# The Effects of Salt Solutions on the Features of Neurofilament Networks

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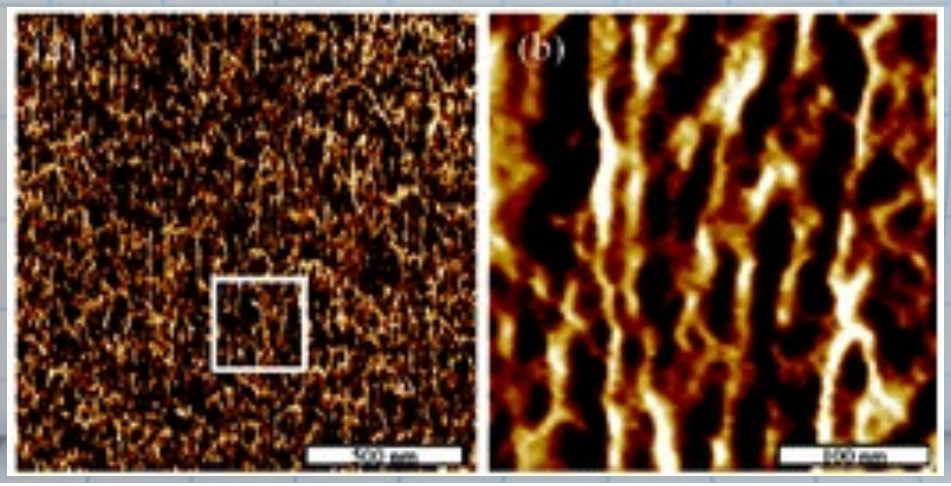
# What are Neurofilament Networks?

- These are a major part of the myelinated axons of spinal neurons.
- They are made up of Low, Medium and High molecular mass proteins.
- They appear as rods with sidearms that will connect to other Neurofilaments

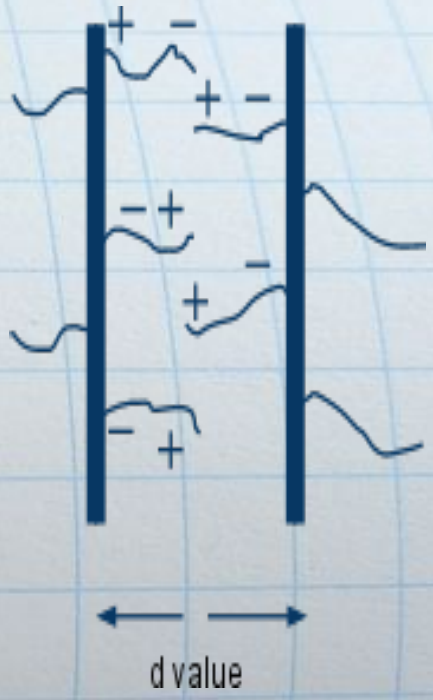


# Why Study Neurofilament Networks?

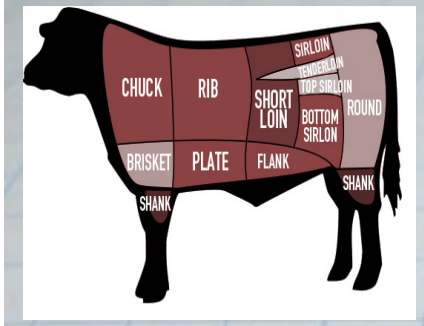
- Neurofilament networks are degraded by diseases such as ALS and Alzheimer's.
- This research hopes to understand how to reform and vary features of neurofilmament networks in a lab setting.
- We hope that in time we can learn to fine tune these networks features.

# Goals of My Research

- Purify samples of Neurofilaments from bovine spinal cords.
- Create Neurofilament sample that will readily form networks.
- Investigate how the presence of buffer (100 miliMolar NaCl) affects how tight the neurofilaments form networks (d value) at different concentrations.



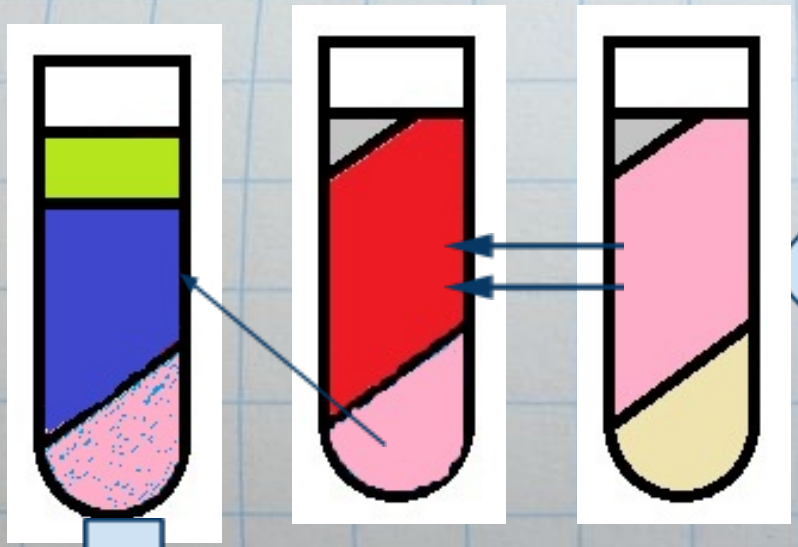
# Purification of Neurofilament proteins




1st Spin- Spinal cord and Buffer

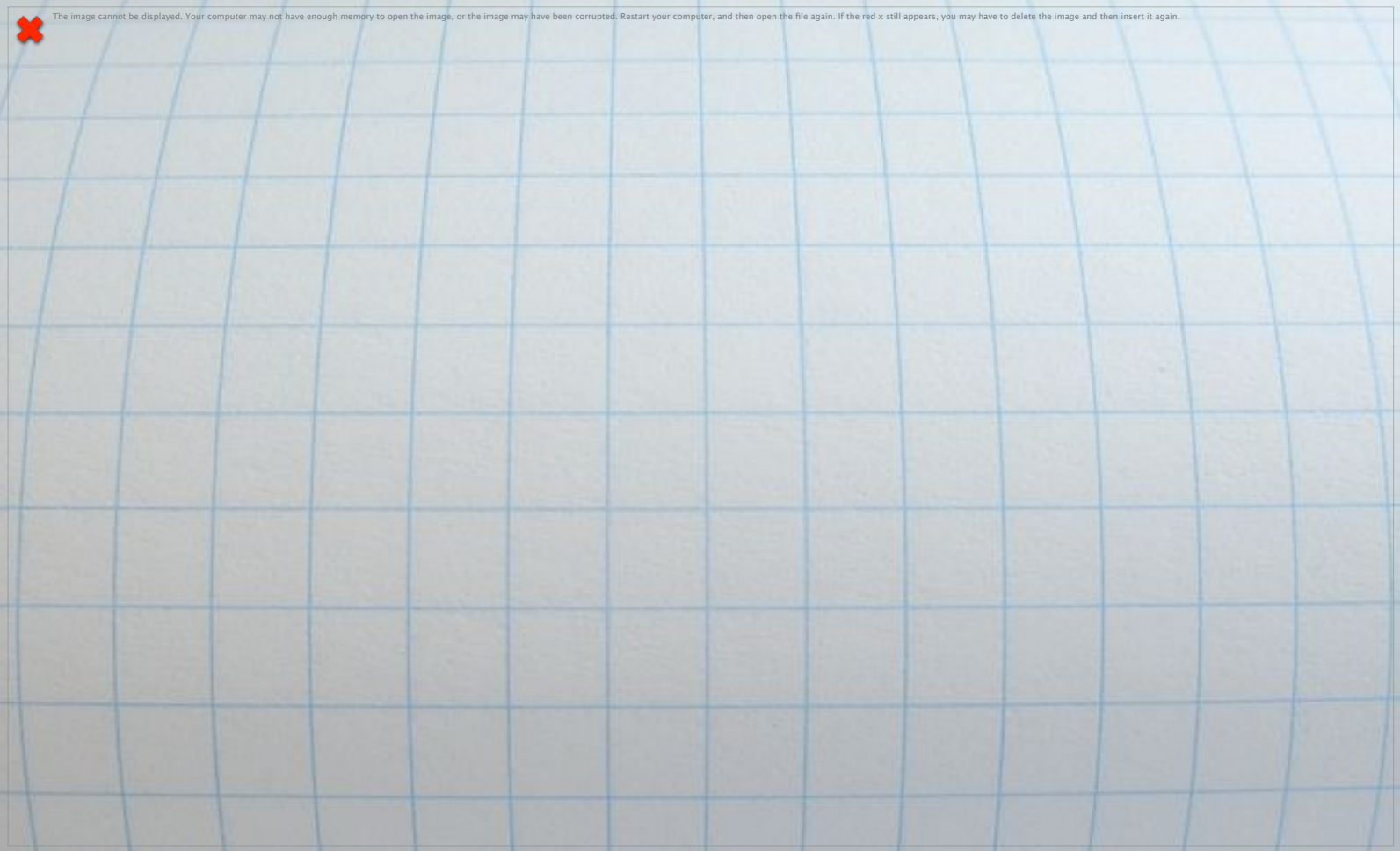
2nd Spin- Spinal Mixture and Glycerol Buffer

