General Biology Laboratory Booklet

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A Member of The Pennsylvania Alliance for Design of Open Textbooks

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About the Author

Dr. Martilias Farrell is a Visiting Professor of Biology at Cheyney University of Pennsylvania. His research spans the application of emerging technologies—including those at the intersection of biology, medicine, and computation—to deepen our understanding of the natural world and improve the human experience. Equally committed to teaching, Dr. Farrell designs scientific learning experiences that are accessible, resource-conscious, and grounded in the principles of rigor and reproducibility. He believes that good science doesn’t require expensive equipment—just sharp thinking, disciplined execution, and a deep curiosity about how the world works. Through his lab manuals and instruction, he encourages students to question boldly and investigate purposefully, regardless of where they begin.

About the Cover

TIP: Ask Gemini to refine images

With image generation in Gemini, your imagination is the limit. If what you see doesn't quite match what you had in mind, try adding more details to the prompt. The more specific you are, the better Gemini can create images that reflect your vision.

The striking Romanesco, often mistaken for an exotic hybrid, is actually a testament to centuries of selective breeding from wild cabbage, the same ancestor as broccoli and its closer relative, cauliflower. Originating in 16th-century Italy, farmers cultivated this unique cultivar for its distinctive chartreuse florets, arranged in mesmerizing logarithmic spirals. This fractal geometry, where each bud mirrors the overall conical structure, arises from a developmental quirk where flower buds repeatedly initiate but fail to fully bloom, a process amplified in Romanesco. Thus, this vegetable, shaped by human intervention, beautifully reveals the underlying mathematical order inherent in natural growth patterns.

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Note to the Reader

Dear Reader,

Thank you for checking out my lab manual. This booklet is designed to be used by the instructor – the students should not receive a raw copy of this booklet. Instead, you should be able to print out the pages (either on paper or to a new PDF that the student can download) that are specific to the student laboratory activity. Each laboratory activity should be an even number of pages, so you can maximize double sided printing. Most of the laboratory activities include "Notes for the instructor", which the students should not receive. In addition, there is some appendix content that will be helpful.

As a scientist that has performed research in laboratories that have ranged in funding support, I feel it's important to demonstrate that good science can be performed without lavish budgets. Indeed, the pivotal facet of science is reproducibility, and modern biomedical science has painted itself into a corner through published work that is inherently incapable of being reproduced due to the costs involved. Therefore, you should be able to perform these activities in this booklet with minimal resources. My institution's teaching labs contain microscopes, prepared microscope slides, and anatomical models. Hopefully your institution considers these things the "basics" of undergraduate teaching labs and has provided this equipment. Some of the labs described require more equipment or consumables, but I have provided links to Amazon items that can be considered.

In addition to wherever you have found this manual, I will also maintain versions on GitHub: General Biology Laboratory Booklet, and Human Anatomy and Physiology Laboratory Booklet. I welcome pull requests or any other forms of collaboration.

While these labs are relatively simple, they do seem to challenge the students and intrigue them. I feel it is important to emphasize to the students that science does not need to be fancy. Instead, it needs to be rigorous and reproducible, and scientists need to be disciplined in their work.

In the future, I hope to establish a virtual mega lab, where some of the data collected from the investigatory labs can be combined and ultimately published.

I wish you the best in your teaching.

Sincerely,
Martilias Farrell, PhD

Microscopic Life, Laboratory Notebooks and the Scientific Method

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Introduction

The scientific method is a fundamental aspect of biology, with observation and documentation playing crucial roles. In this lab, we will explore and document the microscopic life found on our campus using microscopes and collected samples. The overarching goal is to observe, record, and analyze microbial life over time.

Laboratory Notebook Guidelines

Each time you conduct research, make an entry in your laboratory notebook using the following format. Reference the "Lab Notebook Format" document in the appendix for further details.

1. Date – Every lab entry must be dated
2. Title – Provide a descriptive title
3. Hypothesis / Goal / Objective
   - Briefly state the purpose of the experiment, including relevant background for context.
4. Methods
   - List protocols, calculations, reagents, and equipment.
   - Stop writing here—subsequent sections will be completed as lab work proceeds.
5. Observations
   - Document all occurrences, planned or unplanned.
   - Record raw experimental data.
   - Tape in additional information or reference the data’s location.
6. Data Analysis
   - Process raw data, create graphs, and provide interpretations.
7. Discussion / Conclusions
   - Summarize findings and implications.

Lab Activity: Observing Microscopic Life

Sample Collection & Naming Scheme

1. As a class, establish a standardized naming scheme for sample collection locations on campus.

    - Obtain a campus map and overlay a grid to designate sample sites.
2. In your notebook, document the sample location in the Methods section.
3. Record environmental data, including the following:
    - Outdoor temperature
    - Weather conditions
    - Recent rainfall history
    - Sample temperature

Macroscopic Observations

Before using the microscope, examine the sample with the naked eye:

- Is it cloudy?
- Are there visible particles?
- Can you see any movement?

Microscopic Observations

1. Preparing the slide

- Use a plastic pipette to collect a small sample (just a drop).
- Place a drop on a microscope slide.
- Return the remaining sample to its container.
- Cover the drop with a coverslip.
- Document whether the sample was taken from the top or bottom of the container.

2. Using the microscope

- Start with the lowest power objective (4x).
- Adjust the lighting using both the iris and the lamp. A small opening in the iris allows for improved contrast.
- Identify any moving organisms and track them using stage adjustment knobs.
- Examine the edges of the coverslip—many microorganisms tend to gather there. Hypothesize why this might be the case.

3. Exploring higher magnifications

- Increase magnification to locate and examine moving microorganisms.
- Take note of stationary objects and practice distinguishing between biological specimens and dust particles.
- Draw sketches of observed microorganisms. Some students like to take pictures with their cells phones to share. You can do this, but you also need to sketch in your notebook or find a way to print the images and transfer them to your notebook.

- **DO NOT use the oil lens**. The oil lens is messy, and it doesn't really provide any benefit for this experiment.

4. Additional sample exploration

- Prepare a second slide from the same sample.
- Select a different section of the sample to observe additional features.

Post-Lab Cleanup and Documentation

1. Microscope maintenance
   - Remove the slide before returning the microscope to the shelf.
2. Slides
   - Discard slides in a glass / sharps container.

Post Lab Questions

Use these questions below to help guide your discussion section in your lab notebook. Please make your entry in the lab notebook contain complete sentences; I should be able to make sense of the notebook entry by itself. Note that you don't have to answer all these questions; instead, these are just some ideas to get you thinking about your work.

1. What types of microorganisms or structures did you observe? How did their appearance change under different magnifications?

2. Did you notice any patterns in movement? Were certain organisms more active than others? What might explain these differences?

3. How did the environmental conditions (temperature, recent weather, etc.) relate to what you observed in your sample?

4. Did your observations change depending on where in the sample (top, middle, bottom) you collected your drop? What does this suggest about the distribution of microscopic life?

5. What challenges did you encounter when using the microscope? How did you overcome them?

6. How did using phase-contrast microscopy affect what you were able to see compared to standard bright-field microscopy?

7. If you used oil immersion, what additional details were visible compared to lower magnifications?

8. Why do you think microorganisms tend to congregate at the edges of the coverslip?

9. Based on your observations, how might environmental changes (e.g., temperature shifts, rainfall, pollution) affect the types and abundance of microscopic life in future samples?

10. If you were to expand this study, what additional tests or methods would you use to learn more about the organisms in your sample?

11. If you could redo this experiment, what would you do differently to improve your observations or data collection?

12. How does this lab connect to broader biological concepts, such as microbial ecology, water quality, or the scientific method?

Read “Microscopic Life, Laboratory Notebooks and the Scientific Method (Instructor Notes)”

Ecosystem Observation and Sample Collection

Part I: Background and Procedures (for Your Records)

Introduction

Biology is not confined to the laboratory; it is the living world around us. The most fundamental skill for a biologist is the ability to observe, ask questions, and seek answers in the natural world. In this exercise, you will act as a field biologist to explore a local ecosystem.

Foundational Concepts

- **Ecosystem Components:** An ecosystem is a community of biotic factors (living or once-living parts, like plants, animals, and fungi) interacting with their physical environment of abiotic factors (non-living components like sunlight, water, and soil).
- **Habitat and Niche:** A habitat is where an organism lives. A niche is that organism's role within the ecosystem, including what it eats and how it interacts with its environment.
- **Ecological Data:** We use qualitative data (descriptive observations) and quantitative data (numerical measurements) to study ecosystems. A quadrat is a standard-sized frame used to collect quantitative data on species abundance and distribution.
- **Energy Flow and Adaptations:** Energy flows from the sun to producers, then to consumers. Each organism has adaptations (inherited traits) that help it survive in its environment.

Safety Protocols

- **Proper Attire:** Closed-toe shoes are mandatory.
- **Awareness:** Stay with your group and be aware of your surroundings.
- **Leave No Trace:** Do not disturb the environment or leave anything behind.
- **Handle with Care:** Do not touch unknown plants or animals.

Procedures

Procedure 1: Ecosystem Observation

1. **Terrestrial Site:** At your first location, observe silently. Record abiotic and biotic factors in your Lab Report.
2. **Aquatic Site:** At the second location, repeat the observation process, noting properties of the water and the life within it.

Procedure 2: Quadrat Study

1. At the terrestrial site, mark a 1 meter square using string.
2. **Count:** Tally the individuals of 2-3 plant species. You can use apps on your phone to identify different species (one app is Plant Net, but others are available).
3. **Estimate:** Estimate the percent of the ground covered by different materials (grass, soil, etc.).
4. Record all numbers in your Lab Report.

Procedure 3: Behavioral Observation

1. Find an animal to observe without disturbing it.
2. Record your initial observations of the animal. Record your observation at time point 0:00 in the Lab Report.
3. Observe for a continuous 5 minute period.
4. Record your final observations at time point 5:00 in your Lab Report.

Procedure 4: Sample Collection

1. **Soil Core:** At a designated spot, use a spade and ruler to excavate a 5x5x5-inch cube of soil. Place it carefully in a labeled box.
2. **Water Sample:** Collect a jar half-full of water from the aquatic site, including some bottom sediment. Seal and label it.
3. Give your samples to your instructor.

Part II: Prelab Assignment (To Be Completed and Turned in Before Lab)

Name: [Name]

Date: [Date]

Instructions: Before coming to lab, read through the Background and Procedures (Part I) and answer the following questions.

1. What is the difference between a biotic factor and an abiotic factor? Give an example of each.

2. Explain the difference between an organism's habitat and its niche.

3. What is the purpose of a quadrat?

4. Why is it critical to wear closed-toe shoes for this lab?

Part III: Lab Report (To Be Completed and Turned In)

Name: [Name]

Date: [Date]

Data and Observations

1. Qualitative Observations

Qualitative Observations

Location	Abiotic Factors Observed	Biotic Factors Observed
Terrestrial Site		
Aquatic Site		

2. Quantitative Quadrat Data

Quantitate Quadrat Data

Species Name	Number of Individuals
Species 1:	
Species 2:	
Species 3:	

Quantitative Quadrat Data 2

Component	Estimated % Ground Cover
Grasses/Lawn	
Other Plants	
Bare Soil/Rock	

3. Behavioral Observation Log

Animal Observed: [Animal Species and Number]

Quantitative Quadrat Data 3

Time Stamp	Behavior Observed
0:00	
5:00	

Analysis & Conclusions

Answer the following questions in complete sentences.

1. **Compare Sites:** How did the abiotic factors differ between the two sites, and how might this affect the organisms living there?

2. **Analyze Data:** What was the most common plant in your quadrat? Hypothesize why it is successful in this spot.

3. **Interpret Behavior:** What did your observed animal spend most of its time doing? How does this behavior help it survive?

4. **Adaptations:** Describe a key adaptation of one organism you observed.

5. **Energy Flow:** Identify a producer, a primary consumer, and a decomposer you observed. Draw a simple diagram showing the energy flow.

6. **Field Work Value:** What is one thing you learned outside that you couldn't have learned from a textbook?

Soil Core Analysis

Part I: Background and Procedures (for Your Records)

Introduction

This lab focuses on the detailed analysis of the terrestrial soil core collected during the field exercise. Your team will carefully dissect the core, identify its distinct layers, and sort its contents. This will reveal the hidden structure and composition of the ground beneath your feet.

Foundational Concepts

- **Soil Horizons:** Soil is organized into layers called horizons.
  - **O Horizon (Organic):** The top layer, composed of decaying organic matter (detritus).
  - **A Horizon (Topsoil):** A mix of decomposed organic matter (humus) and minerals. Most roots and soil organisms are found here.
  - **B Horizon (Subsoil):** A deeper, mineral-rich layer with less organic matter.
- **Categorizing Components:** To analyze the core, you will sort its contents into standardized categories: Live Plants, Detritus, Rocks & Gravel, Live Invertebrates, and Man-Made Objects.
- **The Soil Ecosystem:** The soil core is a miniature ecosystem. The distribution of its components reveals how the ecosystem functions.

Safety Protocols

- **Lab Safety:** No food or drink at the lab bench.
- **Hygiene:** Avoid touching your face while handling soil. Wash your hands thoroughly with soap and water after the lab.
- **Respect for Life:** Handle any live invertebrates with care.

Procedures

Procedure 1: Core Examination & Stratification

1. Place your soil core in a lab tray.
2. Observe the core from the side to identify the different layers (horizons).
3. In your Lab Report, sketch the core, labeling the visible horizons. Measure and record the approximate thickness of each layer.

Procedure 2: Core Dissection & Component Sorting

1. Carefully separate the O Horizon from the rest of the core. Then, separate the A Horizon from the B Horizon (if visible).

2. **For each horizon separately,** sift through the material.
3. Use forceps and a magnifying glass to find and sort all visible components into labeled sorting containers.
4. Count or estimate the quantity of items in each category for each horizon and record the data in your Lab Report.

Part II: Prelab Assignment (To Be Completed and Turned in Before Lab)

Name: [Name]

Date:[Date]

Instructions: Before coming to lab, read through the Background and Procedures (Part I) and answer the following questions.

1. What are the three main soil horizons, and what is a key feature of each?

2. Why is it important to sort the contents of the soil into standardized categories?

3. What is the most important safety precaution to take after handling the soil?

Part III: Lab Report (To Be Completed and Turned In)

Name: [Name]

Date:[Date]

Data and Observations

1. Soil Core Profile

Sketch your soil core. Label the O, A, and B horizons. Note the thickness and describe the color/texture of each.

- Sketch:

- Descriptions:

2. Component Analysis Data

Component Analysis Data

Component Category	O Horizon (Top Layer)	A Horizon (Topsoil)	B Horizon (Subsoil)
Live Plants (roots, etc.)			
Detritus (dead leaves, etc.)			
Rocks & Gravel			
Live Invertebrates			
Man-Made Objects			

Analysis & Conclusions

Answer the following questions in complete sentences.

1. **Compare Horizons:** In which horizon did you find the most life? The most detritus? Explain why this makes sense.

2. **Connect to the Field:** How do the abiotic components inside the core relate to the abiotic factors you observed at the surface in the previous lab?

3. **The Role of Decomposers:** Why are decomposers essential for the health of the producers (plants) in this ecosystem?

4. **Human Impact:** Did you find any man-made objects? What does this tell you about the ecosystem?

5. **Hypothesize for the Microscope:** Which horizon do you hypothesize will have the most microscopic life? Explain your reasoning.

Microscopic Analysis

Part I: Background and Procedures (for Your Records)

Introduction

This lab focuses on the microscopic investigation of the soil and water samples you collected. Your team will use dissecting and compound microscopes to explore the hidden world of microorganisms that inhabit these ecosystems. This will connect the large-scale patterns you observed in the field to the microscopic life that drives them.

Foundational Concepts

- **Microscope Types:**
  - **Dissecting Microscope:** For lower magnification, 3D viewing of larger objects like detritus and small insects.
  - **Compound Light Microscope:** For high magnification viewing of cells and microorganisms.
- **The Microscopic Community:** Your samples are teeming with life. Look for fungi (thread-like hyphae), protists (amoebas, paramecia, algae), and microinvertebrates (nematodes, rotifers).
- **Wet Mount Preparation:** To view specimens with a compound microscope, you will place a sample in a drop of water on a slide and cover it with a coverslip.

Safety Protocols

- **Microscope Care:** Always carry the microscope with two hands. Start and end on the lowest power objective.
- **Glassware Safety:** Handle slides and coverslips carefully.
- **Lab Hygiene:** Wash hands thoroughly after the lab.

Procedures

Procedure 1: Dissecting Scope Survey

1. Place a small piece of leaf litter (detritus) from your O horizon in a petri dish.
2. Observe under the dissecting microscope. Look for fungal hyphae and tiny invertebrates.
3. In your Lab Report, sketch one significant observation.
4. Repeat with a small clump of soil from your A horizon.

Procedure 2: Microscopic Soil Analysis

1. Prepare a wet mount of a tiny piece of decomposed material from your O horizon.
2. Observe under the compound microscope, starting on low power. Scan for microorganisms.
3. In your Lab Report, sketch one representative microorganism.
4. Repeat this process, creating new wet mounts for your A Horizon and B Horizon samples.

Procedure 3: Microscopic Aquatic Analysis

1. Prepare a wet mount using one drop of water from the bottom of your aquatic sample jar.
2. Observe under the compound microscope.
3. In your Lab Report, sketch at least two different types of microorganisms you observe.

Part II: Prelab Assignment (To Be Completed and Turned in Before Lab)

Name: [Name]

Date:[Date]

Instructions: Before coming to lab, read through the Background and Procedures (Part I) and answer the following questions.

1. When would you use a dissecting microscope instead of a compound microscope?

2. What is the purpose of a "wet mount"?

3. Name two types of microorganisms you might find in your samples.

Part III: Lab Report (To Be Completed and Turned In)

Name: [Name]

Date:[Date]

Data and Observations: Microscopic Observations

For each row: draw (or insert) what you see under the microscope, label identifiable parts, and note the sample source and total magnification.

Microscopic observation entries. One row per observation.

Scope Type / Sample	Horizon	Magnification (X)	Drawing, Labels & Notes
Dissecting – Detritus Sample	O	[e.g., 10X]	[Draw/insert detritus. Label fragments, roots, organisms.]
Compound – O Horizon Sample	O	[e.g., 40X]	[Draw/insert fine structures. Label hyphae, cells, etc.]
[Scope & sample]	[Hrzn]	[MagX]	[Draw & label observed parts; add notes.]
[Scope & sample]	[Hrzn]	[MagX]	[Draw & label observed parts; add notes.]

Analysis & Conclusions

Answer the following questions in complete sentences.

1. **Compare Samples:** In which sample did you find the most biodiversity (greatest number of different types of organisms)?

2. **Confirm Hypothesis:** Refer to your hypothesis from the soil sample dissection lab. In which soil horizon did you find the most microscopic life? Was your hypothesis correct? Explain.

3. **The Fungal Network:** Describe what you saw on the detritus under the dissecting scope. How does this relate to the niche of fungi?

4. **Life in Water:** Describe one organism from the aquatic sample and an adaptation it has for living in water.

5. **Connecting the Labs:** How does the microscopic life you saw today explain the decomposition of detritus into topsoil that you observed in the soil sample dissection lab?

Biochemical Analysis of Environmental Samples

Part I: Background and Procedures (for Your Records)

Introduction

This lab focuses on the biochemical investigation of your environmental samples. Your team will prepare extracts from your soil horizons and test them for the presence of major macromolecules: carbohydrates, proteins, and lipids. This will reveal the chemical foundation of your ecosystem.

Foundational Concepts

- **Nutrient Cycling:** Decomposers break down complex organic matter, releasing simpler molecules (nutrients) into the soil and water for plants to use. Today, you are taking a chemical snapshot of this cycle.
- **Chemical Indicators:**
  - **Benedict's Solution:** Tests for simple sugars (requires heat).
  - **Iodine Potassium Iodide (I2KI) Solution:** Tests for starch.
  - **Biuret Reagent:** Tests for protein.
  - **Sudan IV:** Tests for lipids.
- **Preparing an Extract:** To test for molecules in the soil, you must create an extract by mixing the soil with water and filtering out the solid particles, leaving a liquid containing the dissolved chemicals.

Safety Protocols

- **Lab Safety:** Goggles must be worn at all times.
- **Chemical Safety:** Biuret reagent is caustic. Handle it with care.
- **Hot Plate Safety:** Use test tube holders to handle hot test tubes.
- **Hygiene:** Wash hands thoroughly after the lab.

Procedures

Procedure 1: Preparing the Sample Extracts

1. Label two beakers: "O Horizon" and "A Horizon."
2. Place a small amount of soil from each horizon into the corresponding beaker. Add about 50 mL of distilled water to each and stir vigorously.
3. Filter each mixture through a funnel with filter paper into a clean, labeled test tube to collect the liquid extract.

Procedure 2: Performing the Biochemical Tests

1. For each of the four tests, set up a row of test tubes for your samples: Water (Negative Control), a Known Positive Control, O Extract, A Extract, and Aquatic Sample.
2. **Simple Sugars:** Add 2 mL of sample and 1 mL of **Benedict's solution** to each tube. Heat in a boiling water bath for 3-5 minutes. Record color change.
3. **Starch:** Add 2 mL of sample and 3-4 drops of **Iodine (I2KI) solution** to each tube. Record immediate color change.
4. **Protein:** Add 2 mL of sample and 1 mL of **Biuret reagent** to each tube. Record color change after 2-3 minutes.
5. Lipids: Add 2 mL of sample and 5 drops of **Sudan IV dye** to each tube. Look for a reddish-orange layer or droplets.

Part II: Prelab Assignment (To Be Completed and Turned in Before Lab)

Name: [Name]

Date:[Date]

Instructions: Before coming to lab, read through the Background and Procedures (Part I) and answer the following questions.

1. In your own words, what is nutrient cycling?

2. Why do we need to make a liquid "extract" from the soil?

3. Which indicator tests for protein? Which one tests for starch?

4. Why do we use positive controls?

Part III: Lab Report (To Be Completed and Turned In)

Name: [Name]

Date: [Date]

Data and Observations: Biochemical Data

Record the final color of each test. Write "+" for a positive result and "-" for a negative result.

Biochemical Data and Observations

Sample	Benedict's Test (Simple Sugars)	Iodine Test (Starch)	Biuret Test (Protein)	Sudan IV Test (Lipids)
Water (Neg. Control)				
Glucose (Pos. Control)				
Starch (Pos. Control)				
Albumin (Pos. Control)				
Oil (Pos. Control)				
O Horizon Extract				
A Horizon Extract				
Aquatic Sample				

Analysis & Conclusions

Answer the following questions in complete sentences.

1. **Summarize Findings:** Which macromolecules were most abundant in the O Horizon? In the A Horizon?

2. **The Decomposer's Role:** How do your results demonstrate the process of nutrient cycling by decomposers? (Hint: Think about complex vs. simple molecules in each layer).

3. **Life in the Water:** What macromolecules did you find in your aquatic sample? How might they have gotten there?

4. **Connecting the Labs:** In the microscopy lab, you saw fungi on the detritus. Fungi release enzymes. Which test results from today provide evidence of this enzymatic action? Explain.

5. **The Big Picture:** Explain how the presence of producers (like trees, from the field lab) leads to the presence of the molecules you found in the soil today.

Enzyme Lab I

This section begins the series of enzyme laboratory experiments. In the first lab, you will examine how a varying concentration of amylase affects the starch digestion rate of the amylase enzyme. You will create serial dilutions of amylase and measure reaction time using an iodine potassium iodide (12KI) test. The I2KI test is a colorimetric assay, in which the variation in color of the solution ranges from a dark blue-black to a yellow-amber. The dark blue-black color indicates a high concentration of a starch. The yellow-amber means there is a low concentration of the starch. This kind of assay is a workhorse for many professional laboratories, as it allows a scientist to measure the activity of incredibly small things in a robust and simple way. Additionally, we will use densitometry analysis to quantify these changes, and this type of analysis is another workhorse of professional laboratories. As such, we will continue to use these methods for many laboratory experiments, so get used to it!

Materials

- Test tube rack
- 10 standard test tubes
- Wax pencil / marker
- Assay plate (96 well microplate)
- Distilled water (flask & beaker)
- Rinse water
- 5-mL graduated cylinder
- 1% starch solution
- 2% amylase solution
- Buffer solution (pH 6.8)
- 12KI solution (this can stain clothing and skin. Wear proper PPE!)
- Pipettes:
- 5-mL pipettes with pump
- Micropipettes and tips (or disposable dropper pipettes)

Introduction

This experiment examines how varying amylase concentrations affect starch digestion rate. You will create serial dilutions of amylase and measure reaction time using an iodine (12KI) test. Make a lab notebook entry for this experiment.

