Lab Script

Pre labs

Q6) Biological cell has internal compartments. radii 0.3-0.5μm
Estimate diffusion constant according to

$\eta = 2 \times 10^{3} kg / ms$

motion not hindered by other components.

$\Rightarrow D = \frac{K_B T}{6\pi \eta R}$, Let $T = 300K$

$K_B = 1.38 \times 10^{-23}$

for $R = 0.3\mu m = 0.3 \times 10^{-6} m$

$\hookrightarrow D = \frac{1.38 \times 10^{-23} \times 300}{6\pi \times 2 \times 10^{-3} \times 0.3 \times 10^{-6} m} = \frac{4.14 \times 10^{-21}}{1.13 \times 10^{-8}} = 3.66 \times 10^{-13} m^2/s = 0.366 \mu m^2/s$

for $R = 0.5\mu m = 0.5 \times 10^{-6} m$

$\hookrightarrow D = \frac{4.14 \times 10^{-21}}{6\pi \times 2 \times 10^{-3} \times 0.5 \times 10^{-6} m} = 2.20 \times 10^{-13} m^2/s = 0.220 \mu m^2/s$

$\therefore D \approx 0.22$ to $0.37 \mu m^2/s$

Q7) Reynolds Number Re for a cell with $L = 1\mu m$ swimming $20\mu m/s$ in a fluid with viscosity 10^{-3} Kg/ms & density 10^3 Kg/m³.
Cell is Turbulat or Laminar?

$\Rightarrow Re = \frac{\rho L v}{\eta}$ (to find Turbulant or Laminar)

Given $L = 1\mu m = 10^{-6} m$, $v = 20 \mu m/s = 2 \times 10^{-5} m/s$, $\eta = 10^{-3} Kg/ms$

$\rho = 10^3 Kg/m^3$

$\rightarrow Re = \frac{10^3 \times 10^{-6} \times 2 \times 10^{-5}}{10^{-3}} = \frac{2 \times 10^{-8}}{10^{-3}} = 2 \times 10^{-5} < 10^{-3}$

(Low $\Rightarrow$ Laminar)

Q8) E coli sol. of optical density at 600nm of $OD_{600} = 1.0$
This corresponds to 2×10^8 cells/mL?

Want ~10 cells in FOV, dilute? By what?

⇒ Expected number is $N = VC$

$V_{view} = A_{FOV} \times h$

$= (N_x \times N_y) \times S \times h \rightarrow$ Thickness

↑ pixels ↗ ↑ Calibration (μm/px)

$= (1288 \times 964) \times 0.1\mu m/px \times 5\mu m$

Field Size = $N_x S \times N_y S = 128.8\mu m \times 96.4\mu m$

Area = $1.29 \times 10^4\ \mu m^2 \times h$

Vol = $6.21 \times 10^4\ \mu m^3 = 6.21 \times 10^{-8}\ mL$

∴ $N = (2 \times 10^8)(6.21 \times 10^{-8}) \approx \underline{12.4}$

∴ Yes dilute as now we see 12 cells

Dilution factor = $\frac{12.4}{10} = \underline{1.24}$

∴ $\underline{1.2\times}$ (1 part culture & 0.2 part medium)

$\underline{1:2}$

Lab 2 Session 3: Microscopy and Cell Motility (Live Cells & Bacterial Motion)

Date: _____ Feb 2026 Lab Partner: Nathan Unhorn
Recorder: Ahilan Kumaresan

II. APPARATUS

Refer to Session 1, Section II (pg. 2)

Additional items for Session 3:

Item	Description
E. coli HCB1274	Tumble-disabled mutant strain
E. coli DH5α (optional)	Wild-type strain
S. cerevisiae	Baker's yeast
HT1080 prepared slides	Fixed human cells (stained/unstained)
Sugar, warm water	For yeast activation

III. VARIABLES

Type	Variable	Description
Independent	Cell type	E. coli, Yeast, HT1080
Dependent	x(t), y(t)	Cell position over time
Dependent	v	Swimming velocity
Dependent	D_eff	Effective diffusion coeff
Control	Temperature	Room temp (~21°C)
Control	Frame rate	Match to cell speed
Control	Objective	100x oil immersion

LAB 1 Session 3

I. GOALS

1. Observe and measure size of eukaryotic cells:
   - Yeast (S. cerevisiae): expect ~5-10 μm diameter
   - HT1080 human cells: expect ~20-50 μm diameter

2. Prepare E. coli (HCB1274α mutant) sample with ~10 cells in FOV

3. Capture 60+ second video of bacterial motion at appropriate fps

4. Track bacterial trajectories using MTrack2

5. Compare bacterial motion to bead Brownian motion:
   - Is motion diffusive or ballistic?
   - Calculate effective diffusion coefficient
   - Compare MSD slope to beads

6. Determine if bacteria show directional (swimming) motion vs
   random Brownian motion

5. CELL SIZE - PROCEDURES and NOTES (Sec. 3.3, p. 9)

5.1 Yeast growth and wet-mount preparation

1) Prepared yeast culture in Falcon tube (refer to Sec. 3.3, p. 9):

Take clock and thermometer for room.

