Modelling the Movement of *Salmonella* in Gastrointestinal Mucus

Ardalan Rahmatidehkordi (1654312)
Stavros Greer (172487)
Sanjir Inam Salsabil (1731113))

1 Introduction

Salmonella bacteria in intestines must penetrate the gastrointestinal mucus layer to reach and infect the host epithelium. Unlike viruses, which travel via Brownian motion, these bacteria accomplish this by swimming through the mucus layer to reach the epithelium. The body responds to this by having antibodies attach themselves to the surface of the *Salmonella* bacteria, attempting to slow and/or stop each individual *Salmonella* bacterium[1].

In this paper, the movement of *Salmonella* bacteria in fresh, undiluted gastrointestinal mucus from mice will be examined. This will be accomplished by examining 993 readings of different bacteria particle movements from the previous study done by *Schroeder et al.* These readings are from twenty seven different videos where the movement of individual *Salmonella* bacterium were tracked, with their x and y coordinates measured in pixel coordinates of $dx = 0.156\mu m$ and the time measured in frames of $dt = 0.0667s$[3], as seen in Figure 1. From these measurements, three questions will be answered. First, the swim speed of a *Salmonella* bacterium will be determined. Second, we will determine when a *Salmonella* bacterium will penetrate the mucus barrier. To accomplish this, a model will be created to simulate the movement of *Salmonella* bacteria in mucus, and the mean time of first passage of a bacterium through the mucus barrier will be estimated. Third, using this model, the proportion of bacteria particles that are mobile vs immobile will be estimated.

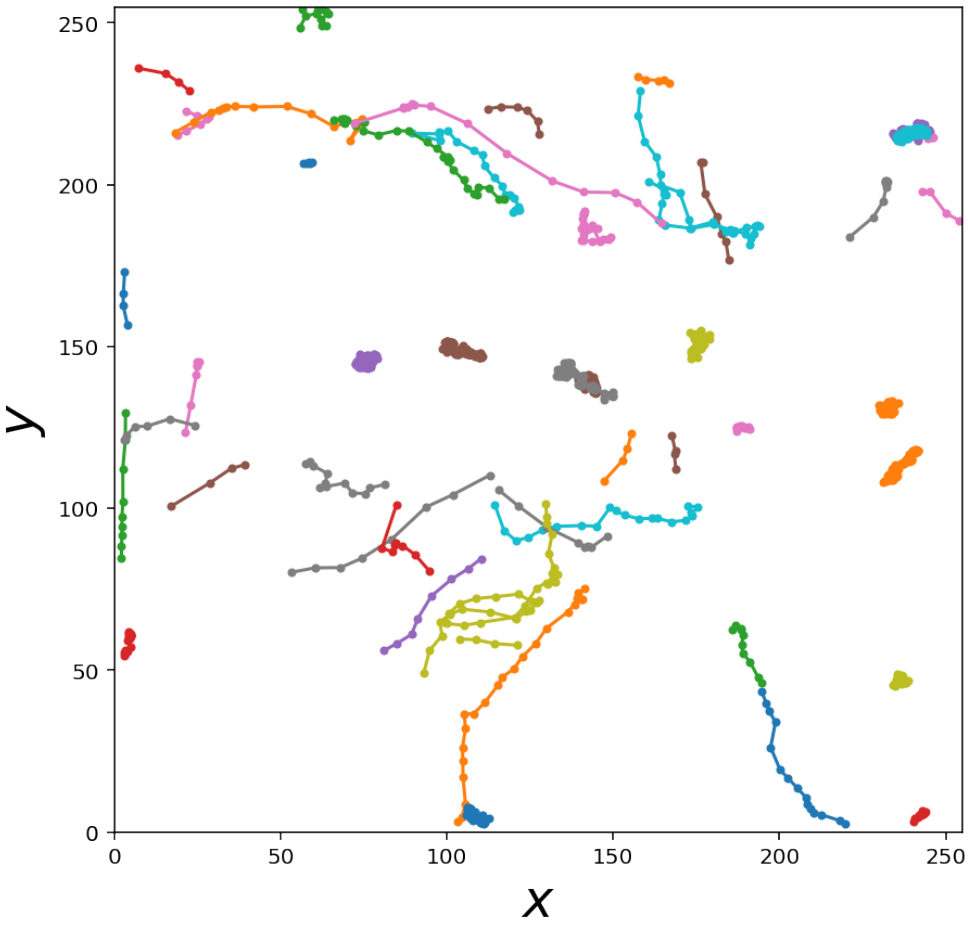

Figure 1: A plot showing the movements of all bacteria particles in a single video recorded in the study

2 Formulation of Mathematical Model

The first goal of this paper is to estimate the swim speed of a single *Salmonella* bacterium. This is done by first assuming that all bacteria have the same mean swim speed. To find the swim speeds of the bacteria, we take the differences in positions at each recorded time step for each bacterium, as seen in equation 1.