3rd Spin- Spinal Gel and Sucrose Gradient

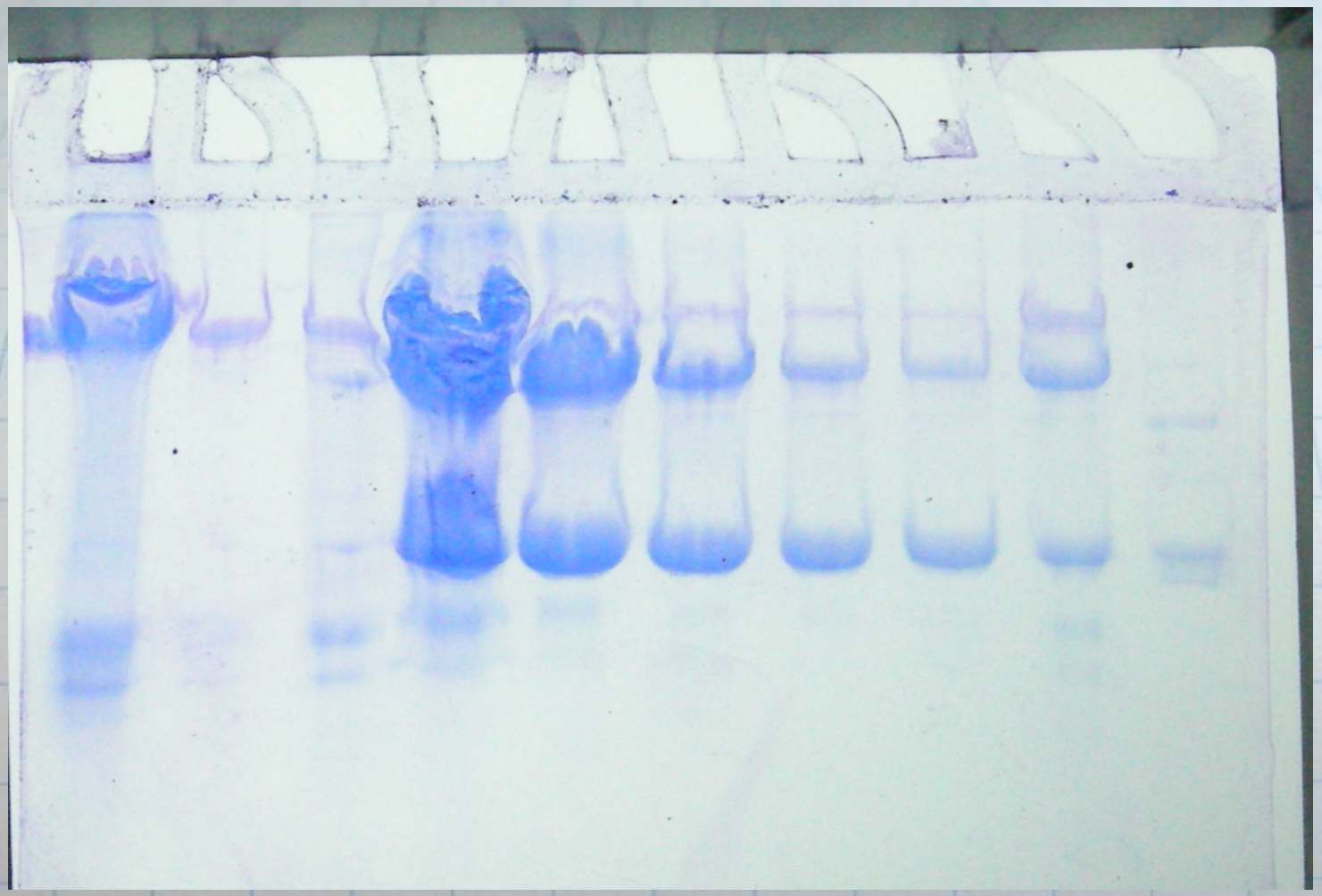


Retain bottom gel

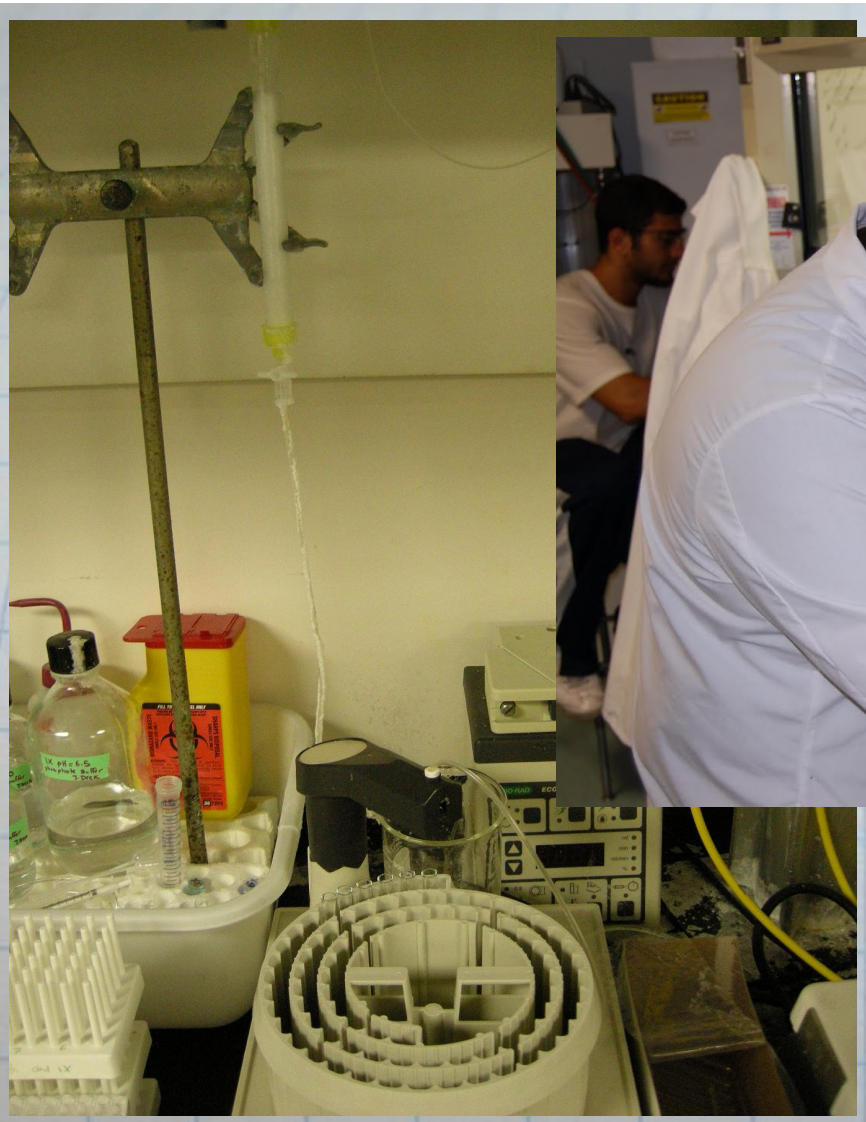
# Protein Column to Seperate Low, Medium and High Mass Proteins