Tube volume: 15 mL Falcon
V_water (warm, body temperature) = 10 mL
m_sugar added = 2 g
m_yeast added = 2 g
Stirred gently for _____ s and left to sit for _____ min
Time when bubbling started _____

(Write them like you did for Brownian motion; this is

Note: labscript footnote says amounts do not need to be exact, but I recorded what we actually used.

2) Wet mount (thin sample without spacer - "wet mount" in Sec. 3.3):

Volume of yeast mixture on slide: _____ µL
Coverslip: _____ × _____ mm, #1 thickness (0.13-0.17 mm)

Any extra dilution? (yes/no)
If yes, yeast : water = 1 : _____

Cell size s
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– Cell type (yeas
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– Preparati
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– Cell linear size along major a

3) Brief notes on first visual check at low magnification (10× or 40×):

Density of cells: _____
Are individual cells resolvable _____
Any clumps / bubbles / debris? _____

5.2 Imaging yeast cells

Objective used for size measurement: _____× (write mag and NA if printed on lens)

Kohler illumination: set up as in Protocol: Microscope Setup (same as previous session).

Image acquisition settings (Vision Assistant):
Camera: FLIR BlackFly BFS-U3-_____
Exposure time t_C = _____ s
Gain or brightness settings: _____

Yeast image filenames
(store in Data/2026-__-__/Yeast/):
yeast_img_01.tif (path: _____)

5.3 Yeast size measurement, formulas and slots

Calibration used here:

pixel size = ____ µm/pixel ± ____ µm/pixel

yeast_img_02.tif (path: ____

(Copied from calibration section done previously; eyepiece magnification irrelevant, as already encoded in labscript Sec. 3.1.)

Short notes per image (crow r

For each cell, measured number of pixels along major axis:

Image 2 notes: ____

Image 3 notes: ____

Cell 1:

image = ____

N pixels major = ___px

L major = N pixels major × pixel size =

= _____ m

Yeast

Cell 2

image = _____

N_pixels_major = _____

$\bar{x}$

L_major = _____

$\bar{m}$

$\bar{C}$

l 3:

image = _____

_____

_____

N_pixels_major =

_____

___ px

L_major = _____

_____

5.4 HT1080 cell imaging (still Sec. 3.3, p. 9)

HT1080 slide description from labscript:
"HT1080 cells are epithelial cells derived from fibrosarcoma cells ... fixed mount,
either unstained or stained blue."

Slide label and type used today:
Fixed HT1080, unstained / stained blue (circle one)
Label text on slide:

_____

Imaging:

Objective: _____×
Camera and illumination settings similar to yeast (note any differences):

Exposure: _____s
Aperture setting: _____
Notes on contrast / color:

_____

HT1080 images (store in Data/2026-__-__/HT1080/):

HT1080_img_01.tif (path: _____)

Thilan

6. BACTERIAL MOTION - PROCEDURES and NOTES (Sec. 3.4, pp. 9-10)

6.1 Overview from labscript (write in notebook for context)

From Sec. 3.4 "Bacterial motion":

- Beads are passive (Brownian), bacteria are active materials with self-propulsion.
- E. coli normally run at ~constant velocity for ~1 s, then tumble to choose a new direction.
- Mutant strain HCB1274 has tumble disabled (run-only).
- Wild-type strain DH5α shows full run-tumble.
- "The experiment proceeds exactly as in Sec. B for polystyrene beads."

6.2 Dilution estimate (Prelab 8 logic)

Given (from labscript Prelab 8):

$OD_{600} = 1.0$ $\quad$ 2×10^8 cells/mL

Goal: about 10 cells in field of view (FOV).

First, compute FOV volume:

$N_x = 1288$ px, $N_y = 964$ px (camera)

Must Take multiple values & find uncertainty.

px_{Bac} → pixel size Bacteria (Objective) = _____ μm/px = $\frac{\text{distance on micrometer (μm)}}{\text{no.s of px}}$

$d_x = N_x \times Px_{Bac} = 1288 \times$ = _____ μm

$d_y = N_y \times Px_{Bac} = 964 \times$ = _____ μm

$d_z \approx$ 5 μm (This is what Lab Script told us
Better ways to find This ???)

$\Rightarrow$ Vol.view = $d_x \times d_y \times d_z$

= _____ μm^3

= $\mu m^3 \times 10^{-12}$ = _____ mL

Now, for a target of ~ 10 cells in View

$$C_{Need} = \frac{N_{Target}}{Vol.view} \left(\frac{cells}{mL}\right)$$

$\Rightarrow$ $C_{Stock} = 2 \times 10^8$ cells/mL

$$\text{dilution factor} = \frac{C_{stock}}{C_{Need}}$$

$\therefore$ We choose; V_{stock} = _____ μL, V_{Total} = _____ μL

our Dilution = $\frac{V_T}{V_{Stock}}$ =

Observation: