

# **An Investigation of Light and its Uses in Analysis of the Immunological State**

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## I. Science Background

- a. The teacher should have the following understandings prior to teaching this unit.
  - i. Teacher should have a basic understanding of the immune response, interaction of T cells and B cells, and how they destroy a virus. (A lot of good information can be found at <http://bit.ly/1HjxZcH> and continuing on with further videos on how the immune response works.) In addition refer to the text about Basic Concepts of the Immune System at <http://www.medicine.mcgill.ca/physio/vlab/immun/backg.htm>.
  - ii. Teacher should know how a Bohr model is interpreted and its role in understanding light.
  - iii. Teacher should understand basic light equations.

## II. Student Outcomes

- a. Science Concepts
  - i. Bohr model of atoms
  - ii. Basic light equations and calculations
  - iii. T cell & B cell response to viruses and their interactions with each other
  - iv. The workings of fluorophore staining of lymph nodes
  - v. Fluorescence microscopy
  - vi. Flow cytometry
  - vii. Graphing using a bar graph
  - viii. Writing conclusions using data from experiments
- b. NGSS Alignment
  - i. **HS-PS4-1 Use mathematical representations to support a claim regarding relationships among the frequency, wavelength, and speed of waves traveling in various media.** [Clarification Statement: Examples of data could include electromagnetic radiation traveling in a vacuum and glass, sound waves traveling through air and water, and seismic waves traveling through the Earth.] [Assessment Boundary: Assessment is limited to algebraic relationships and describing those relationships qualitatively.]
  - ii. **HS-PS4-4 Evaluate the validity and reliability of claims in published materials of the effects that different frequencies of electromagnetic radiation have when absorbed by matter.** [Clarification Statement: Emphasis is on the idea that photons associated with different frequencies of light have different energies, and the damage to living tissue from electromagnetic radiation depends on the energy of the radiation. Examples of published materials could include trade books, magazines, web resources, videos, and other passages that may reflect bias.] [Assessment Boundary: Assessment is limited to qualitative descriptions.]
  - iii. **HS-PS4-5 Communicate technical information about how some technological devices use the principles of wave behavior and wave interactions with matter to transmit and capture information and energy.\*** [Clarification Statement: Examples could include solar cells capturing light and converting it to electricity; medical imaging; and communications technology.] [Assessment Boundary: Assessments are limited to qualitative information. Assessments do not include band theory.]
- c. Recommended Placement of Unit
  - i. Integrated into unit on the atom in general, academic and honors chemistry.
    1. During this unit, the Bohr model of the Hydrogen atom, electrons, and light equations are discussed.
    2. These lessons are a direct application to the use of light and its properties in the biological world.
- d. What Students Will Learn
  - i. The mathematical relationships between energy, wavelength and frequency
  - ii. Understand Bohr's model of the hydrogen atom
  - iii. Interpret the electromagnetic spectrum
  - iv. Understand how instruments in the immunological field use the principles of light to make new discoveries
  - v. Explain the basic immune system and why scientists study T cells and B cells

- e. Relevance to Students' Lives
  - i. Cross curriculum focus between chemistry and biology concepts
    - 1. Many times students learn about the basic immune system as a system in biology and about light in chemistry but never learn about the connections between the two along with the continued research that is being conducted in this field.
  - ii. Students have a misconception that when they come into contact with a virus or bacteria they should start to feel ill right away. This idea of needing time for the body to respond and build up an immune response is discussed in this unit and shown in the diagram students draw along with their results in both experiments.

### **III. Learning Objectives**

- a. Calculate frequency, wavelength, and energy using mathematical light and energy equations.
- b. Correctly interpret Bohr's diagram of the hydrogen atom.
- c. Be able to explain how a photon of light is generated in an atom and why different colors are emitted.
- d. Understand the basic immune system response.
- e. Compare and contrast the basic workings of and uses for Flow Cytometry, Fluorescence Microscopy, and Two Photon Microscopy in the field of immunology.

### **IV. Time Requirements**

- a. Three and a half 90-minute block class periods
- b. Or five 45-minute class periods

### **V. Advance Preparation**

- a. Assembly of flow cytometer model along with worksheets / handouts.

### **VI. Materials and Equipment**

- a. Worksheet for light equations and Bohr model.
- b. Flame Test Demonstration
  - i. Cotton Balls
  - ii. Tongs
  - iii. Lighter / Matches
  - iv. Salts (\_sodium chloride, calcium chloride, strontium chloride, lithium chloride, potassium chloride, etc.\_)
  - v. Ethanol
  - vi. Matches
- c. Worksheet for flame test demonstration.
- d. YouTube Video: "How T Cells Work" or "Your Immune System: Natural Born Killer – Crash Course Biology #32" <http://bit.ly/137lccA>
- e. Diagram of Immune System on the microscopic cellular level. (diagram and video link shown below) <http://bit.ly/1zNqJVn>
  - i. White paper
  - ii. Two different colored pencils
  - iii. Pen / pencil
  - iv. White board / document camera (to show students how to draw the diagram)
- f. Diagram of Immune Response (handout / students draw their own)
- g. Fluorescence Microscopy Activity Materials
  - i. Three different sets of cell color by number sheets
  - ii. Highlighters
  - iii. Black light
  - iv. Regular markers of coordinating colors with highlighters

- v. Direction sheet
- vi. Projector / whiteboard
- h. Flow Cytometer
  - i. Large test tube of width that colored glass bead can fit
  - ii. 90% corn syrup + 10% water solution
  - iii. Colored glass beads (red, green, blue, yellow)
  - iv. Cardboard box with one end cut off
  - v. White paper
  - vi. Flashlight (able to be compactly focused)
  - vii. Markers
  - viii. Direction sheet
  - ix. Data sheet / analysis sheet

## VII. Student Prior Knowledge and Skills

- a. Students should have a basic understanding of
  - i. The idea that we have an immune system, and there are smaller cells that make up our immune response.
  - ii. Atoms are made up of protons, neutrons, and electrons.
  - iii. Different atoms are made up of different amounts of subatomic particles.
  - iv. Elements are placed on the periodic table in terms of their subatomic particles.

## VIII. Daily Unit Plans

- a. Day 0
  - i. Homework: Students watch teacher-made video on how to interpret the Bohr Model of the Hydrogen Atom (<http://bit.ly/1DTbdHx>) and another on an Introduction to Energy and Light Equations ([https://www.youtube.com/watch?v=oJ8FLJTzRWU&list=PLIFJ5oDa\\_F14ZjiFn3U62Y5Zx-vA\\_x7Bf&index=4](https://www.youtube.com/watch?v=oJ8FLJTzRWU&list=PLIFJ5oDa_F14ZjiFn3U62Y5Zx-vA_x7Bf&index=4)) and take respective notes. (Attached Documents “Video: Bohr Model of Atom & Bohr Model of Hydrogen Atom” and “Video: Energy & Light Equations”)
  - ii. Modification – Students learn about the Bohr model the day prior in class via direct instruction.
- b. Day 1 (90 minute block)
  - i. Students practice interpreting the Bohr Model of Hydrogen Atom along with completing practice problems of mathematical light equations  $E=h\nu$ ,  $c=\lambda\nu$  along with converting nanometers to meters. Inform students of quiz on this tomorrow. (Attached Documents “Bohr’s Model of Hydrogen Atom” and “Light and Energy Class Work”)
    - 1. Modification – this can be done as homework from the direct instruction from Day 0 prior to coming to class on Day 1.
    - 2. Modification – Alternative light equation sheet can be given without two-step problems of converting wavelength to energy.
  - ii. Students watch demonstration on flame tests of different chemical compounds further reinforcing that different electron transitions emit different wavelengths of light. After demonstration, students complete post lab questions. (Attached Document “Flame Test Demonstration”)
  - iii. Present question to students: How do you think the idea of light can be used in the human body? Discuss fluorophores
  - iv. Homework: Watch video “Crash Course Biology #32” <http://bit.ly/1OqKEeq> or Video “How T Cells Work” <https://www.youtube.com/watch?v=klxmiTuRydw>. Students to answer question “What is the role of B Cell / T Cell / Dendritic Cell in the immune system?”
- c. Day 2 (90 minute block)
  - i. Take quiz on mathematical light equations. (I wrote questions on board and had students do on their own sheets of paper.) Teacher discretion. Example questions provided below.