Procedure

1. Preparation of Amylase Dilutions (Test Tube Set I)

1. Label the test tubes:
   - Use a permanent marker to number five standard test tubes from 1 to 5.
2. Add distilled water:
   - Using a 5-mL graduated pipette, dispense 4 mL of distilled water into each test tube.
3. Prepare serial dilutions:
   - Follow the dilution steps below, ensuring thorough mixing by capping the tube and inverting it three times (just turn the tube upside down and then right side up) after adding solutions:
   - Tube 1: Add 4 mL of the original 2% amylase solution and mix well.
   - Tube 2: Transfer 4 mL from Tube 1 into Tube 2 and mix.
   - Tube 3: Take 4 mL from Tube 2, transfer it to Tube 3, and mix.
   - Tube 4: Take 4 mL from Tube 3, add it to Tube 4, and mix.
   - Tube 5: Take 4 mL from Tube 4, add it to Tube 5, and mix thoroughly.

Amylase Dilutions

Tube	Amylase %
1	1.00%
2	0.333%
3	0.125%
4	0.067%
5	0.032%

2. Prepare Reaction Test Tubes (Set II)

1. Label another set of five tubes (1–5).
2. Transfer 2 mL from each tube in Set I to the corresponding tube in Set II. Set I is no longer needed.
3. Add 2 mL of pH 6.8 buffer to each tube in Set II and mix.

3. Prepare the Assay Plate

1. Add 50 ul of 12KI to five rows of a test plate (one row per enzyme concentration).
2. Choose 4 wells and fill with 50 ul of I2KI. These will be your controls.
3. Make a sketch of the assay plate in your lab notebook as this will help you with analysis.

4. Conduct the Reaction

1. Begin with tube 5 (lowest concentration of amylase):
    - Add 1 mL of 1% starch solution and mix (invert). Start timing immediately (Time 0). This is the start of the reaction: the amylase will start breaking down the starch.
    - Use a micropipette to transfer 50 ml of the mixture to the test plate immediately. (Time 0). This is called “sampling.”
    - Repeat sampling every 10 seconds in new compartments, using a new pipette tip every time. Repeat this until the entire row is full (12 wells, so 120 seconds).
2. Repeat for tubes 4, 3, 2, and 1.

5. Control Wells

1. In 2 of your control wells, add 50 ul of 1% starch solution. This will serve as the maximum signal for your analysis because there is no enzyme present. These wells should be the darkest of the wells.
2. In the other 2 control wells, add 50 ul of water. This will serve as the minimum signal for your analysis because there is no starch present. These should be the lightest of the wells.

Results & Analysis

1. Record Data

To capture the data for this experiment, we will take images of the assay plates you created. You can either use a gel imager (your instructor may be the one that does this for you), a plate reader (if your lab is equipped with one), or you may construct an imaging device using your cell phones and some carboard boxes (see appendix). This completes the laboratory activity for Day 1 of this experiment.

1. Graph Your Data (Day 2).

You will extract the data from the images using image analysis software like ImageJ to perform a densitometry analysis. You will use ImageJ (or a modern equivalent, such as FIJI) to essentially measure how dark the wells on your plate are.

Please follow the instructions in the attached packet (or in the PDF provided). You will submit your graphs to your instructor on your online platform or via email. You can perform this analysis on your own time or during the next laboratory session. It is recommended to wait until the next session in case you run into issues with the process.

Read “Enzyme Lab I (Instructor Notes)”

Enzyme Lab II – Modifiers of Enzyme Rate

The influence of other molecules on the enzyme activity of amylase.

Instructions

In today's lab, you will be doing something like you did last time (measuring the activity of the amylase enzyme as it breaks down cornstarch using the color change of I2KI). However, this week we won't be investigating the effect of enzyme concentration on this rate. Instead, we will be measuring whether the presence of other molecules (a modifier) can modify the enzyme activity. This is called "screening for activity," or, in other words, a "drug screen".

To do this, we will need 1 concentration of enzyme, 0.125%. In the interest of time, this has been prepared for you.

Your instructor will have a selection of modifiers you can choose from, including spice extracts, synthetic sweeteners, and the like.

Therefore, in your reaction tubes labeled 1-5, you should have the following:

Reaction Tube Contents

Tube #	Ph 7 buffer	Enzyme	Modifier
1 (control)	2.0 mL	2 mL	0
2 modifier A	1.9 mL	2 mL	100 µL
3 modifier B	1.9 mL	2 mL	100 µL
4 modifier C	1.9 mL	2 mL	100 µL
5 modifier D	1.9 mL	2 mL	100 µL

Prepare your reactions tubes as indicated above—add the ph7 buffer, the enzyme, and the modifier. Record in your notebook how you are setting things up and which modifiers you have selected (in other words, don't just copy the table above. Instead of "Modifier A", write in the actual modifier you added). In general, make a proper notebook entry! Remember, someone should be able to reproduce your work from your notebook entry.

Prepare your assay plate. Using a micropipette set at 50 µL, fill 5 rows of wells with 50 µL of I2KI solution. Also fill the control wells (at least 4, a "0" and a "max" in duplicate, as in the first enzyme experiment. How many controls can you think of?).

When your tubes and your plate are ready, start with the control tube. Add 1 mL of the 1% cornstarch solution, cap, and invert twice to mix. Immediately transfer 50 µL of reaction to the first well in the assay plate. 10 seconds after adding the starch, transfer 50 µL of your reaction to the second well in the plate. After another 10 seconds, transfer 50 µL of your reaction to the third well in the plate. Repeat until all 12 wells are completed for that reaction tube.

Now, move on to tube #2. Add 1 mL of 1% cornstarch and then perform the same sampling as above.

When you are done, we will image your plate as we did in the Enzyme 1 and quantify using ImageJ. You can perform this quantification with the remainder of lab time or outside of class (How to use ImageJ to quantify images).

Read “Enzyme Lab II – Modifiers of Enzyme Rate (Instructor Notes)"

Enzyme Lab III – Concentration Effect Curve

Instructions

In a prior lab, you performed a screening. That is, you took a bunch of different things and tried to find whether one of them would modify the activity of the amylase enzyme. As it turns out, you found a modifier! Or, at least, someone in your class did. This week, you will do 2 things: you will reproduce that finding (a key for robust science is confirmation), and you will also explore the concentration dependence of that effect. This is called many things depending on the context (a dose-response curve, a concentration-effect curve, etc.) but the primary idea is the same. We have two molecules, our enzyme and our modifier, and they interact with each other. How they interact with each other can influence their concentration-effect curve. In a more advanced version of this laboratory, the data we obtain from these kinds of experiments can be used to determine some fundamental aspects of how these molecules are interacting.

To pick your concentrations, you need to consider your results from your prior lab. If your modifier caused the enzyme to speed up—that is, your assay plate row went from dark to light very quickly (or was never dark to begin with)—that means that your modifier was great at speeding up the enzyme. This result means you need to test lower concentrations of the modifier. On the other hand, if your modifier caused the enzyme to slow down—your row went from dark to light very slowly—you should test higher concentrations of spice.

To do this, we will need 1 concentration of enzyme, 0.125%. In the interest of time, this has been prepared for you.

Therefore, in your reaction tubes labeled 1-5, you should have the following:

Reaction Tube Conents

Tube #	Ph 7 buffer	Enzyme	Modifier
1 (control)	2.0 mL	2 mL	0
2 concentration 1	1.9 mL	2 mL	100 µL
3 concentration 2	2.0 mL – x ul	2 mL	x µL
4 concentration 3	2.0 mL – y ul	2 mL	y µL
5 concentration 4	2.0 mL – z ul	2 mL	z µL

Prepare your reactions tubes as before—add the ph7 buffer, the enzyme, and the modifier. Record in your notebook how you are setting things up and which modifier and concentration you have selected (be sure to record the concentration of enzyme!!). In general, make a proper notebook entry!

Do note that you need to add different amounts of the modifier solution, and you therefore need to add different amounts of the ph7 buffer. For x, y, and z, for example, you could choose 10, 30, and 300µL. Therefore, you would need to calculate how much ph7 buffer you need. The 10 µL concentration tube would need 1990 µL (you can use the P1000 micropipette and set it to 1000 first and then 990).

Prepare your assay plate. Using a micropipette set at 50µL; fill 5 rows of wells with 50 µL of I2KI solution. Also, prepare your control wells.

When your tubes and your plate are ready, start with the control tube. Add 1 mL of the 1% cornstarch solution, cap, and invert twice to mix. Immediately transfer 50 µL of reaction to the first well in the assay plate. 10 seconds after adding the cornstarch, transfer 50 µL of your reaction to the second well in the plate. After another 10 seconds, transfer 50 µL of your reaction to the third well in the plate. Repeat until all 12 wells are completed for that reaction tube.

Now, move on to tube #2. Add 1 mL of 1% cornstarch and then perform the same sampling as above.

When you are done, we will image your plate as we did in the Enzyme 1 and quantify using ImageJ. You can perform this quantification with the remainder of lab time or outside of class (How to use ImageJ to quantify images).

Here are some questions to help guide the discussion session of your lab notebook entry. Remember to write your lab notebook discussion session in complete sentences.

1. What effect did the modifier have on amylase activity at different concentrations? Was the effect consistent across the concentration range?

2. Were your results consistent with your expectations from the previous lab’s screening? If not, what factors might have contributed to the differences?

3. What sources of error could have affected your data, and how might these errors influence your interpretation of the results?

4. How would you improve this experiment if you were to repeat it or extend it further?

Planaria Locomotor Lab

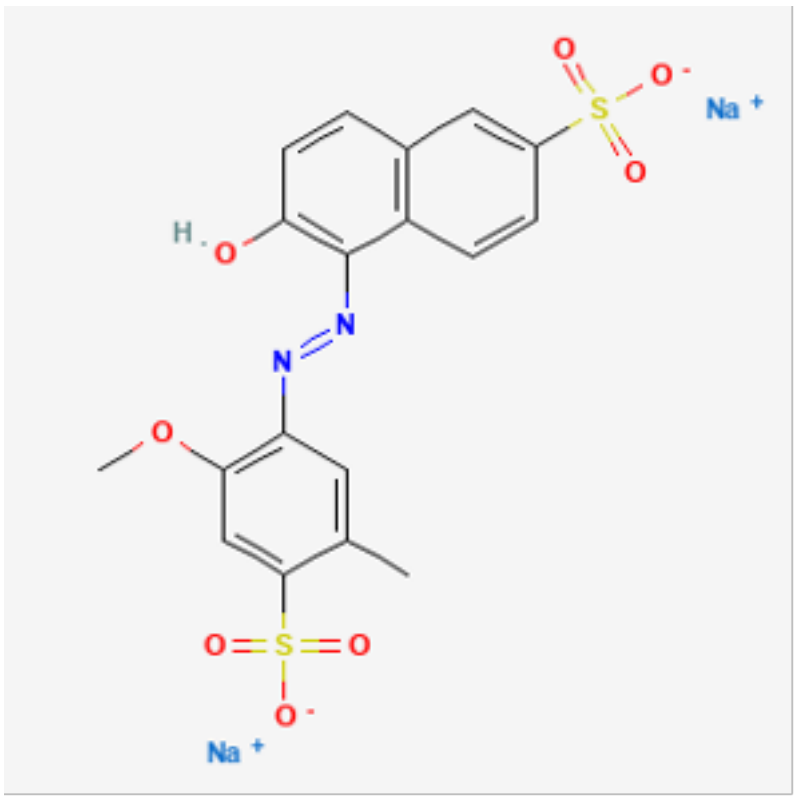
Introduction

Planaria are freshwater flatworms known for their remarkable regenerative abilities. They are also a convenient model organism for studying the effects of environmental contaminants due to their simple nervous system and sensitivity to changes in their environment.

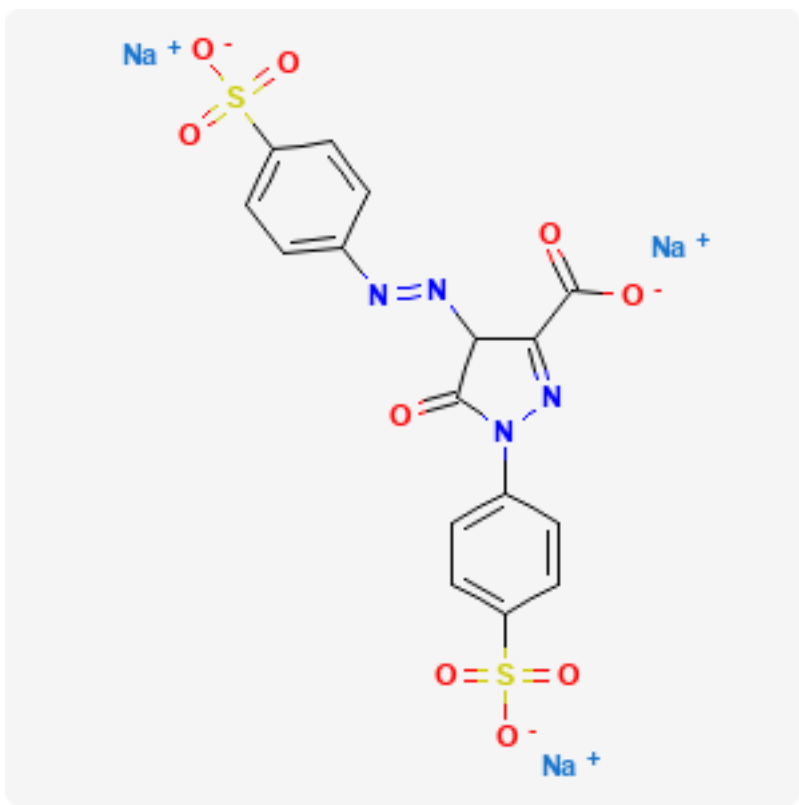
Red-40, also known as Allura Red AC, is a synthetic red dye widely used in processed foods, beverages, and cosmetics. Made from petroleum, it gives products a vibrant red color. While it is generally considered safe for consumption by the FDA, some studies suggest potential links to ADHD and allergies in sensitive individuals. The European Union approves Allura Red AC as a food colorant, but the local laws of some EU countries ban these food colorants.

Yellow-5, also known as tartrazine, is a synthetic bright yellow dye commonly used in processed foods, drinks, medications, and cosmetics. Made from petroleum byproducts, it creates an appealing color but can trigger allergic reactions in some people, especially those with aspirin sensitivity. While it is generally considered safe by regulatory bodies, some studies suggest potential links to hyperactivity in children.

In today’s experiment, we will be investigating the effects of pure food dye on the locomotor activity of planaria. We will be comparing the effects of Red-40 to Yellow-5.



Red-40



Yellow-5

Hypothesis

Red-40 exposure or Yellow-5 exposure will modify planarian locomotor activity compared to a control group.

Materials

- Planaria
- 15 cm petri dish
- Spring water
- Red-40 concentrated solution
- Yellow-5 concentrated solution
- Timer (use your phone)
- Graph Paper

WARNING – THESE ARE CONCENTRATED SYNTHETIC FOOD DYES. THEY WILL STAIN CLOTHES AND SKIN. WEAR GLOVES, LAB COATS, AND GOGGLES.

Procedure

In general, the procedure is to place a 15 cm petri dish on a piece of graph paper, add water or experimental solution, and then place the planaria in the center of the dish. Then count the number of grid lines crossed for 5 minutes. You consider a grid line crossed if the planaria's "tail" (end opposite of its eye-spots) crosses over a line.

Control Condition: Place petri dish on graph paper. Add 100 mL of room-temperature spring water to petri dish. Add planaria to center of dish. Start timer. Count the number of grid lines crossed by marking a tally in your notebook. Observe for 5 minutes. At the end of 5 minutes, add your tallies and record the number in your notebook.

Experimental Condition: Place petri dish on graph paper. Add 100 mL of room temperature spring water containing a concentration of red food dye to petri dish. Add planaria to center of dish. Start timer. Count the number of grid lines crossed by marking a tally in your notebook. Observe for 5 minutes. At the end of 5 minutes, add your tallies and record the number in your notebook. Scientists always perform each condition multiple times, so remove your used planaria and obtain a new one. Triplicate is considered a minimum, but you are free to perform as many replicates as you like (minimum of 3).

CONSIDERATIONS!!!: You want to wait until the planaria is "settled" (they stretch out, get a grip on the dish, and then start moving). In general, if you wait just 5 seconds (count in your head to 5), then start the timer, you should be good. However, don't wait for the planaria to start moving. Just count to five and then start recording their behavior. A lack of initial motor activity may be due to the experimental conditions.

When you are done with a planaria, place them in a separate "used" jar. Some students realized that if we use the same planaria over again, this may be influencing our data in unintended ways.

DON'T MOVE THE DISH FOR A BETTER VIEW! This movement will alter the behavior of the planaria. You need to move to get a better view. The planaria may glide on the walls. You can still count the lines crossed.

Data

Record your data in your lab notebook. Create a heading for your condition. You should mark a tally for every line the tail of the planaria crosses. An example:

Control condition (100 mL spring water)

||||||||||||||||||||||||||||||||||

Experimental condition (some concentration food dye in 100 mL spring water)

||||||||||||||||||||||||||||||||||||||||||||||||||

Analysis

Create a table in your notebook.

Locomotor Recording

Condition	Total # gridlines crossed
Control	
(Condition 1) – write your condition here	
(Condition 2)	
Etc…	

Results

Describe your results. “The planaria exposed to (some amount) of (chemical) moved (more/less) than the control condition. Also indicate any other behaviors you saw in the planaria.

Discussion

Record your thoughts on the experiment as well as notes for next time (what you would change), etc. Here are some post-lab questions that can help guide your discussion. If you decide to use these in your discussion, remember to include them in a full sentence. Don’t just write the answer to the questions.

1. Based on your data, did exposure to red-40 or yellow-5 affect planarian locomotor activity compared to the control? How do you know?
2. How consistent were your results across replicates? Were there any noticeable trends or unexpected variations?
3. Were there any challenges in counting the locomotor activity of planaria? How might these challenges affect the reliability of your results?
4. Did you notice any other behavioral changes in the planaria (e.g., erratic movement, clumping, curling)? How might these behaviors be interpreted?
5. How could using the same planaria across multiple conditions impact the results? Why is it important to use different individuals for each trial?
6. If you were to repeat this experiment, what changes would you make to improve data accuracy and reliability?
7. How do your findings relate to potential effects of synthetic food dyes on other organisms, including humans?
8. Given that food dyes are generally regarded as safe by regulatory agencies, what additional studies or evidence would be needed to evaluate their impact on living organisms?

Read “Planaria Locomotor Lab (Instructor Notes)”

Planaria Regeneration Screen

Objective

To determine whether various compounds affect the regenerative ability of planaria.

Background

As mentioned in a previous lab, planaria are known for their regenerative abilities. If you cut them in half, the head half will grow a new tail, and the tail half will grow a new head. This ability is directly related to the cell cycle. Studying the cell cycle is an important component of cancer biology because cancers cells are cells that have a dysfunctional cell cycle system. In today's lab, we will explore whether we can identify some substances that interfere with the cell cycle by measuring the ability of planaria to regenerate in the presence of these substances.

Lab Protocol

Materials

- Live planaria
- Dissection microscope
- Dissecting tools (scalpel, forceps, razor blade)
- 12-well plates
- Various test compounds (including controls)
- Planaria water or suitable culture medium
- Pipettes and disposable tips
- Gloves and safety equipment

Procedure

Day 1:

1. **Plate Preparation:**
    - Label the 12-well plate to indicate different test compounds and controls.
    - Make a sketch of the plate in your lab notebook, indicating which wells have which experimental conditions.
    - Fill each well with planaria water and the appropriate compound at the designated concentration.

2. **Planaria Preparation and Cutting:**
    - Obtain planaria. They should be kept in a jar on ice. The ice slows down their movement to make them easier to cut.
    - Carefully place planaria on a dissecting dish under a dissection microscope.
    - Using a scalpel, cut each planarian into three pieces (head, midsection, tail).
    - Transfer each piece into a separate well using forceps.
3. **Incubation Period:**
    - Maintain plates under appropriate conditions for 7 days.
    - Do not disturb the planaria during this period.

Day 2:

1. **Observation and Data Collection:**
    - After 7 days, use the dissection microscope to examine each planarian piece.
    - Record whether regeneration has occurred, using a binary scoring system:
        - **Regenerated**: Eyespots and normal morphology present.
        - **Not Regenerated**: No eyespots or abnormal morphology.
    - Record data individually, then compile class data for analysis.
2. **Data Analysis and Comparison:**
    - Compare regeneration rates across different compounds in the class dataset.
    - Identify trends or compounds that significantly impact regeneration.

Post-Lab Questions

Here are some post-lab questions to help guide your discussion:

1. Why is it important to use a control group when testing the effects of compounds on regeneration?

2. How might differences in planaria size or cutting technique affect the results of the experiment?

3. What are some potential sources of error in this experiment, and how could they have impacted the results?

4. How could the experimental design be improved to increase accuracy or reliability?

Read “Planaria Regeneration Screen (Instructor Notes)”

Concentration - Dependent Effects of Screen Hits on Planaria Regeneration

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Objective

To determine the concentration-dependent effects of selected compounds on the regenerative ability of planaria.

Background

Like the enzyme experiment, last week you performed a screen to identify active substances, and this week you will explore the relationship between the concentration and the effect of the substance. Performing a concentration-effect analysis allows us to characterize the substance and functions as a sort of control. In other words, if we see the same effect at all concentrations tested, this could indicate that some of our underlying assumptions are wrong, and we need to re-think our experiment (or we just need to test more concentrations!). However, if we see a relationship between concentration and the effect, this tells us that something interesting is happening.

Lab Protocol

Materials

- Live planaria
- Dissection microscope
- Dissecting tools (razor blade)
- 12-well plates
- Identified test compounds (from the initial screen)
- Planaria water or suitable culture medium
- Pipettes and disposable tips
- Gloves and safety equipment

Procedure

1. Plate Preparation

- Label the 12-well plate to indicate different concentrations of the test compounds and control wells. You may have 3 compounds and want to do 3 different concentrations, or you may have 1 compound and want to do 9 different concentrations. You need to maintain 3 wells for controls.

- Prepare a serial dilution of each compound to create a range of concentrations.
- Fill each well with planaria water and the designated compound concentration.

2. Planaria Preparation and Cutting

- Carefully place planaria on a dissecting dish under a dissection microscope. The planaria should be in a jar on ice. The cold slows them down for easier cutting.
- Using a razor blade, cut each planarian into three pieces (head, midsection, tail).
- Transfer each piece into a separate well, using a transfer pipette.

3. Incubation Period

- Maintain plates under appropriate conditions for 7 days.
- Do not disturb the planaria during this period.

4. Observation and Data Collection

- After 7 days, use the dissection microscope to examine each planarian piece.
- Record whether regeneration has occurred using a binary scoring system:
  - **Regenerated**: Eyespots and normal morphology present.
  - **Not Regenerated**: No eyespots or abnormal morphology.
- Record data individually, then compile class data for analysis.

5. Data Analysis and Concentration Curve

- Compare regeneration rates across different concentrations of each compound.
- Create a concentration-response curve to assess the relationship between compound concentration and inhibition of regeneration.
- Identify possible threshold concentrations where effects become significant.

Report Guidelines

Format

Your report should include the following sections:

1. Introduction

- Briefly explain planarian regeneration and why studying concentration effects is important.
- State the objective of the experiment.

2. Methods

- Describe how the experiment was conducted, including how serial dilutions were made, how planaria were cut, and how regeneration was assessed.

3. Results

- Present individual and class data in a clear format (tables, graphs, etc.).
- Use the binary scoring system to indicate whether regeneration occurred.
- Include a concentration-response curve.

4. Discussion

- Compare regeneration rates between control and experimental groups at different concentrations.
- Discuss possible explanations for observed trends.
- Address any sources of error or limitations.

3. Do you think our scoring system is adequate for this kind of experiment? How about our single timepoint?

5. Conclusion

- Summarize key findings, including any observed concentration-dependent effects.
- Suggest possible follow-up experiments.

6. References (if applicable)

- Cite any external sources used.

Read “Concentration - Dependent Effects of Screen Hits on Planaria Regeneration (Instructor Notes)”

Appendix A: Notes for the Instructor

Microscopic Life, Laboratory Notebooks and the Scientific Method (Instructor Notes)

This is meant to be a mid-early-stage laboratory activity for general biology. The students should have completed an intro to microscopy laboratory activity prior to this lab (the one with the newsprint "e," colored fibers, etc. You can find these on the internet, though I may include in this book eventually). You will need to obtain some sample containers. I used some old plastic containers I had left over from one of those cook-at-home meal services. You could also use 50 mL conical vials.

