Simulation and Fitting of FRAP Data in Python

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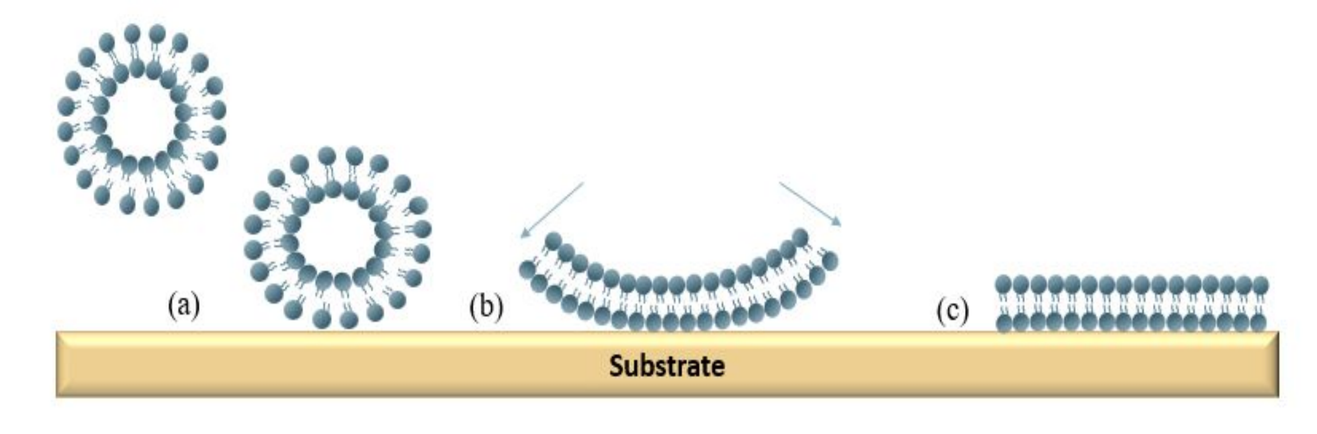
What are Supported Lipid Bilayers?

- Supported lipid bilayers (SLBs) - a mimic of the cell membrane formed on a substrate such as glass
- Spontaneously forms a nice, homogenous surface



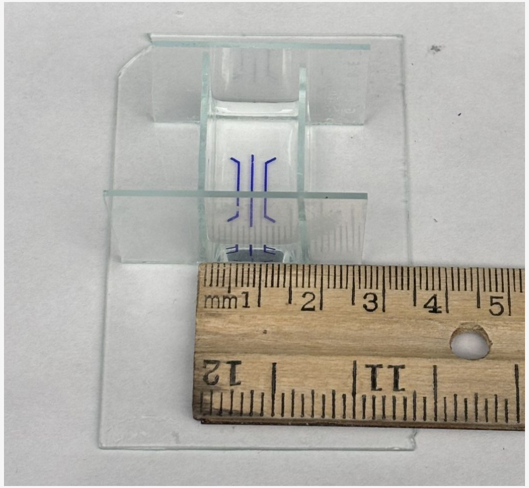
Significance:

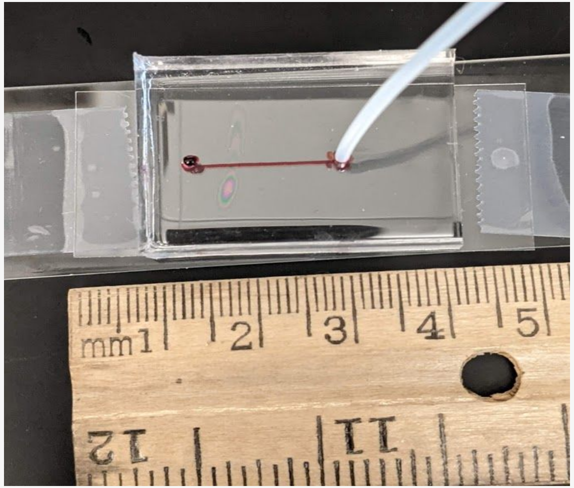
- biosensor
- diagnostics
- surface coating

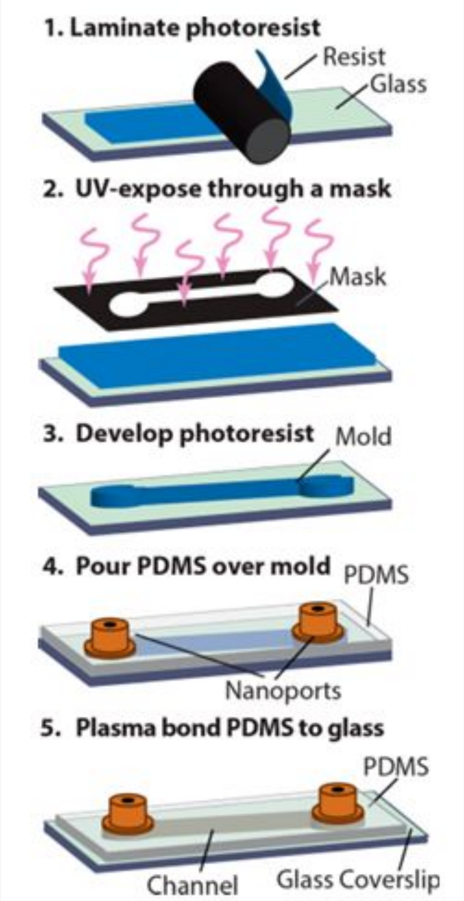
Jackman et al., Langmuir. **2020**, 36, 6, 1387-1400
Sut et al., Apmt. **2021**, 25, 2351-9407

