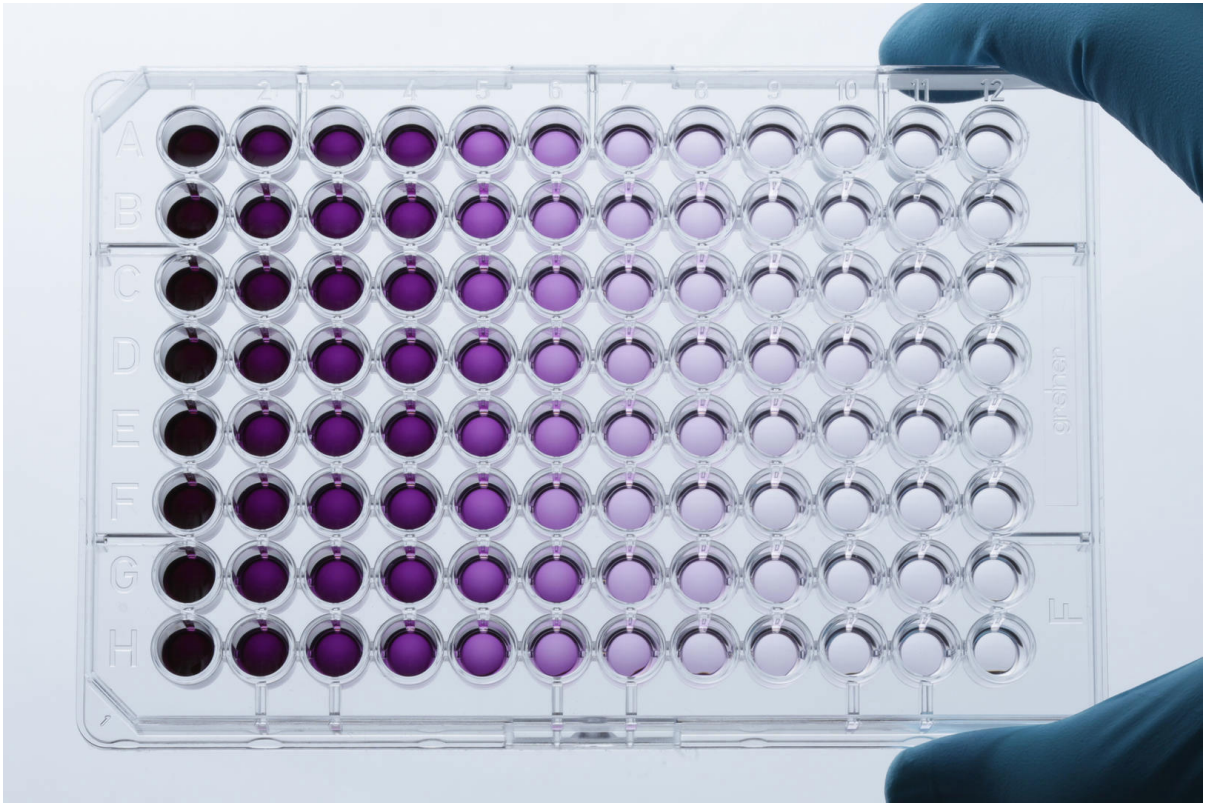
Beer-Lambert law relates absorbance and solution concentration linearly

- **Intensity** I - measure of how much light
- **Transmittance** T - ratio of transmitted light vs. incident light at a wavelength λ
  - $T = \frac{I}{I_0}$
- **Absorbance** A - capacity of a substance to absorb light at a wavelength λ
  - $A = -\log_{10}(T) = -\log_{10}\left(\frac{I}{I_0}\right)$

- **Beer-Lambert law** - absorbance varies linearly with solution concentration and path length
  - $\boxed{A = \varepsilon b C}$
  - ε - extinction coefficient
  - b - path length
  - C - solution concentration