1. Calculate the wavelength of light with a frequency of  $7.5 \times 10^{12}$  1/s.
2. Determine energy of light if its wavelength is 525 nm.
3. How much energy is required for an electron to move from  $n=1$  to  $n=3$ ?
  - a. To scale questions, I would give my general chemistry students questions 1 & 2 the way they are but break #3 down into smaller steps (a. What wavelength of light is absorbed when an electron moves from  $n=1$ - $n=3$ ? b. What frequency will this light have? c. What energy will this light need to absorb?). I would give my honors chemistry students only the 3 questions as they are.
- ii. Discuss the immune system. Probe students with question: How can the use of fluorescence and the microscopic nature of the immune system be combined to better our understanding?
  1. Draw diagram of immune response. (30 minutes) <http://bit.ly/1Hjvwiz> (Video of my explanation)
    - a. Need white paper, colored pencils, pen/pencil (Attached Documents "Diagram of the Immune Response – Teacher Copy" and "Diagram of Immune Response in a Lymph Node" student copy)
    - b. Explain the roles of B cells and helper T cells and how they interact with a virus causing an immune response. Have students draw a similar diagram on paper. Include cell function and placement during infection and no infection. Vocabulary words to define: B cell, Th cell, epitope, virus, phagocyte, MHCII (major histocompatibility complex), antibody, dendritic cell, cytokine, activated Th cell.
    - c. Explain how B cells and activated helper T cells interact together to kill the virus. Have students draw a similar diagram on paper.
- iii. Briefly discuss 3 instruments immunologists used in lab.
  1. Flow Cytometer – used to sort cells
  2. Fluorescence Microscopy – used to see where cells are located
  3. Two Photon Microscopy – used to see how cells interact in real time
- iv. Detailed discussion on Fluorescence Microscopy. (Attached Document: "Lymph Node Staining for Analysis via Fluorescence Microscopy," "Lymph Node Fluorescence Microscopy - Teacher Copy", and "Lymph Node Fluorescence Microscopy" student copy)
  1. Discuss idea again of fluorescence & fluorophores. (Contained in lab)
  2. Draw diagram of three states of an immune response (healthy, initial, heightened response)
  3. Explain how to stain cells & then "stain" paper cells. (Contained in lab)
    - a. Depending on time, may want to make coloring sheets easier to do / less cells to color.
  4. View stained paper cells under UV (black) light & discuss results. Which ones have response? Which ones are healthy? Students answer post lab questions.
- d. Day 3 (90 minute block)
  - i. Discussion on Flow Cytometer. (Attached Documents "Flow Cytometry Experiment: Lab Set-Up" and "Using Flow Cytometry to Determine the State of Immune Response in a Host")
    1. Complete experiment with made model of flow cytometer.
    2. Have students complete post lab questions and graph
- e. Day 4 (half class period 45 minutes)
  - i. Discussion on Two Photon Microscopy.
    1. Discuss why a Two Photon Microscope would be used.
    2. Discuss how microscope works.
  - ii. Creation of a 3-way Venn Diagram of three microscopes. Compare how microscopes work, what they are used for, and properties of light learned of thus far. (Attached Document: "Venn Diagram of Flow Cytometry, Fluorescence Microscopy, and Two Photon Microscopy" )

**IX. Summative Assessment**

- a. Students will create a 3-way Venn diagram of the three microscopes detailing how they work and why each is used.
- b. Students will hand in their conclusions / post lab questions from the two experiments.

**X. Student Section (following pages)**

## VIDEO: Bohr Model of Atom & Bohr Model of Hydrogen Atom

Bohr Model of Atom –

Row 1

Row 2

Remember

Electrons are represented as

Row 1 has \_\_\_\_\_ elements therefore has \_\_\_\_ electrons max on ring.

Row 2 (&3)

Row 4

Each element has

Example 1. Beryllium “Be” atomic #4

Example 2. Chlorine “Cl” atomic #17

Your Try: Sodium

### Bohr Model of Hydrogen Atom \*\*On Your Reference Table\*\*

When you add energy to an atom –

When electrons relax back down –

Diagram of Hydrogen atom

Example:

Told as a transition from  $n=3$  to  $n-1$ .

Follow line down to wavelength spectrum. Wavelength = 103 nm which is UV light.

You try: Transition of  $n=2$  to  $n=1$ . What is the wavelength of light emitted?

## Video: Energy & Light Equations

Light Wave Diagram

As wavelength \_\_\_\_\_ frequency \_\_\_\_\_.

Spectrum

Speed of Light =

Example 1. What is the frequency of light if light has a wavelength of 450 nm?

Energy of Light

Energy is measured in Joules = J

$E = h\nu$                        $h$  = Planck's Constant =

Example 1. What is the energy of light with a  $\lambda=450$  nm?

You Try: Find energy of light with a  $\lambda=500.0$  nm.

## Bohr's Model of the Hydrogen Atom

1. What wavelength of light is emitted when an electron relaxes from  $n=4$  to  $n=2$ ?
2. What wavelength of light is emitted when an electron relaxes from  $n=5$  to  $n=3$ ?
3. An electron moves from  $n=3$  to  $n=5$ . Is energy emitted or absorbed?
4. An electron moves from  $n=2$  to  $n=1$ . Is energy emitted or absorbed?
5. What electron transformation(s) cause(s) red light to be emitted?
6. What electron transformation(s) cause(s) UV light to be emitted?

## Light and Energy Class Work

Complete the following problems on your own paper.  
Use your reference packet!

### Useful Equations

$$c = \lambda \nu$$

$$c = 2.998 \times 10^8 \text{ m/s}$$

$$E = h\nu$$

$$h = 6.626 \times 10^{-34} \text{ J}\cdot\text{s}$$

$$1\text{m} = 1 \times 10^9 \text{ nm}$$

1. Draw the Bohr Models for Hydrogen, Nitrogen & Aluminum.
2. Light with a wavelength of 525nm is green. Calculate the frequency for this green light.
3. Calculate the energy (in J) for a photon of green light from the previous question.
4. UV radiation has a frequency of  $6.8 \times 10^{15} \text{ 1/s}$ . What is the energy (in J) for a photon of UV light?
5. What is the wavelength and frequency of a photon with an energy of  $1.4 \times 10^{-21} \text{ J}$ ?
6. A ruby laser produces red light that has a wavelength of 500 nm. Calculate its energy in joules (J).
7. As frequency increases, wavelength \_\_\_\_\_. Explain why.
8. What is the wavelength of light emitted as an electron moves from  $n=5$  to  $n=1$ ? In meters?
9. What is the energy of light for the previous question?
10. What kind of light is emitted?
11. Which color of visible light has the longest wavelength? The shortest?
  - a. What are the frequencies that correspond to these colors of light?

## Flame Test Demonstration

**Purpose:** You will use the flame tests to determine the identity of the cation in an unknown solution based on its characteristic color in flame.