Extensions of this lab can be used as a "filler" lab. Students can perform this lab during week 3, and then later do the same activity in week 8, etc., to observe the changes in the microscopic life over time. Some modification of the document may be necessary. Microsoft .docx versions of this document should be available online.

Supplies

- I assume that your lab already has microscopes, slides, and coverslips. Disposable transfer pipettes are listed in the other labs in this book.
- "Onwon 10 Pieces Empty Clear Plastic Makeup Sample Containers"

Return to Microscopic Life, Laboratory Notebooks and the Scientific Method.

Enzyme Lab I (Instructor Notes)

This laboratory activity is perhaps the most involved and the most expensive of the labs described in this booklet. However, I would argue that these activities most closely resemble those of an actual professional biomedical research laboratory. Furthermore, most of these supplies are one-time purchases that provide at least 3 laboratory sessions for a given semester and should be re-usable for multiple years. Finally, the assay described can be modified and expanded for additional laboratory sessions.

Due to the advanced nature of these labs, they could also be considered for a biochemistry course, though I did perform these labs in a general biology section.

I try to design these labs so that all the supplies can be obtained from Amazon because going through the institutional purchasing process can be so mind numbing. I include links to specific products, but in case they go out of stock, I include the product name.

Supplies

Assay plate

The key is to get a 96 well plate that is flat bottom and clear. While you don't need a plate that is tissue-culture treated the cheaper options on Amazon end up being treated. Here is a non-treated one:

- "Diamond SureGro Multiple Well Plate, 96 Well, Flat Bottom, Non-Treated, Sterile, Individually Wrapped, 50/Box"

Amylase enzyme

You only need 1 of these; I bought two and barely used the first one.

- "Amylase Enzyme 1 Oz"

To make 2 % amylase solution, you add 2 grams of amylase per 100 ml of water, or 1 gram in 50 mls. You get the idea.

Test tubes

You can probably use any test tubes you have around, but I like to use falcon tubes. These can be washed and re-used if you need to.

- "Konohan Conical Centrifuge Tubes Sterile Plastic Test Tubes with Screw Caps Polypropylene Conical Container with Graduated Marks and Bottom(200 Pack,Blue Cap,15 ml)"

Micropipette

This purchase is where things can get expensive if you don't have these. This juncture is also where you decide how precise you want these activities to be and how realistic the experience. There are nice, cheap sets on Amazon that work well:

- "Huwazine Micropipette kit, 0.5-10ul 10-100ul 100-1000ul, Adjustable Single Channel Pipette, 3 Pieces"

Of course, you can always use the disposable droppers. You may get curves that are a bit messy though. In general, these are super cheap and are good to have around the lab.

- "300PCS 3ML Plastic Transfer Pipettes, YSSHUI Disposable Pipette Transfer Pipettes for Essential Oil Pipette Makeup Tool300PCS 3ML Plastic Transfer Pipettes"

You'll notice that one of the steps is to load the 96 well plate with the same volume of the iodine solution. Of course, you could purchase or find a multichannel pipettor, but I think repetitive pipetting is good for the student. It gives them a chance to master the technique and the manipulation of the micropipette device. Make sure they are doing it properly though.

Water

You can buy distilled water in jugs from your grocery store.

1 % Starch solution

For this experiment, you can use mostly any starch. I bought cornstarch from the grocery store. The instructions below essentially describe making a very exact gravy. For more advanced labs, you could assign this activity as part of the lab, including as little information as you think your students can handle. For instance, you could just tell them to make 200 ml of a 1% starch solution.

To prepare 200 mL of a 1% starch solution using cornstarch, follow these steps:

Materials Needed

- Cornstarch (food-grade is fine)
- Distilled water
- Beaker (250 mL or larger)
- Graduated cylinder (for measuring water)
- Stirring rod or magnetic stirrer (can also just stir with a spoon.)
- Hot plate or microwave

Procedure

1. Weigh the starch
    - 1% solution means 1 g of starch per 100 mL of water.
    - For 200 mL, weigh 2 g of cornstarch using an analytical balance.
2. Make a slurry
    - In a small container, mix 2 g of cornstarch with about 10–20 mL of cold distilled water to make a smooth paste (slurry).
    - Stir well to prevent clumping.
3. Heat the water
    - Heat 180–190 mL of distilled water in a beaker until it's near boiling (~80–90°C) but not vigorously boiling.
4. Combine & stir
    - Slowly add the starch slurry into the hot water while stirring constantly.
    - Continue heating and stirring until the solution becomes clear or slightly translucent (indicating starch is fully dissolved).
    - Avoid overboiling, which can cause degradation.
5. Cool & adjust volume
    - Allow the solution to cool to room temperature.
    - Add distilled water to bring the final volume to 200 mL if needed.
6. Storage
    - Use the solution fresh, as starch solutions can degrade over time.
    - If storing, keep it in a clean, covered container in the refrigerator for up to 24 hours. Stir before use.

Iodine-Potassium Iodide Solution

You can also use Lugol’s solution if you happen to find some in the lab.

- “Iodine-Potassium Iodide Solution, Laboratory Grade, 500 mL”

Return to Enzyme Lab I

Enzyme Lab II – Modifiers of Enzyme Rate (Instructor Notes)

This lab is mostly the same as the first enzyme lab except for the addition of the modifiers. For my lab, I had a work-study student create spice extracts by boiling common spices in water and then filtering through coffee filters. Other ideas for modifiers include various salts you can find in the lab and artificial sweeteners, such as xylitol, that you can buy on Amazon.

- Xylitol: "NOW Foods, Xylitol, Pure with No Added Ingredients, Keto-Friendly, Low Glycemic Impact"

Return to Enzyme Lab II – Modifiers of Enzyme Rate

Planaria Locomotor Lab (Instructor Notes)

Generally, planaria are easy to work with. You can use a disposable transfer pipette to move them from one vessel to another. It takes some getting used to, but you generally just pull them up into the transfer pipette, trying to keep them in the lower part of the pipette, acting quickly so that the planarian doesn't attach to the walls of the pipette. You then squirt them into your next vessel. It is possible to dislodge them from the pipette wall by pipetting some liquid up and down, but it is best to just avoid this scenario.

For these experiments, the liquid in the petri dish should be deep enough for the planaria to move around. Too deep, though, and the planaria will end up gliding on the walls of the dish. The planaria may end up on the walls of the dish regardless, so just get used to finding ways to count the locomotor activity in this orientation.

A jar of planaria from Carolina Biological will last about a week without any intervention (except the initial loosening of the lid). You can keep the same culture going for a while by feeding it (hard-boiled egg yolk, little bits) and changing the water regularly (50% exchange, like taking care of fish).

When working with planaria, you want to use spring water from the grocery store for all your liquids in the experiment.

Instead of the tally method, you could get those hand-held clicker counters, but they aren't cheap.

Materials

As elsewhere in this manual, I provide Amazon as a source for these materials due to simplicity.

Food Dyes

I chose to use food dyes because a student was interested in studying food dyes, but you can pick whatever kind of chemical you want to investigate or can get ahold of. Be careful though — you may want to test the substance before having your students perform the experiment.

- "Fd & C Dye Lake Red, Red No. 40 Allura Red AC, 50 Gram Poly Bottle"

The first time I did this experiment, I obtained a large quantity of Red-40 from this company: https://flavorsandcolor.com/ and the same company below for the Yellow-

- "FD&C Yellow #5 Lake HD 38-42% 227 Grams"

Large Petri Dish

- "Plastic Petri Dish, 150x15mm, Polystyrene, 1 Compartment, 3 Vents, Sterile, Karter Scientific (Pack of 10)Plastic Petri Dish, 150x15mm, Polystyrene, 1 Compartment, 3 Vents, Sterile, Karter Scientific"

Planaria

- "Planaria, Living Culture, Contains Enough Material For A Class Of 30"

Transfer Pipettes

You may need to cut some plastic off the tip of these to make the pipettes large enough to transfer the planaria.

"300PCS 3ML Plastic Transfer Pipettes, YSSHUI Disposable Pipette Transfer Pipettes"

Graph Paper

You just need some 1 cm graph paper (though it doesn't matter if the squares as 1 cm, you just need something around that scale, and everyone needs to be using the same kind of graph paper). I like to put the graph paper inside plastic sheet protectors because liquids splash everywhere.

Return to Planaria Locomotor Lab

Planaria Regeneration Screen (Instructor Notes)

The planaria need to be well fed before this experiment, as this pre-feeding helps fuel the regeneration process. Food should be withdrawn prior to the experiment, though, as cutting the planaria when they have food particles in their system isn’t a great idea.

You will want to prepare the substance solutions that the students can choose from. As with the other screening experiments, you can use whatever you can get your hands on. Spice extracts made from spices at the grocery store or food dyes may be a good starting point. This way the students can just add the pre-made solutions directly to their plates.

Return to Planaria Regeneration Screen

Concentration - Dependent Effects of Screen Hits on Planaria Regeneration (Instructor Notes)

This lab is essentially the same as last week’s, except now the students need to create a serial dilution of their substances.

Return to Concentration - Dependent Effects of Screen Hits on Planaria Regeneration

Appendix B: Lab Notebook Format Entry Guide

Remember: One of the goals of a laboratory notebook entry is to provide someone in the future the ability to reproduce your work. Reproduction is the bedrock of science.

Date: [Date of Experiment]

Title: [Title/Name of the Experiment]

Objective

Briefly describe the purpose of the experiment. What are you aiming to learn or discover? This is where you would include a hypothesis. Provide some background on the experiment. You might include relevant theory, past studies, or any foundational information that's relevant to the experiment. A good sentence framework is “To determine whether [Blank], this experiment will do [Blank]”

Materials and Equipment

- [List all the materials you'll be using]
- [e.g., Microscope, Slide, Solutions A, B, C...]

Procedure

1. [Step-by-step description of the experimental procedure]
2. [e.g., Prepare a slide with a drop of Solution A...]

Data and Observations:

[Use tables, charts, or descriptive paragraphs to record your observations. Make sure to include measurements and units.] (The following is an example of a table. Not all experiments need it)

Example Table

Time (min)	Measurement (unit)	Observation
0	[Value]	[Observation]
5	[Value]	[Observation]
...	...	...

Analysis

Analyze your data (this is where graphs can go).

Discussion/Conclusions

Discuss the results of your experiment. Were there any patterns? Any data points that stood out as unexpected? Use any calculations or statistical methods if needed. Interpret your results in the context of the experiment's objectives. Were your hypotheses supported? What might explain any anomalies in the data? What did you learn from the experiment?

Recommendations for Future Experiments

Any suggestions or modifications for future repetitions of this experiment? Were there any limitations or potential sources of error?

References

[List any sources you referred to, both for the background and for any methods or analyses. Ensure citations are in a consistent format.]

Appendix C: DIY Imager

For the enzymology experiments, I realized that our lab has equipment (a gel imaging system) that not all teaching labs may have. (And they can be pricey, even on eBay.) For these cases, I have provided a simple DIY imaging device assembly idea that uses students' smart phones. (This also keeps them off their phones!) Here is the final product:

DIY Imager Final Product (Image by Author)

As you can see, we are using a smart phone on top as the camera.

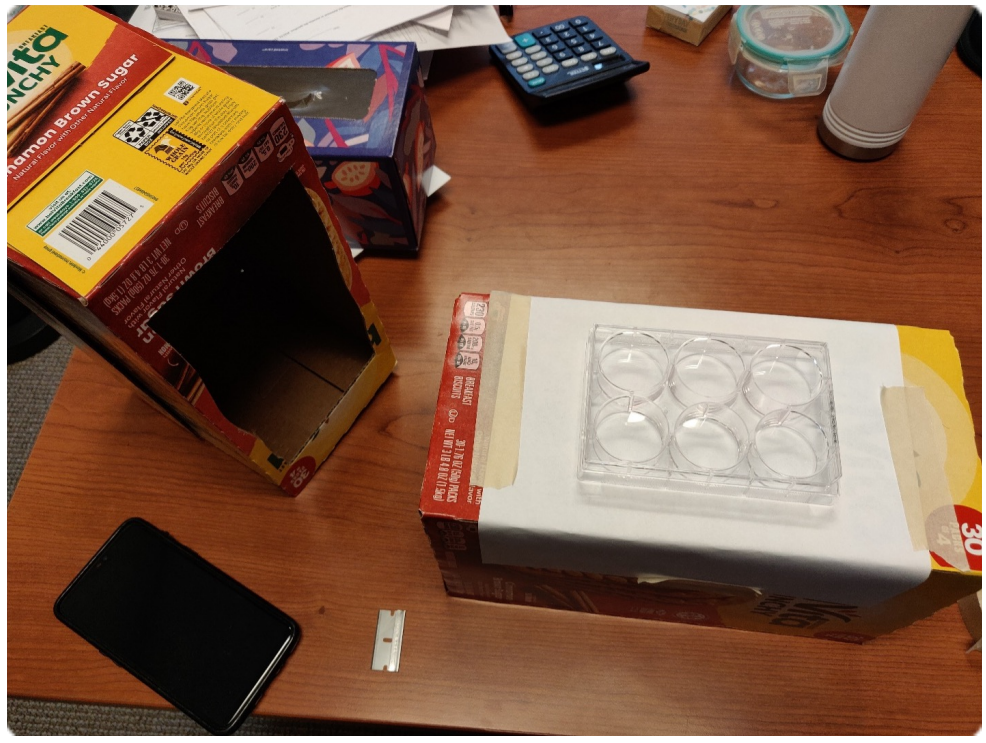
Step 1

Start with 2 boxes. I have these boxes here, as I go through a lot of these biscuits (the students love them as well). The box on the right has a smaller hole cut, and this will hold the thing being imaged. You may need to adjust your hole size depending on your material. The box on the left is the top box (currently upside down).

Step 1 (Image by Author)

Step 2

Tape a piece of paper to the box, covering the sample hole. You can see my 6 well dish is sitting on the hole that is covered by the paper.



Step 2 (Image by Author)

Step 3

Cut a small hole in the top box, on the opposite side of the big hole. This is the camera hole.

Step 3 (Image by Author)

Step 4

Stack the top box with the big hole on top of the box with the sample holder. Take a smart phone, turn the flashlight on, and place it in the lower box, with the light facing up.

Step 4 (Image by Author)

Step 5

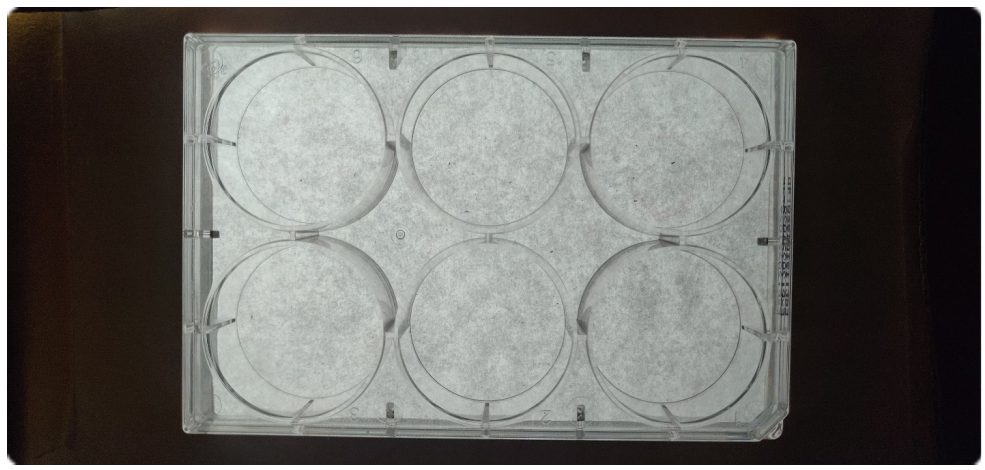
Place a smart phone on top of the top box, with the camera lined up to picture through the small hole made. Voilà! You have a DIY imaging box.

Step 5 (Image by Author)

Example Image

And here is the resulting image from the above setup. As you can see, there are some improvements that can be made. For instance, you could probably pick up some plastic sheeting from a hardware store to use instead of paper (this may get rid of that gray blobby effect seen above). Also, a bundle of Christmas lights may work better than a phone flashlight as a light source (this approach could get hot though). You may also need to adjust the size of your box depending on what you are imaging.

Regardless, you now have some images to use for densitometry analysis. This is also a great time to introduce students to the importance of consistency in scientific experimentation. In other words, this equipment may not be perfect, but if you keep everything controlled (the light source, the camera settings, etc.), you can obtain useful data.

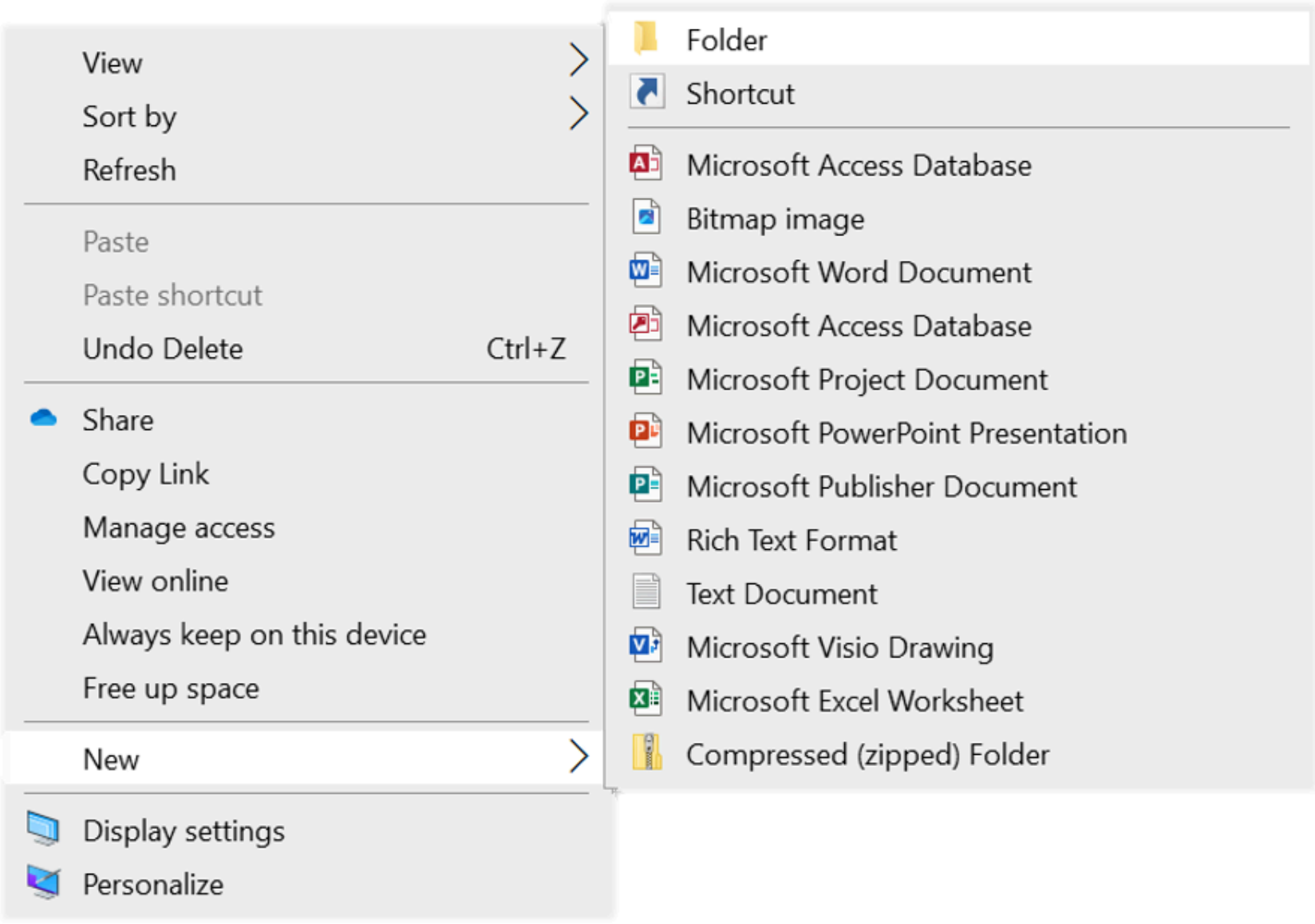


Example Image (Image by Author)

