

# Lab 2 Session 1: Mobility and Mortality (Exploring Microscope and Imaging)

Date: 3rd Feb 2026

Lab Partner: Nathan Unhru

Recorder: Ahilan Kumaresan

## Table of Contents

1. Goals
2. Apparatus
3. Sketch
4. Variables
5. References
6. Procedure
7. Post lab reflections

## I. Goals

1. Learn operation of upright bright-field microscope and achieve proper Kohler illumination
2. Calibrate the 100x oil-immersion objective using stage micrometer ( $\pm 2\%$  uncertainty target)
3. Prepare diluted sample of 1  $\mu\text{m}$  polystyrene spheres with  $\sim 20$  beads in field of view
4. Capture 60-second video of Brownian motion at 30 fps (1800 frames minimum)
5. Extract particle trajectories and compute diffusion coefficient D
6. Compare measured D to Stokes-Einstein prediction within stated uncertainties

## II. Aparatus

Item	Description
Software	Vision Assistant, ImagesJ 1.54f
Immersion oil	$n = 1.518$
Stage micrometer	1 mm / 100 div (10 $\mu\text{m}/\text{div}$ )
Camera	FLIR BlackFly U3-13Y3M
Objective	10 $\times$ , 40 $\times$ & 100 $\times$ oil

### III. Variables

Variable Type	Variable	Description
Dependent	Time	Frame number $\times$ interval
Dependent	$x(t), y(t)$	Bead position
Control	$\Delta x, \Delta y$	Displacement Of Polystyrene
Control	Bead Size	Water
Control	Frame Rate	30 or 60 fps

### IV. References

#### Primary Lab Documents:

1. MM-LabScript-microscopy.pdf (PHYS 332 Microscopy and Cell Motility lab script)  
Variable Type  
Dependent
2. "Background: General Aspects of Cell Motion" document  
Depend
3. Protocol: Microscope Setup
4. Protocol: Acquiring Movies with Vision Assistant
5. Protocol: Tracking Particles (MTrack2)
6. Protocol: Making Sample Chambers.

#### Textbooks:

Hughes, I. and Hase, T. Measurements and their Uncertainties, Oxford (referenced for Ch. 2.9, 5—8)

#### Online Resources:

1. Nikon Microscopy U ([microscopyu.com](http://microscopyu.com)) — Kohler illumination tutorial
2. ImageJ documentation (for MTrack2 plugin)

3. Added a new folder on GitHub:

Home Folder: Lab2-Mobility-and-Motility

<https://github.com/Ahilan-Bucket/phys332W-sfu/tree/main/Lab2-Microscopy-and-Motility>

## V. Setup

Image 1: Important elements in this lab



Calibration slide

Image 2: Close up of Translational Stage

## VI. Detailed Procedure

Time:

Quick check

Check:

Focus

a) Kohler Illumination Setup Procedure:

(Following Protocol: Microscope Setup)

1. Turned on illumination lamp and allowed 5 min warm-up
2. Placed blank slide on stage, focused with 10 × objective
3. Closed field diaphragm until edges visible in FOV
4. Adjusted condenser height until field diaphragm edges sharp
5. Centered condenser using centering screws
6. Opened field diaphragm to just outside FOV
7. Adjusted aperture diaphragm to 70% of objective NA for optimal contrast

Note: Field diaphragm controls illumination area; aperture diaphragm controls NA (and thus resolution/contrast tradeoff).

## B. Camera Calibration with Stage Micrometer

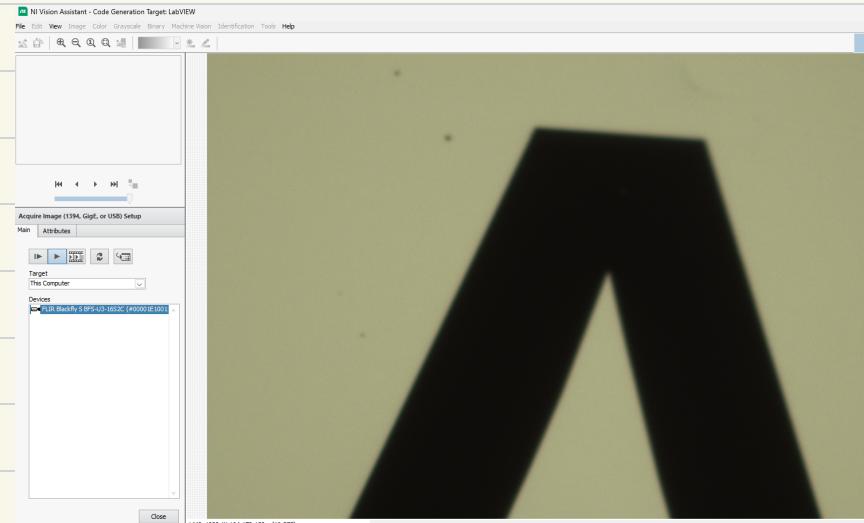
### Procedure:

- 2:30 PM
1. Placed stage micrometer into the sample holder, translated a bit with xy knob
  2. Focused and adjusted illumination lamp
  3. Applied the given oil on the stage
  4. Rotated 100x oil objective into position (parfocal - minimal refocus needed)
  5. Launched Vision Assistant, LabVision > Image Acquire > Second Acquire.

Error! Our Lab Accusion is not detecting the Microscope, the TA mentions this is a recurring issue for this station. So we will document this troubleshooting.

Solution: Just restart and now it works. That was underwhelming, we were not able to understand why the error occurred.