Microfluidics

- polydimethylsiloxane (PDMS)-based
- Simultaneous imaging of different bilayers
- Controlled environment to conduct experiments





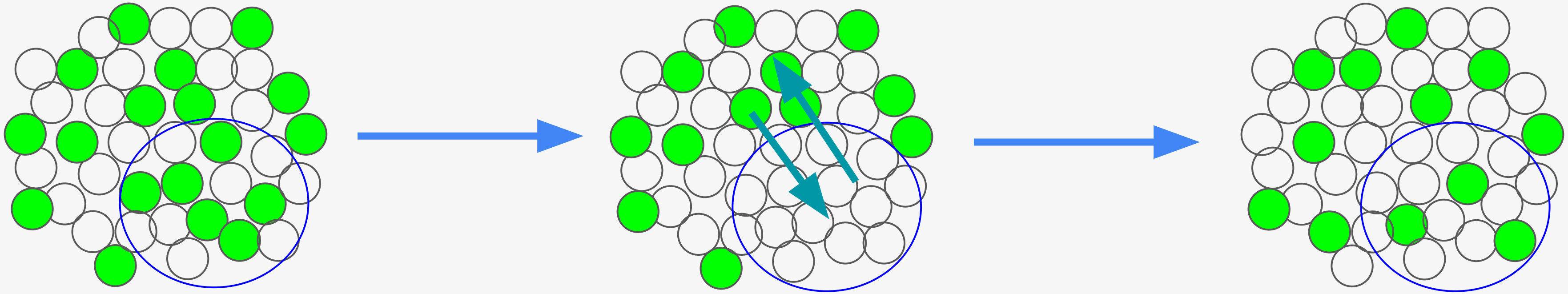


Robison et al., Anal. Chem. **2014**, 86, 9, 4157-4163

FRAP analysis

Fluorescence Recovery After Photobleaching (FRAP)

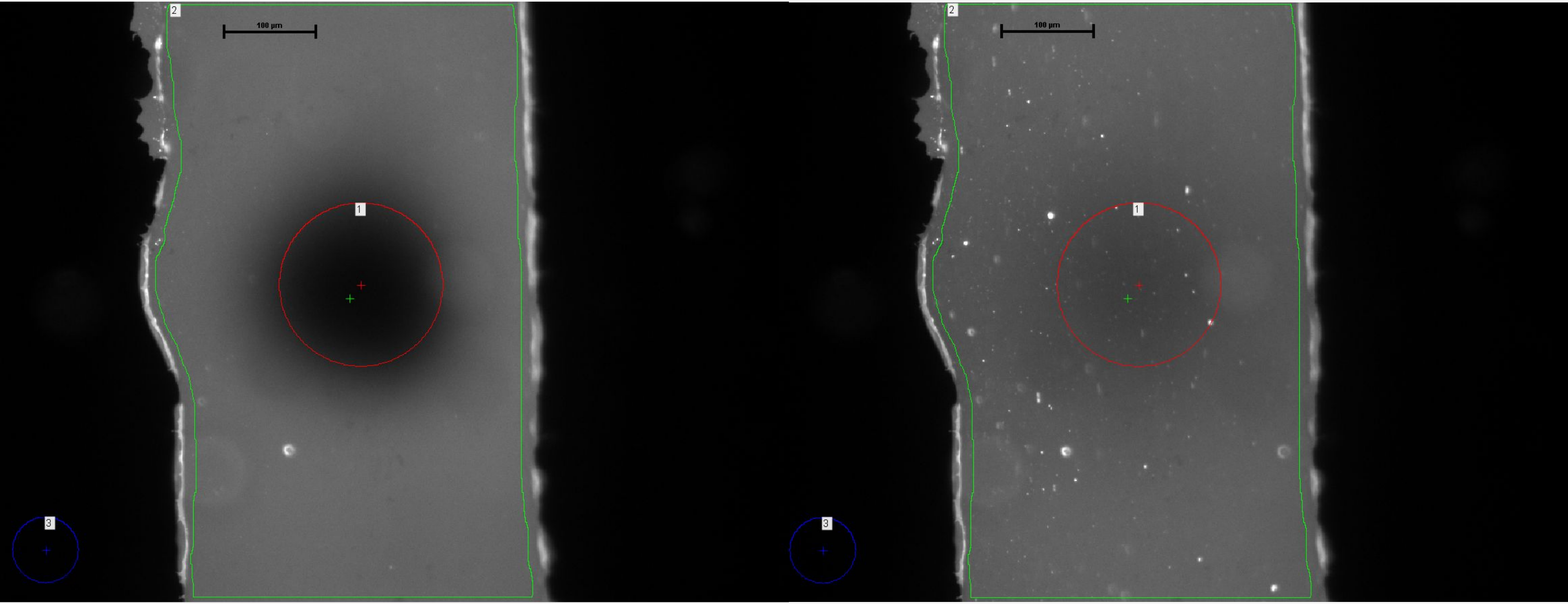
- Part of a lipid bilayer is shot with a fine laser
- This destroys the fluorescence of the bilayer at that location
- If bilayer is fluid, then the fluorescence will recover at that position



Axelrod, D, et al. *Biophys J* **1976**, *16* (9), 1055–1069.