Appendix D: Using ImageJ To Quantify Images

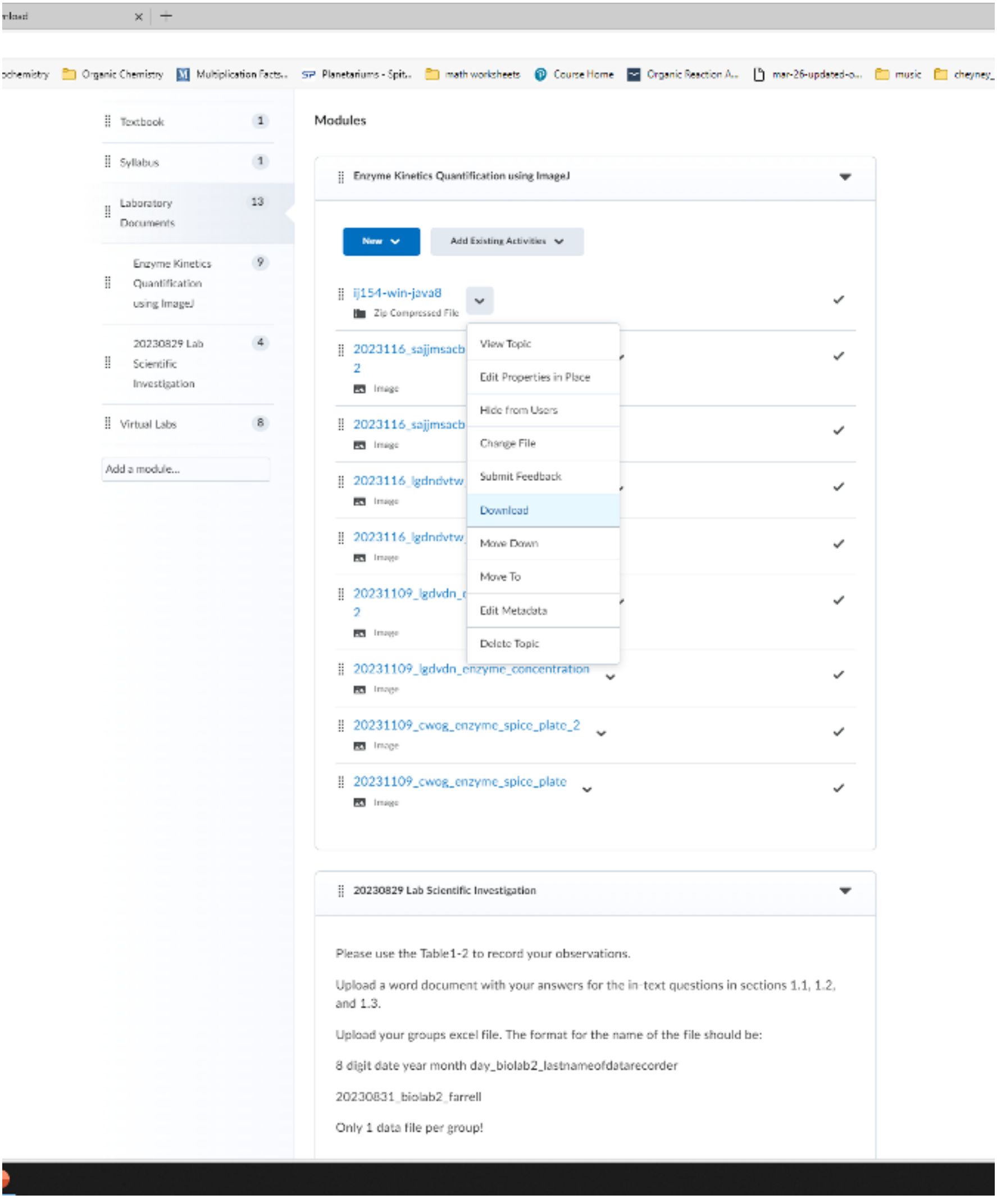
Quantification Process

1. First, on your desktop, right click and select “New” and then “Folder.” Name the folder “Biolab.”



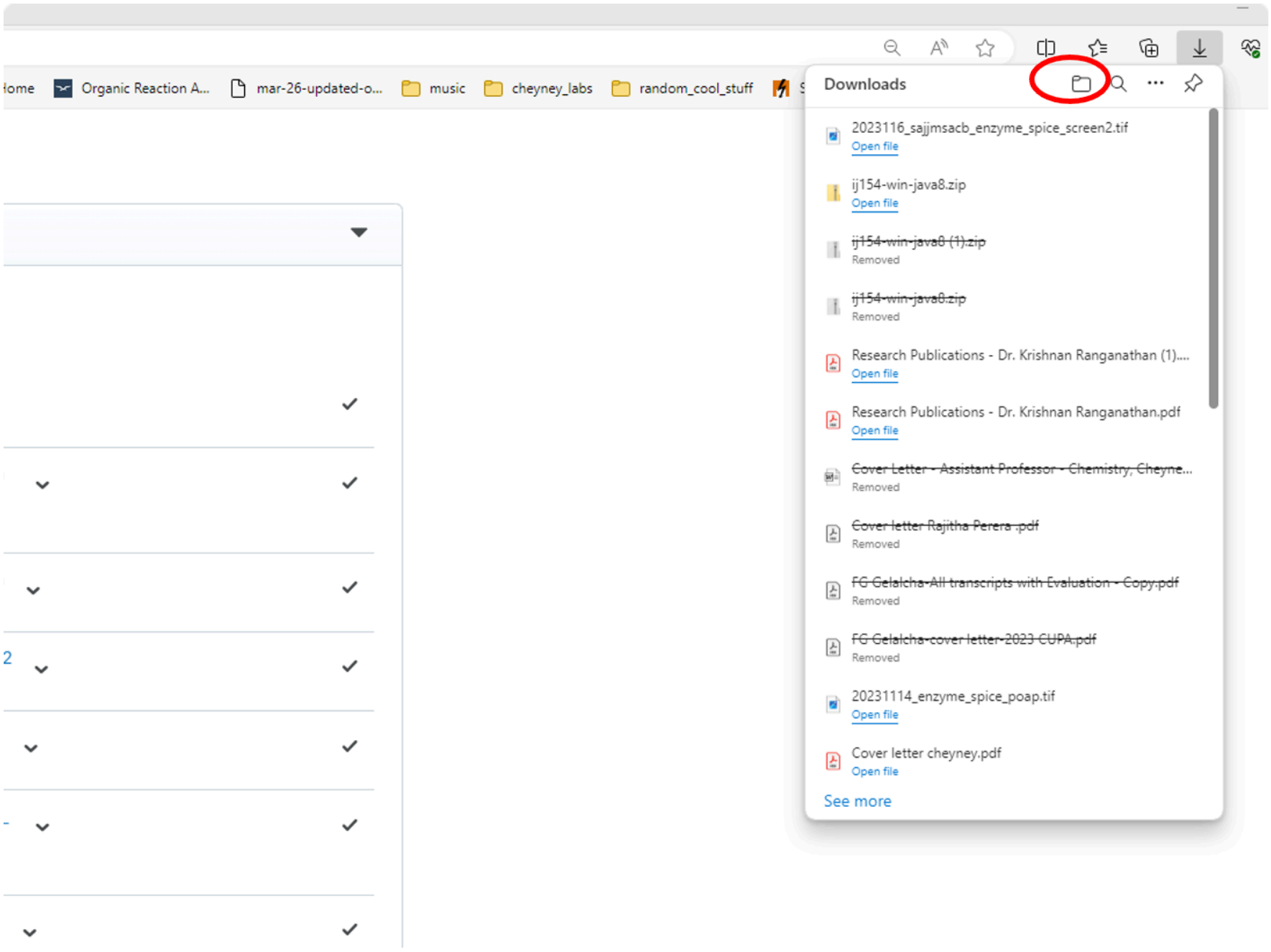
Step 1

2. Screenshot of a Windows desktop context menu with the 'New' submenu expanded, showing options to create new items such as Folder, Shortcut, Microsoft Office documents, and other file types.



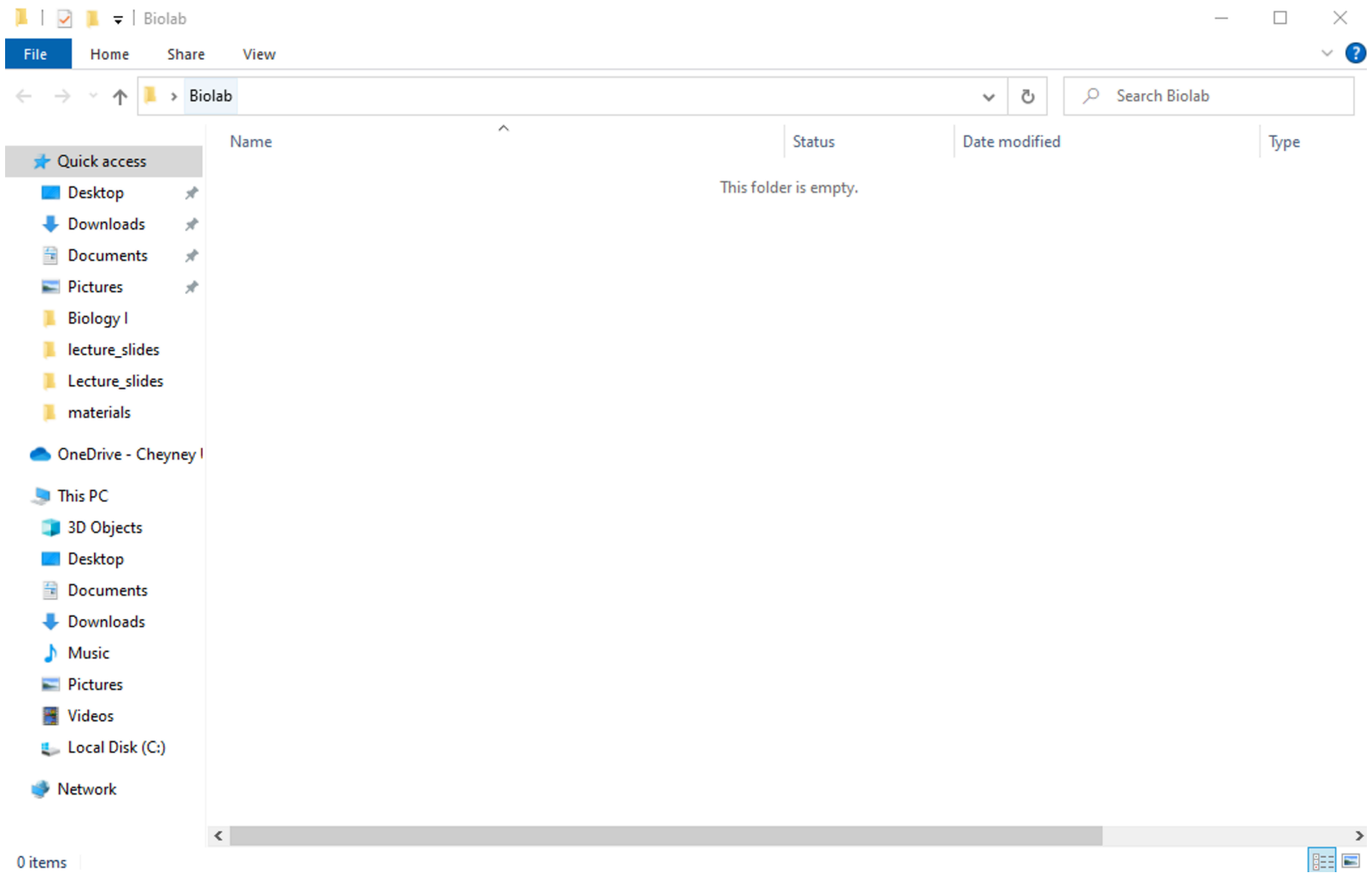
Step 2

3. On the top right of your browser, you will see a download status. Click on the folder icon to open the “Downloads” folder.

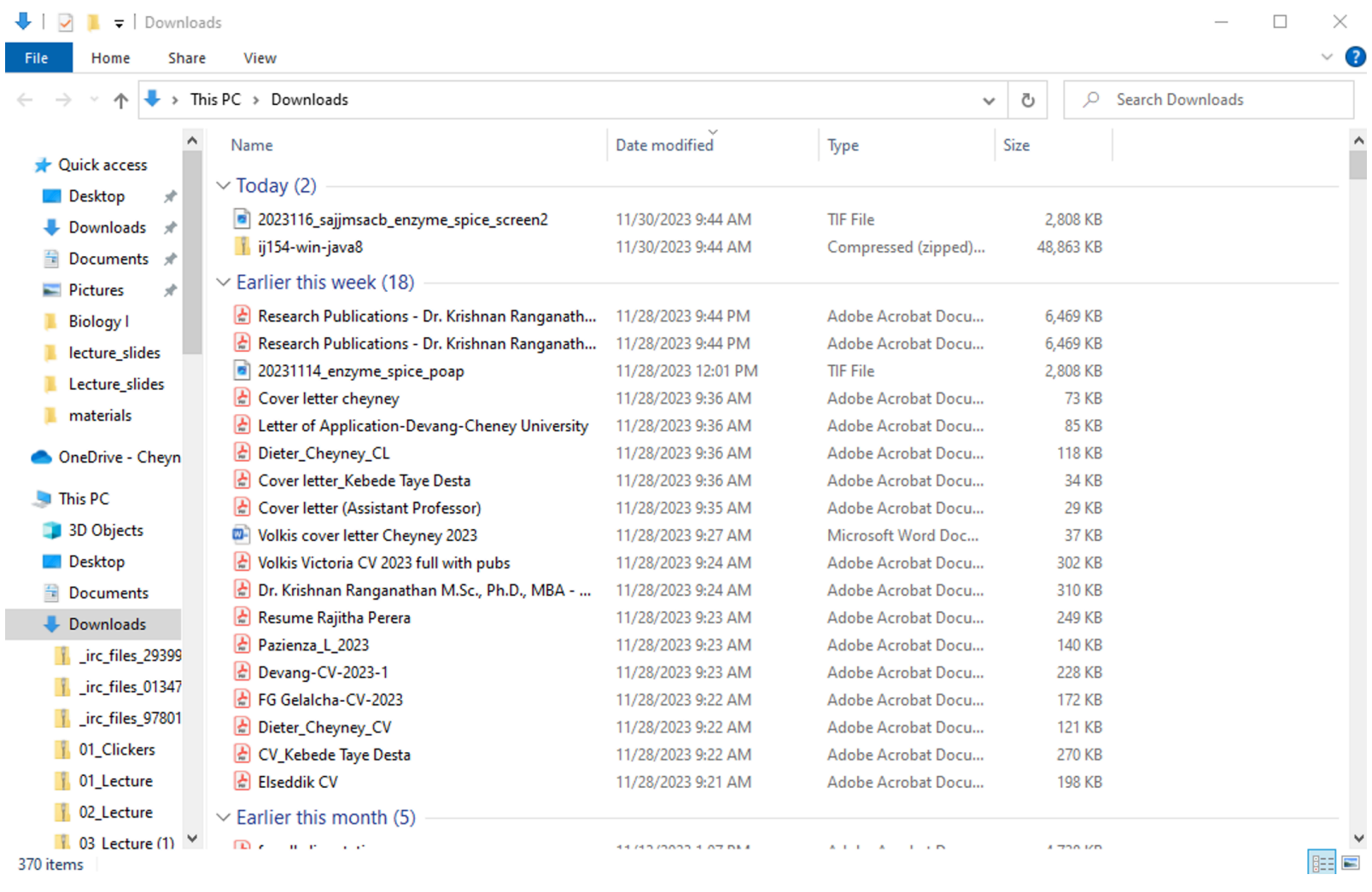


Step 3

4. Get back to your desktop (you can hit win + D) and open your biolab folder. You can resize it and try to get them open side by side like this. Drag over the image file you downloaded.

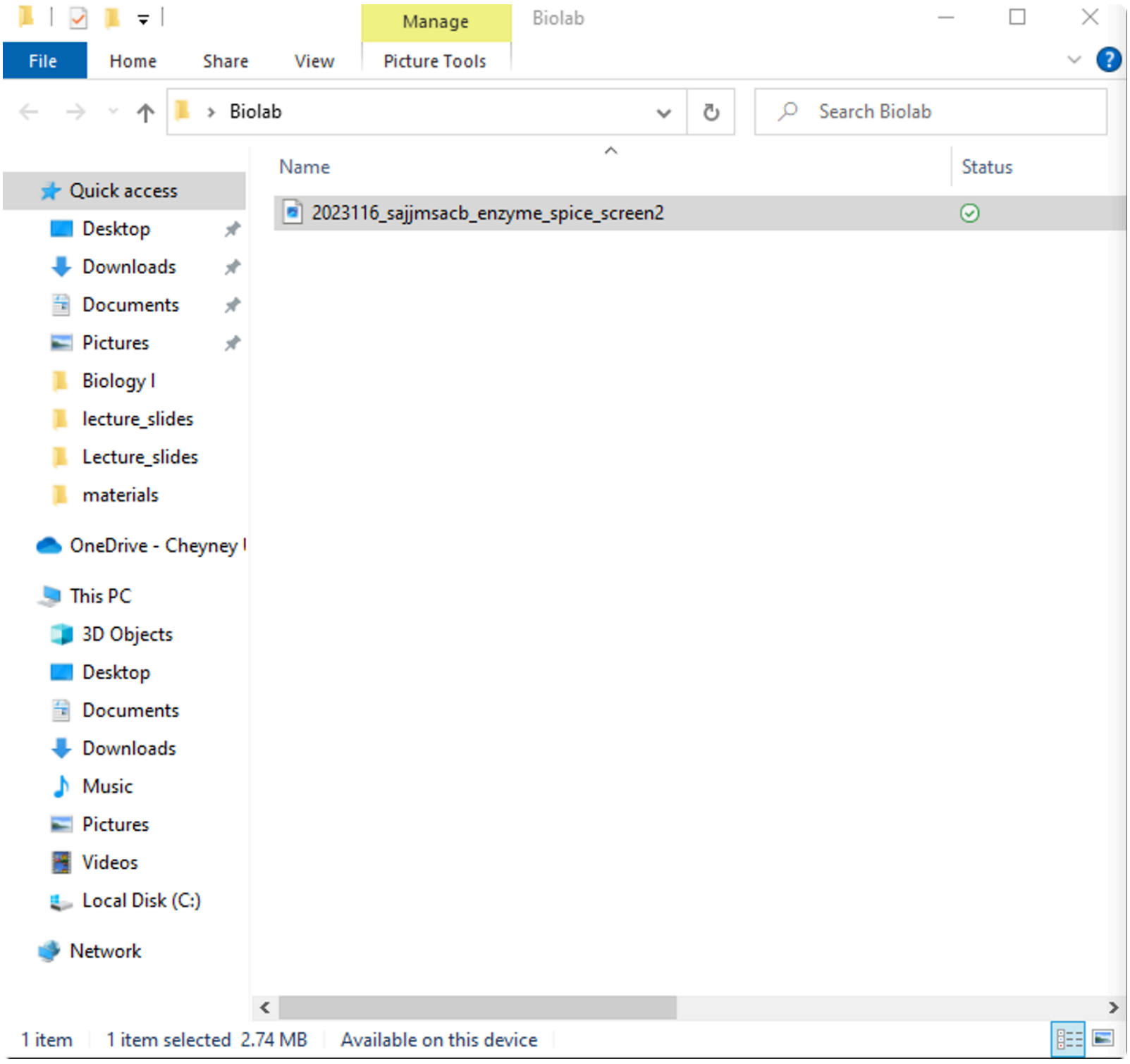


Step 4a

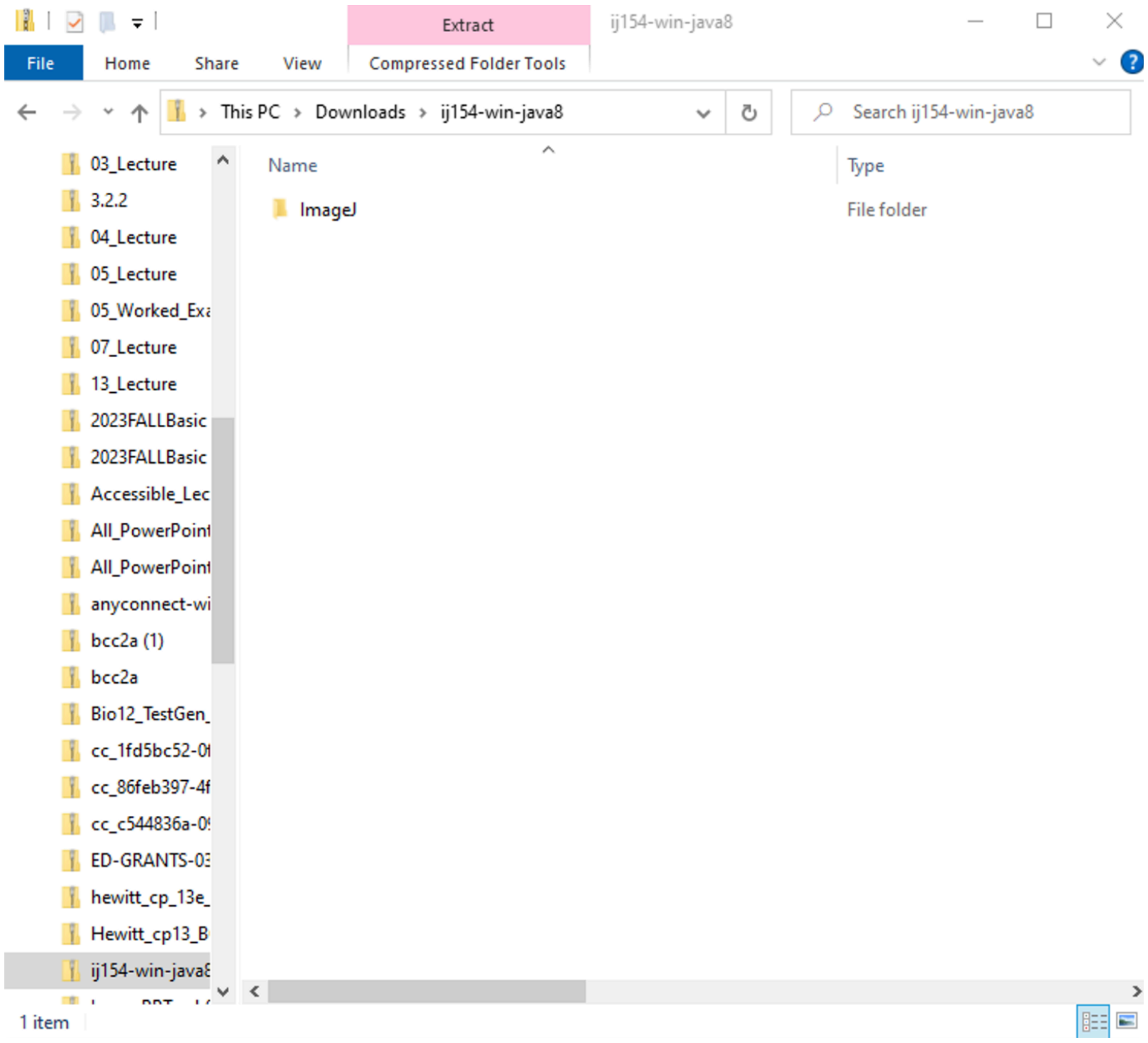


Step 4b

5. Next, double click on the ij154-win-java8 file. It will open to look like the image on the right. Drag the “ImageJ” folder over to your Biolab folder. It will start extracting. It will take about 30 seconds.

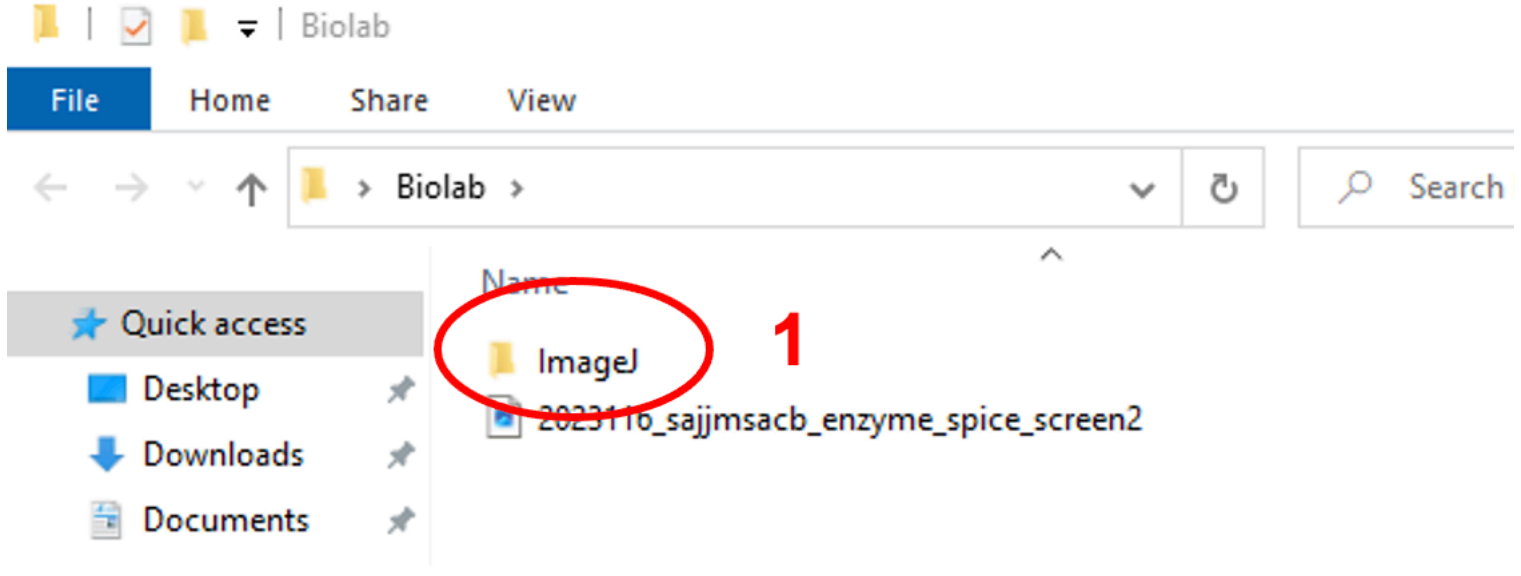


Step 5a

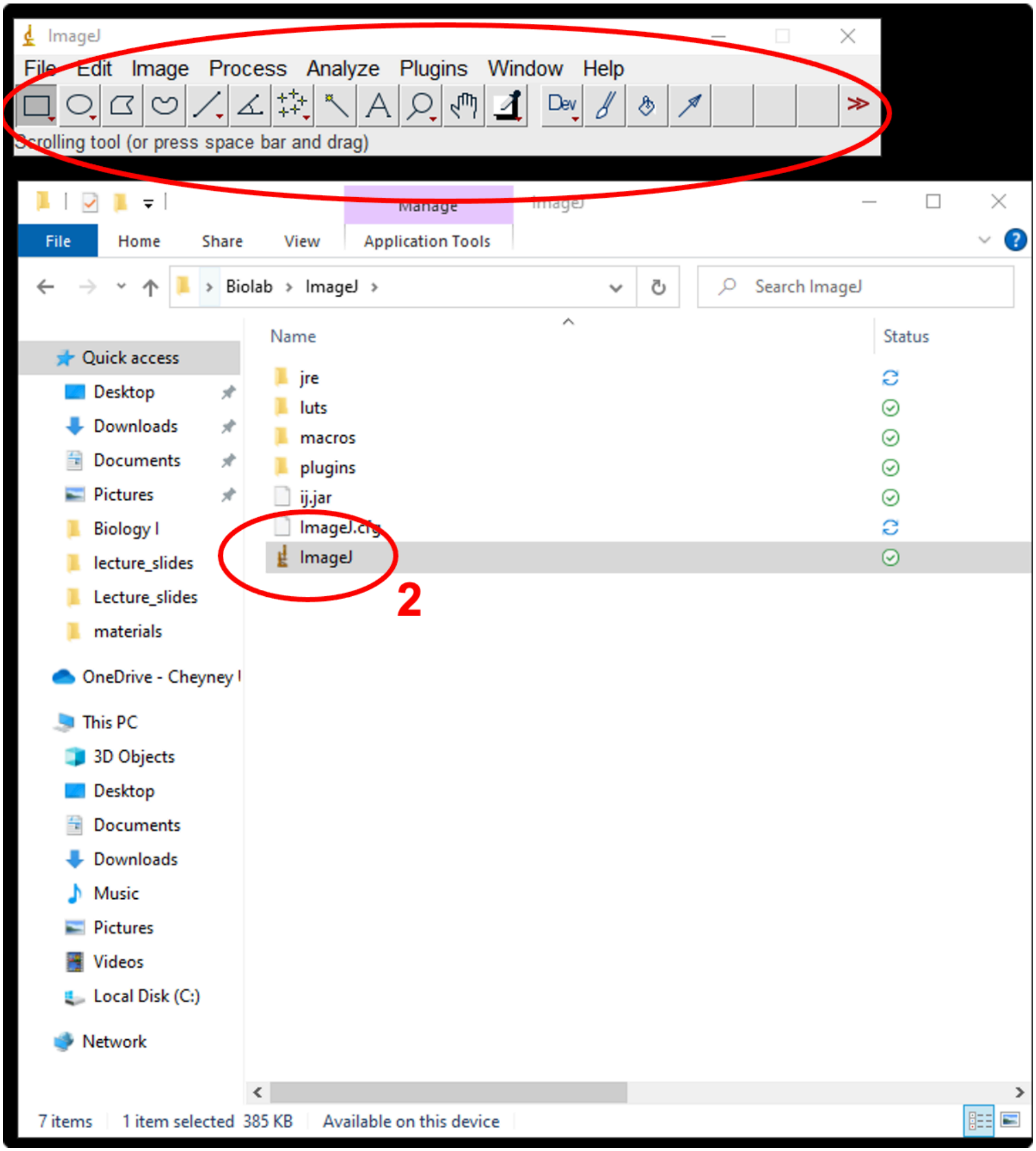


Step 5b

6. Double click on the ImageJ folder. It will open as on the right. Then double click on the ImageJ application (with the little microscope). Then the ImageJ application will open (that's the thing at the top). Hooray, ImageJ is open!