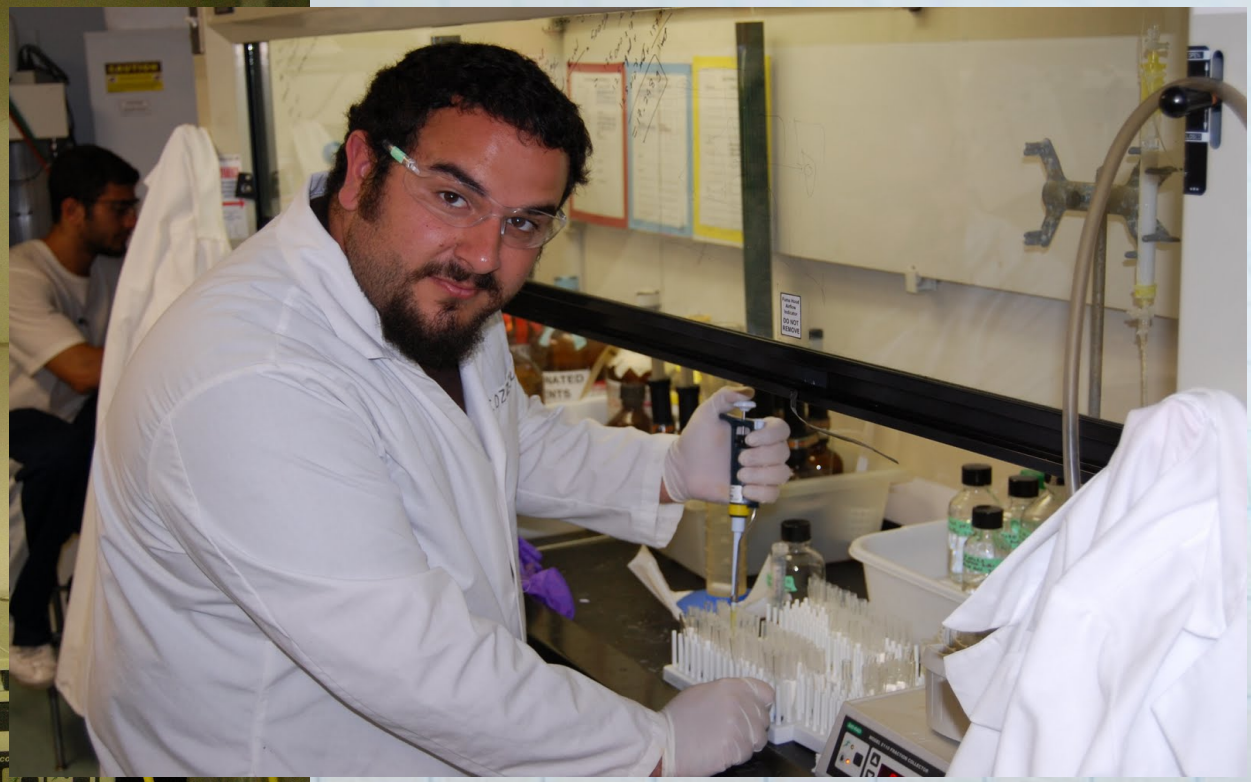



We ran two columns, and therefore the two gels. We want samples enriched in one type of subunit.

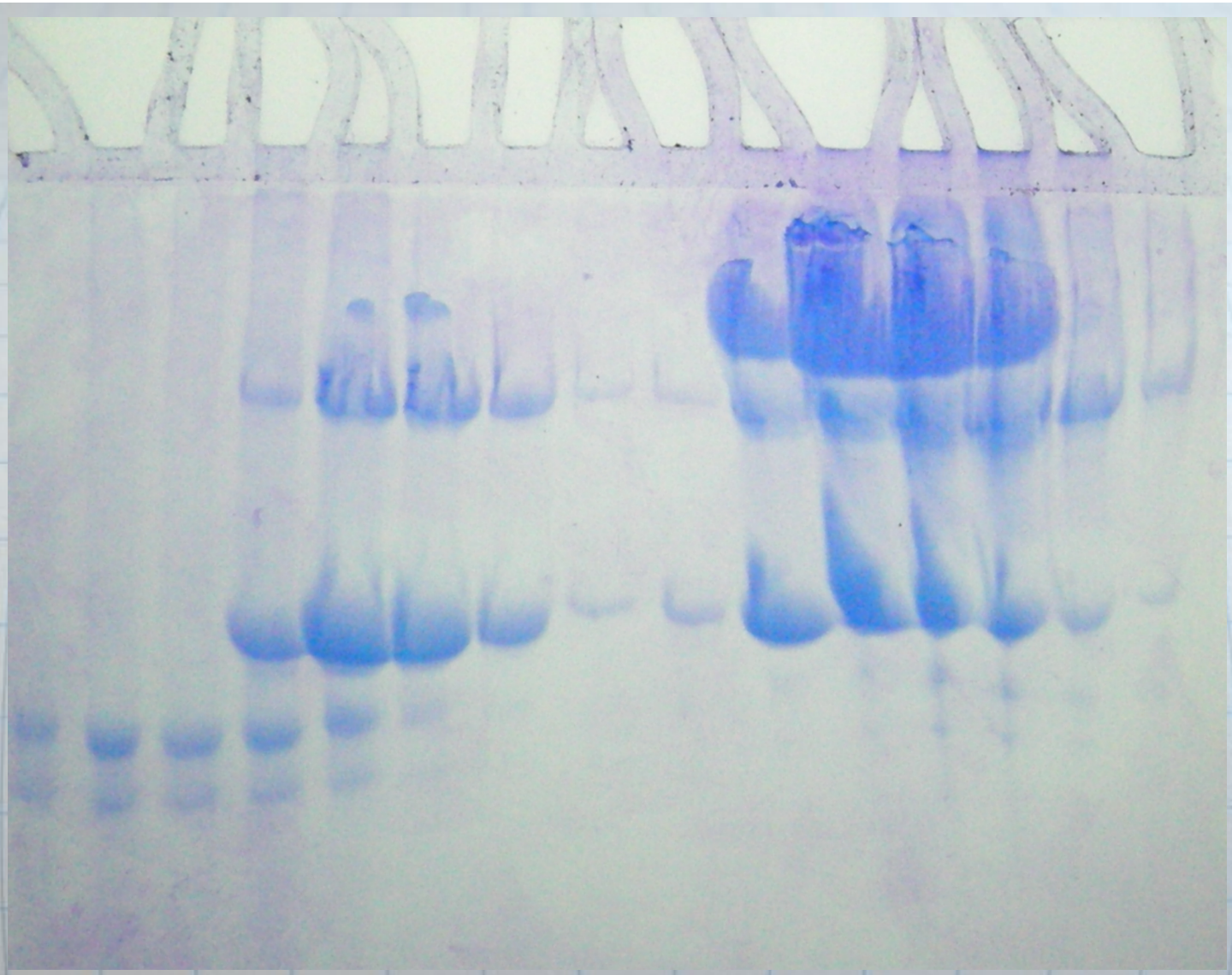


Gel showing separation of High and contaminants from Low and Medium. We kept samples represented by wells 4-9.



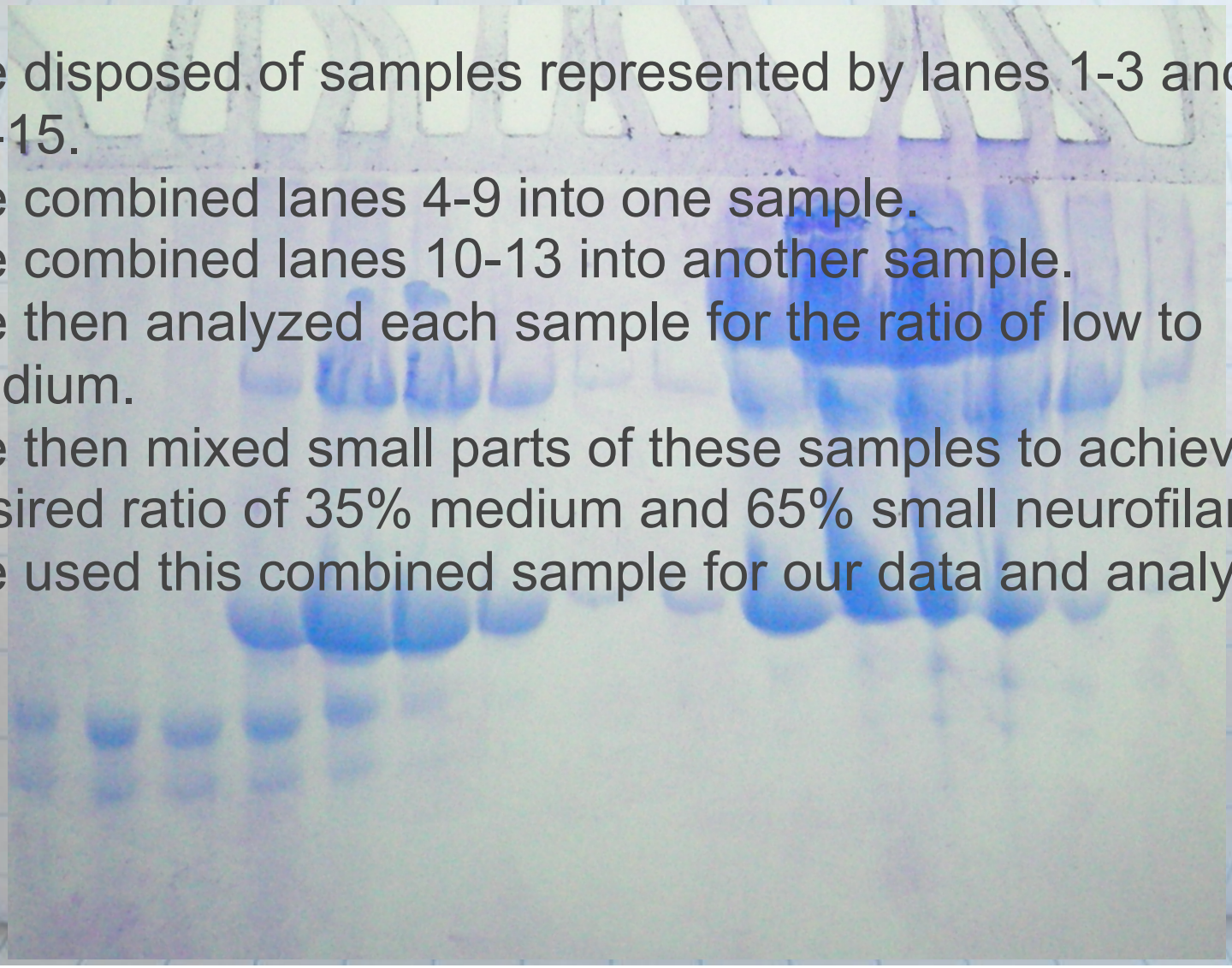


We ran another Column to further separate the Low and Medium proteins.We had to periodically check the fractions for proteins using Bradford dye.