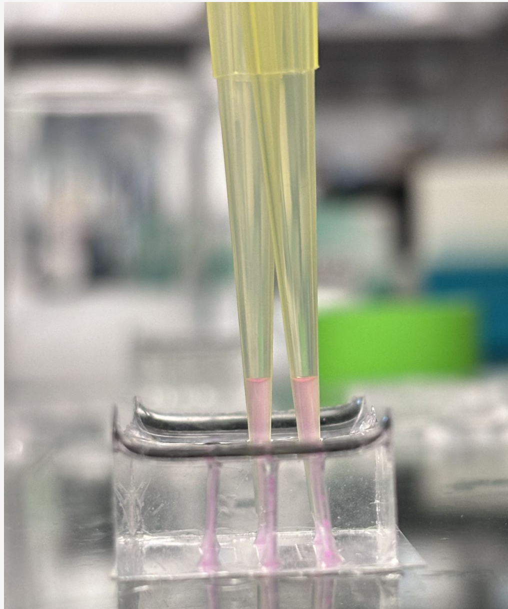
Lorén, N, et al. *Biophys* **2015**, *48* (3), 323–387.

Typical FRAP - before & after



Research Question

- Many experiments desire the knowledge of the fluidity of their SLB in the system they are in.
- Therefore, we want to be able to control and measure the fluidity of SLBs in microfluidic devices.
- We currently use the EasyFRAP software to determine the fluidity with poor accuracy
- Can we use a simulation method with Python to more accurately analyze FRAP data to obtain diffusion coefficients?



Goals

- Create code for a simulation of our experimental data to obtain diffusion coefficients
- Use our approach for multiple FRAP experiments
- Create an easy-to-use framework for future students

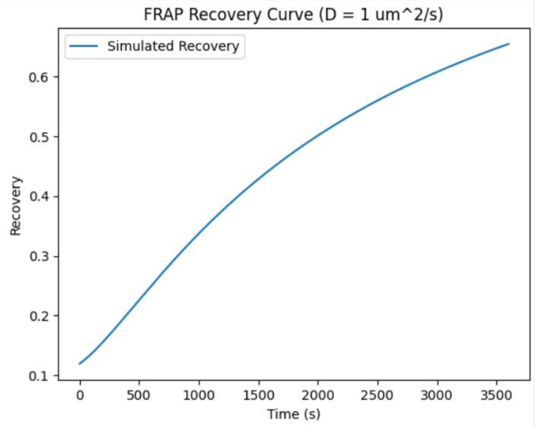
Diffusion Differential Equation

- We are looking to find the diffusion coefficient D which obeys the diffusion equation: $\partial C(r,t)/\partial t = D\nabla^2 C(r,t)$
    - D represents the speed at which the lipid bilayer moves
- We can use Laplace’s equation with the finite difference method to simulate diffusion over time

$$\nabla^2 f(i,j) = f(i+1,j) + f(i-1,j) + f(i,j+1) + f(i,j-1) - 4f(i,j)$$

Methodology

- 1) Run a simulation on an initial condition for an experiment
- 2) Track intensity data for our experiment through time
- 3) Compare the two to get the correct D value



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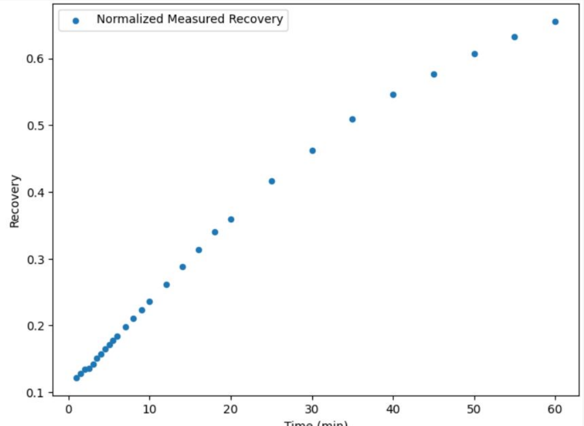