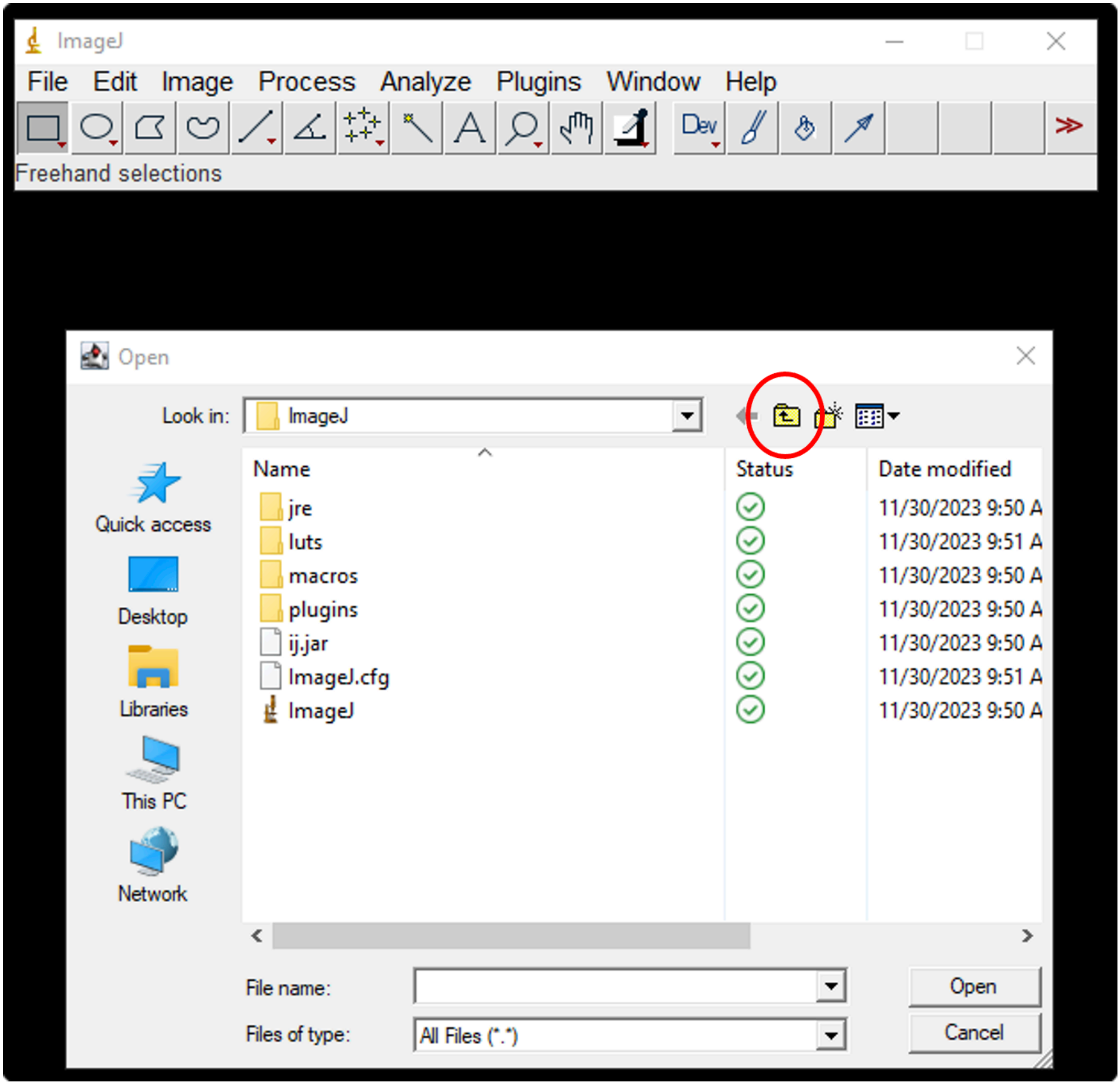


Step 6a


ImageJ
File Edit Image Process Analyze Plugins Window Help
Scrolling tool (or press space bar and drag)
Manage
ImageJ
File Home Share View Application Tools
Biolab › ImageJ ›
Search ImageJ
Quick access
Desktop
Downloads
Documents
Pictures
Biology I
lecture_slides
Lecture_slides
materials
OneDrive - Cheyney I
This PC
3D Objects
Desktop
Documents
Downloads
Music
Pictures
Videos
Local Disk (C:)
Network
Name
Status
jre
luts
macros
plugins
ij.jar
ImageJ.cfg
ImageJ
2
7 items
1 item selected 385 KB
Available on this device

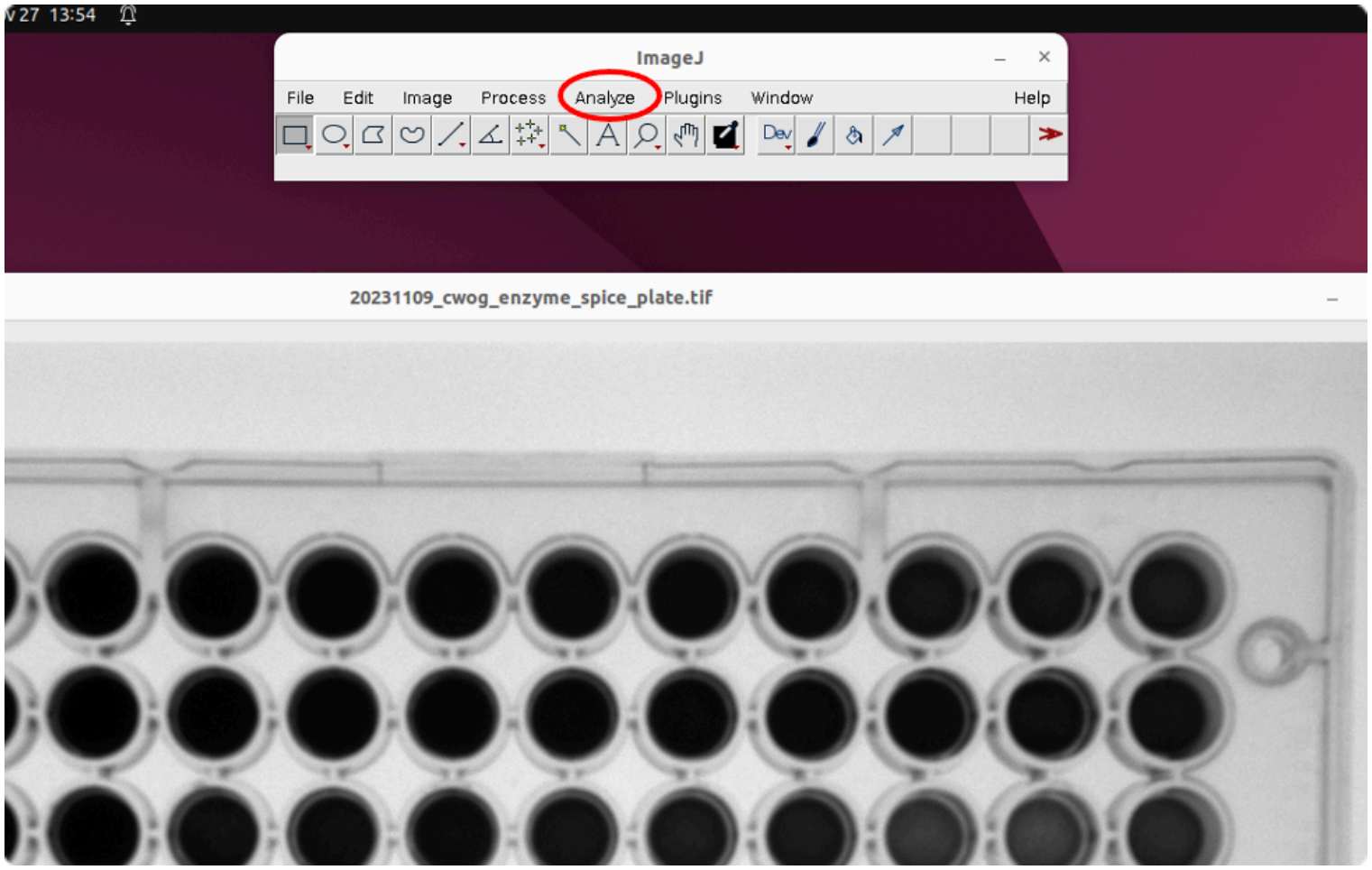
Step 6b

7. Screenshot of the ImageJ software interface showing the menu bar and tool icons. The bottom section displays the 'Open' dialog box with folders and files listed in the 'ImageJ' directory. A red circle highlights the view settings icon in the top-right corner of the dialog box.



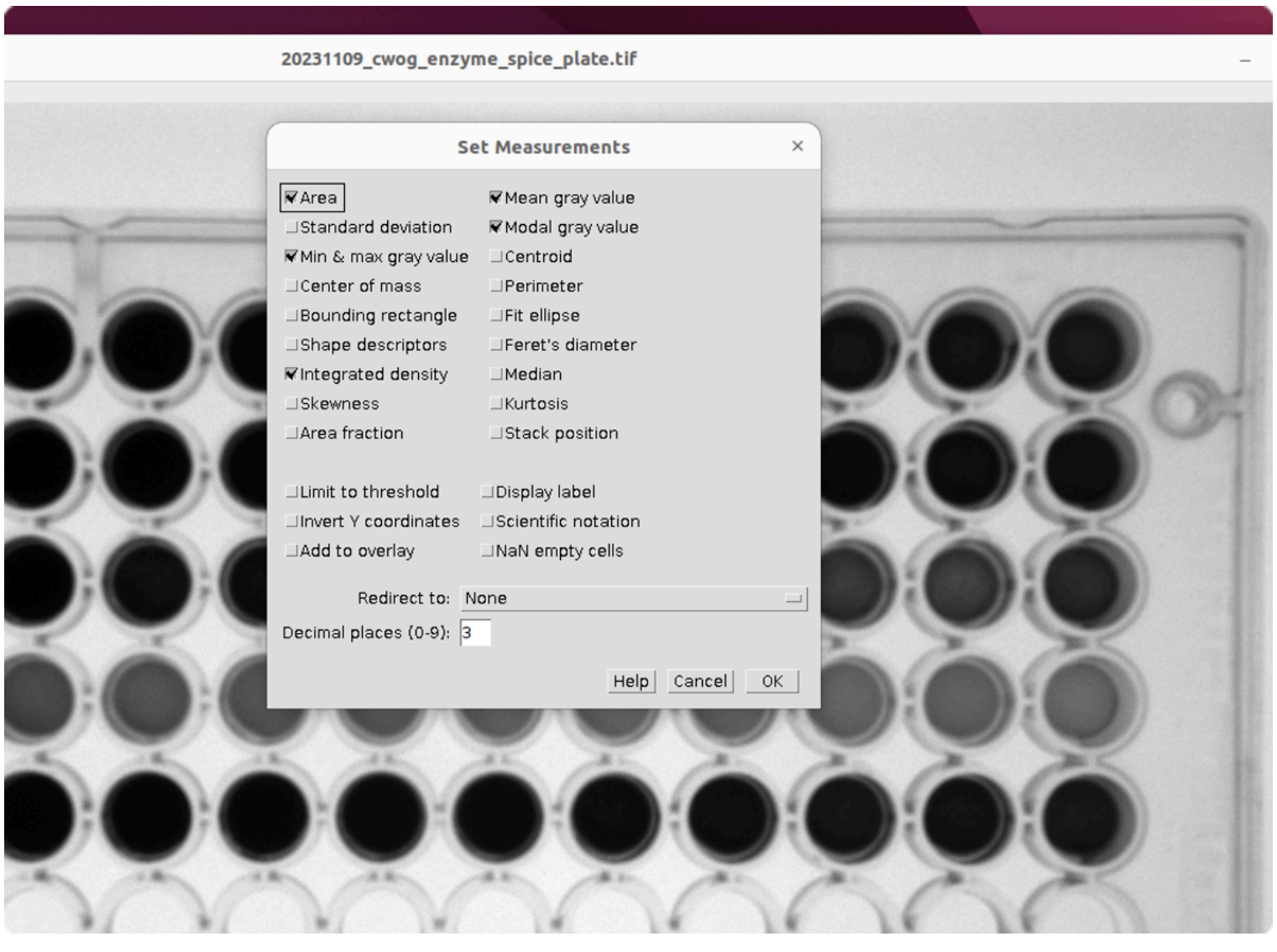
Step 7

8. Go to “Analyze,” then “Set Measurements.”



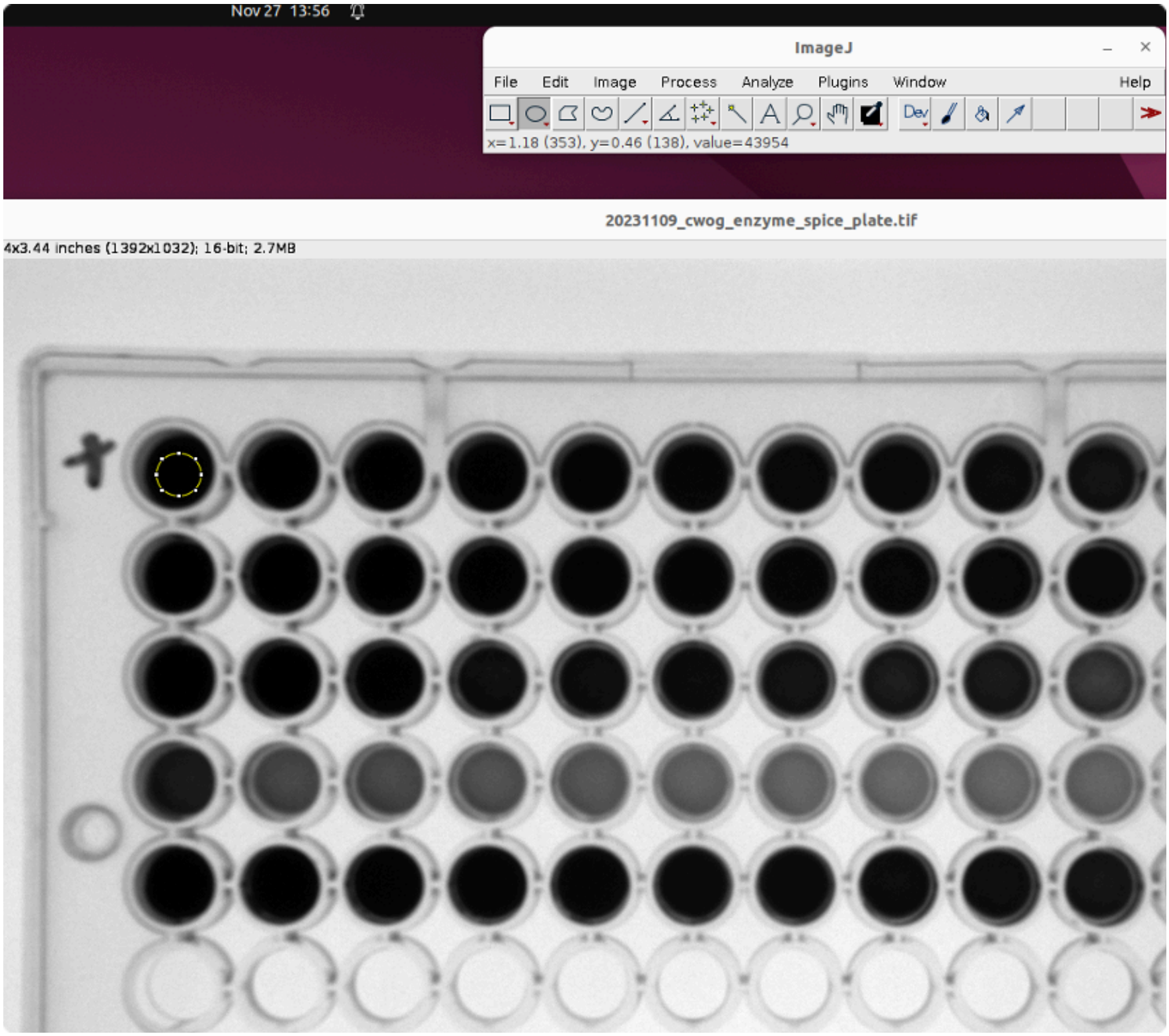
Step 8

9. Make sure the same boxes are checked as in this image. Then hit “OK.”



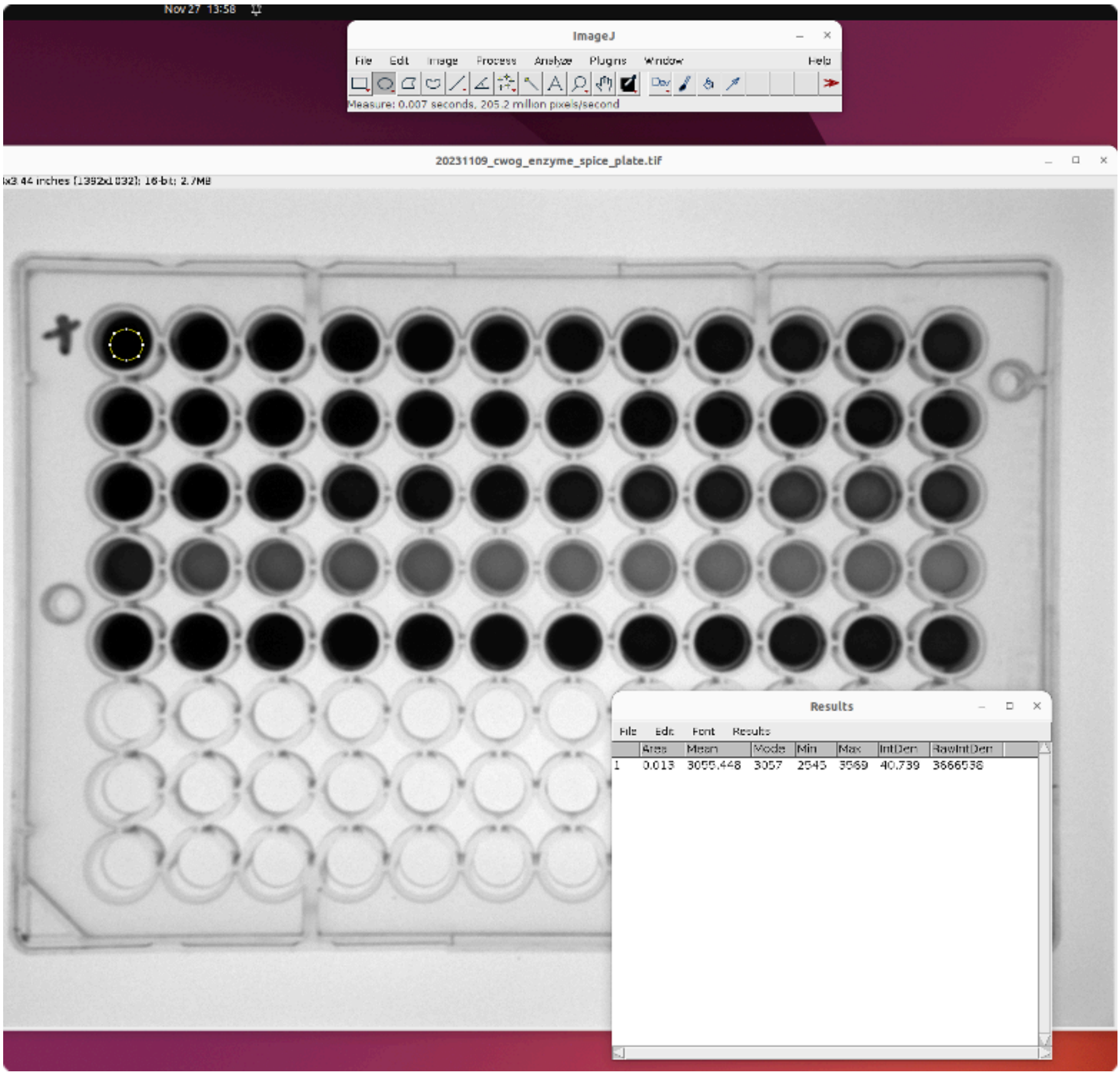
Step 9

10. Select the circle tool and then draw a circle in the first well (you can also use the square. Just be sure that whatever you draw only covers the dark parts in the well) It helps to magnify the image so your circle doesn't have to be so tiny. You can go to "Image" → "Zoom" → "In". You'll have to resize the window to get your whole plate in view again.