**Materials:** Lighter, 6 small test tubes, test tube rack, tongs, 6 cotton swabs, 0.1M NaCl, 0.1M CaCl<sub>2</sub>, 0.1M LiCl, 0.1M CuCl<sub>2</sub>, 0.1M KCl, unknown solution

**Data:**

Solution	Cation	Flame Color
Unknown		

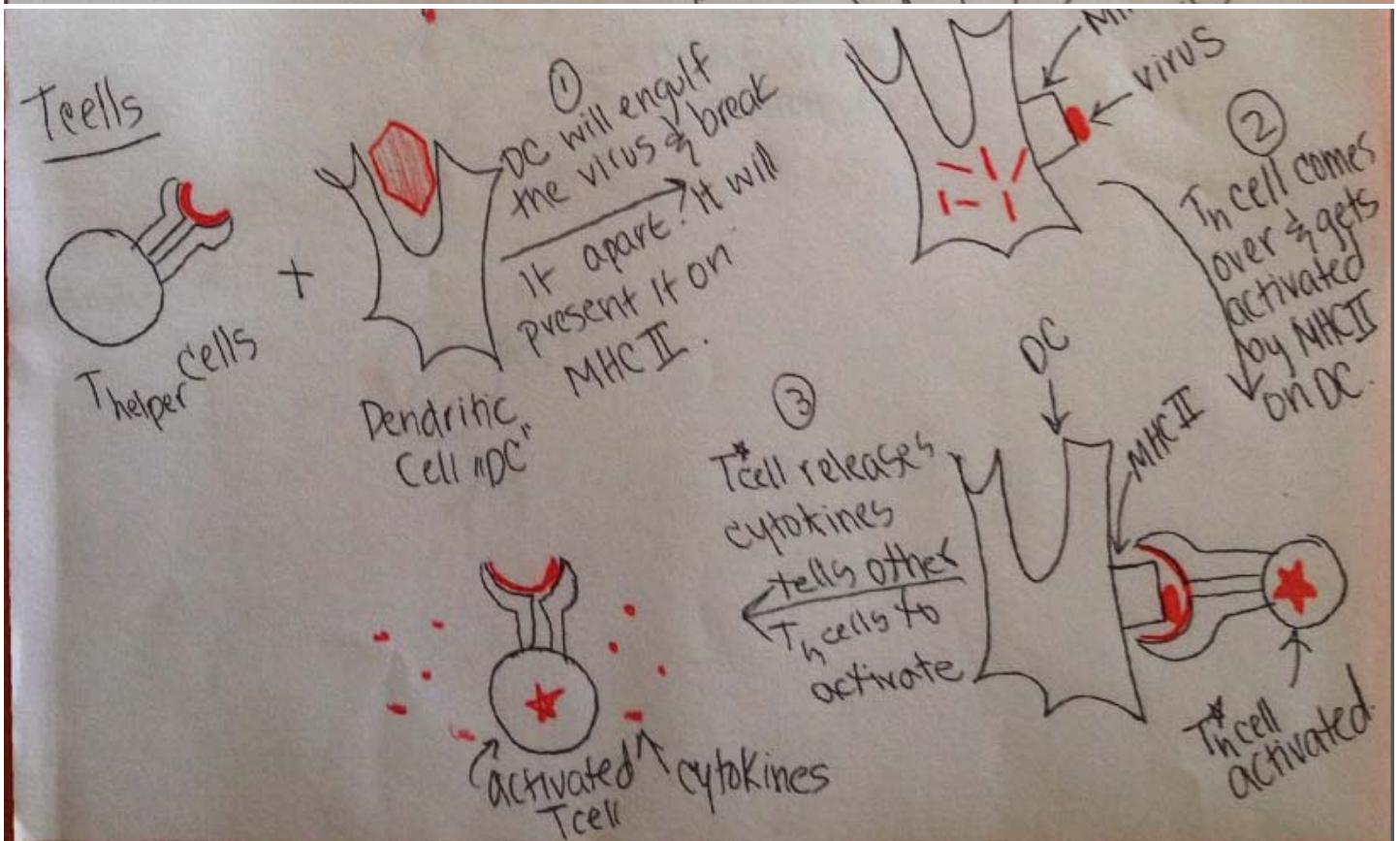
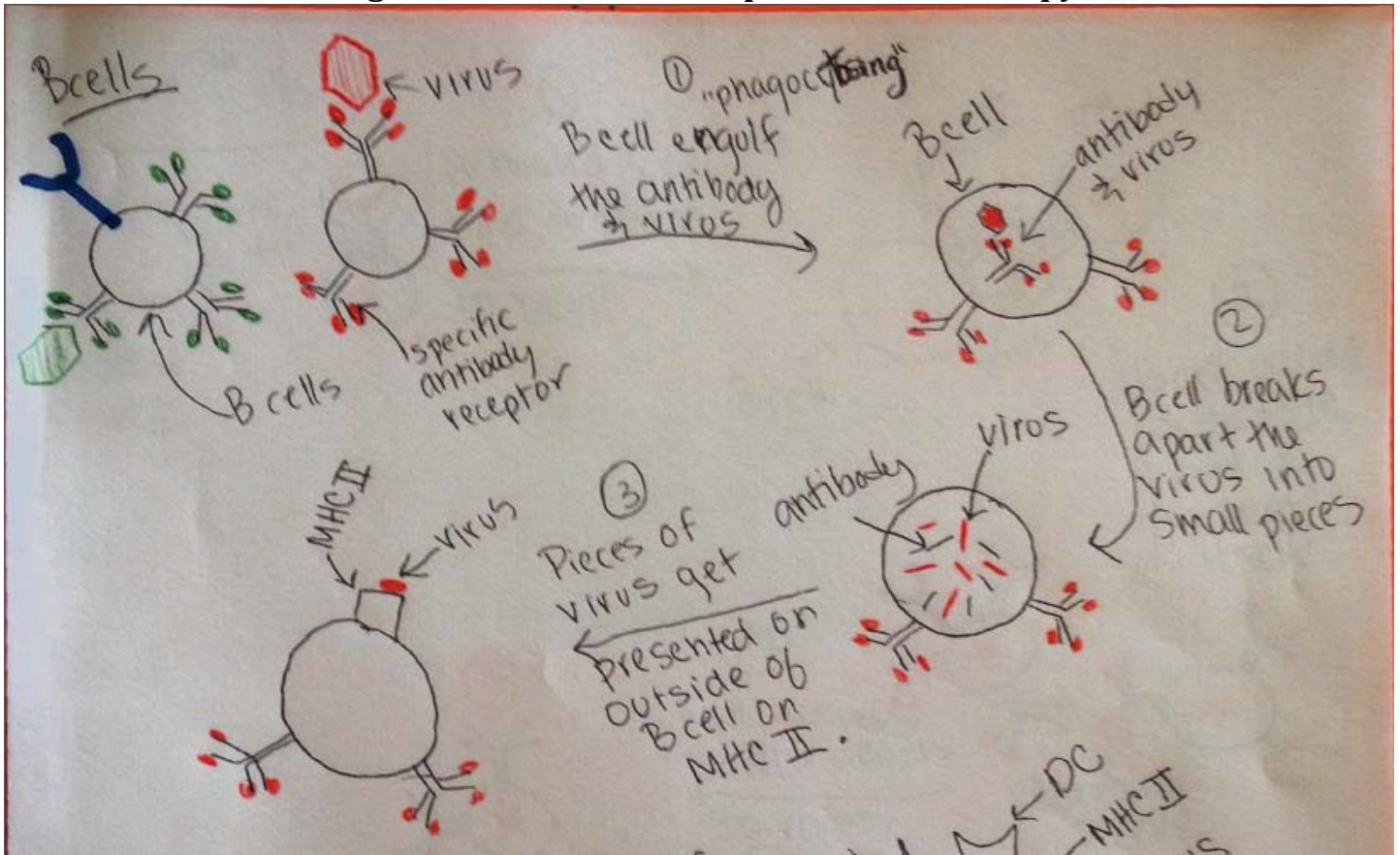
**Conclusion:**

1. Identify the cation of your unknown and provide an approximate wavelength (using your reference packet) for the color of visible light of your flame.
2. Using your wavelength in number one, calculate the frequency of light produced by your cation in the flame.
3. Select one other cation (with a different color flame), provide an approximate wavelength, and then calculate the frequency.
4. Each salt produces a unique color. Would you expect these results based on the modern view of the atom? Explain your answer.
5. Fireworks contain gunpowder and other chemicals to produce the wide array of colors. What element must one include to produce crimson red fireworks? Yellow? Green?