Gel Shows fractions had large amounts of Low and Medium Mass Proteins

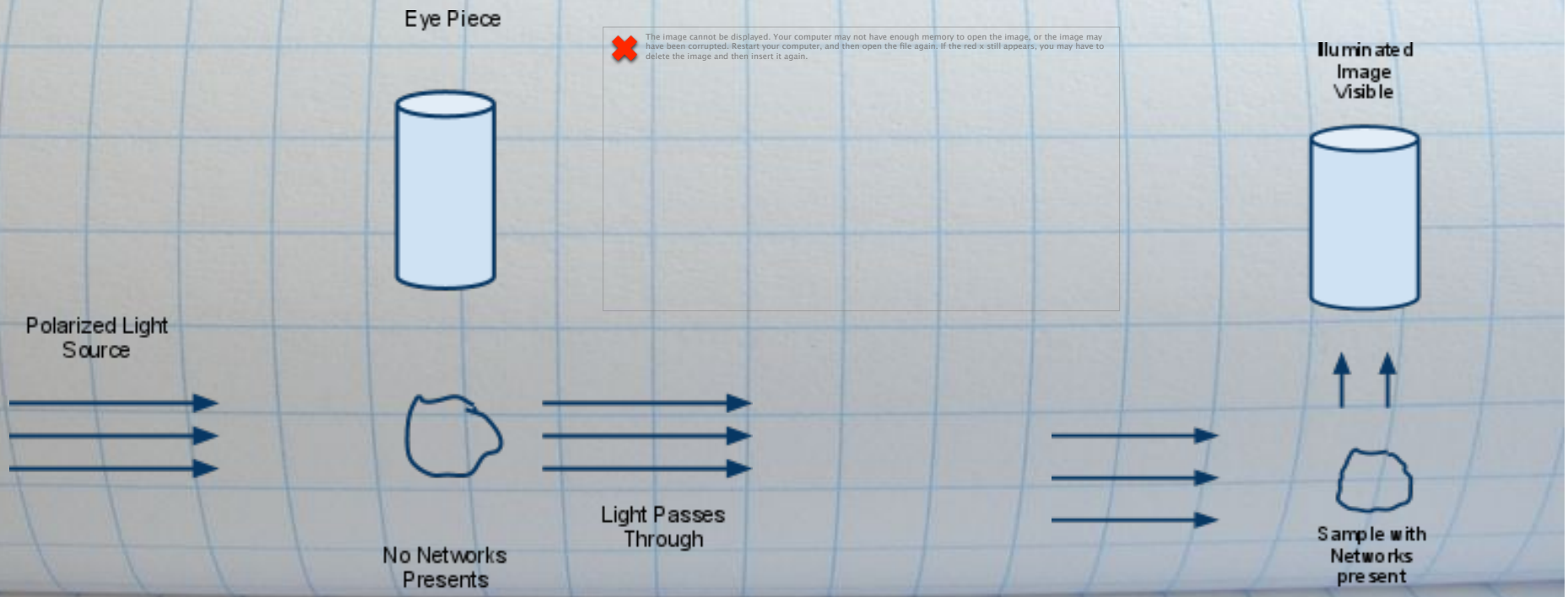
# Results from Second Column

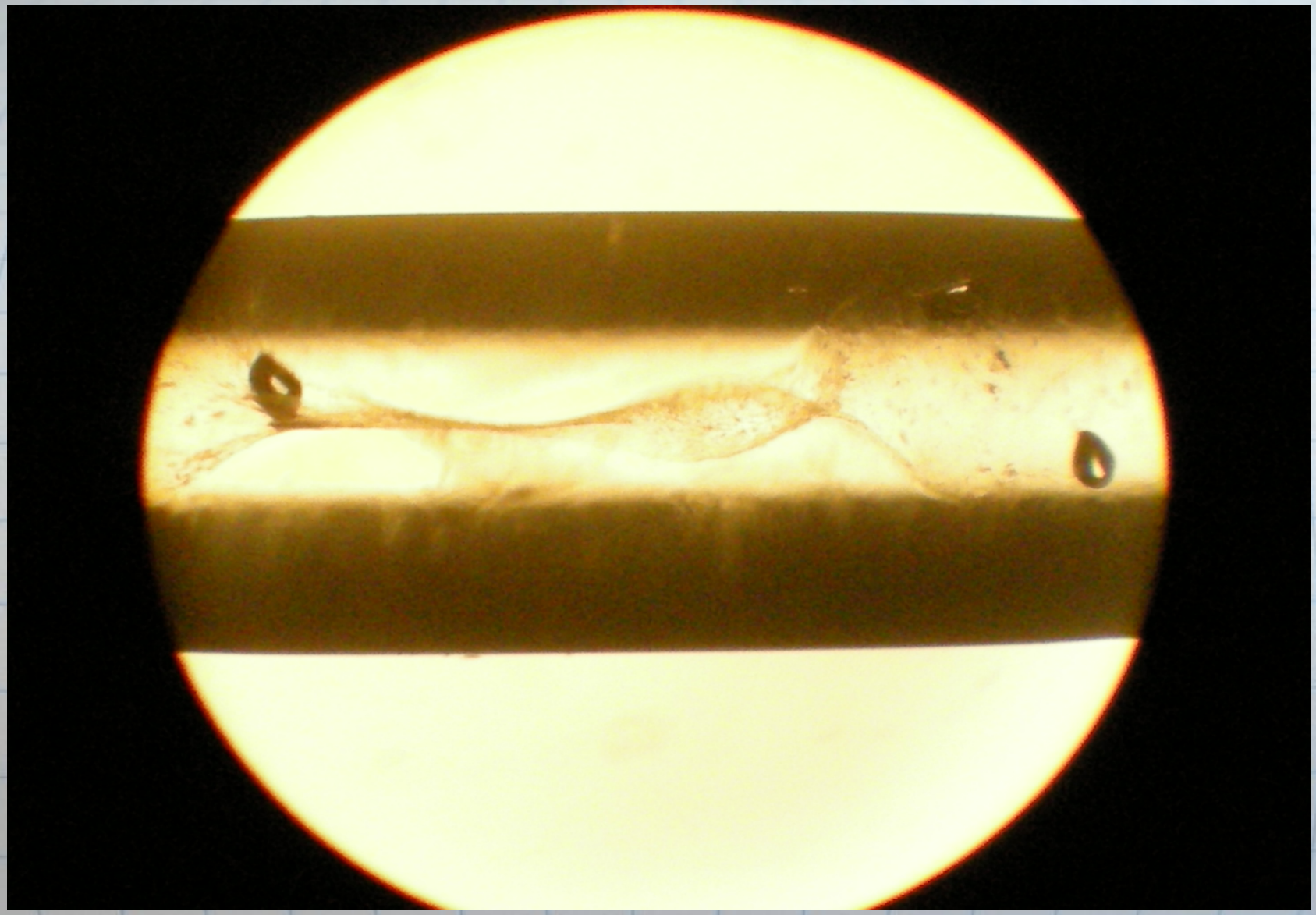


- We disposed of samples represented by lanes 1-3 and 14-15.
- We combined lanes 4-9 into one sample.
- We combined lanes 10-13 into another sample.
- We then analyzed each sample for the ratio of low to medium.
- We then mixed small parts of these samples to achieve a desired ratio of 35% medium and 65% small neurofilaments.
- We used this combined sample for our data and analysis.