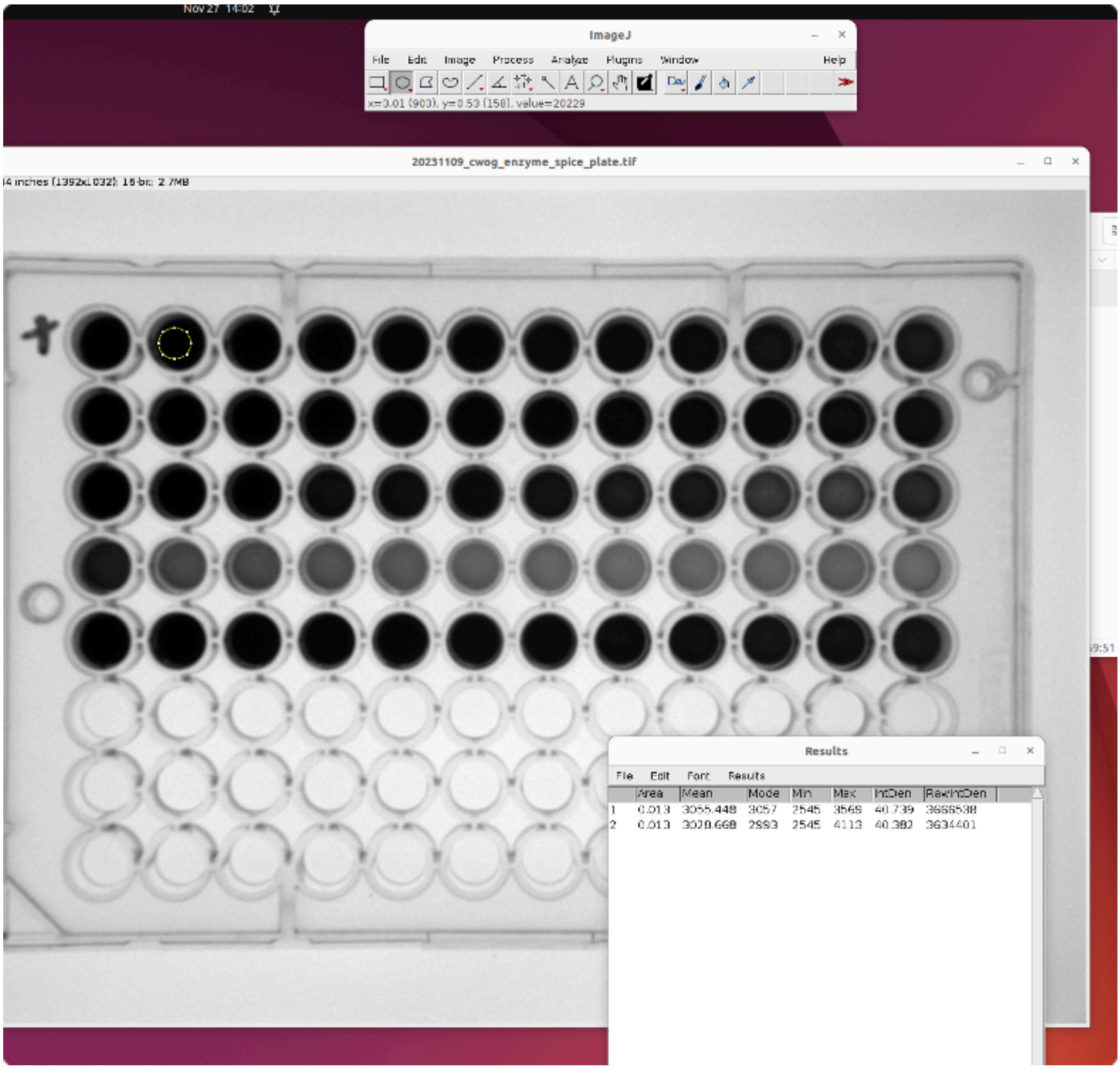
Step 10

11. From the analyze menu, select “Measure.” A little “results” window will appear, with your measurement.



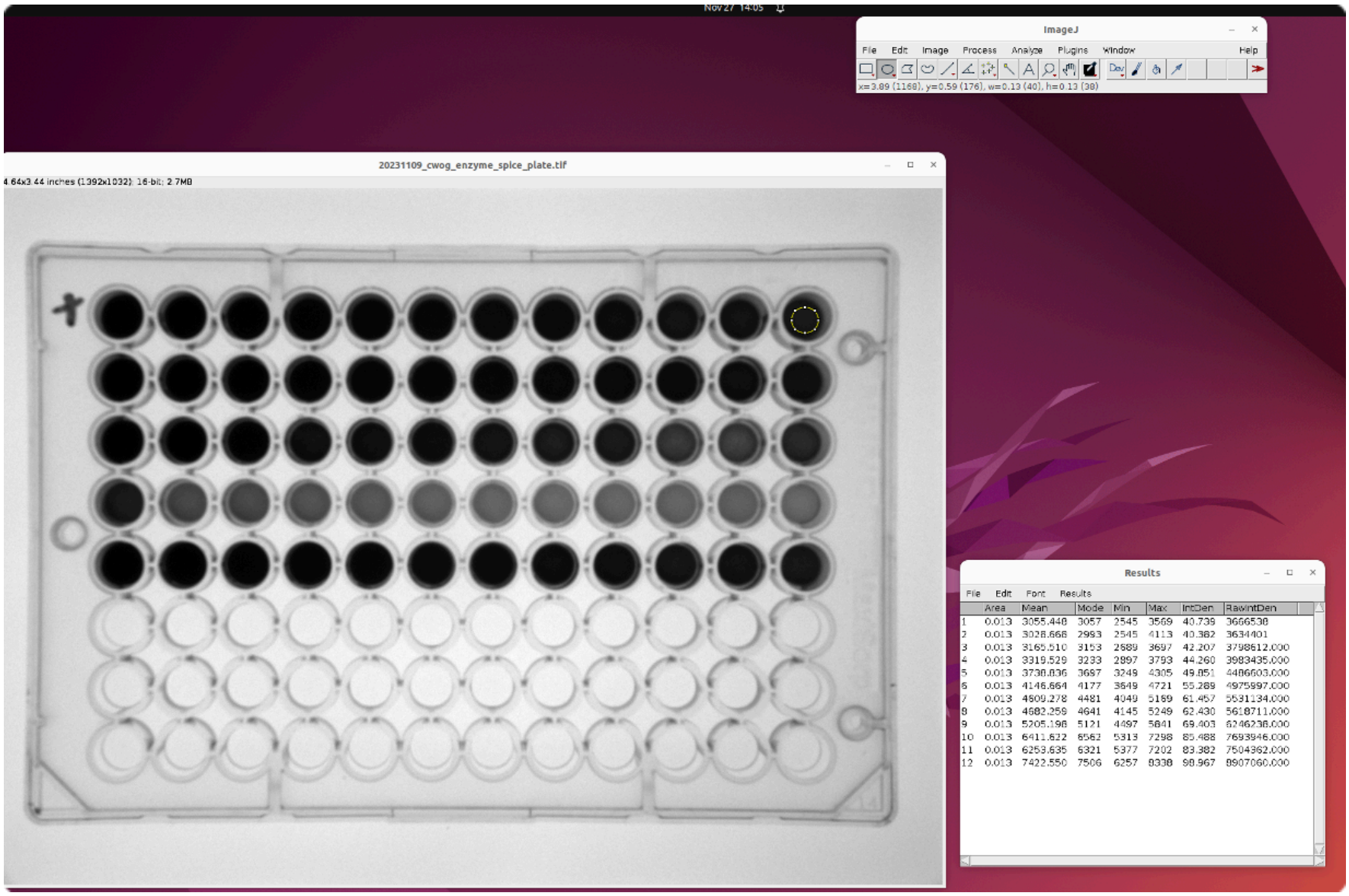
Step 11

12. (This is step 1 of the acquisition cycle.) Next, drag the circle to the next well. Then measure again. The new measurement will appear in the results window. It’s very important to keep the same circle. This way we are recording the same amount of signal in the image.



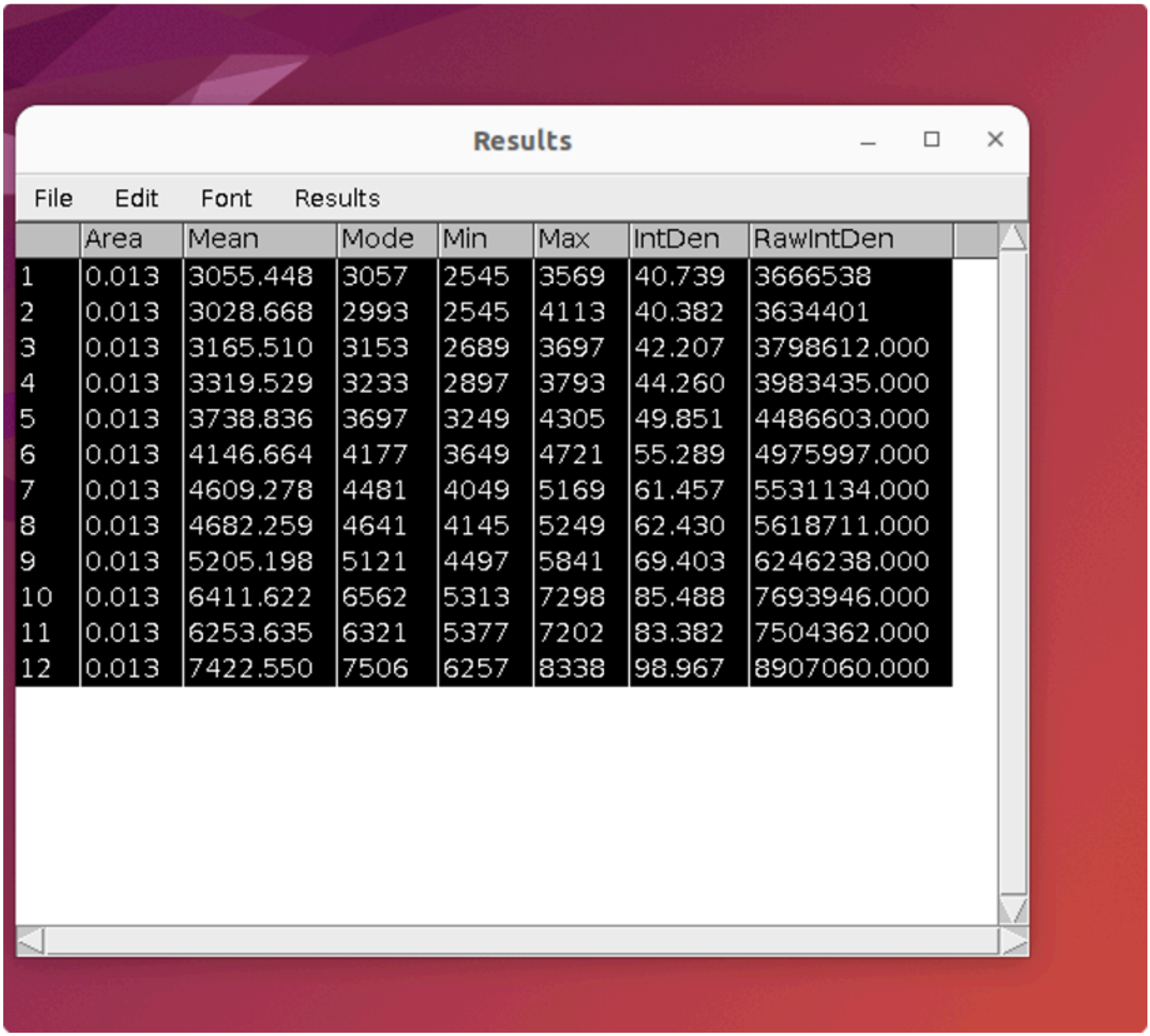
Step 12

13. Repeat this process for all of the wells in a given row. You can speed things up by using the keyboard shortcut Ctrl + M to make a measurement



Step 13

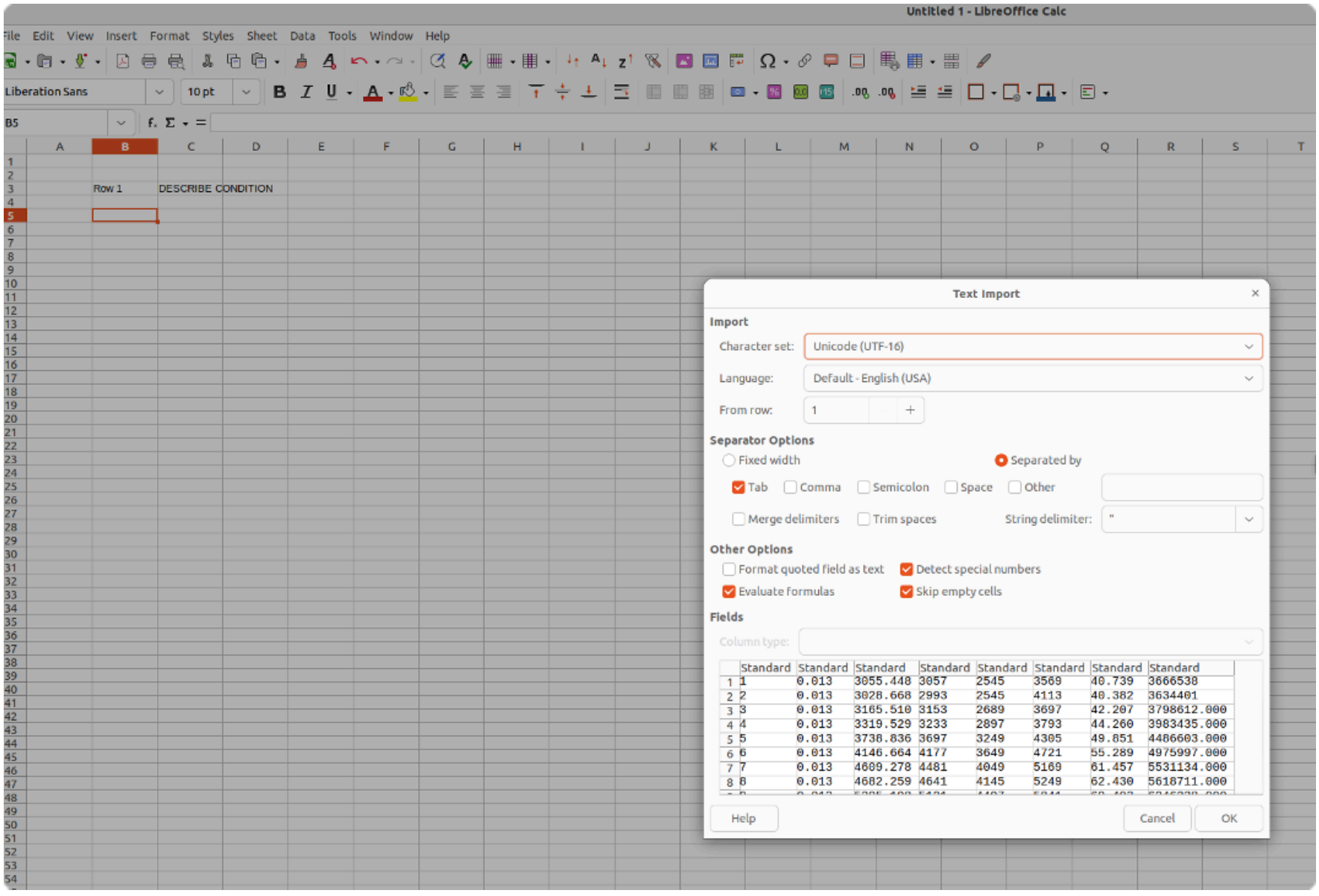
14. In the results window, select all and then copy into the clipboard (edit → select all, edit → copy). Keyboard = Ctrl + A, Ctrl + C. You can also save the file as a .CSV, but that is clunkier IMO.



	Area	Mean	Mode	Min	Max	IntDen	RawIntDen
1	0.013	3055.448	3057	2545	3569	40.739	3666538
2	0.013	3028.668	2993	2545	4113	40.382	3634401
3	0.013	3165.510	3153	2689	3697	42.207	3798612.000
4	0.013	3319.529	3233	2897	3793	44.260	3983435.000
5	0.013	3738.836	3697	3249	4305	49.851	4486603.000
6	0.013	4146.664	4177	3649	4721	55.289	4975997.000
7	0.013	4609.278	4481	4049	5169	61.457	5531134.000
8	0.013	4682.259	4641	4145	5249	62.430	5618711.000
9	0.013	5205.198	5121	4497	5841	69.403	6246238.000
10	0.013	6411.622	6562	5313	7298	85.488	7693946.000
11	0.013	6253.635	6321	5377	7202	83.382	7504362.000
12	0.013	7422.550	7506	6257	8338	98.967	8907060.000

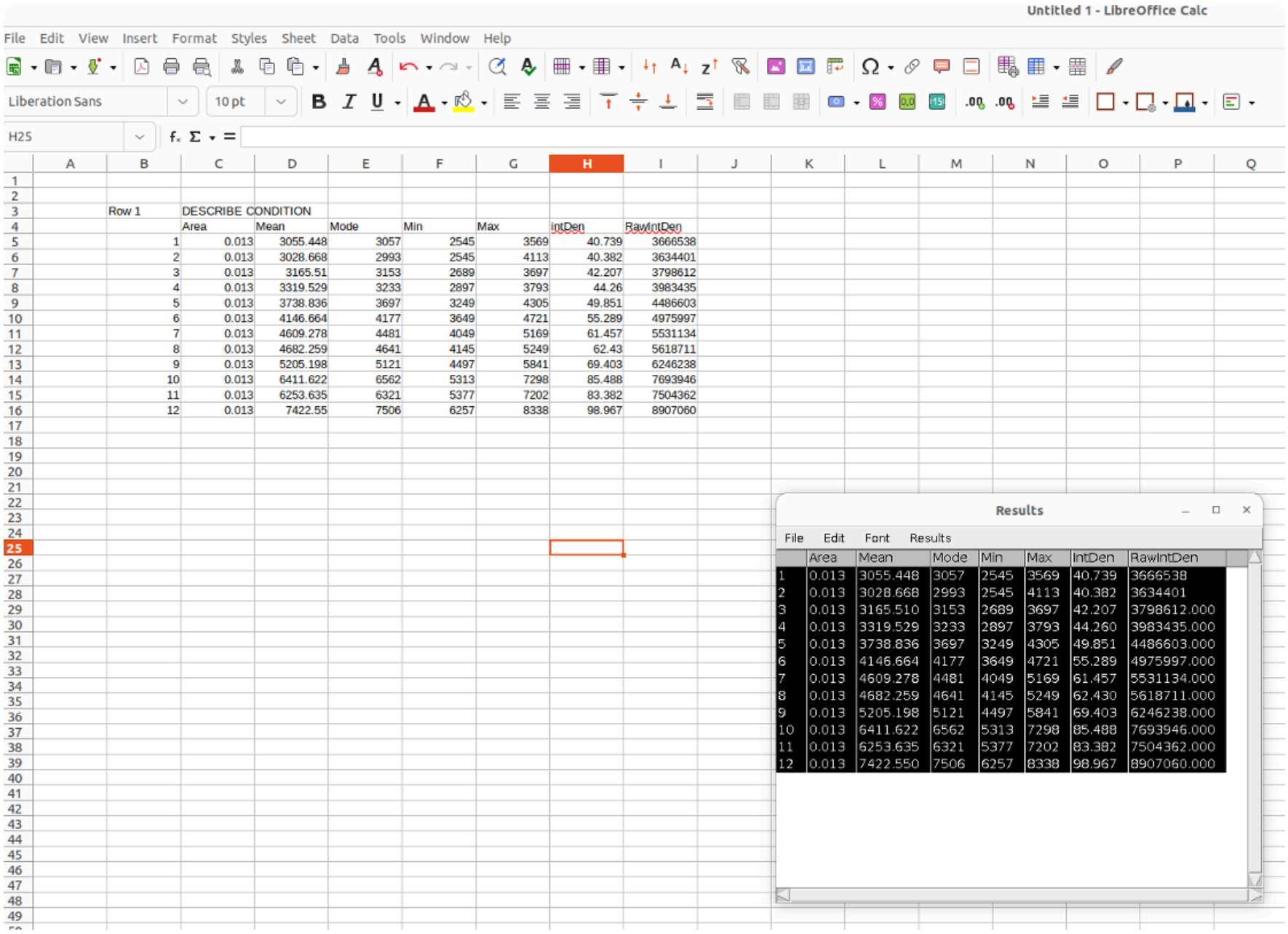
Step 14

15. Open your spreadsheet software (Excel). (Hit windows key, type Excel, select the Excel app). In a cell near the top, write “Row 1” and then describe the condition. The select a cell 2 down and paste the data in (Edit → paste, or Ctrl + V). A text import should appear. The defaults are fine, just hit “OK.”



Step 15

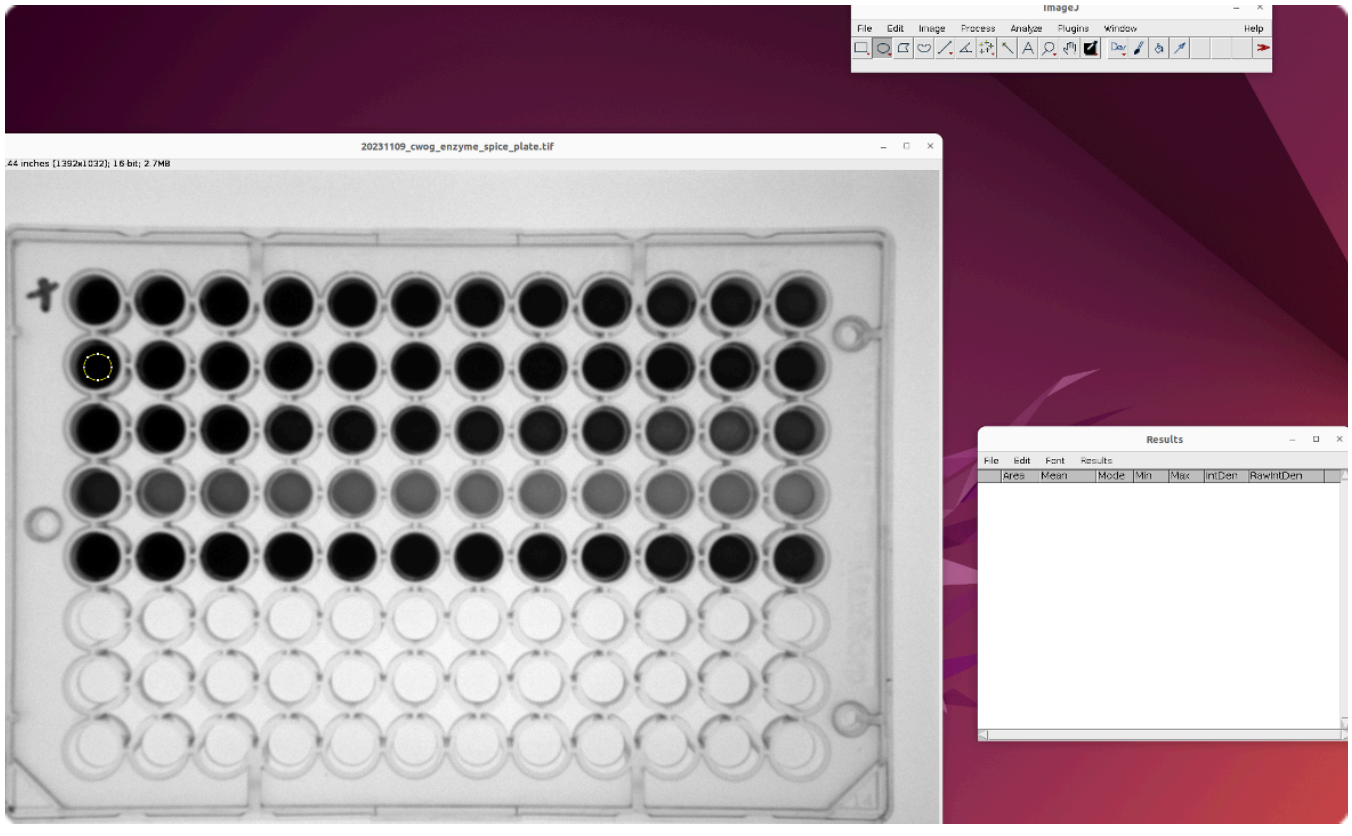
16. Get your results window in view again and put the column names in your spreadsheet.



Step 16

17. Clear your results in the results window by going to Results → clear results. Don’t save the measurements—we already got them in the spreadsheet file.

18. Now repeat the process for the remaining wells. Remember to drag the circle (the process restarts on step 12 of these instructions).



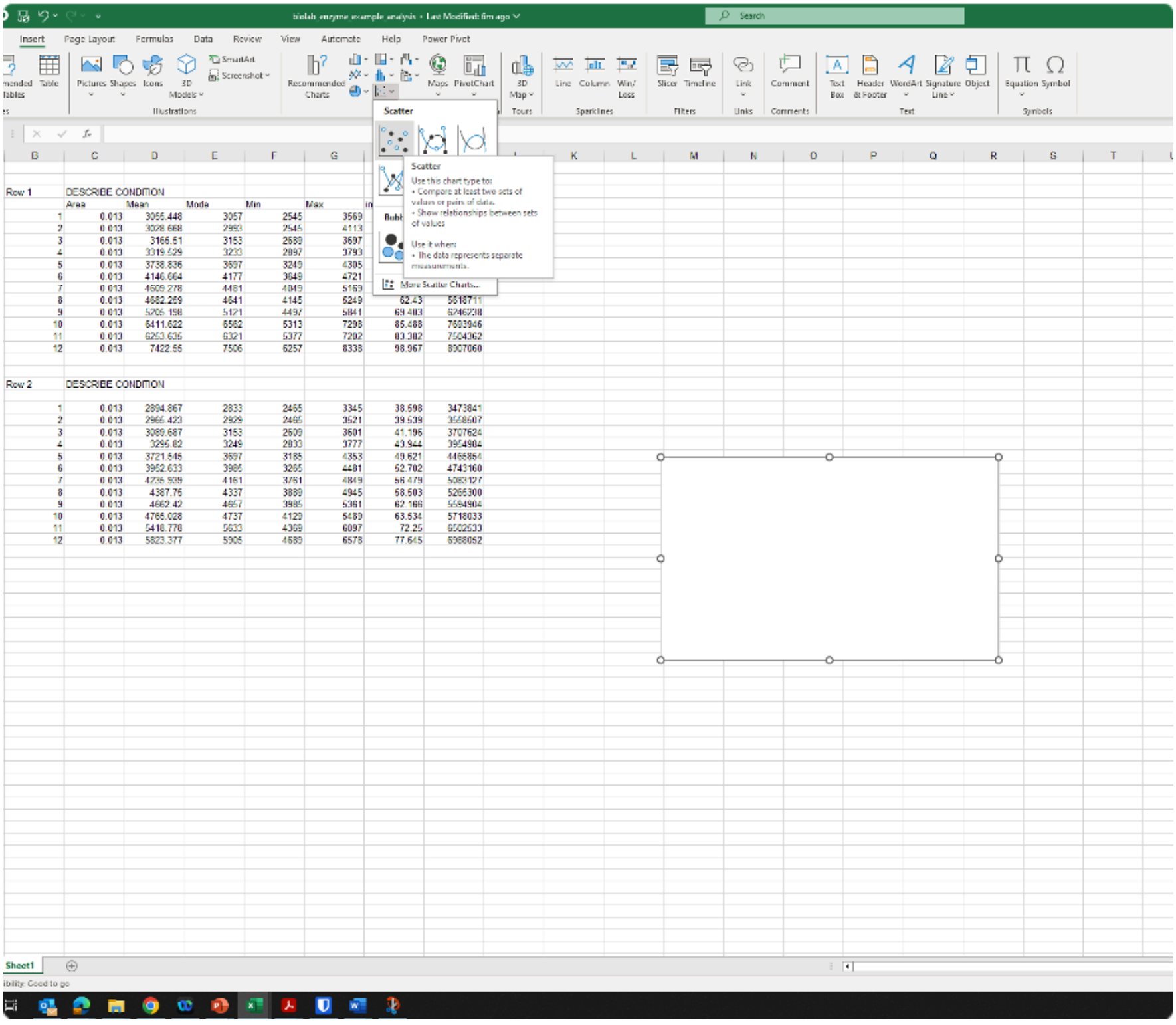
Step 18

19. After gathering the data for all your wells (also get the data from the control wells if you made them), you can start graphing the data in excel. Click on the little dot plot icon in the Charts menu in the Insert menu.