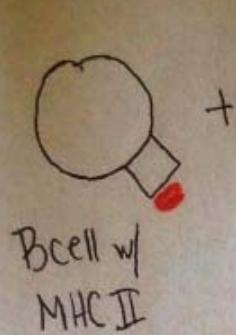
**Teacher Directions:**

Teacher picks up cotton swab with tongs. Dip cotton swab into solutions of ~ 1M solutions of salt + ethanol (true concentration does not really matter, just dissolve enough salt into ethanol so that a color is produced when flame is made). Using a lighter, ignite the cotton swab. Students will record the salt, cation making color, color of light emitted and the wavelength of light.

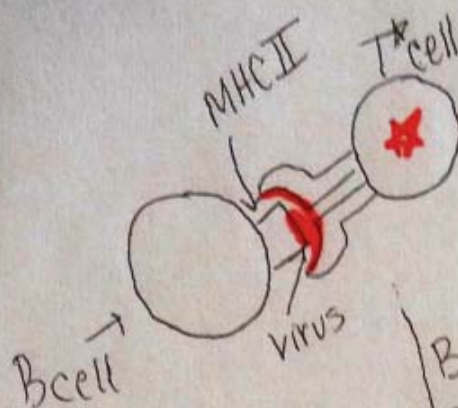
# Diagram of the Immune Response - Teacher Copy



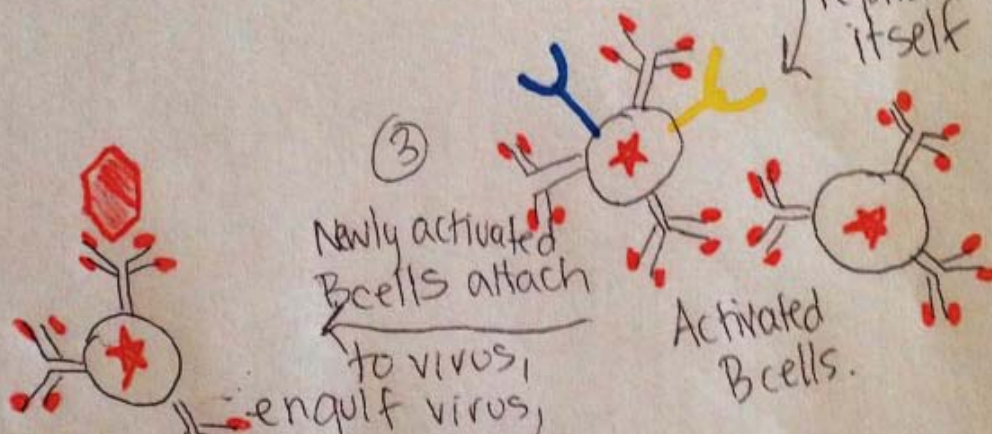
Bcells +  $T_h^*$  cells



①  
Activated  $T_h$  cell  
bind to MHC II  
on Bcell.



②  
Bcell becomes  
activated &  
start to  
replicate  
itself



Activated  
Bcells.



# Diagram of Immune Response in a Lymph Node

# Lymph Node Staining for Analysis via Fluorescence Microscopy

## Purpose:

The purpose of this lab is to simulate the staining of lymph node cells and the subsequent analysis using fluorescence microscopy. Students will learn how an immunologist stains lymph node cells with varying fluorescence antibodies to determine their location at different time points during an immune response to an infection.

## Background:

A **fluorescence microscope** is any microscope that uses fluorescence to generate an image. To prepare a lymph node to be viewed under a fluorescence microscope it must first be stained with fluorescence tags in the form of antibodies attached to cells that have fluorescence, called fluorophores. A lymph node is soaked in a sucrose solution and frozen at -80°C. Then it is cut on a cryostat into slices that are 6 - 8 microns (about 1 cell layer) thick. Next, the lymph node is "fixed," a solution is placed on the cell that in essence freezes all cells in place so they are stopped in their position. Once this is complete, an immunologist stains for the cells of interest.

We will look to see what stage of infection our lymph node is at. This is dependent on the amount of activated T cells and B cells present in the lymph node as well as their anatomical location. A **healthy lymph** node does not have any activated T cells or B cells. A lymph node at the **beginning of infection** will have a few activated T cells and B cells interacting at the T cell/B cell border. At the **height of the infection** germinal centers will be apparent with a high concentration of activated T cells and B cells located here.

## Materials:

Lymph node diagram  
Glitter Crayons  
Highlighters  
Colored Pencils  
UV (black) light  
Spot light (bright flashlight)

## Procedure:

1. Follow teacher given directions on when to stain for what cell and how long to let the stain absorb into the cells.
2. "Fix" cell for 10 minutes.
3. Stain all #4 cells with a yellow marker. Wait 10 minutes.
4. Stain all #2 cells with an orange marker. Wait 10 minutes.
5. Stain all #3 cells with a yellow highlighter. Wait 10 minutes.
6. Stain all #1 cells with an orange highlighter. Wait 5 minutes.
7. Go to black light and take photo of your cell.
8. Back at your desk, record observations in data table. How many cells are fluorescing? Where are these cells fluorescing? What does this tell us about the immune response of the cell?