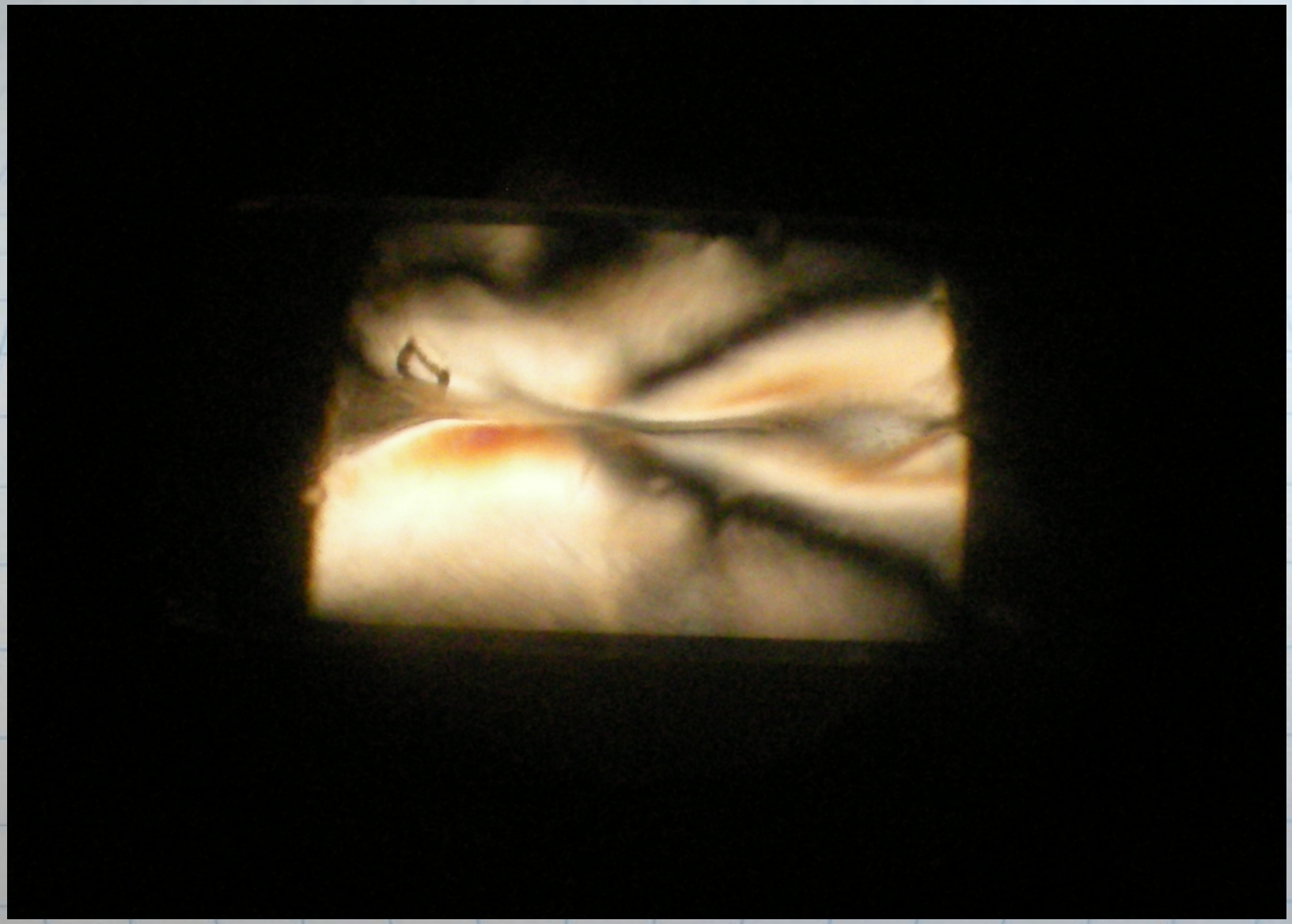
# Data- Polarized Microscopy (Qualitative)

- Use polarized light to identify if the proteins have settled into aligned networks.
- The polarized light will only illuminate the sample if there are aligned networks present to refract the light.

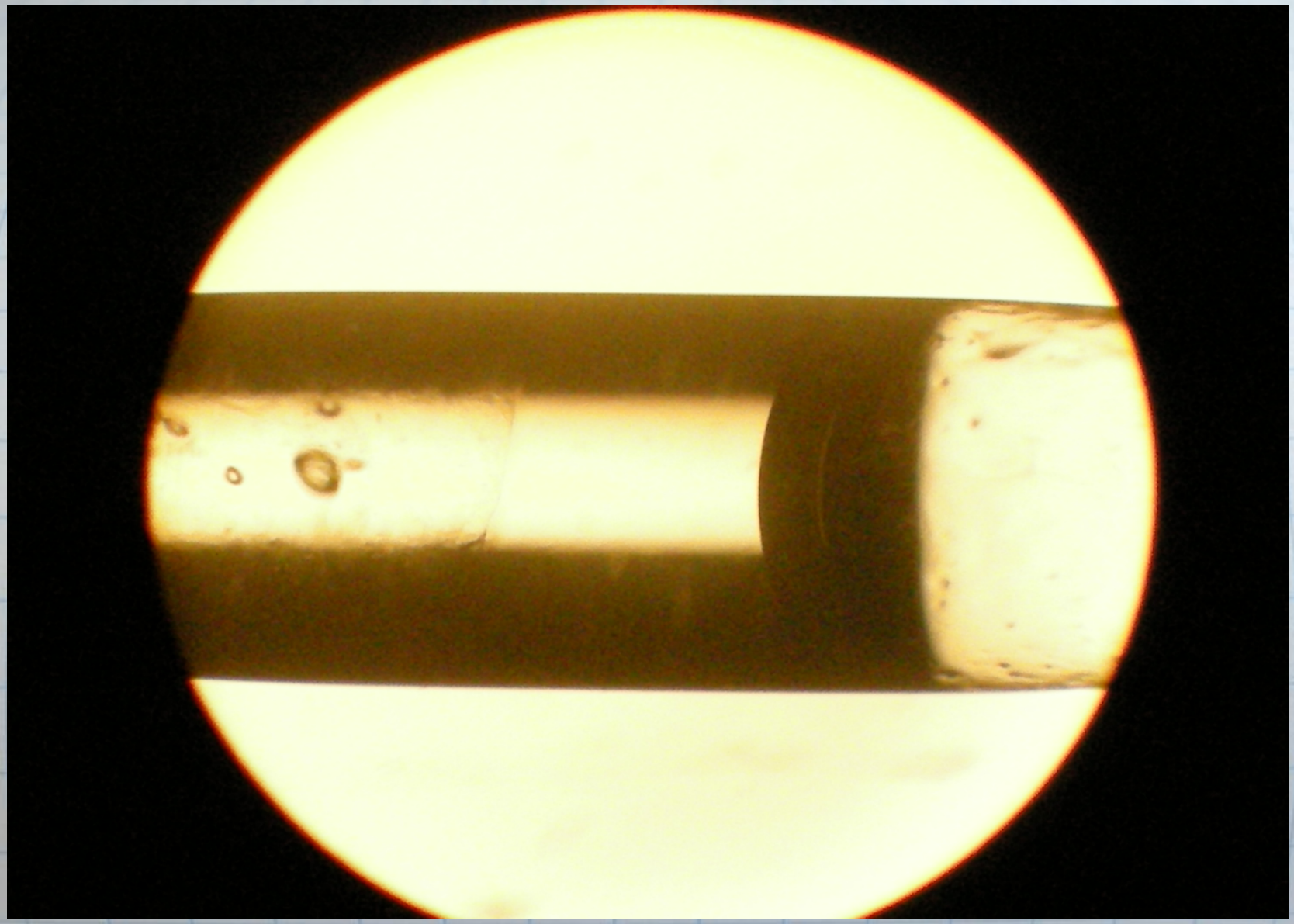




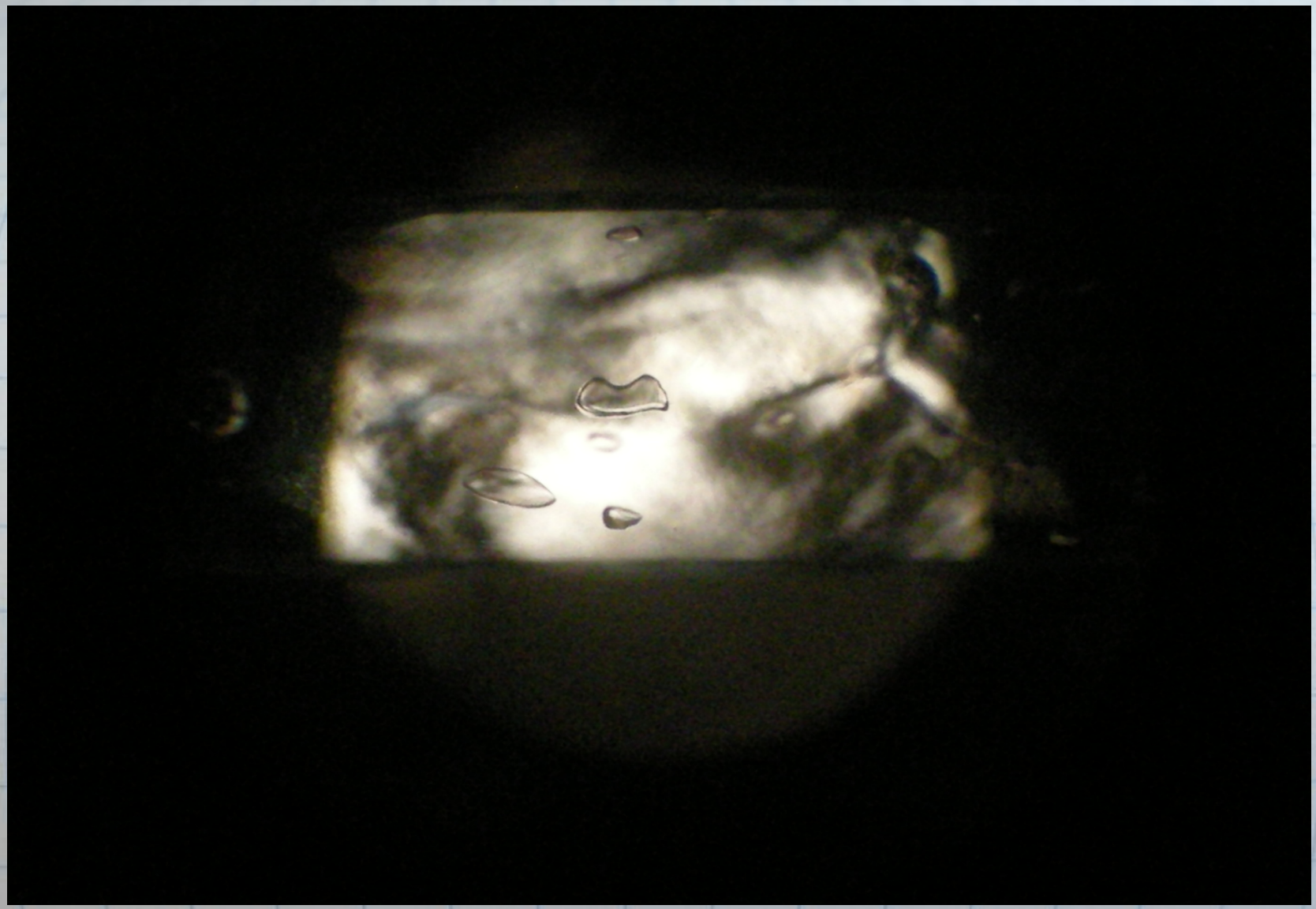
Sample 1- Protein Gel, no buffer, Regular Light