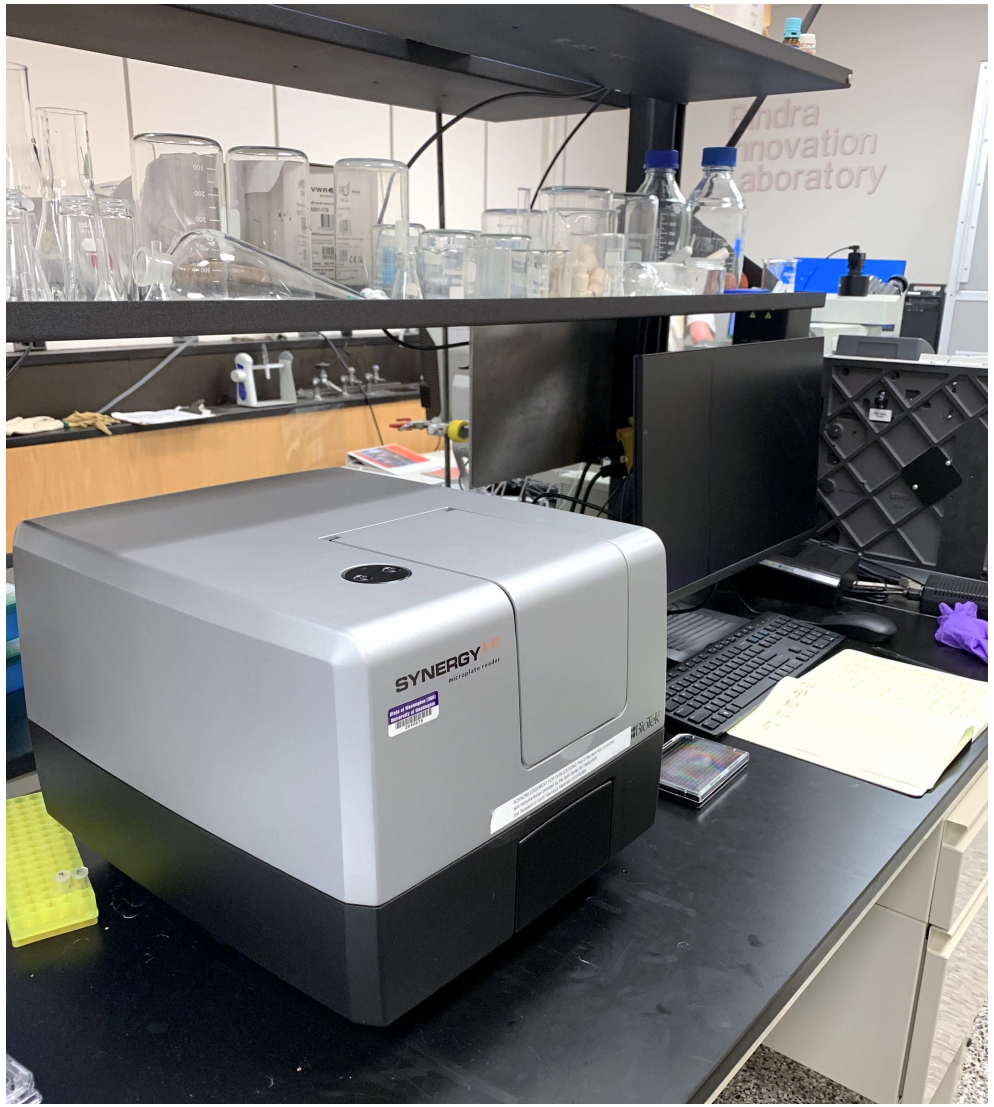
96-well plates contains 200 μL sample of interest in each well

- 200 μL sample in each well
- At least one blank control
- Avoid air bubble (reverse pipetting)
- Label the wells



Microplate reader location, access, and training

- The microplate reader (BioTek Synergy H1) is located in the Bindra Innovation Laboratory (Benson Hall 121).
- Book usage time on shared Google Calendar
- Log usage time on logbook