Image3:  
Screenshot of  
our Working  
Window



Because it was hard to find the grating, we first focused on the letter refer image 3! And then were able to find the ruling

6. Captured image of micrometer scale bars

Calibration Data:

File: Home/Data/2026-02-03/imagecalibration.tif

7. Opened image in ImageJ for calibration measurement.

We selected 8 lines in 100x objective. Edge to Edge. This was the widest possible to reduce the uncertainty.

Physical image length per pixel calibration:  
8 lines has 1168.706 pixels

That is, 73.044 pixels per line

From calibration slide: 0.01mm per line

$$\text{Mm/px} = 6.845177 \times 10^5 \text{ mm} \times 10^3$$

$$= 0.06845177 \mu\text{m / px}$$

$$= 68.45177 \text{ nm/px}$$

Camera model BFS-U3-16S2C-CS

Calculated dilution factor (using prelab method)

20 beads per  $1440 \times 1080$  pixels  $\times 0.0685 \mu\text{m}$  per pixel

= 20 beads per  $98.6 \times 73.98 \mu\text{m} \times 5 \mu\text{m}$

Volume of image:  $36,482 \mu\text{m}^3$  vs  $77,760 \mu\text{m}^3 = 0.46916x$

(compared to prelab)

Density Prelab:  $2.572 \times 10^8 \text{ beads/ml}$

$$\text{No. of Beads} = \text{Vol} \times \text{Conc.}$$

$$20 \text{ beads in } V = 36,482 \mu\text{m}^3 = 3.65 \times 10^{-8} \text{ ml}$$

$$n = \frac{N}{V} = \frac{20}{3.65 \times 10^{-8} \text{ mL}} = 5.48 \times 10^8 \text{ beads/ml}$$

→ Required:  $5.48 \times 10^8 \text{ beads/ml}$

$$\text{Mass conc: } 5.50 \times 10^{13} \text{ g/bead} \times 5.48 \times 10^8 \text{ beads/} \\ \text{mL} = 3.01 \times 10^{-4} \text{ g/mL}$$

Stock: 0.005 g/mL

Initial: 0.005 g/mL

$$\text{Dilution} = \frac{\text{initial}}{\text{final}} = \frac{0.005}{3.01 \times 10^{-4}} = 16.6x \approx 1:17$$

## C. Sample Preparation

2:44 PM  
3.12 PM

### Materials:

Stock concentration: 0.5 wt% (5 mg/mL)

Water

Glass microscope slides (Fisher Scientific)

1 coverslips (22x22 mm, 0.13-0.17 mm thick)

Parafilm spacers (100 µm thick)

### Dilution procedure:

Based on pre-lab calculation, target dilution ~1:30

1. Vortexed stock solution for 30 seconds to disperse aggregates

2. Pipetted 1 µL of stock into microcentrifuge tube

3. Added 30 µL of water (1:30 dilution)

4. Gently mixed by pipetting up and down 10 times, just to get things more uniform

Make sure you wear gloves and not touch the outer side.

## D. Chamber preparation:

(Following Protocol: Making Sample Chambers - thick chamber method)

1. Cut two strips of Parafilm (~3 mm wide, ~20 mm long)
2. Placed strips parallel on glass slide, ~15 mm apart
4. Pipetted 10  $\mu\text{L}$  diluted bead solution between Parafilm strips
5. Gently lowered coverslip onto Parafilm strips
6. Sealed edges with nail polish (quick-dry, 2 min)

### Quick check:

Initial observation with 100x objective showed good bead distribution

Estimated 25-30 beads in FOV - slightly more than target but acceptable

No obvious aggregates or debris visible

Chamber depth: 100  $\mu\text{m}$  (Parafilm thickness)

CONCLUSION: Sample prepared with 1:30 dilution.

Observed 25-30 beads in FOV at 100x. No aggregation visible.

## E. Data Collection - Brownian Motion Video

Environmental conditions:

Room temperature:  $22.3 \pm 0.5^\circ\text{C}$

Acquisition procedure:

1. Switched to 100x oil objective, applied immersion oil
2. Focused on mid-plane of chamber
3. Adjusted illumination for good contrast (beads appear as dark circles)
5. Started Vision Assistant recording
6. Recorded for 120 frames

I'm not too  
sure...

Saving two versions of the files to explore how frame rate affects us ( 5 frame and 1 frame Rate )

Save: home/Data/2026-02-03/1um-1frame.avi  
home/Data/2026-02-03/1um-5frame.avi

Errors notes:

Issue 1: First recording showed drift (all beads moving in same direction)

Cause: Coverslip not fully sealed, causing flow

Solution: Applied more nail polish to edges, waited 5 min

Issue 2: Some beads going in/out of focus

This is expected - chamber is much deeper, even labscript tells us this

Solution: Track only beads that stay in focus throughout recording

CONCLUSION: Successfully recorded 60 s video at 1 fps (60 frames).

## F. Particle Tracking and Trajectory Extraction

Method: ImageJ MTrack2 Plugin  
(Following Protocol: Tracking Particles)

Procedure:

1. Opened AVI file in ImageJ (File > Import > AVI)
3. Applied background subtraction (rolling ball radius = 50 pixels)
4. Ran Plugins > MTrack2
5. Exported trajectories as CSV

Save: home/Data/2026-02-03/1um-1frame.avi  
home/Data/2026-02-03/1um-5frame.avi

Results:

Successfully tracked beads for 120 frames

G. Analysis

## H. Post lab Analysis

Firstly, the Data seems fishy -

I want to recheck the Distillation. Maybe there is an error

We need to do the Calibration error, for that, we must take multiple Data sets of the edge to edge pixel values