$$velocity = \begin{bmatrix} \frac{\Delta x}{\Delta t} \\ \frac{\Delta y}{\Delta t} \end{bmatrix} \tag{1}$$

Since the time intervals, Δt are equal to one pixel, we can simplify as follows

$$velocity = \begin{bmatrix} \Delta x \\ \Delta y \end{bmatrix} \tag{2}$$

Thus, by applying Pythagorean's theorem, we find that the speed of a particle at each time step is as follows in equation 3.

$$speed = \sqrt{\Delta x^2 + \Delta y^2} \tag{3}$$

Following finding the speed of a bacterium at each time step, we can find the average speed of each bacterium. Taking the average of the mean speeds of all the bacteria, we can find the swim speed at which a bacteria travels at, assuming all bacteria move at the same speed.

The second goal of this paper is to develop a stochastic model of *Salmonella* motion and predict the mean first passage time to cross a mucus barrier. This will be accomplished using a random walk Markov Chain, where the next position of the bacterium falls within a Normal distribution around the current position.
Thus, letting $\mathbf{X}_k$ denote the position of a bacterium at the k_{th} time:

$$\begin{aligned} \forall k \in \mathbb{N}_0, \qquad \mathbf{X}_k &= \begin{bmatrix} x_k \\ y_k \end{bmatrix} \\ \forall k \in \mathbb{N}, \qquad \mathbf{X}_k &\sim \text{Norm}(\mathbf{X}_{k-1}, \Sigma) \end{aligned} \tag{4}$$

However, this is a multi-dimensional normal. To simplify this problem, we assume that the mucus stretches out to distances far greater than the bacteria can reasonably travel in the X and Y directions, and that the only way the bacteria can reach the mucus barrier is in the Z direction, which is typically around $100\mu m$ thick[2].Thus, we only need to simulate a random walk in the Z plane.

$$\forall k \in \mathbb{N}, \qquad Z_k \sim \text{Norm}(Z_{k-1}, \sigma) \tag{5}$$

We create a one dimensional line, where the bacteria start exactly in the center of the mucus layer, approximately $50\mu m$ above a barrier created at $z = 0\mu m$, which acts as the barrier the bacteria have to cross to reach the epithelium. A reflective barrier is created at $z = 100\mu m$, where if a bacteria were to cross this point it would be reflected back into the mucus layer. It was assumed that the motion of the bacteria is the same in all directions, so the σ used for our random walk was found using the analyzed data. It was found by first finding the absolute average step size of all particles in the data. Then, since we know that 50% of all step sizes must fall within $\pm|\Delta X|$, the inverse CDF function, seen in equation 6, can be used to find at what proportion of ΔX the standard deviation of the step sizes occurs. This was found to be at $1.4826\Delta X$. Thus, we input this value as our σ in equation 5. We can then run this random walk many times, taking the average time at which bacteria pass the barrier as the mean first passage time.

$$F^{-1}(p) = \inf\{x \in \mathbb{R} : F(x) \geq p\} \tag{6}$$

The third and final goal of this paper is to estimate the fraction of cells which are mobile vs immobile. As seen in figure 2, bacteria which are mobile move across areas which are a factor of 10 larger in size than the areas in which immobilized bacteria move in the same time frame. Using the same simulated Markov Chain random walk as used to estimate the mean first passage time, we can decreased the number of steps each particles takes, and set our barriers to cover 10% of the total area of the mucus layer, from $Z = 45\mu m$ to $Z = 55\mu m$. After simulating the movement of the bacteria, if they stay within this range, they can be considered immobile, and if they exit

this range, they can be considered mobile. Thus, after simulating the movement of many different bacteria particles, we can find the proportion of which are mobile vs immobile.

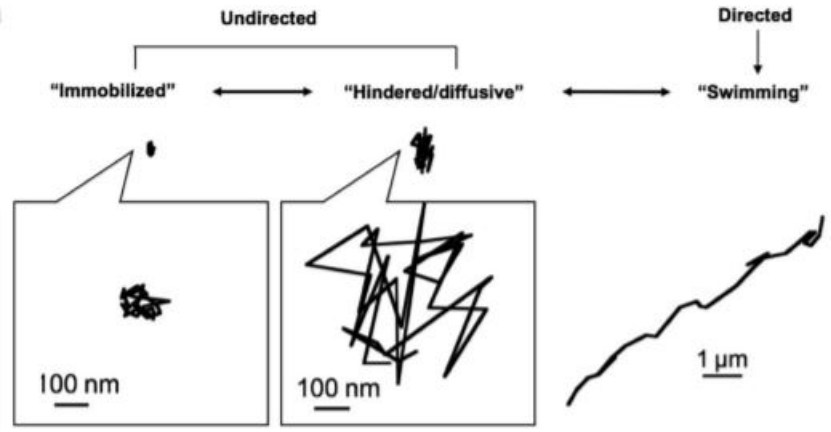

Figure 2: Visualization of the movement patterns of bacteria in mucus[1].

3 Solution of the Problem

By taking the mean speeds of each bacterium using equation 3, we average the mean speeds of all the bacteria particles (see figure 4 in appendix) to find that the swim speed of a single *Salmonella* bacterium is $3.0872\frac{pixels}{frames} = 3.0872\frac{0.156\mu m}{0.0677s} = 7.1138\frac{\mu m}{s}$. Next, we created (see figure 5 in appendix) and ran a random walk simulation based on equation 5, simulating the motion of 1000 bacteria particles through a time period of 100,000 frames (6770 seconds). And example of the random walk motion of a single bacterium is shown in figure 3.

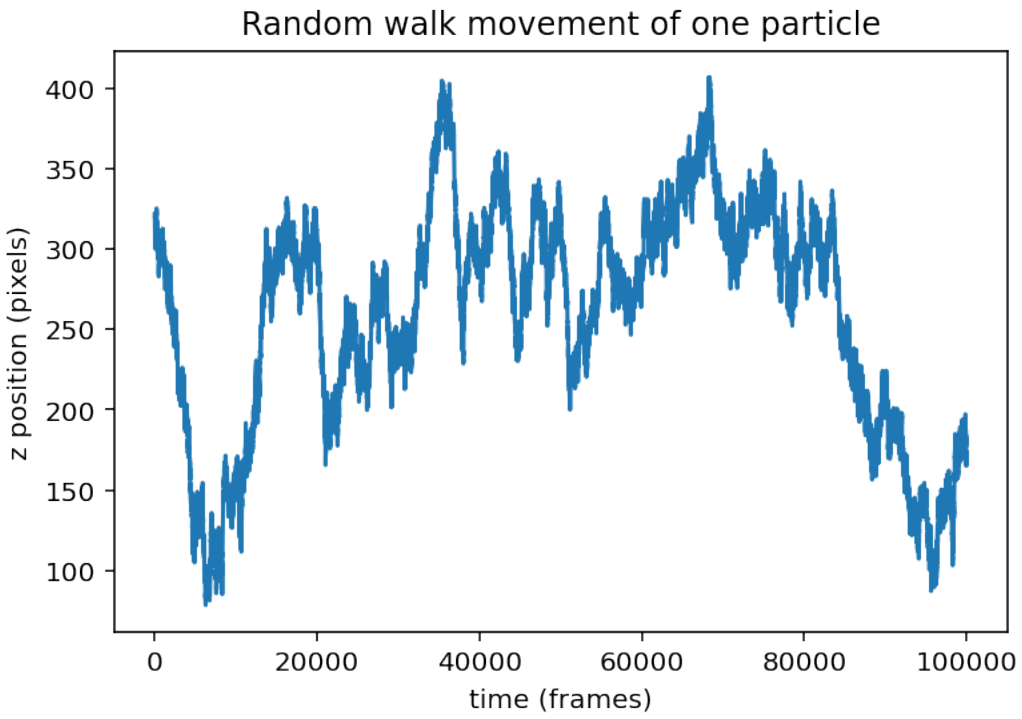

Figure 3: The simulated random walk in the Z axis of one bacterium

Following the simulation of the 1000 bacteria particles, it was found that 365 crossed the barrier at $Z = 0$, and that the average time at which they crossed was $t = 49925.05454545454 frames = 3379.93s$. Finally, the same random walk model was used but with barriers at $Z = 45\mu m$ and $Z = 55\mu m$, and with the time period decreased to 1000 frames. If a bacteria particle remained within this area throughout the end of the simulated time period, it was considered to be immobile. Following the simulation of 1000 bacteria particles, 298 had excited the area, and 702 remained inside.

4 Interpretation of results

Above, we found that the swim speed of a *Salmonella* bacteria in mucus was $7.1138\frac{\mu m}{s}$. This result was recovered from the data taken from the *Schroeder et al.* study, and suggests that if the *Salmonella* bacteria moved in one consistent direction, it could cross the gastrointestinal mucus layer in approximately 14.3 seconds. In addition, we found that the mean time of first passage of the bacteria is 3379.93 seconds, or 56.33 minutes. Interestingly, in a time span of 6770 seconds, only 365, or 36.5% of the bacteria were able to reach the barrier, suggesting that a large portion of the bacteria are immobile. Finally, when testing the mobility of bacteria particles, we found that 298 out of 1000, or 29.8%, were mobile and had moved out of the area required for them to be considered mobile. This is a smaller proportion than the amount of bacteria that crossed the barrier, but that is likely due to the time scale for the barrier passing simulation being a factor of