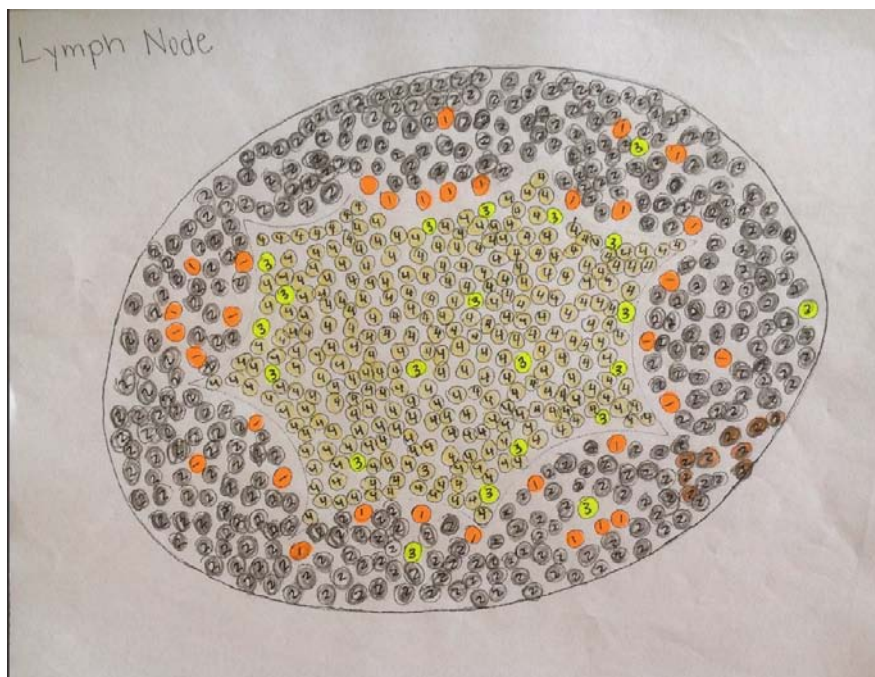
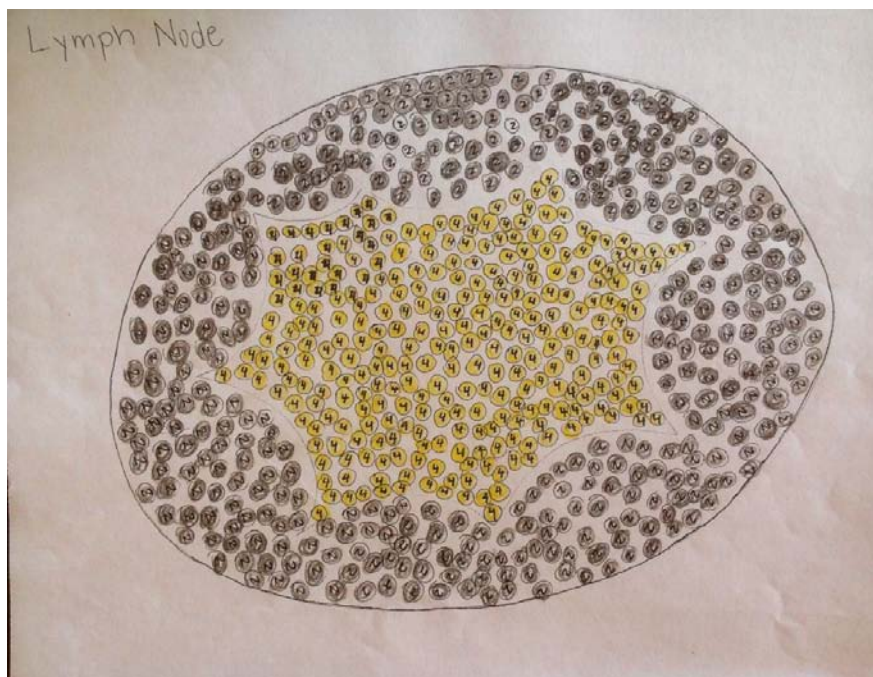
**Data Table:**

Cell Number	Are cells fluorescing?	Probable identification of cell?	Location of fluorescing cells (T Cell Region, B Cell Region, Germinal Centers)	Indication of probable immune response state.
#1				
#2				
#3				
#4				

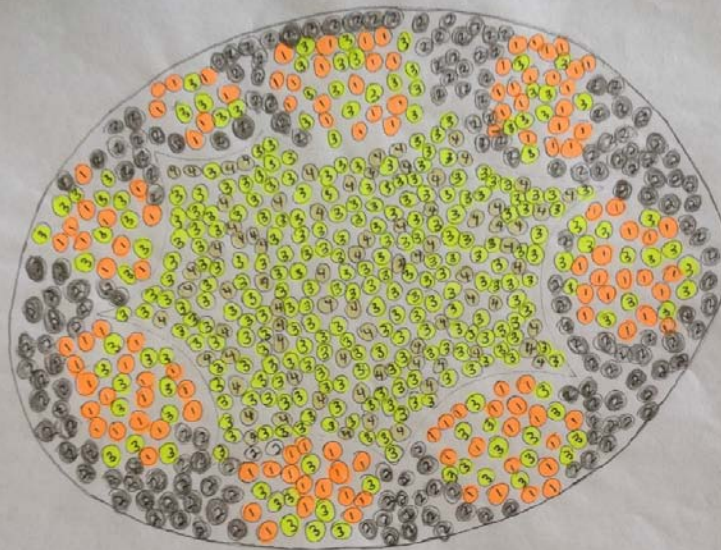
**Analysis & Conclusion:**

Using your data from analyzing your cells under the UV light what stage of the immune response is occurring in your cell? How is this **different** from another stage of the immune response and how is this **similar** to another state of the immune response?