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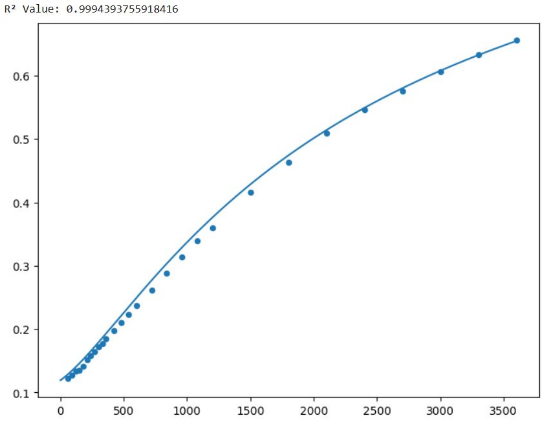
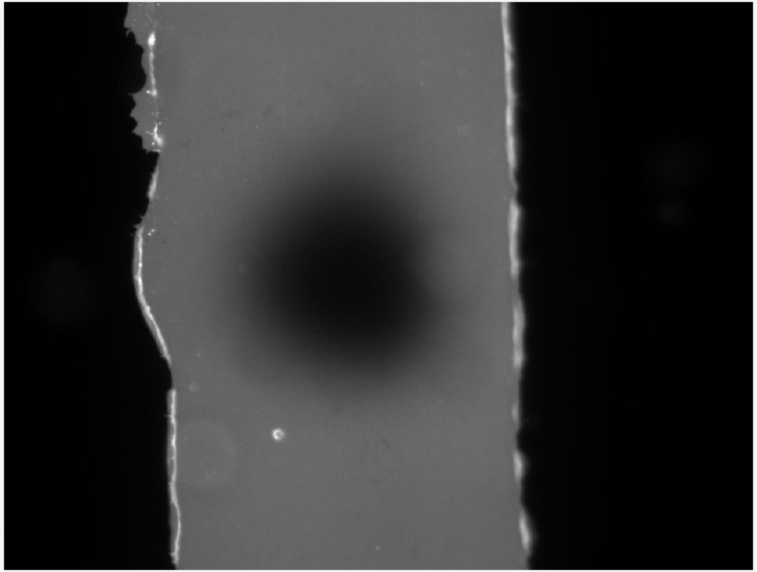


Step 1: Simulate

- We need to be able to set up an initial condition for a situation like this one:
    - Shape of the channel (fluorescent region)
    - Shape of the outside of the channel
    - Shape of the gaussian spot



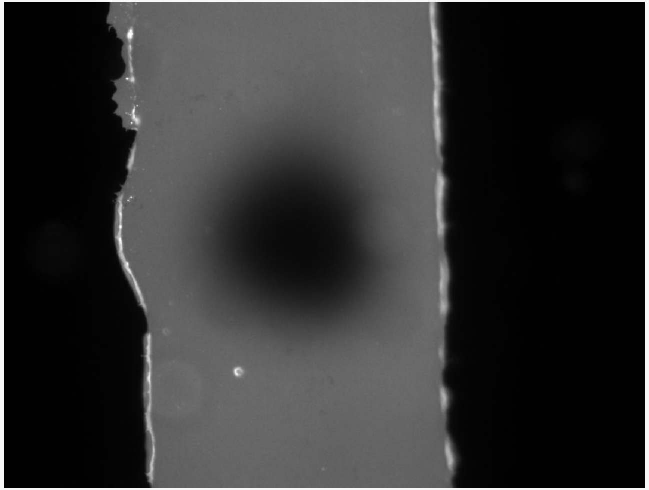
Axelrod, D, et al. *Biophys J* **1976**, *16* (9), 1055–1069..

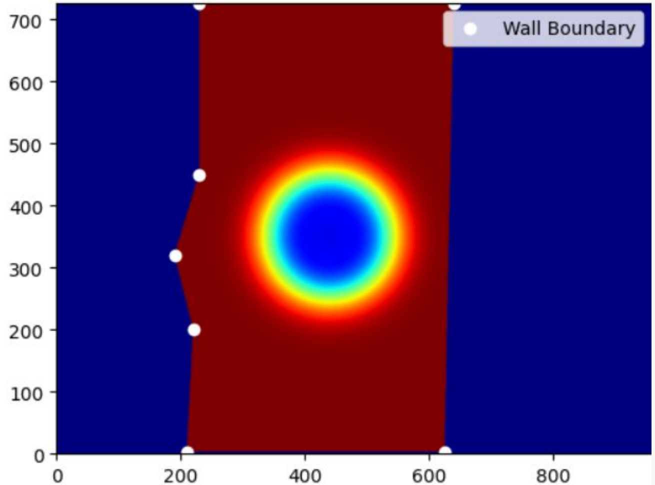
Step 1: Simulation

- Manually plugged in points to make an approximate boundary around our channel
- Inside the boundary: concentration = one
- Outside the boundary: concentration = zero
- Used a nested exponential function to model our laser spot

$$C(r) = C_c + (1 - C_c) \cdot e^{-Pe^{-r^2}}$$

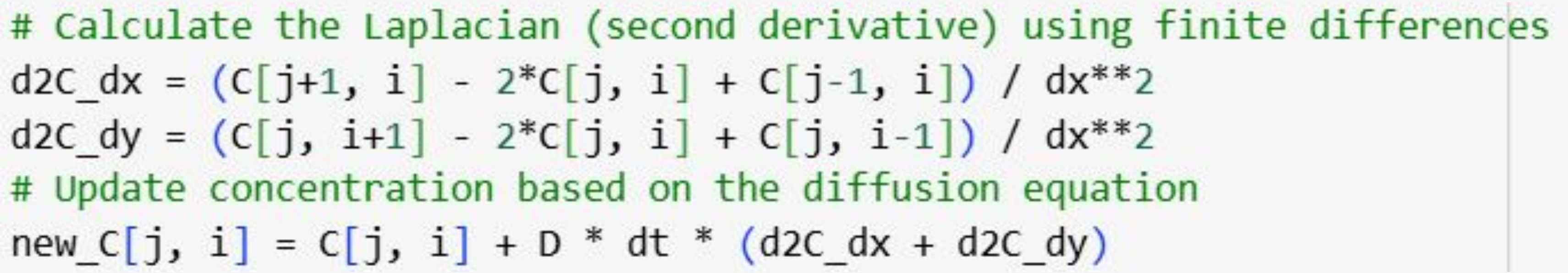
$C_c = \textit{Initial center brightness}$ $P = \textit{laser power}$