Microplate reader startup

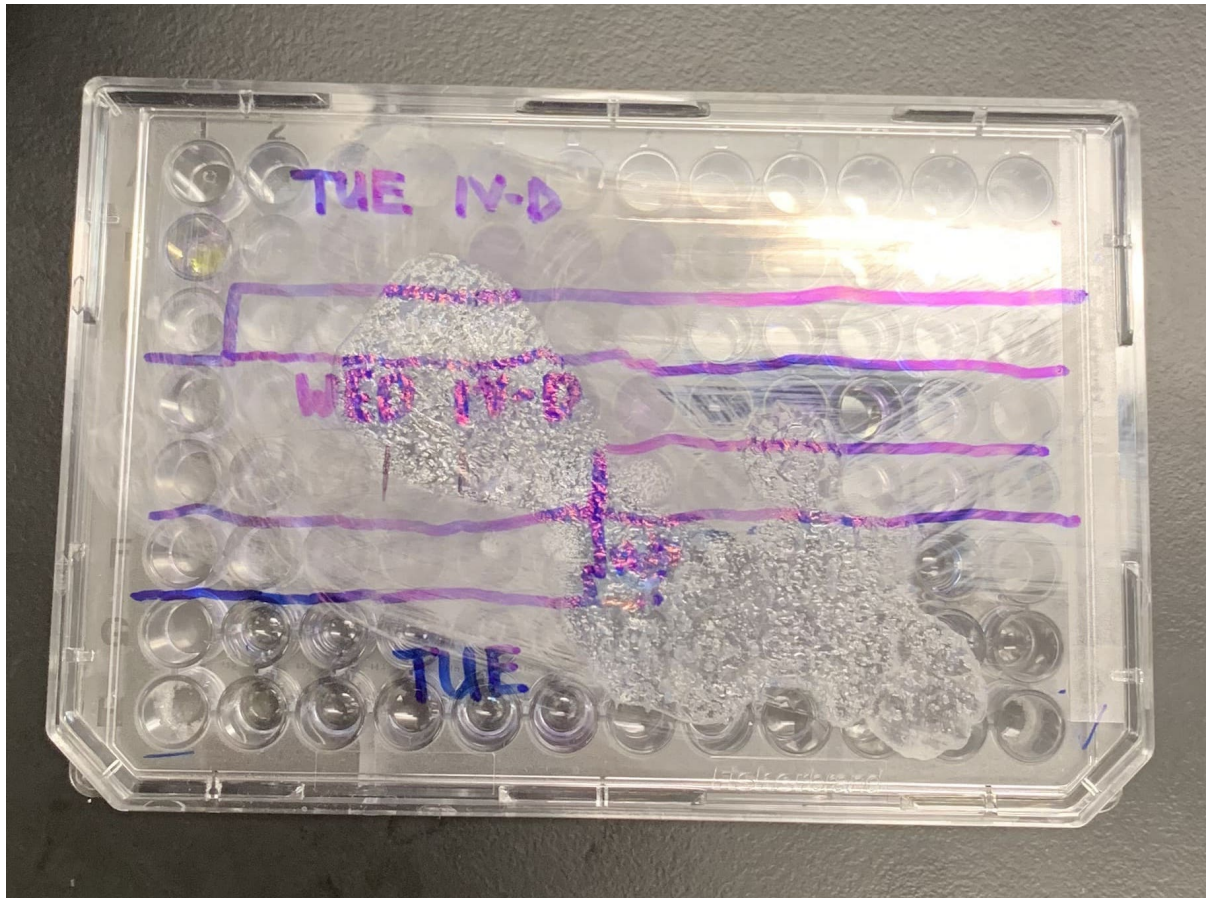
- Turn on the microplate reader
  - Wait for self-diagnosis
  - Create empty Google Sheet
- Open "Gen5 3.09" software

Plate reading settings - absorbance wavelength

- Measurement mode (default)
  - Read method: Absorbance
  - Read type: Endpoint/Kinetic
  - Optics type: Monochromators
- Absorbance wavelength
  - Lab 3-1: CMC by Dye Titration
    - λ = 615 nm for pinacyanol chloride
  - Lab 3-3: Aggregation of Clay
    - λ = 860 nm for turbidity
  - Lab 4-4: Bubble Fractionation
    - λ = 590 nm for crystal violet