## Lymph Node Fluorescence Microscopy – Teacher Copy



Lymph Node

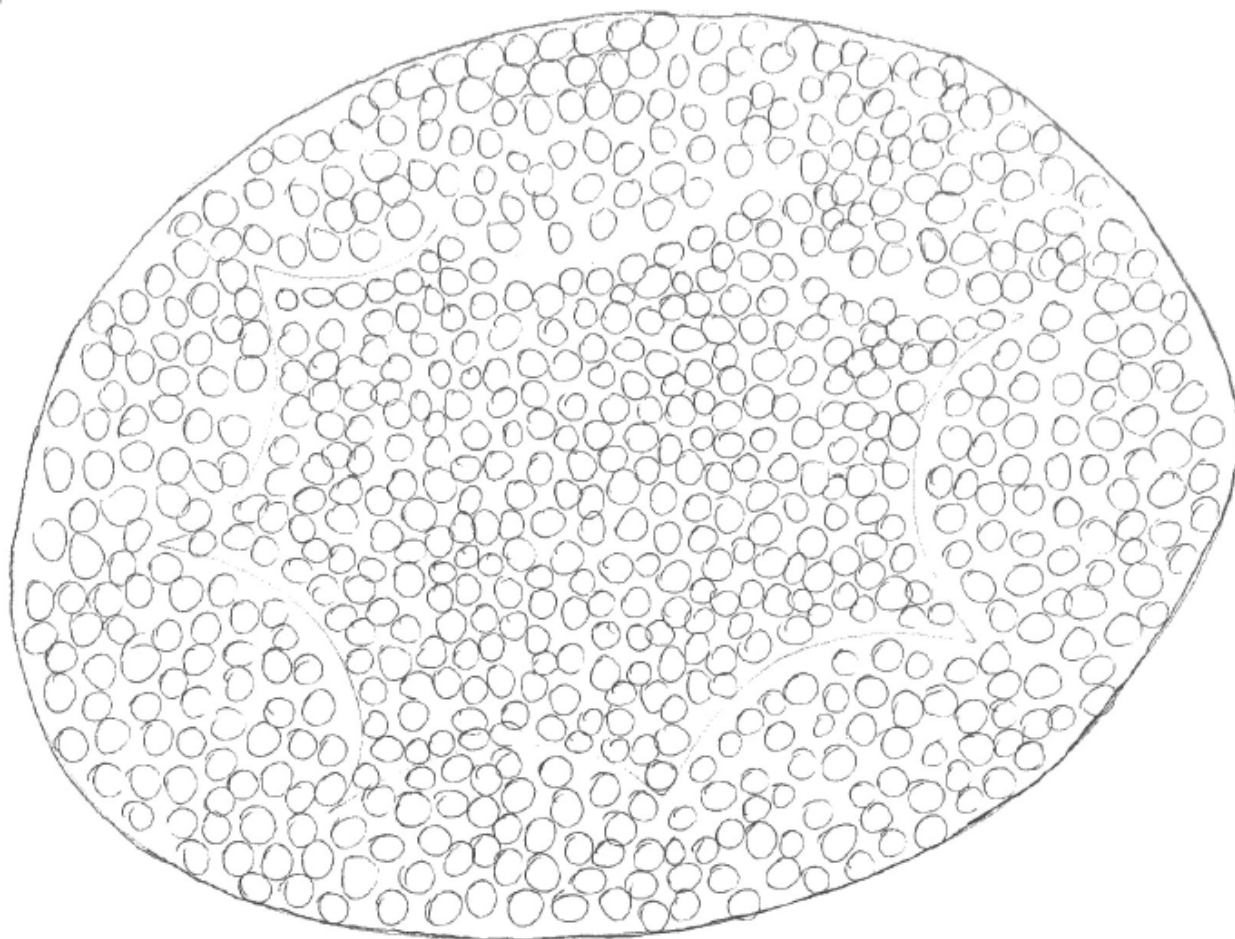


## Lymph Node Fluorescence Microscopy

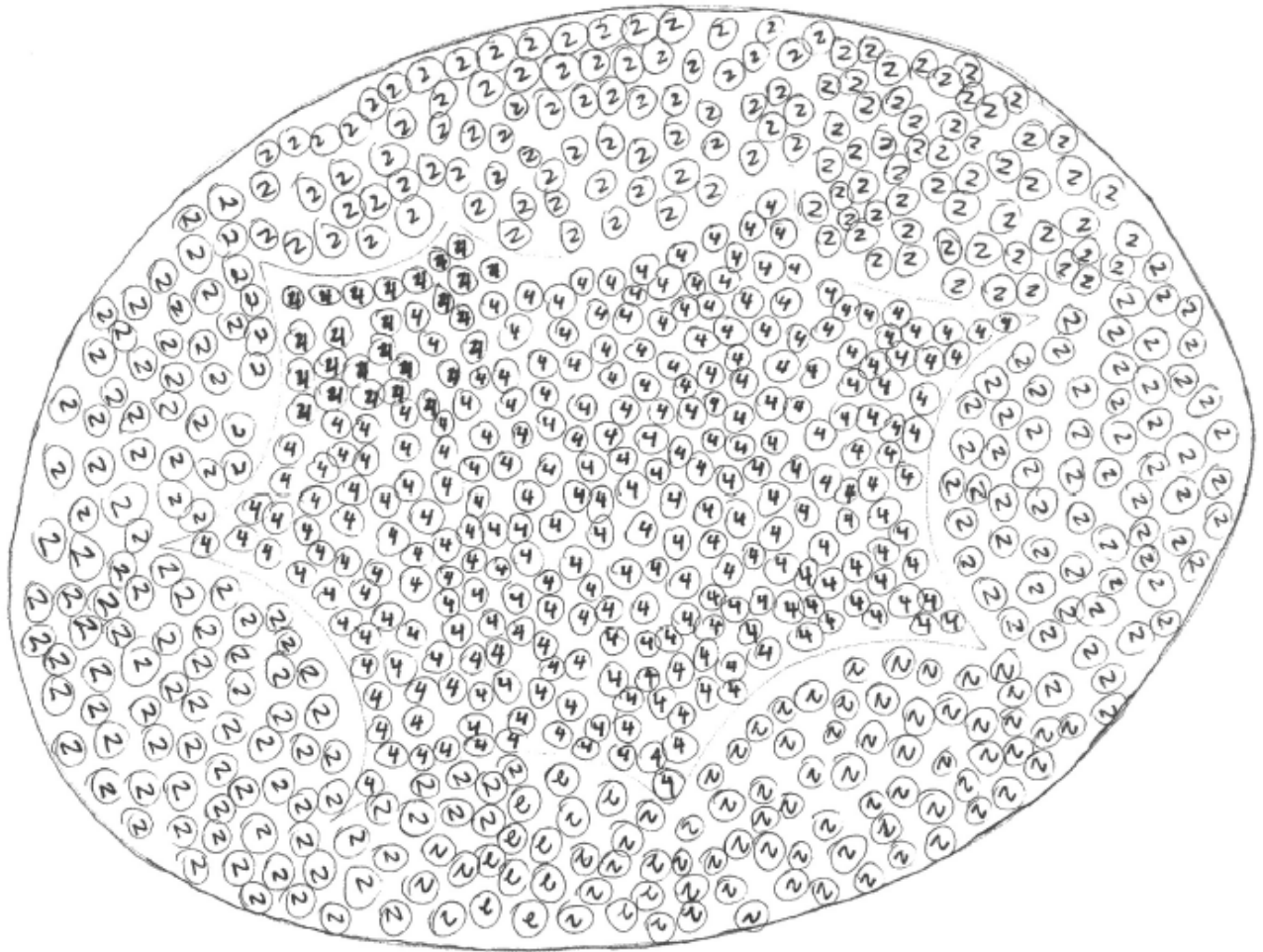
### Lymph Nodes to Color for Lymph Node Staining Lab

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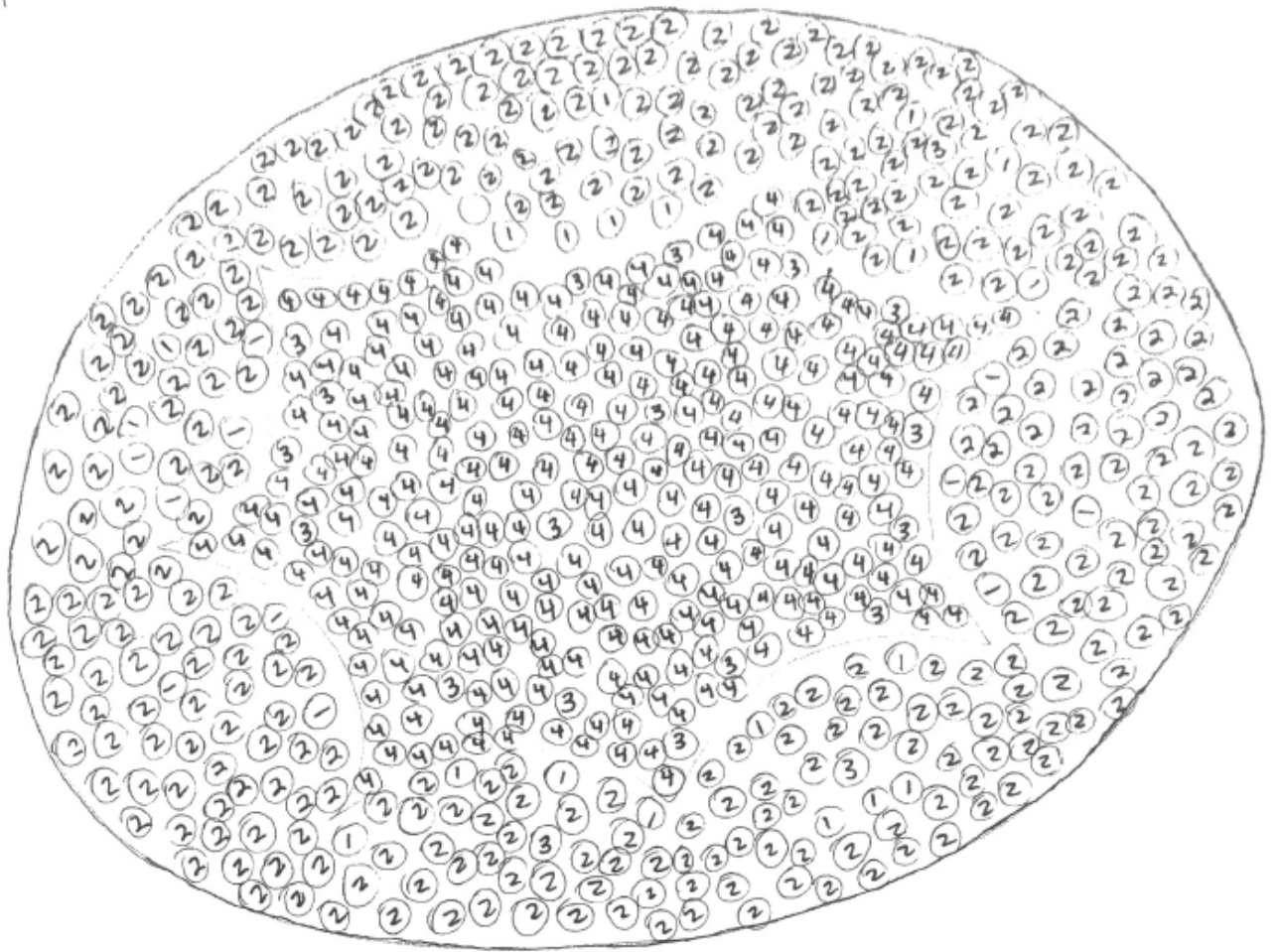
Lymph Node