Step 1: Simulation

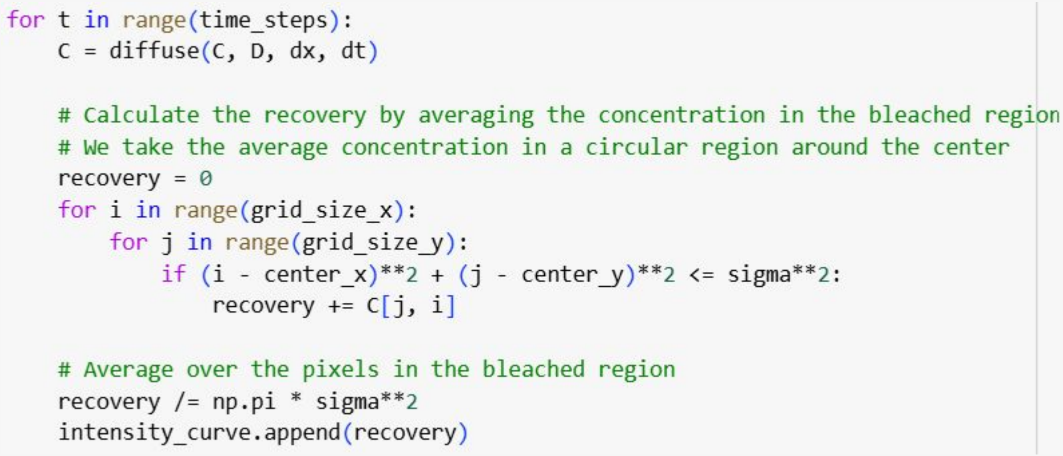
- Now that the initial conditions are realistic, we are ready to simulate.
- Use the finite difference method with Laplace’s equation to get the new concentration for a given time step.



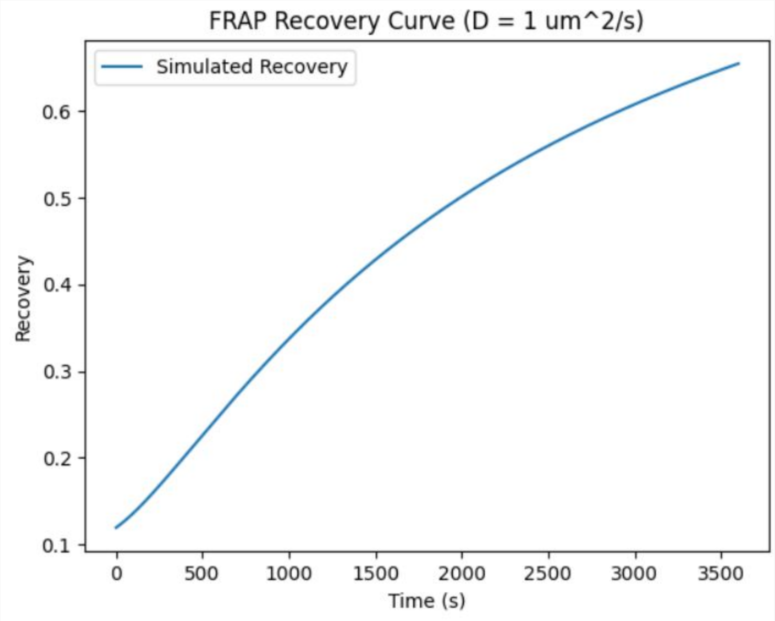
```
# Calculate the Laplacian (second derivative) using finite differences
d2C_dx = (C[j+1, i] - 2*C[j, i] + C[j-1, i]) / dx**2
d2C_dy = (C[j, i+1] - 2*C[j, i] + C[j, i-1]) / dx**2
# Update concentration based on the diffusion equation
new_C[j, i] = C[j, i] + D * dt * (d2C_dx + d2C_dy)
```

Step 1: Simulation

- Run throughout time to get a FRAP recovery curve for a given D value
- The recovery is measured by tracking the intensity of the bleached spot over time