	E	F	G	H	I	J	K	L
	Mode	Min	Max	intDen	RawIntDen			
.448	3057	2545	3569	40.739	3666538			
.668	2993	2545	4113	40.382	3634401			
5.51	3153	2689	3697	42.207	3798612			
.529	3233	2897	3793	44.26	3983435			
.836	3697	3249	4305	49.851	4486603			
.664	4177	3649	4721	55.289	4975997			
.278	4481	4049	5169	61.457	5531134			
.259	4641	4145	5249	62.43	5618711			
.198	5121	4497	5841	69.403	6246238			
.622	6562	5313	7298	85.488	7693946			
.635	6321	5377	7202	83.382	7504362			
2.55	7506	6257	8338	98.967	8907060			
.867	2833	2465	3345	38.598	3473841			
.423	2929	2465	3521	39.539	3558507			
.687	3153	2609	3601	41.196	3707624			

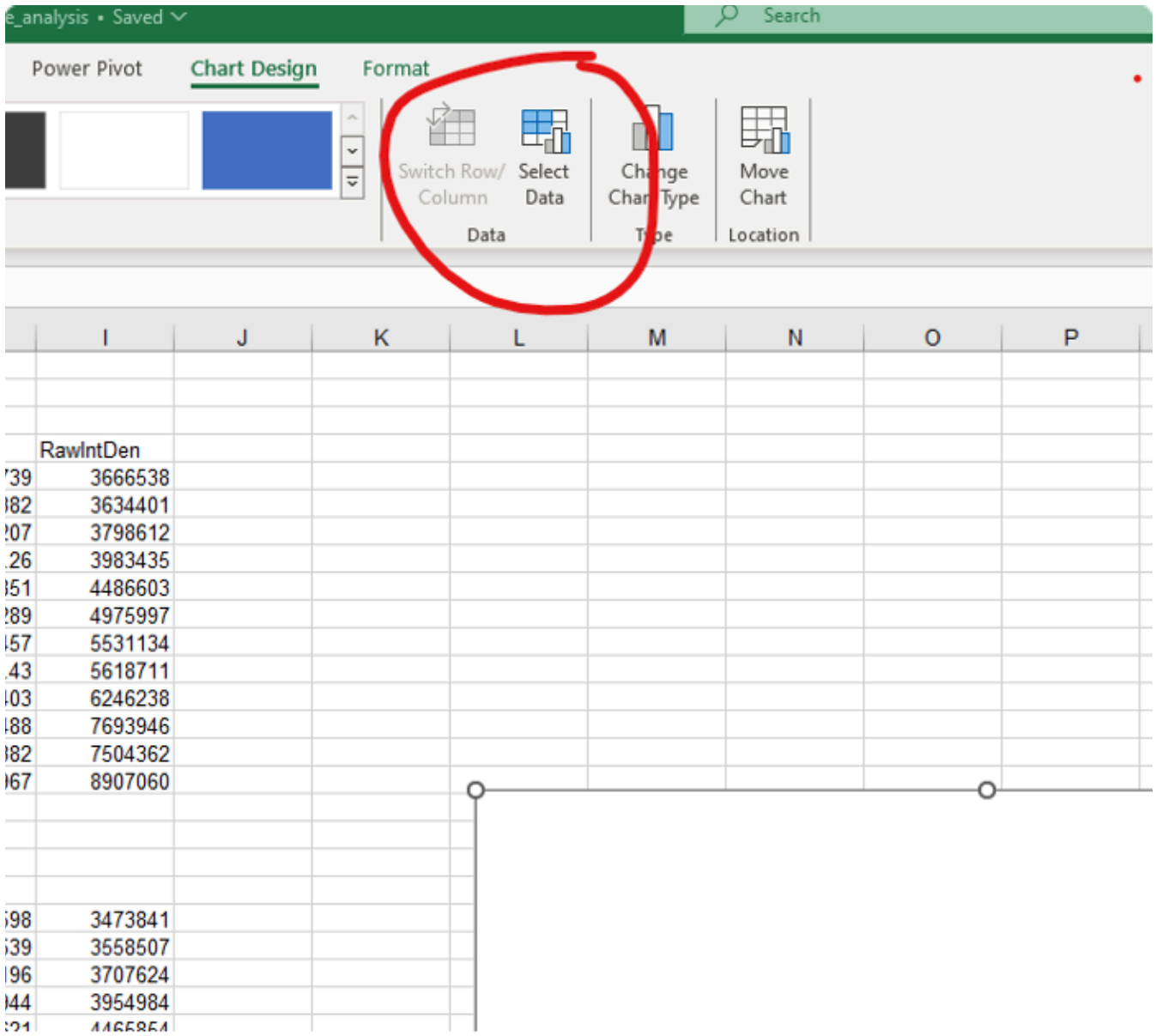
Step 19

20. A menu will appear. Select the Scatter chart type. Click on the square that appeared on your Excel sheet.

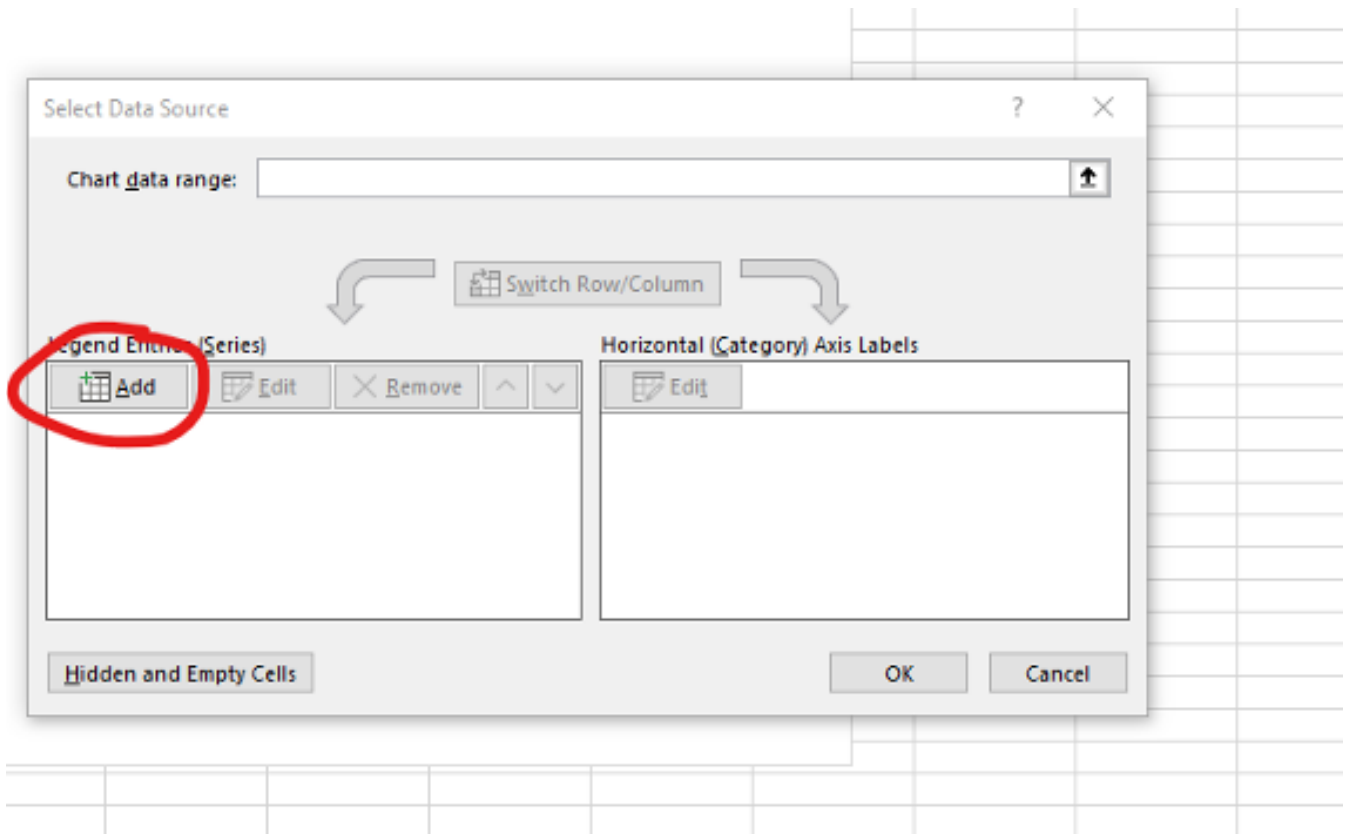


Step 20

21.Click on “Select Data”, and then in the new window, select “Add”

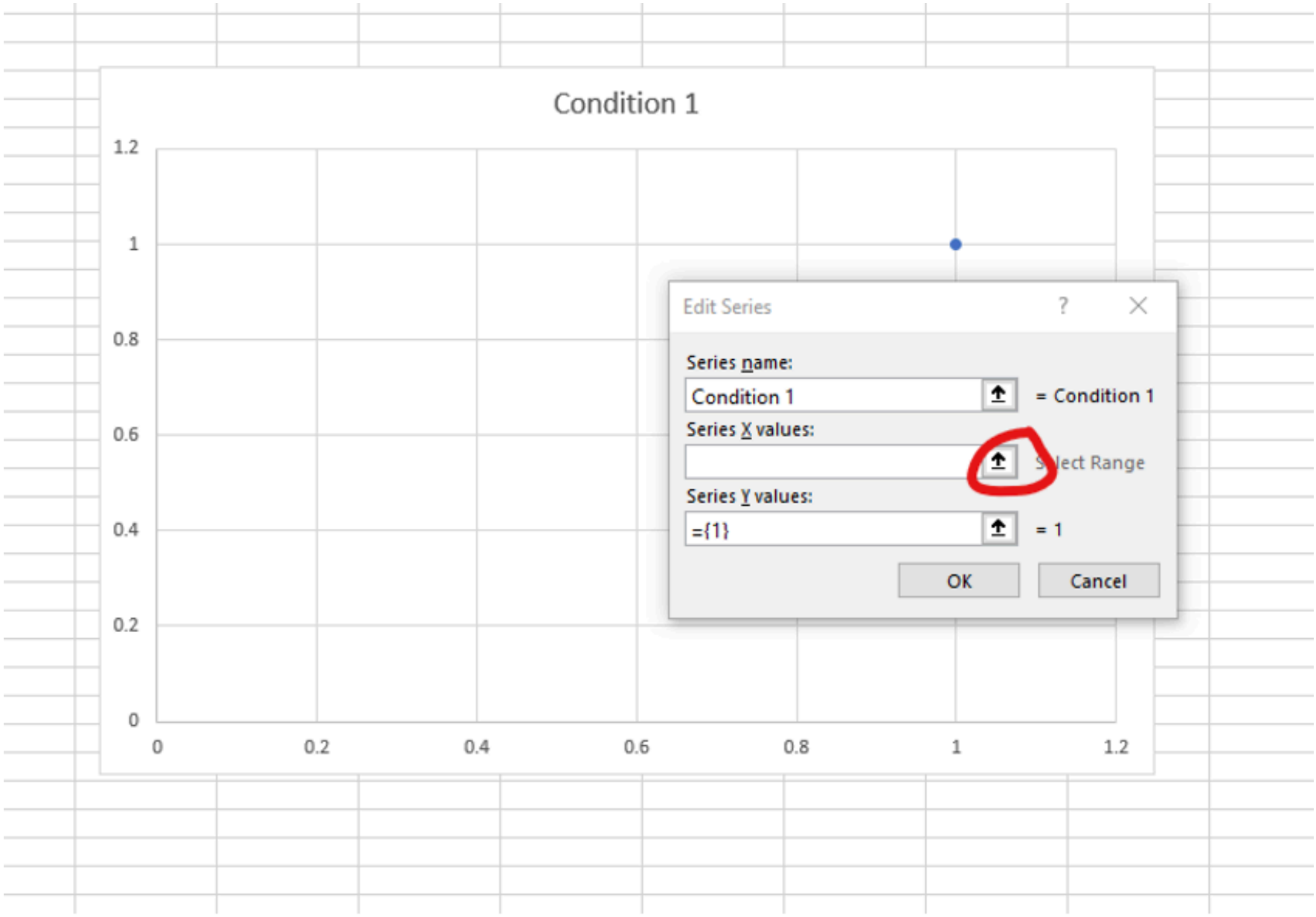


Step 21a



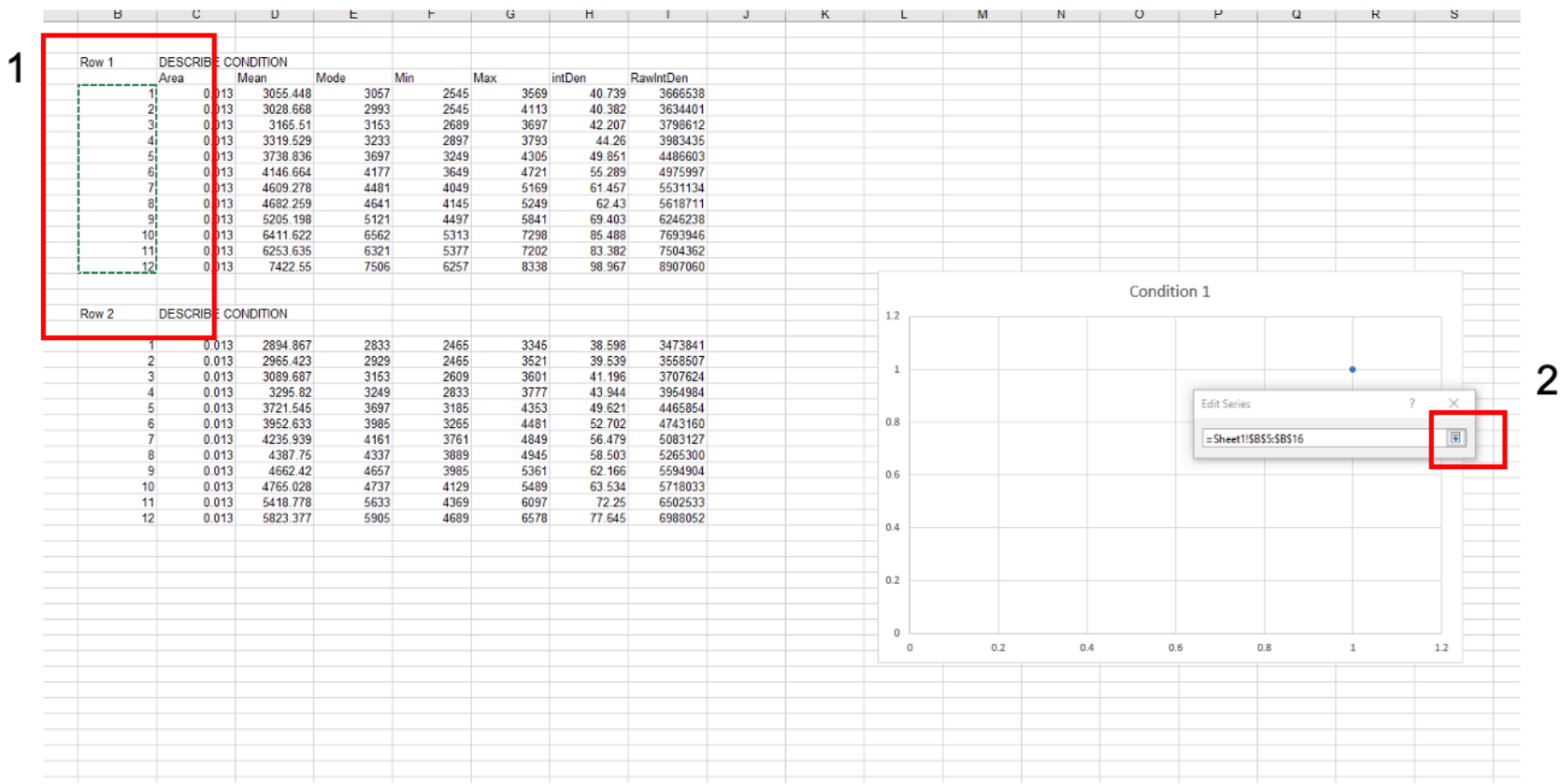
Step 21b

22.Click on the arrow indicated here (the middle dropdown).



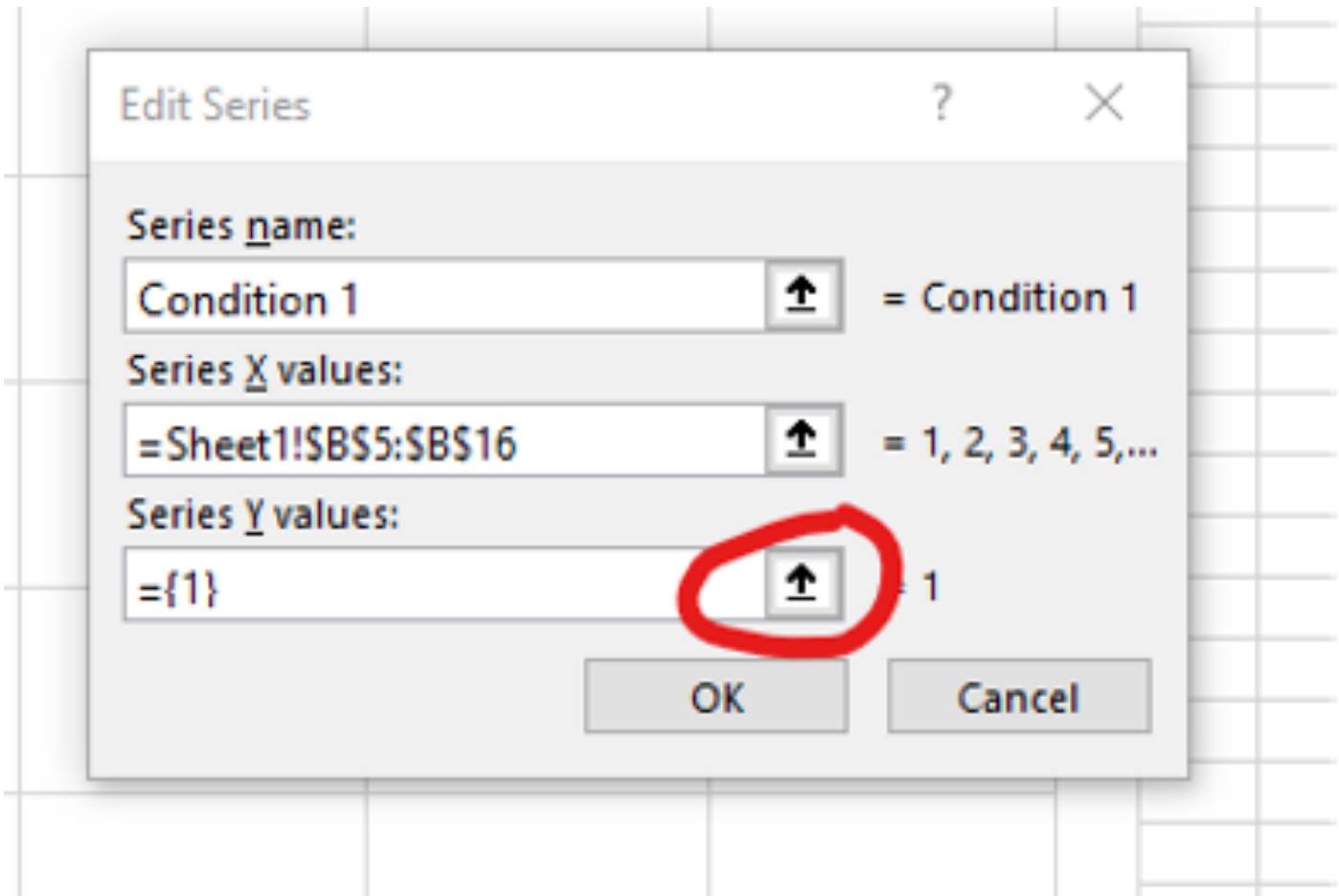
Step 22

23. This little dialogue box will pop up. From your data table, select the entire column with your time stamp data (1 through 12). Click and drag to select it. Then click on the little box on the right.