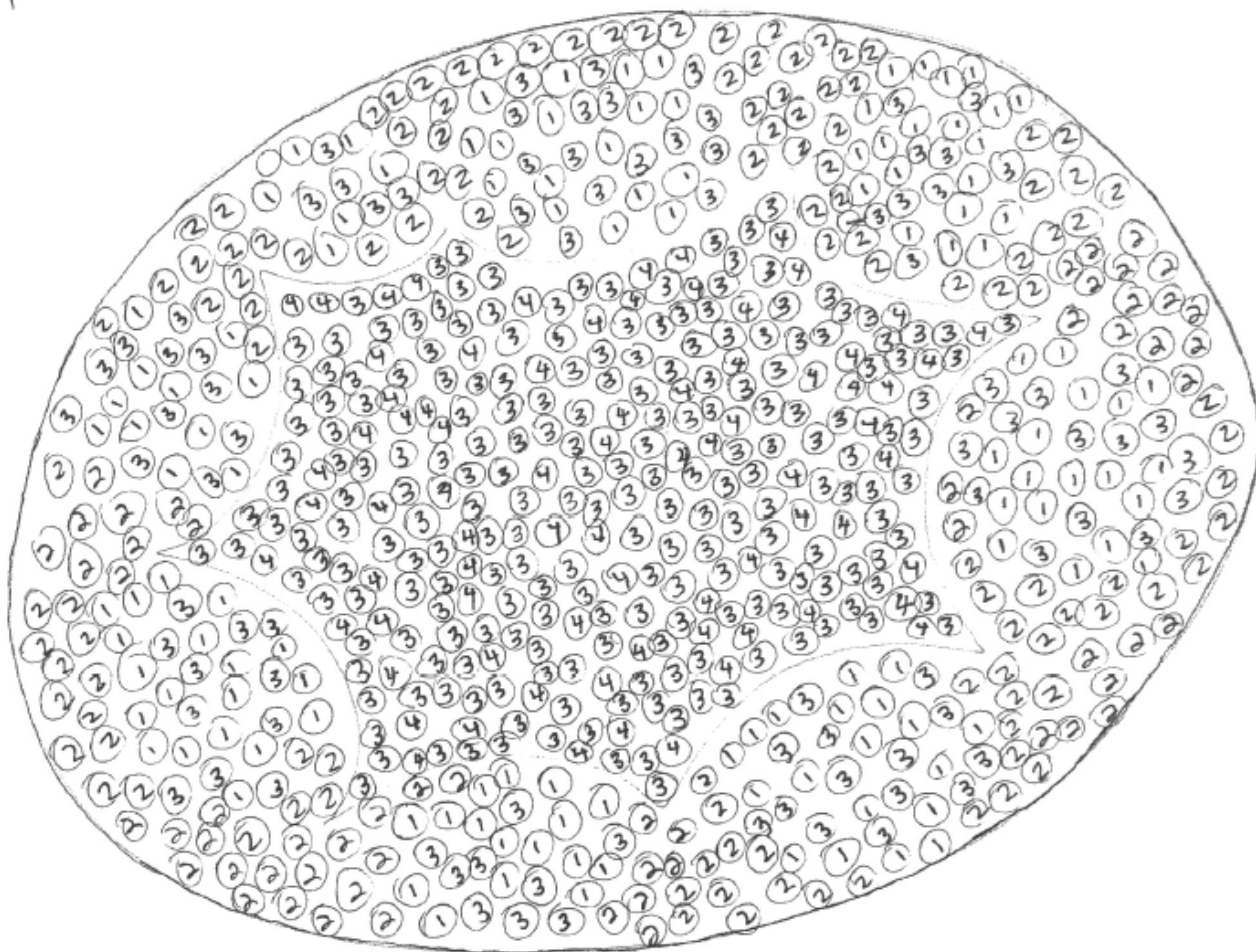
# Lymph Node



# Lymph Node



# Lymph Node



# Flow Cytometry Experiment

## Lab Set-Up



## Using Flow Cytometry to Determine the State of Immune Response in a Host

### Purpose:

Use a flow cytometer to identify the immune response occurring in a lymph node. Through this students will learn about the workings of a flow cytometer and the properties of light that it uses in addition to how fluorophores are used to mark lymph node cells and determine if an immune response is underway.

### Background:

A **flow cytometer** is an instrument used mainly in the field of immunology to analyze or sort fluorophore tagged cells. A **fluorophore** is a fluorescent chemical compound that re-emits light upon excitation. An immunologist will stain lymph node cells with multiple fluorophore markers attached to antibodies to allow him/her to determine what cells are present at different stages of immune responses. In a **flow cytometer** cells are passed in single file by a beam of light that will excite the fluorophore. When a fluorophore is excited it emits a wavelength of light that is detected by a detector that recognizes that particular wavelength of light. This information is then sent to the computer for analysis and varying plots of information are able to be constructed based upon what the immunologist is looking for. Typically data is organized into scatter plots or bar graphs.

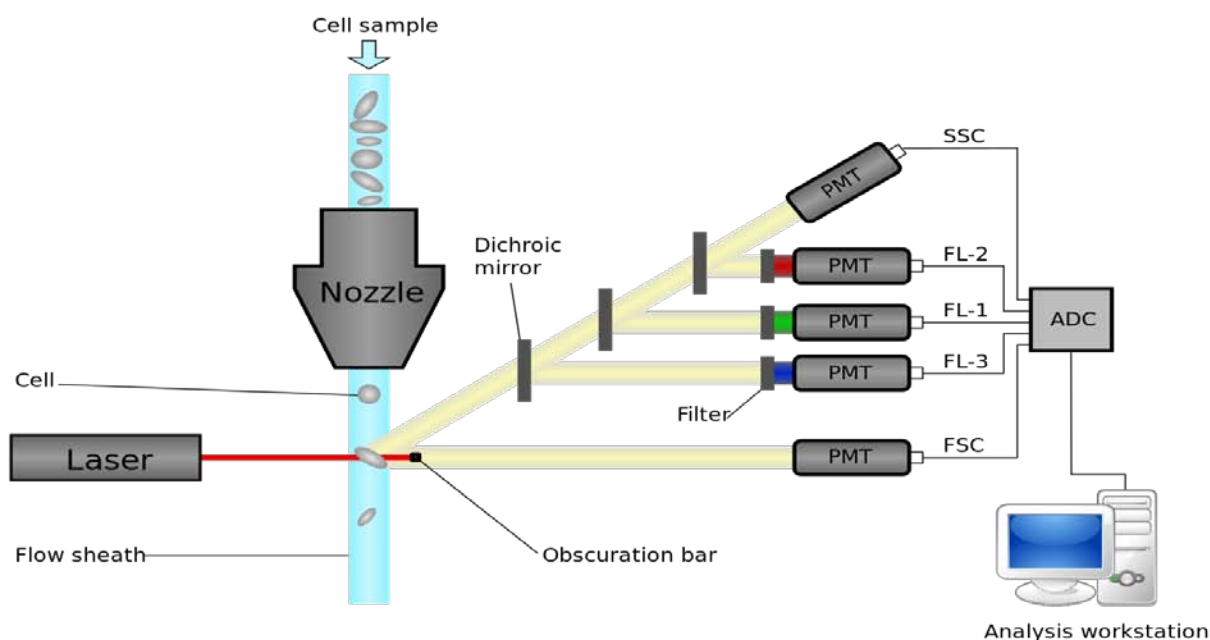


Figure 2: Schematic of a flow cytometer.