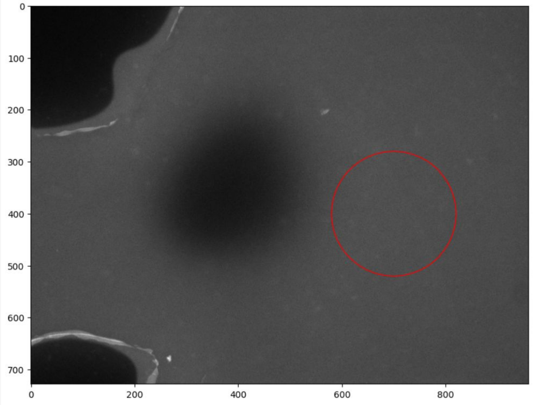


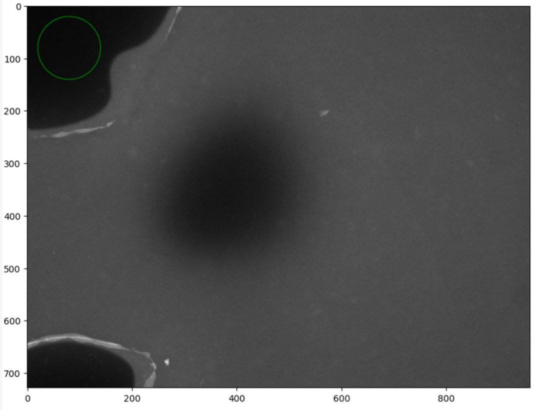
```
for t in range(time_steps):
    C = diffuse(C, D, dx, dt)

    # Calculate the recovery by averaging the concentration in the bleached region
    # We take the average concentration in a circular region around the center
    recovery = 0
    for i in range(grid_size_x):
        for j in range(grid_size_y):
            if (i - center_x)**2 + (j - center_y)**2 <= sigma**2:
                recovery += C[j, i]

    # Average over the pixels in the bleached region
    recovery /= np.pi * sigma**2
    intensity_curve.append(recovery)
```



Step 2: Experimental Data

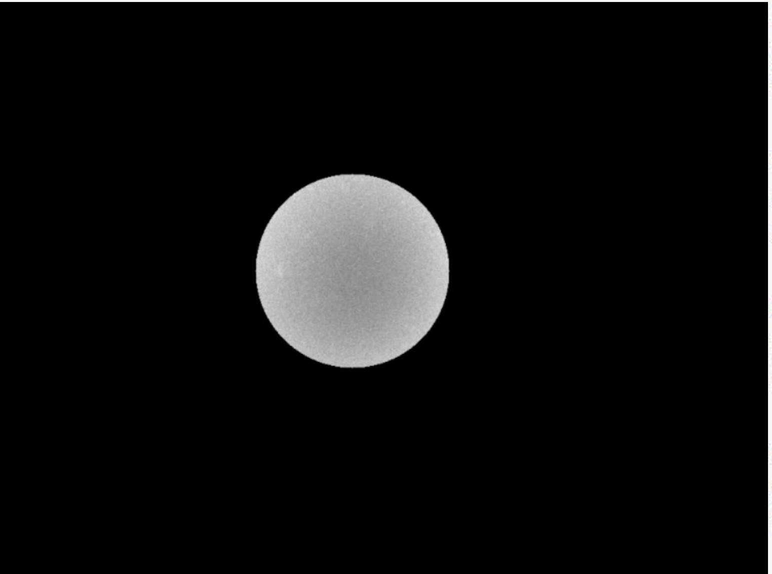
- 28 images per FRAP experiment
- Track intensity of a circular ROI through time
- First we grab a section of unbleached bilayer and a section of background to aid with later normalization

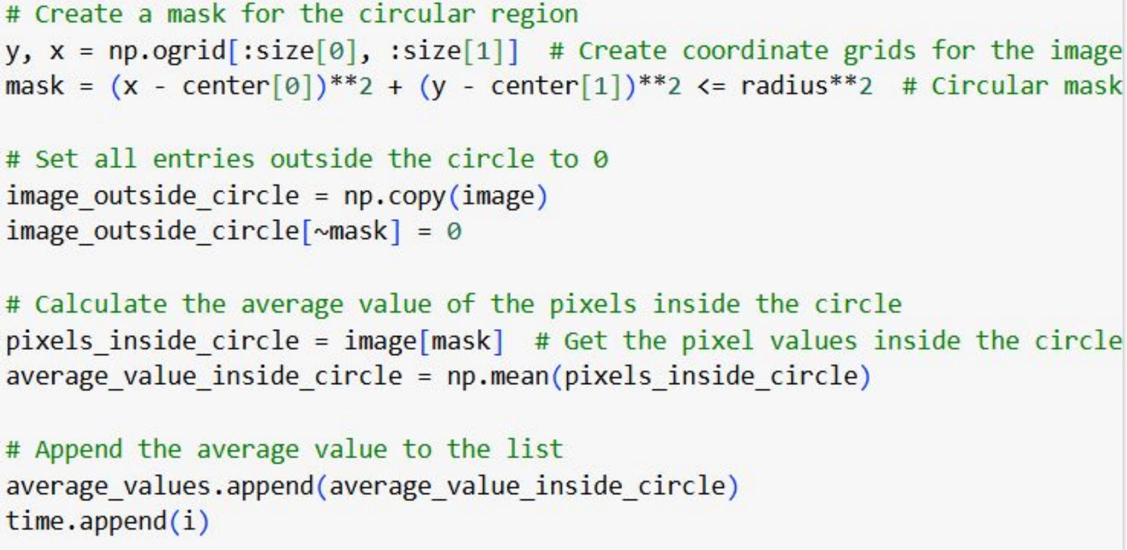




Step 2: Experimental data

- Make a circular mask around the bleached spot
- Obtain the average intensity for the pixels in this region
- Do this for each of the 28 images