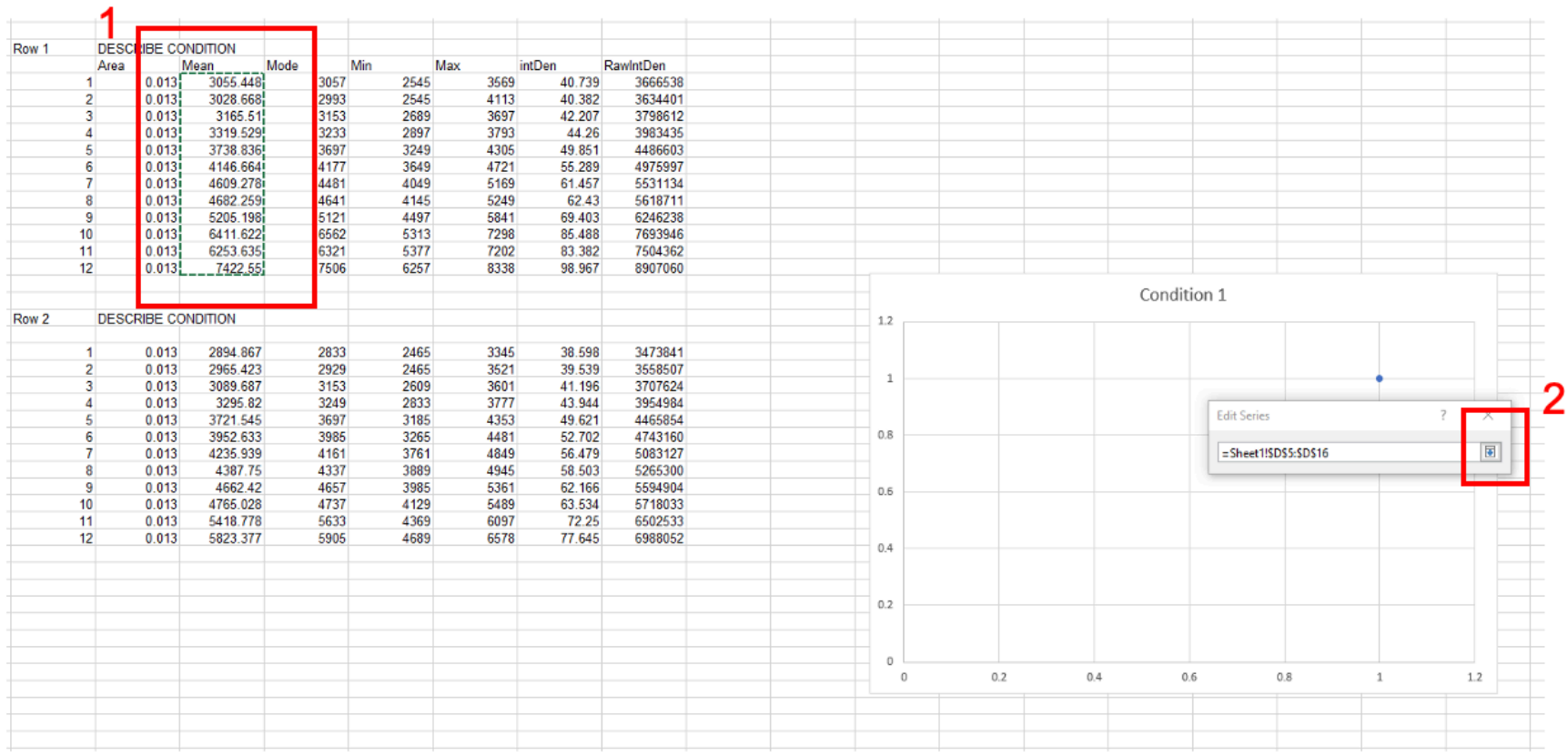
Step 23

24. Then click on the little arrow to indicate the Y-axis data.



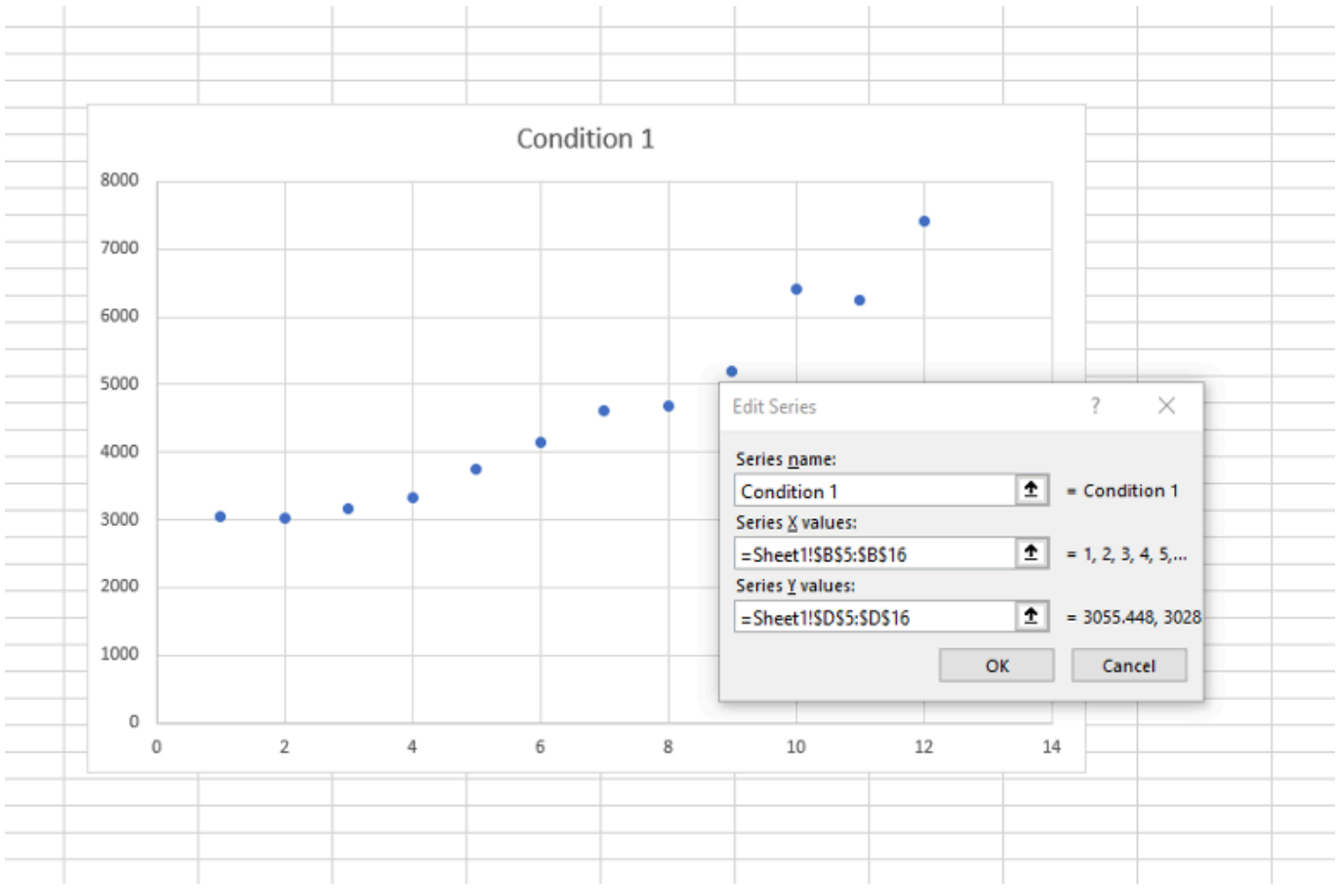
Step 24

25. Like before, select the data from your table. This time select the “Mean” column. Then click the little arrow on the dialogue box. You should see your data appear on the graph!



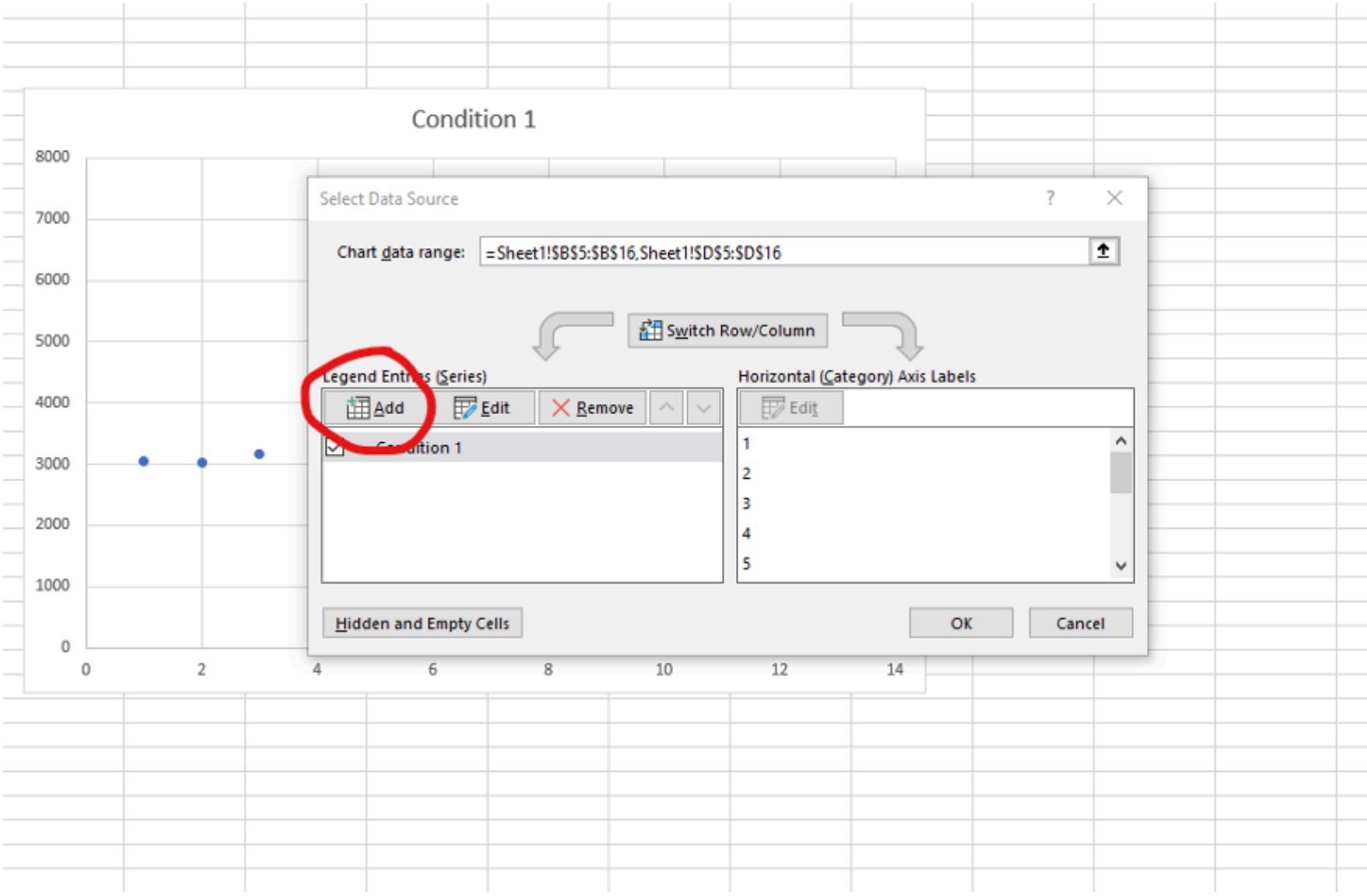
Step 25

26. Hit “OK.”



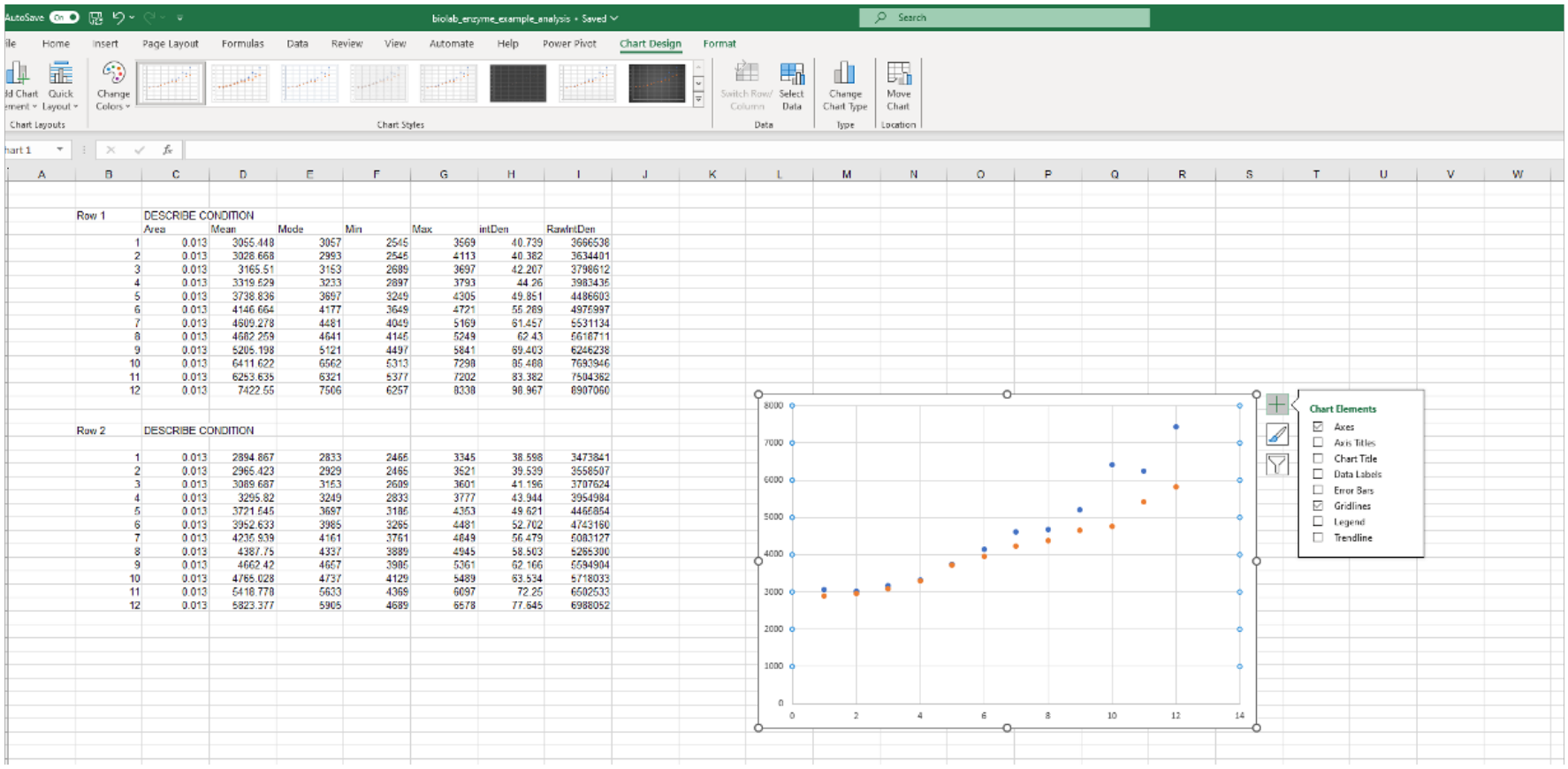
Step 26

27. Add another series. Do this for all your rows. When you are done, hit "OK."



Step 27

28. When you are done, you should have at least 5 sets of data on your graph (I only have 2). You can then click on your graph and then click on the plus sign that shows up. Then check the box for “legend” and you can see which color is which condition.

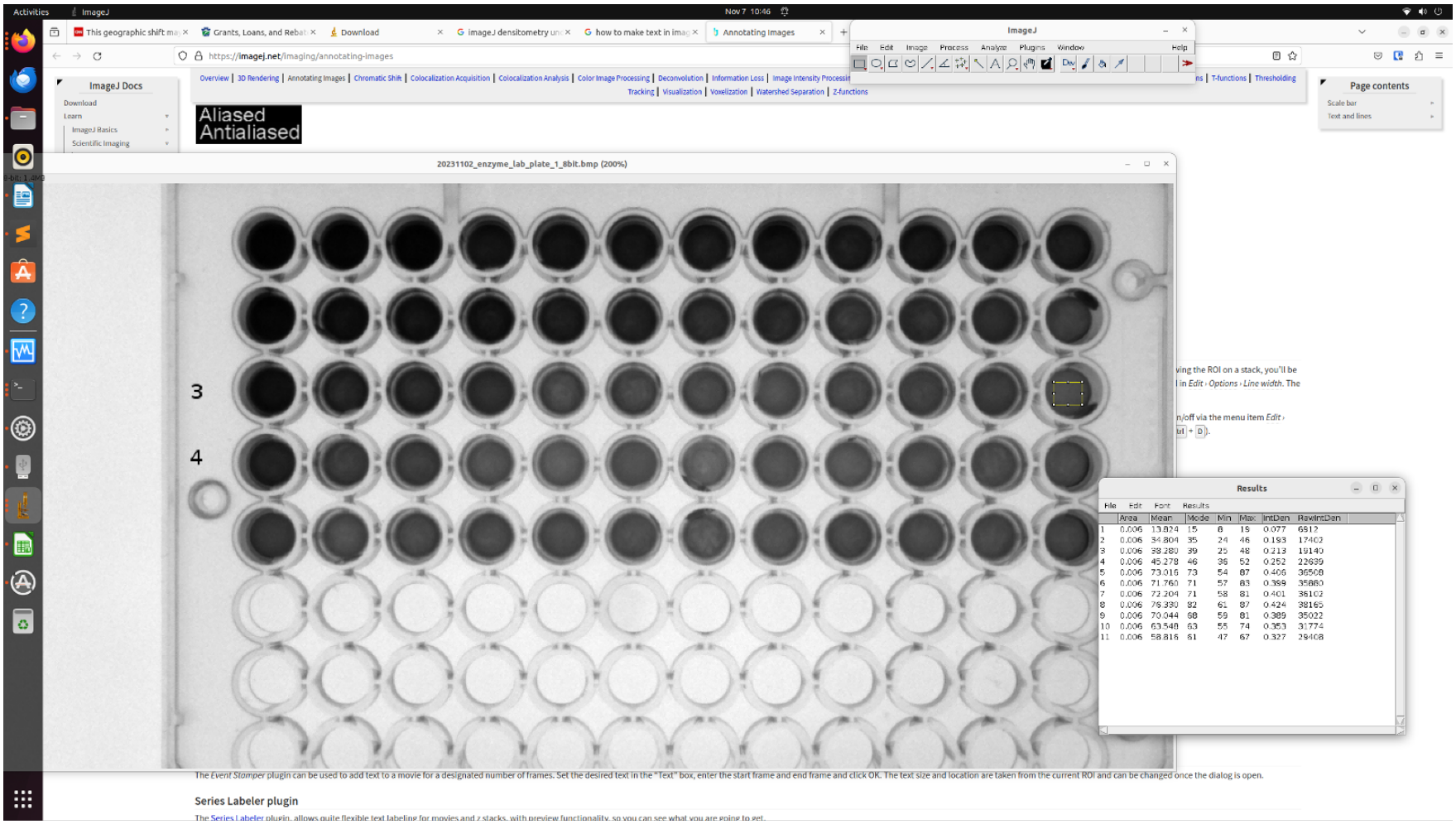


Step 29

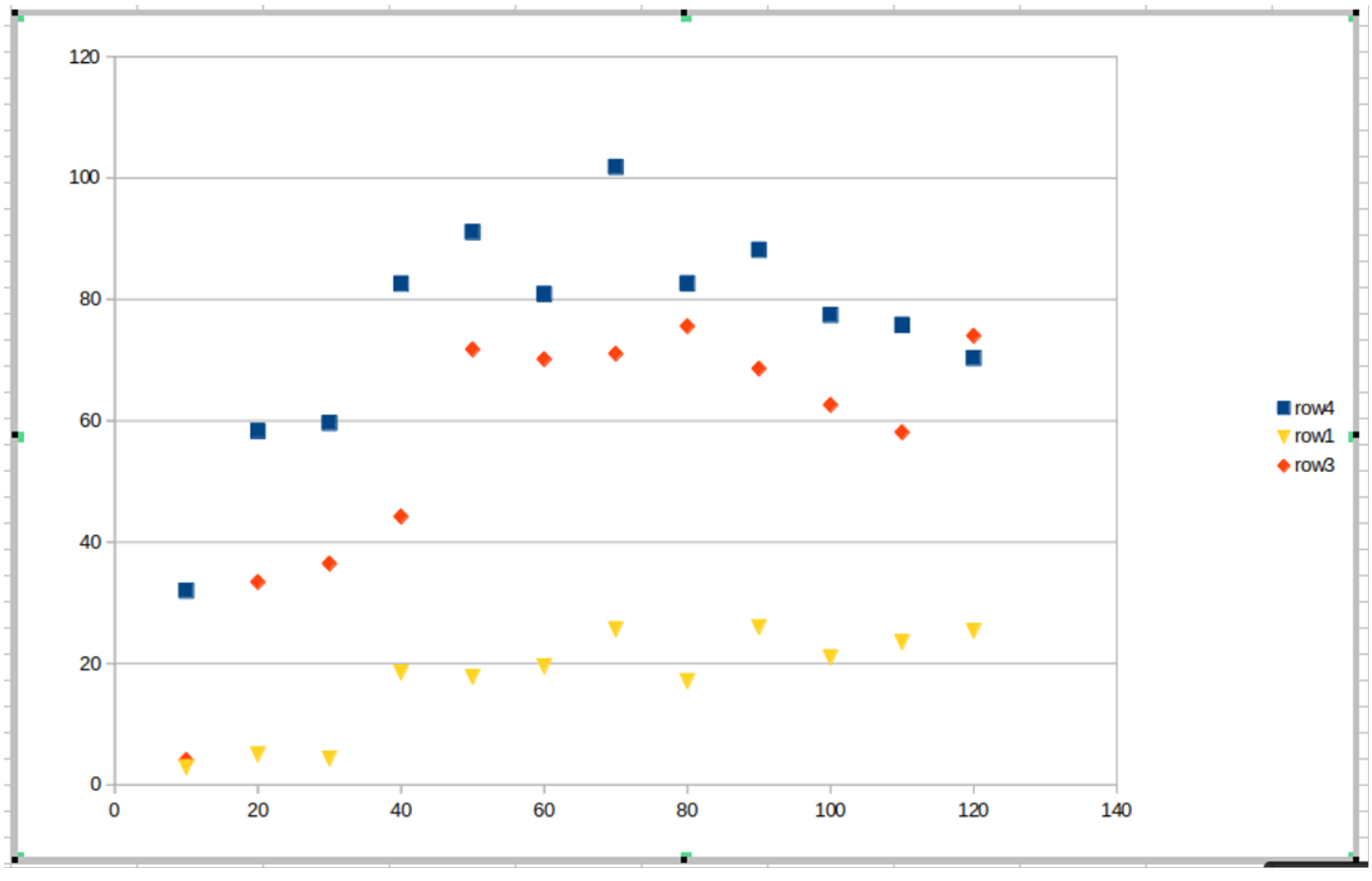
29. After you have completed the graphing of all five conditions, upload your Excel file to D2L Dropbox in the module.

Example Quantification

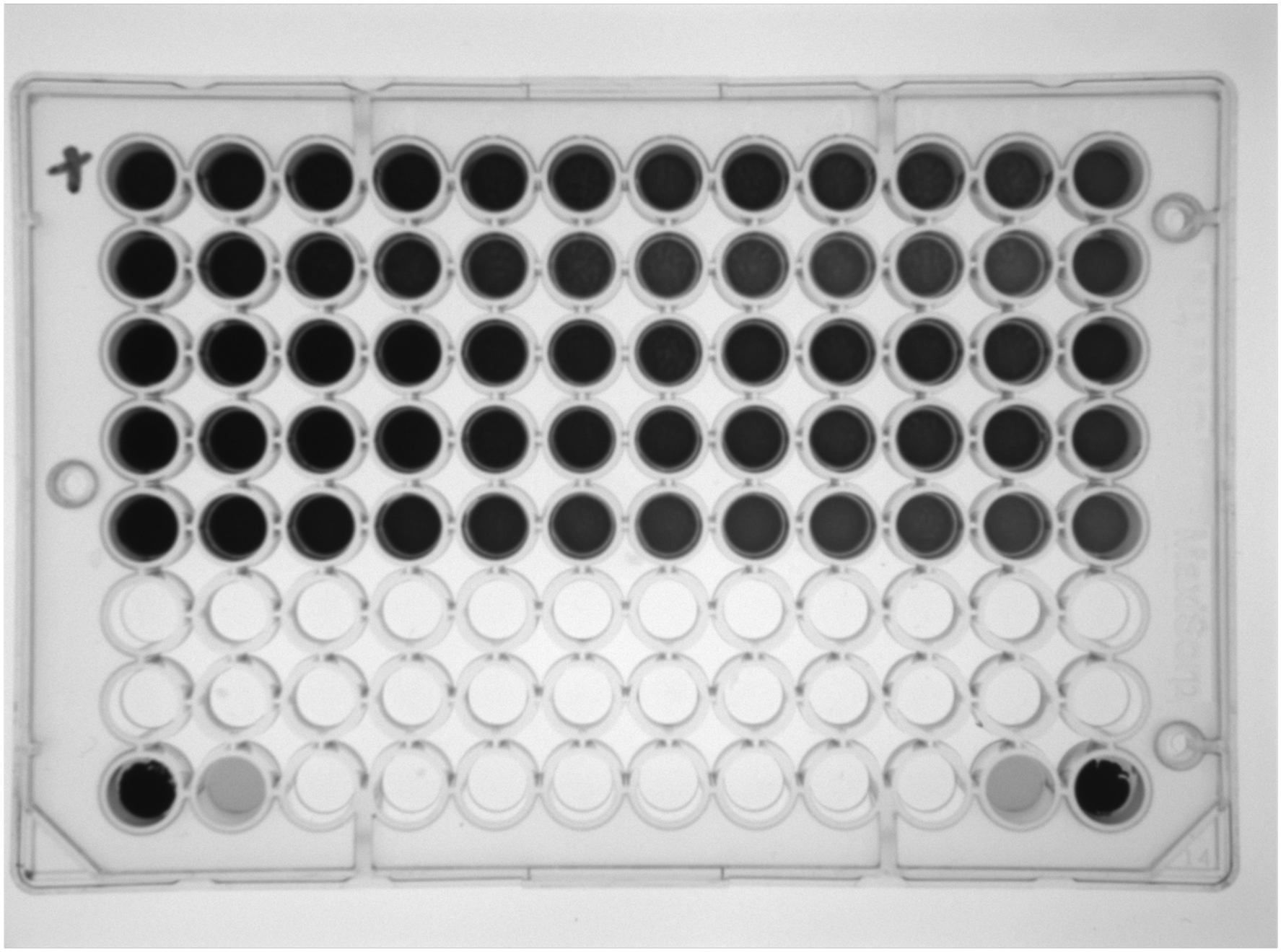
The following slides show examples of this quantification procedure.



Example Procedure Image 1



Example Procedure Image 2



Example Procedure Image 3

Image Citations

Red-40: National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 33258, Allura Red AC Dye. Retrieved July 24, 2025 from https://pubchem.ncbi.nlm.nih.gov/compound/Allura-Red-AC-Dye.

Yellow-5: National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 164825, Tartrazine. Retrieved July 24, 2025 from https://pubchem.ncbi.nlm.nih.gov/compound/Tartrazine.