[https://en.wikipedia.org/wiki/Flow\\_cytometry\\_bioinformatics#/media/File:Cytometer.svg](https://en.wikipedia.org/wiki/Flow_cytometry_bioinformatics#/media/File:Cytometer.svg)

## Materials:

Box (open top and right side) with grid paper inside (flow cytometer & detector)  
Test tube filled with corn syrup (flow cell)  
Brown Bag of glass gems (lymph node's cells)  
Flashlight (beam of light)  
Phone / Camera to video record

## Procedure:

1. Set up the flow cytometer as shown in the figure 2.

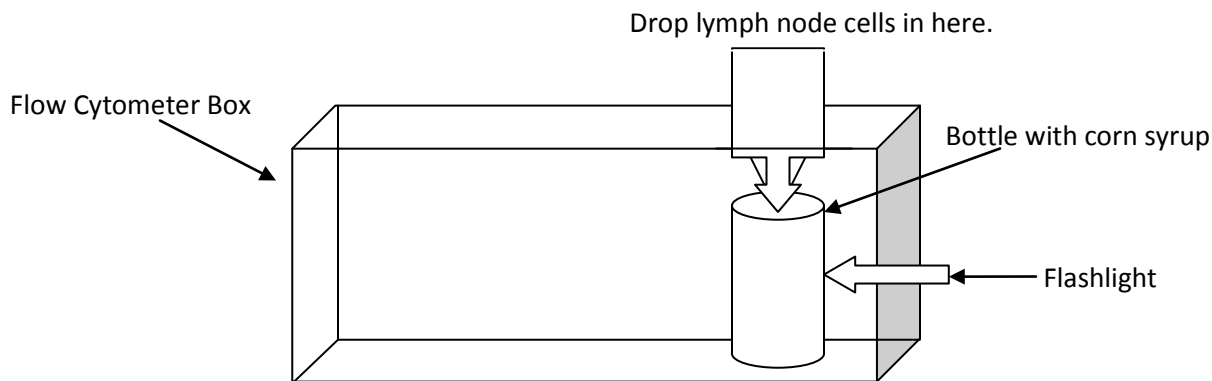


Figure 2: Flow cytometer instrument set up.

2. Pick up a lymph node (brown bag) that has been stained with fluorophores.
3. Assign roles to group members.
  - a. Member One: hold the flashlight 1 cm below corn syrup fill line and 0.5 - 0 cm away from the test tube of corn syrup.
  - b. Member Two: find a good position to record the light being emitted from the fluorophores on the grid paper inside the flow cytometer.
  - c. Member Three: record initial color observations on data sheet.
  - d. Member Four: drop one lymph node cell at a time into the test tube of corn syrup.
4. With all members in place and the classroom lights off / down, slowly start dropping cells one at a time into the flow cytometer.

Data Table: On next page.

### Fluorophore Colors of Tagged Cells

B cells: \_\_\_\_\_

Activated B cells: \_\_\_\_\_

T cells: \_\_\_\_\_

Activated T cells: \_\_\_\_\_

Data Table:

Cell Number	Fluorescence Color(s)	Possible Identity	Light Wavelength(s)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

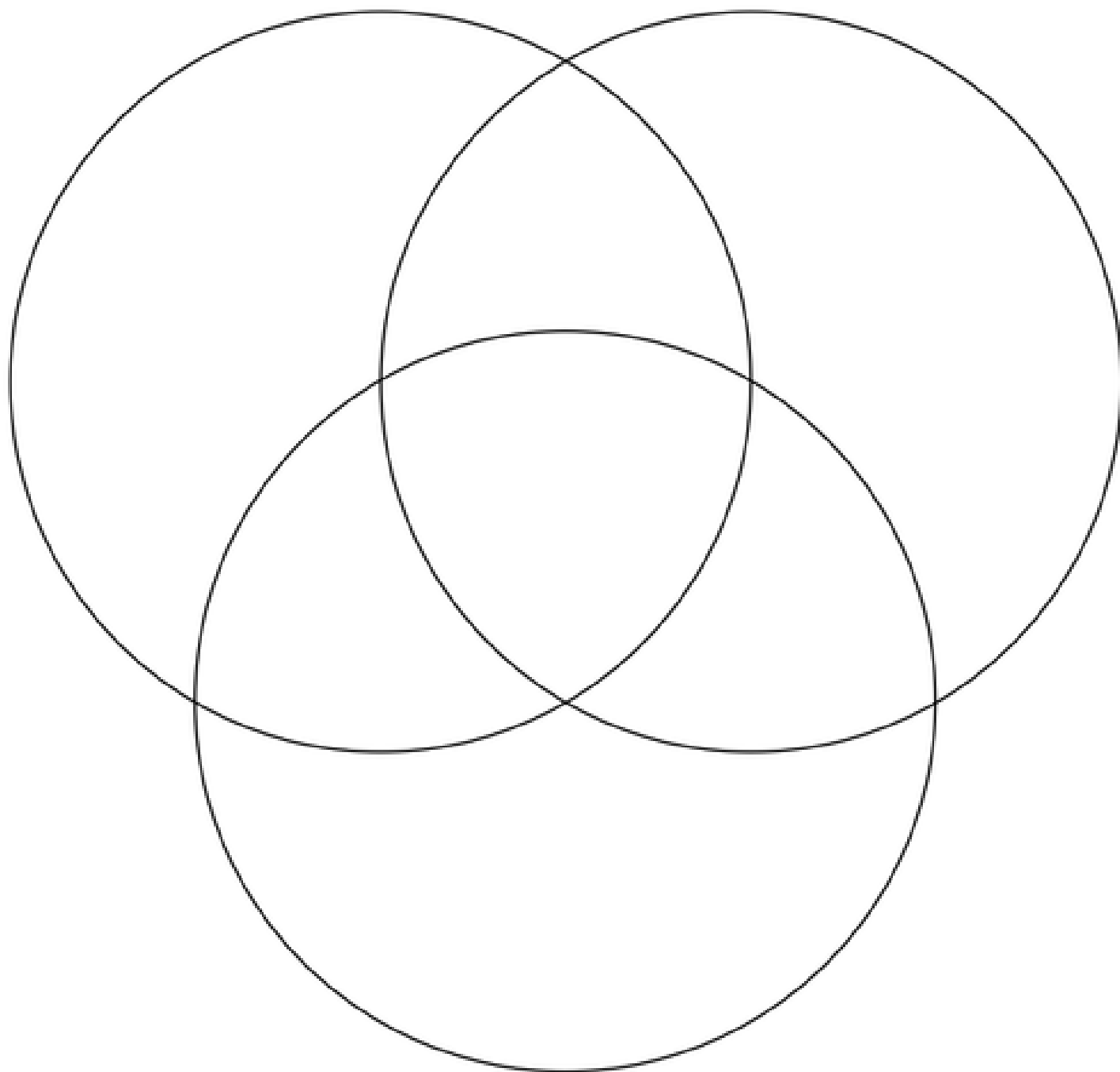
**Analysis:**

On graph paper, create a **bar graph** of number of cells and identity of cells.

**Conclusion:**

On back of graph paper write a conclusion summarizing the data that you collected in the lab and what you believe to be the state of immune response in the mouse's body. Remember to use your data to support your conclusion.

## Venn Diagram of Flow Cytometry, Fluorescence Microscopy & Two Photon Microscopy



## **Text: The Basic Immune System**

Basic Concepts of the Immune System - <http://www.medicine.mcgill.ca/physio/vlab/immun/backg.htm>