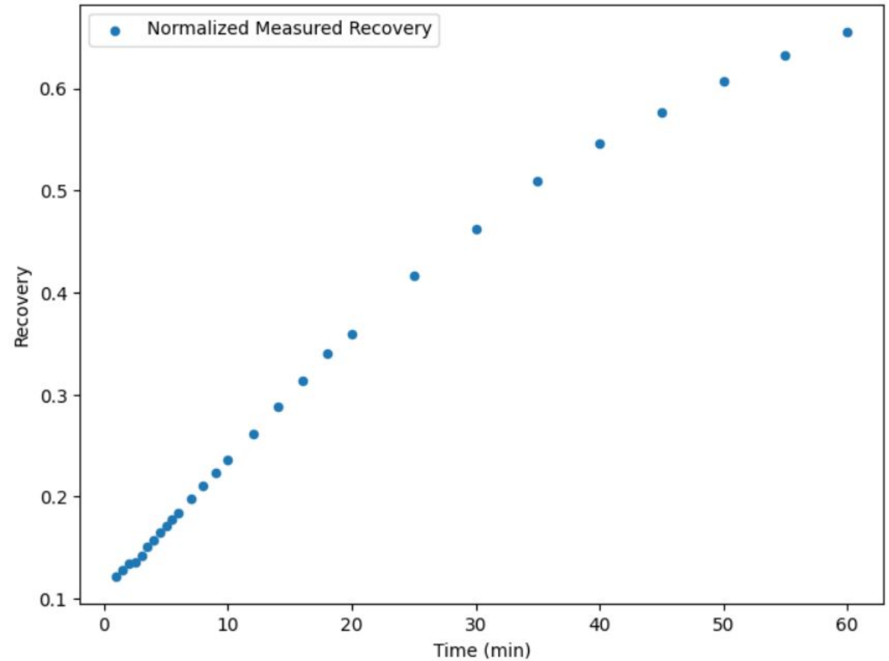




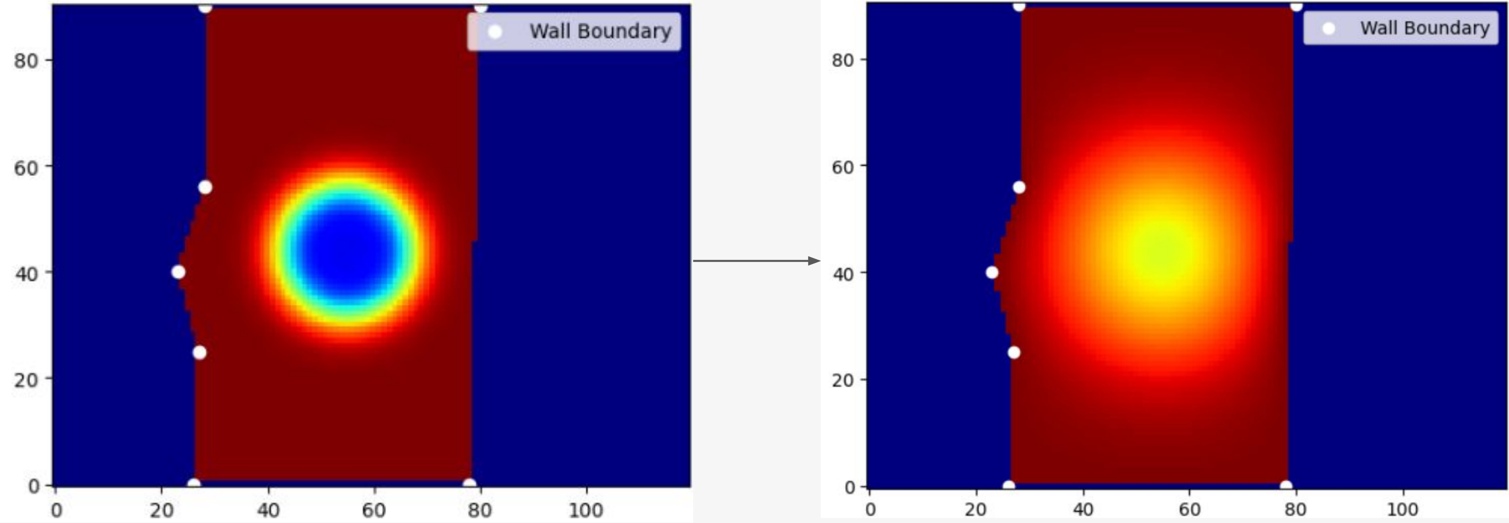
```
# Create a mask for the circular region
y, x = np.ogrid[:size[0], :size[1]]  # Create coordinate grids for the image
mask = (x - center[0])**2 + (y - center[1])**2 <= radius**2  # Circular mask

# Set all entries outside the circle to 0
image_outside_circle = np.copy(image)
image_outside_circle[~mask] = 0

# Calculate the average value of the pixels inside the circle
pixels_inside_circle = image[mask]  # Get the pixel values inside the circle
average_value_inside_circle = np.mean(pixels_inside_circle)

# Append the average value to the list
average_values.append(average_value_inside_circle)
time.append(i)
```