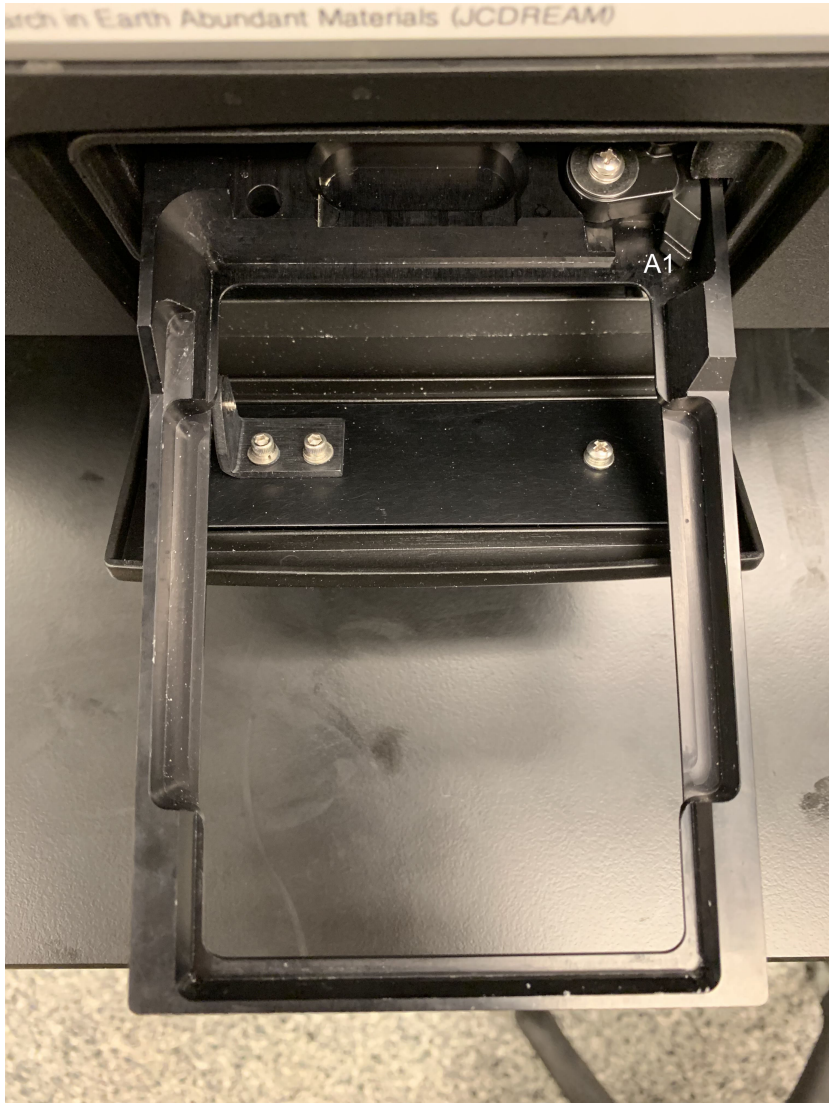
Plate reading settings - plate layout

- Plate Layout
  - Select “Blanks” and “Samples”



Absorbance measurement

- Place the plate into the plate holder
  - A1 well is on the top right.
- Read the plate using computer software
- Remove the plate from the plate holder.



Data recording

- Export the data in both matrix and stats form
  - Use blank-subtracted absorbance

Microplate reader shutdown

- Close all programs on the computer.
- Push the IN/OUT button for the plate holder so the plate holder retracts.
- Push the ON/OFF button for the plate reader so the instrument is turned off.

Absorbance measurements for Surface and Colloid Science Laboratory

- Lab 3-1: CMC by Dye Titration
  - λ = 615 nm for pinacyanol chloride
  - Look for changes in linear trend of absorbance
- Lab 3-3: Aggregation of Clay
  - λ = 860 nm for turbidity
  - Need calibration curve
- Lab 4-4: Bubble Fractionation
  - λ = 590 nm for crystal violet
  - Need calibration curve

References

- Jones, F. E., & Harris, G. L. (1992). ITS-90 Density of Water Formulation for Volumetric Standards Calibration. Journal of Research of the National Institute of Standards and Technology, 97(3), 335–340. https://doi.org/10.6028/jres.097.013
- Koretsky, M. D. (2012). Engineering and Chemical Thermodynamics (2nd edition). Wiley.
- McVay, C. W. (1989). Determination of Density with the Paar Digital Density Meter. https://www.nrc.gov/docs/ML0329/ML032960458.pdf
- Richard Shelquist. (2023). Equations—Air Density and Density Altitude. https://wahiduddin.net/calc/density_altitude.htm