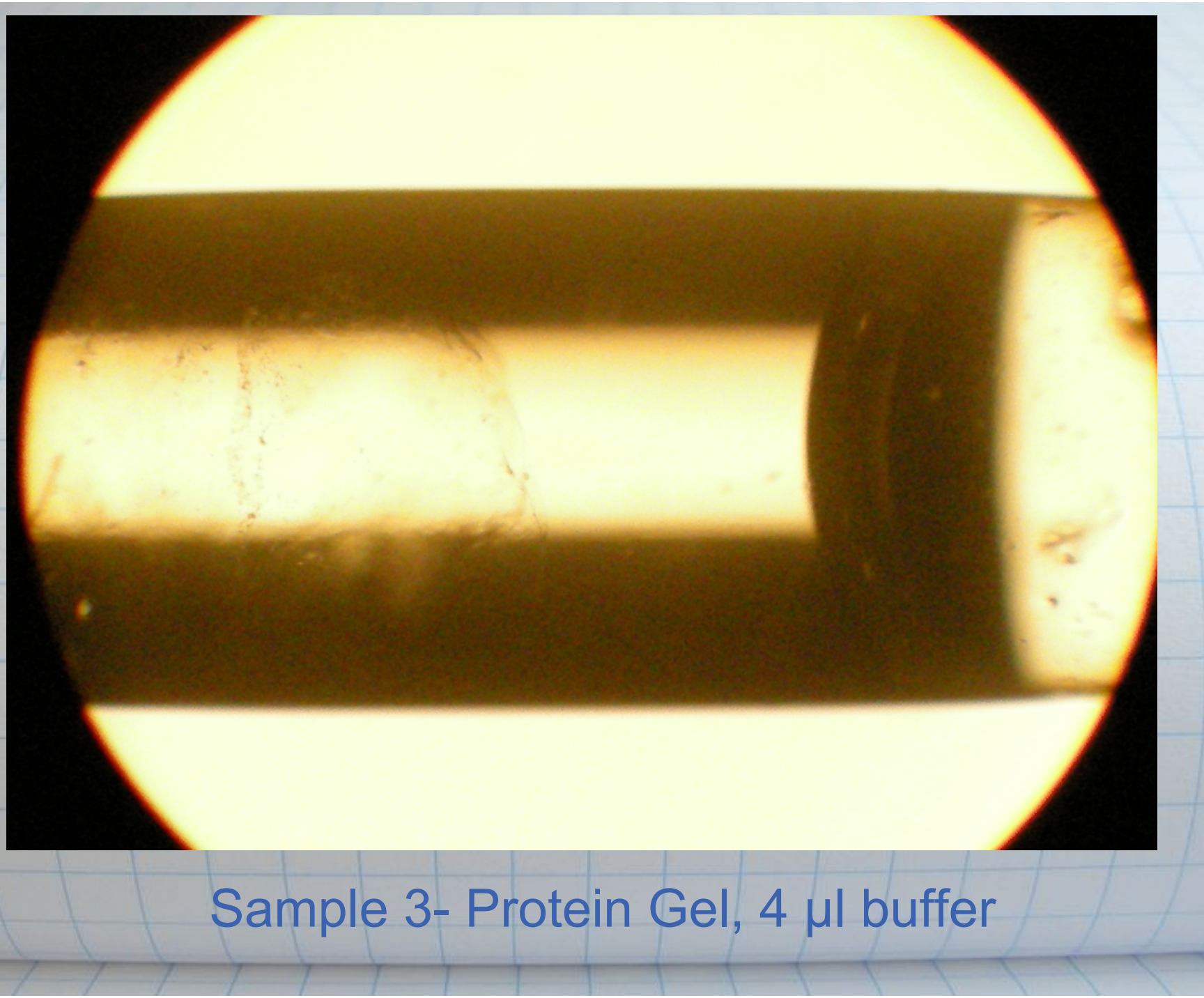
Sample 1: Sample 1- Protein Gel, no buffer, polarized light