100 times larger then the mobility testing simulation. This allowed some slower-moving bacteria to be able to have enough randomized movements in the right direction to pass the barrier. On the shorter timescale, we can see what proportion of bacteria will have truly random or directed motion, and better represent the motions of immobilized and mobilized bacteria. Thus, as a result of this study, we can conclude that the mean *Salmonella* bacteria moves at a speed of $7.1138\frac{\mu m}{s}$, has a mean time of first passage of $3379.93s$ and that 29.8% of bacteria are mobile

5 Critique of the model

This model successfully answers the three questions we set out to answer in this paper. However, it can be improved upon in the future with further time and with a more advanced knowledge of modelling chains. The model used in this paper was quite rudimentary, as it was just a simple random walk which assumed all bacteria particles moved at the same speed and had the same starting position in the mucus layer. In addition, this model only took into account movement in one direction. To expand on this model, we would like to extend it to model the motion of bacteria particles in all three dimensions of movement. In addition, we could have the bacteria particles start at random locations within the mucus layer, not just set them in the middle of the layer as done here.

6 Appendix

```
[4] #Code to find average speed of bacterias, first finding average change in position for each particle

    df=pd.DataFrame()
    speed_df=pd.DataFrame()
    total_means= [] #this will be the list of the average speed of each bacterium.
    full_delta_x = []
    full_delta_y = []

    for filez in files:
      df=pd.read_csv(filez)

      deltax =[]
      deltay=[]

      for bacterium_number, bacterium in df.groupby('particle'):

        deltax.extend(diff(bacterium['x']))
        full_delta_x.extend(diff(bacterium['x']))
        deltay.extend(diff(bacterium['y']))
        full_delta_y.extend(diff(bacterium['y']))


      deltax=array(deltax)
      deltay=array(deltay)

      speed=sqrt(deltax**2+deltay**2) #this is vector of speeds

      maxz=max(df['particle'])
      numbers=zeros(maxz+1)
      for j in arange(0,maxz+1):
        N=array(df['particle']==j).sum() #the number of bacteria with label particle=j
        numbers[j]=N

      numofspeeds=numbers-1

      particles_for_speeds=[]
      for salmonella_num in arange(0,maxz+1):
        particles_for_speeds.extend(np.repeat(salmonella_num,numofspeeds[salmonella_num]))
      particles_for_speeds=array(particles_for_speeds)

      speed_df=pd.DataFrame({'particle':particles_for_speeds,'speed':speed})

      meanz=zeros(maxz+1)
      for particlez, particlezz in speed_df.groupby('particle'):
        meanz[particlez]=mean(particlezz['speed'])

      total_means.append(meanz)
```

```
#Now combine the averages for each bacterium, and take the average speed
concatenated_total_means=concatenate(total_means,axis=0)
average_speed = mean(concatenated_total_means)
average_speed
```

Figure 4: The Python code to simulate find and average the speeds of all particles in the study

```
#Simulate the random walk of bacteria particles through mucus
Nsteps = 100000
Nsims = 1000
sigma = sigma_estimate

z0 = 320 #320 pixels * 0.156 microm/pixels = 50 microm
barrier = 0

time_of_passage = []
for j in range(1,Nsims+1):
    z = zeros(Nsteps)
    z[0] = z0
    for t in arange(1, Nsteps):
        z[t] = normal(z[t-1], sigma)
        if z[t] <= barrier:
            time_of_passage.append(t)
            break
        elif z[t] >= 640: #this is the reflective barrier.
                          #640 pixels * 0.156 microm/pixels = 100 microm
            z[t] = 639

#print(time_of_passage)
first_time_of_passage_estimate = mean(time_of_passage)
first_time_of_passage_estimate , len(time_of_passage)
```

Figure 5: The Python code to simulate the random walk motion of bacteria particles within a mucus layer. Based on equation 5.

References

[1] H. Schroeder *et al.* "LPS-binding IgG arrests actively motile Salmonella Typhimurium in gastrointestinal mucus". In: *Mucosal Immunology* 13 (2020), pp. 814–823.

[2] C. Atuma et al. "The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo". In: *American Journal of Physiology-Gastrointestinal and Liver Physiology* 280.5 (2001). PMID: 11292601, G922–G929. DOI: 10.1152/ajpgi.2001.280.5.G922. eprint: https://doi.org/10.1152/ajpgi.2001.280.5.G922. URL: https://doi.org/10.1152/ajpgi.2001.280.5.G922.

[3] Jay Newby. *Project Salmonella*. URL: https://github.com/newby-jay/SSC_Workshop_2021/blob/main/Project%20Salmonella/Project%20Salmonella.ipynb.