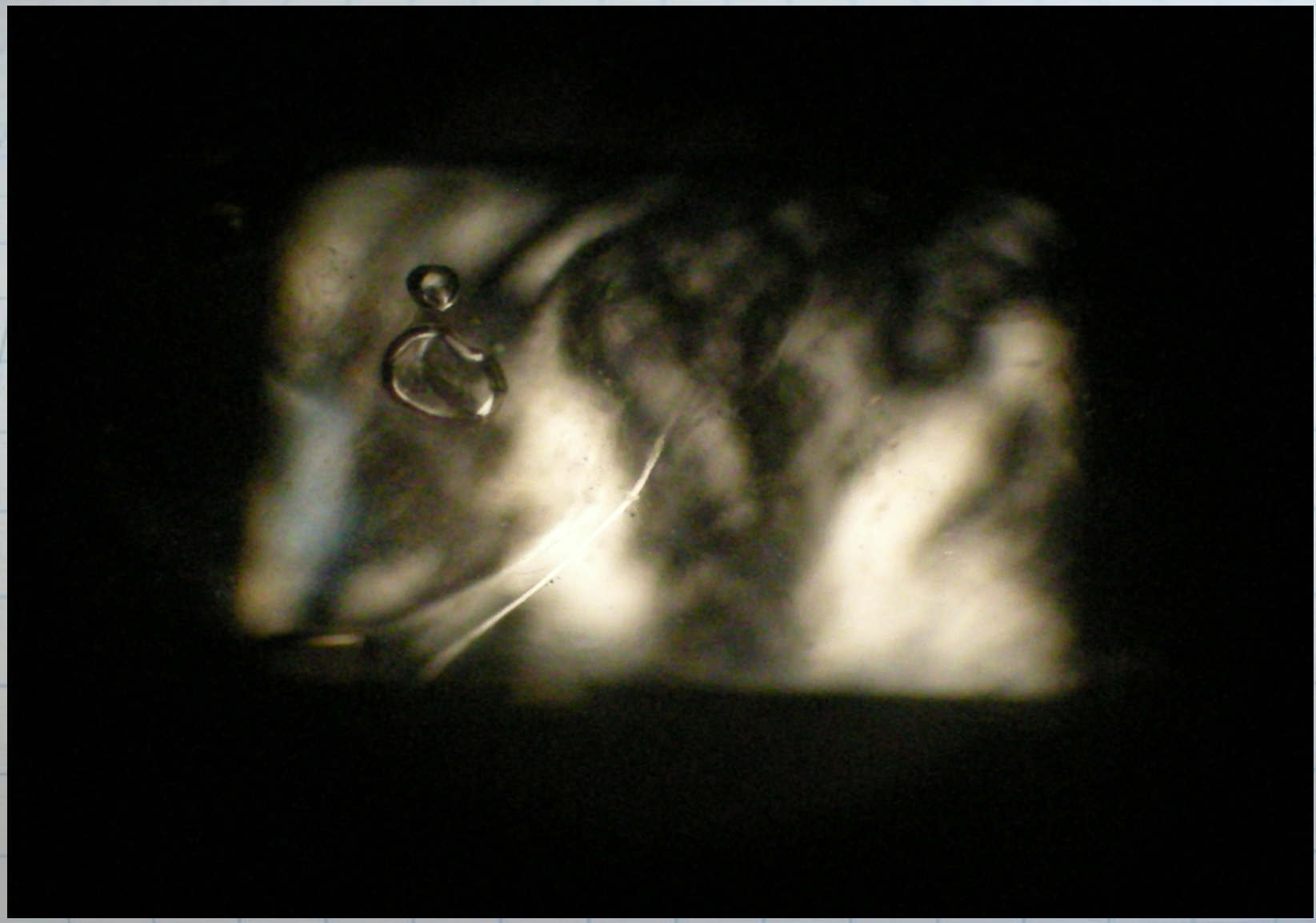
Sample 2- Protein Gel, 2 µl buffer



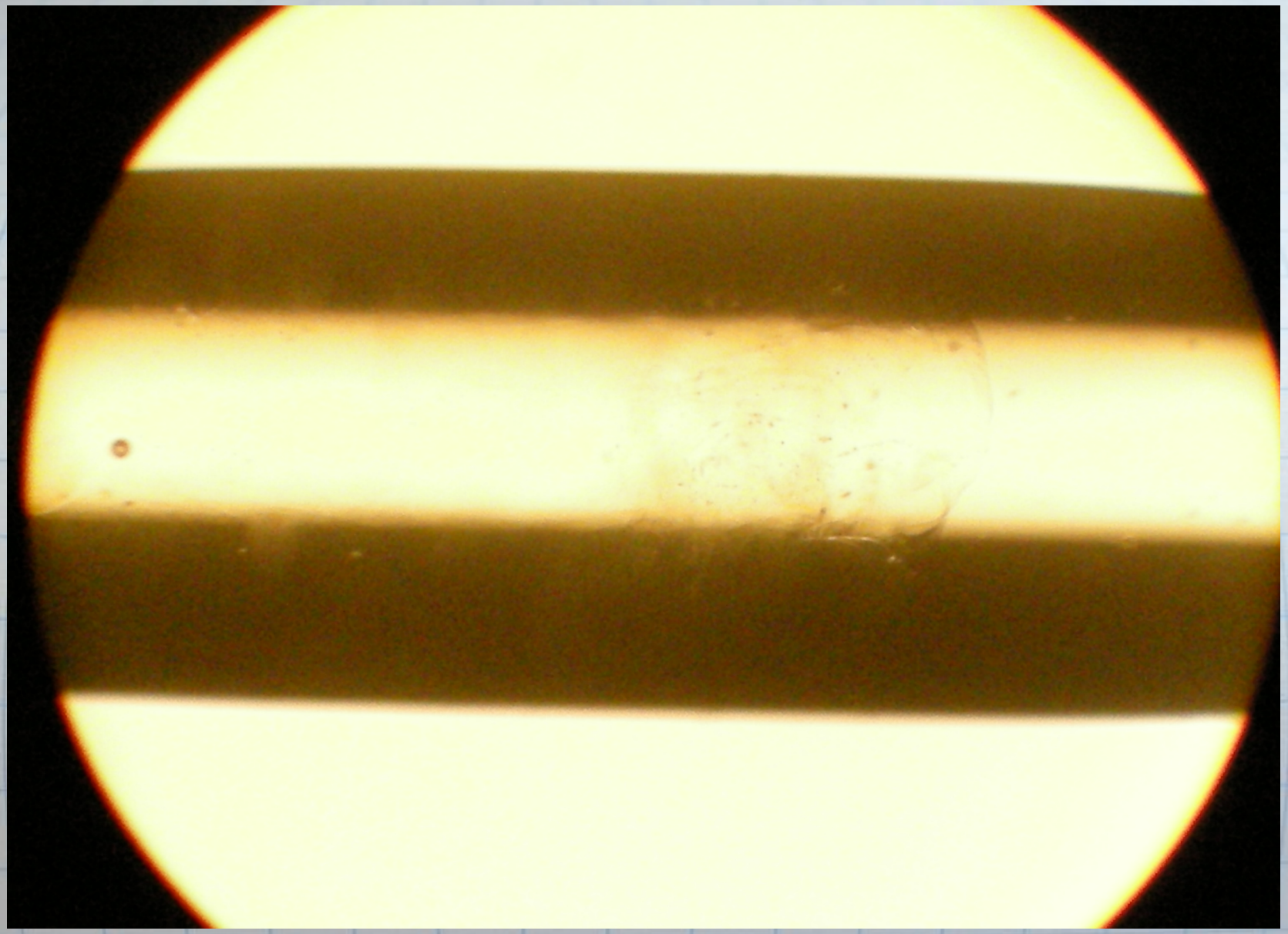
Sample 2- Protein Gel, 2 µl buffer



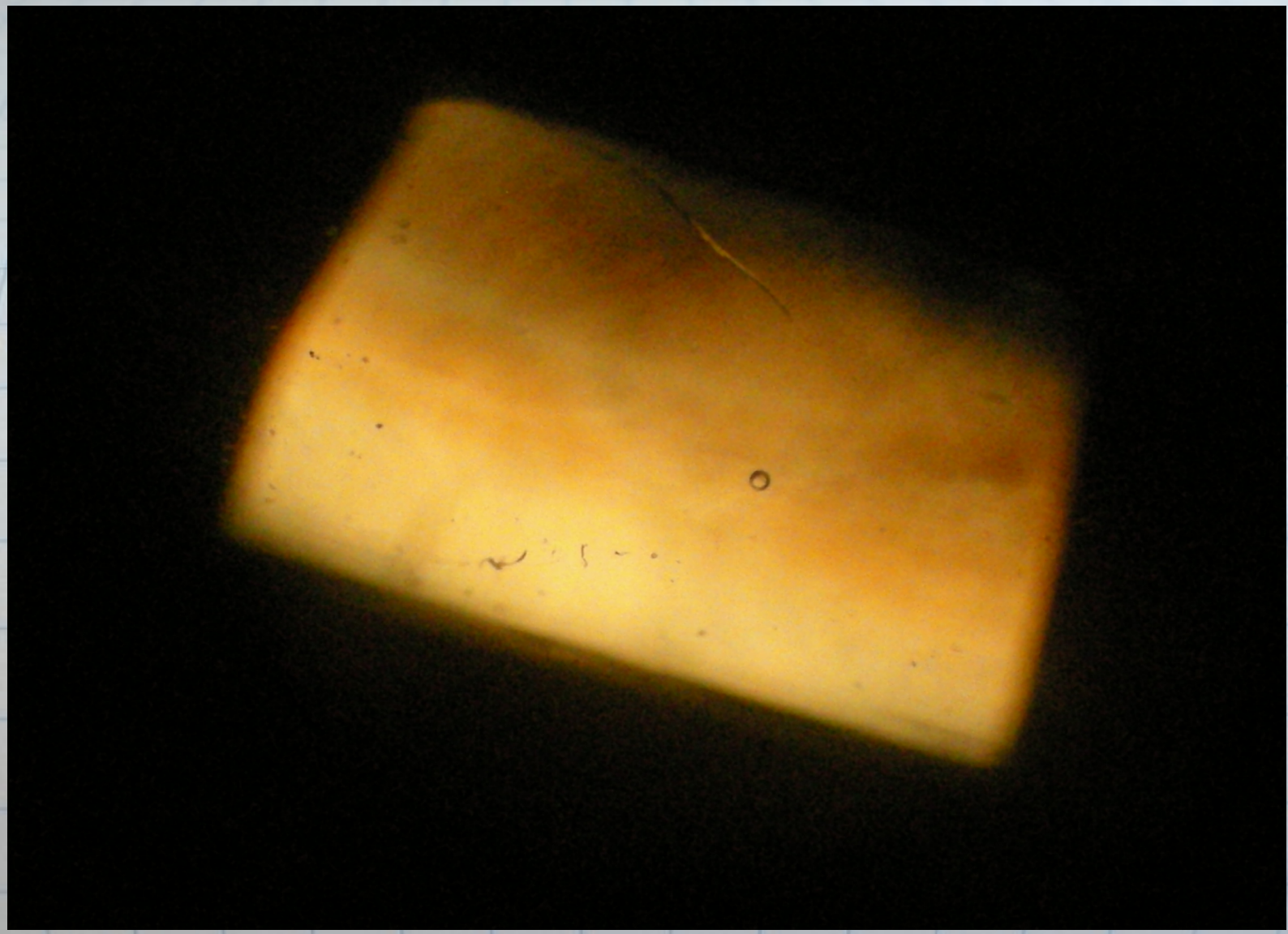
Sample 3- Protein Gel, 4 µl buffer



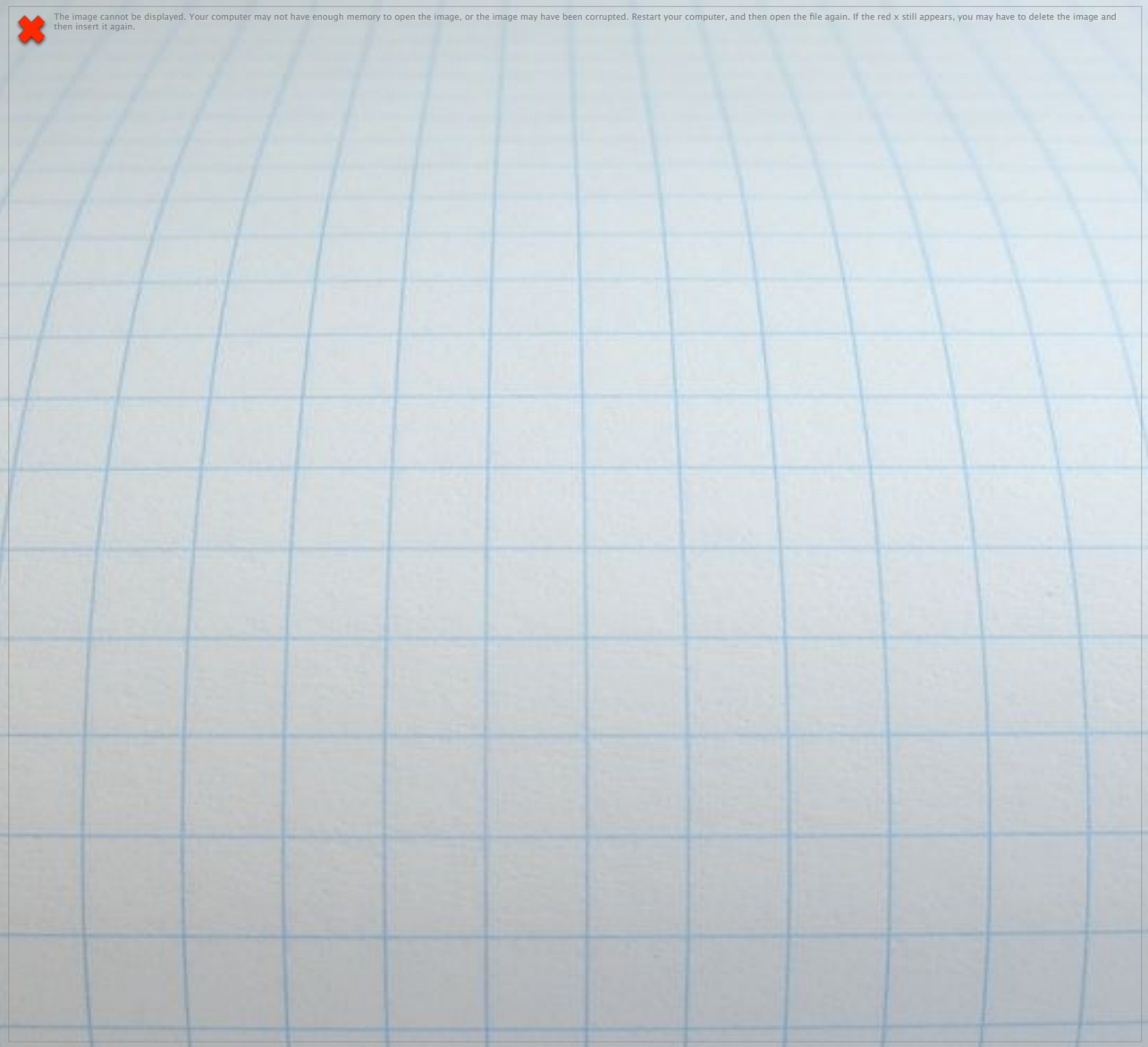
Sample 3- Protein Gel, 4 µl buffer



Sample 4- Protein Gel, 6 µl buffer

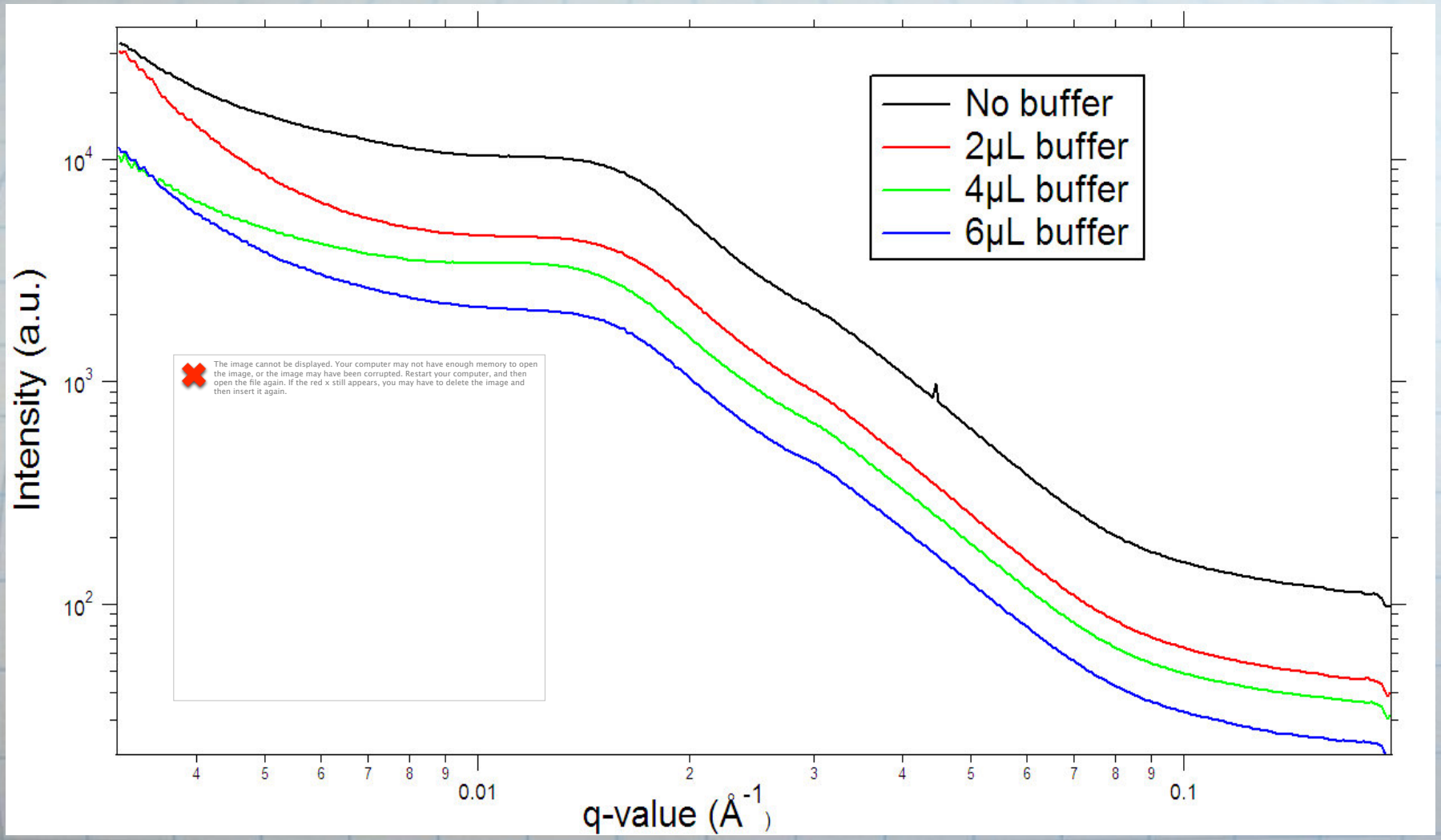


Sample 4- Protein Gel, 6 µl buffer




# X-ray Diffraction - Typical Image of Sample

# Raw Data from X-ray Diffraction




X-ray diffraction allows us to calculate the d value for our samples.

# X-Ray Diffraction Analysis

- Using the q value from the X-ray diffraction we found the d values.
- d= 2 π / q
- q is measure in Å -1
- d is measured in ångströms - Å (10 -10 m )
- Sample 1, no buffer: q = 0.01454 ; d = 432.131 Å
- Sample 2, 2 µl buffer: q = 0.0149 ; d = 421.6903 Å
- Sample 3, 4 µl buffer: q = 0.01385 ; d = 453.6596 Å
- Sample 4, 6 µl buffer: q = 0.01436 ; d = 453.6596 Å

# Experimental Conclusions

- The d values for the 4 samples were similar.
- This means that the neurofilaments formed relaxed networks whose d values were not affected by protein concentration of the sample.

# Bigger Picture

Joanna will continue researching the neurofilament networks by manipulating the types of salts, the concentration of salts, and different proteins to form neurofilament networks.

# Personal Outcomes

- Less intimidated by the "micro side" of Biology.
- "This isn't so bad the first time..." but the repetition for the different variables can be excruciatingly repetitive.
- Ideas for my classroom
  - Using Bradford and other dyes for a "molecules in food" lab.
  - Demonstrations in Solubility with different substances.

# Thanks to...

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