Step 2: Experimental Data

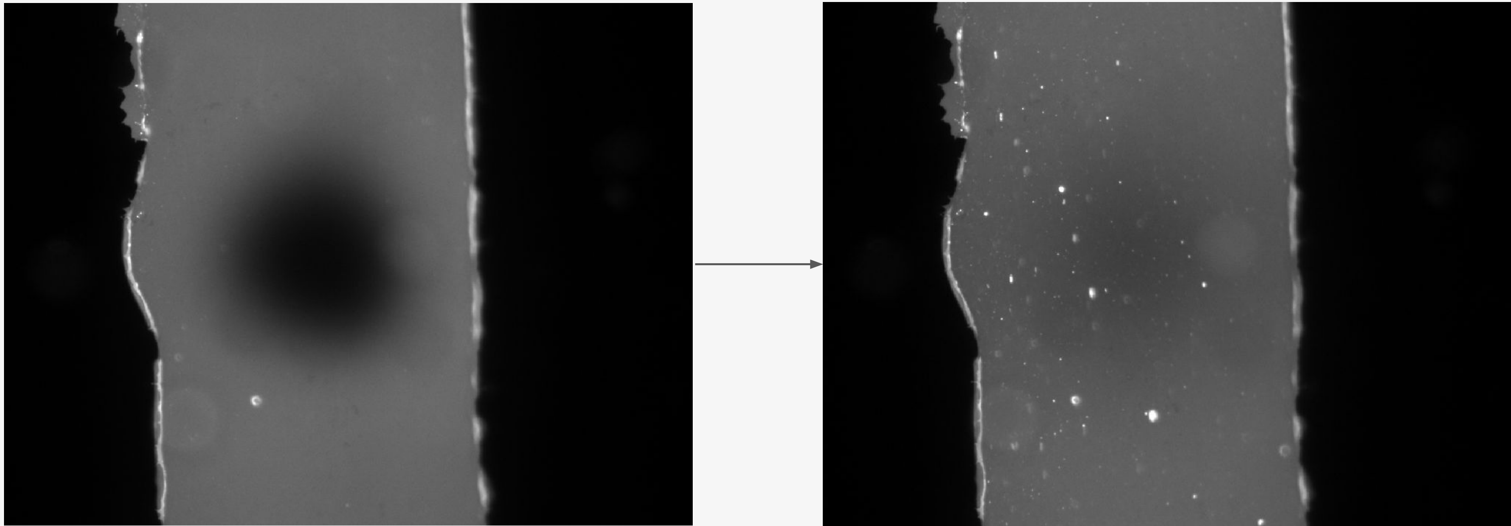
- These intensities give us an unnormalized FRAP recovery curve
- The y-axis is currently based directly on the original image pixel values
- We want the y-axis to range from 0 to 1 so compare it to the simulation.
- Normalizing the data yields us a curve like this



Simulation

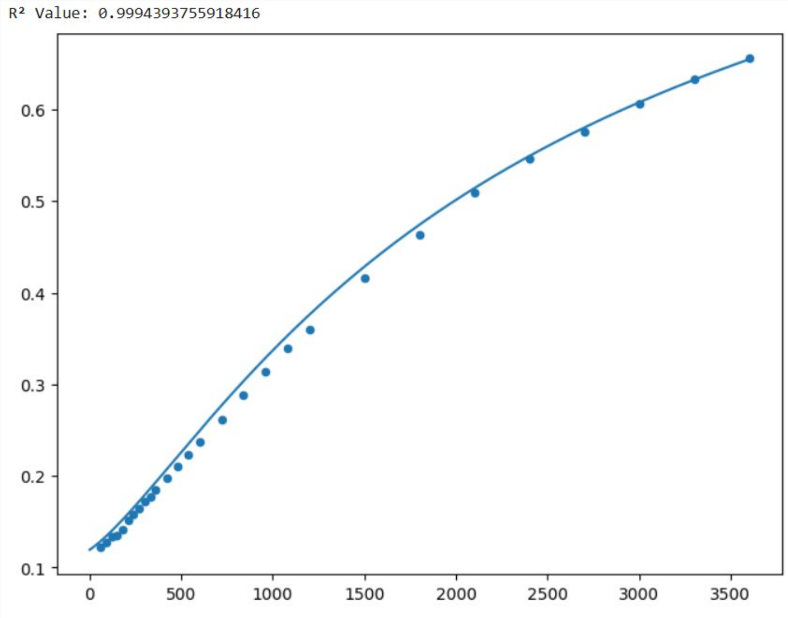


Experiment



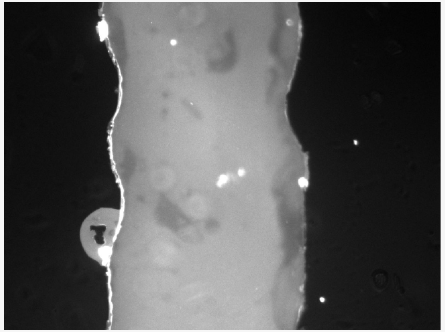
Step 3: Compare

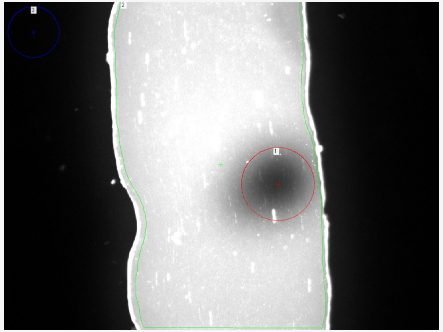
- After plugging in and testing some D values, we find one that overlaps well
- In this case we find that D = 1 μm^2/s
- 3 other FRAP experiments yield similar values of 0.95, 1.1, and 1.3 μm^2/s based on our “eyeball method”


R² Value: 0.9994393755918416

Discussion

- The literature values of the diffusion coefficient for the lipids we use (DOPC and POPC) are also in the neighborhood of D = 1 μm^2/s, giving us confidence in our method.
- There are other experimental factors to consider in our analysis
  - Surface cleanliness
  - Loose vesicles
  - Spot size
  - Bleach time
  - Temperature



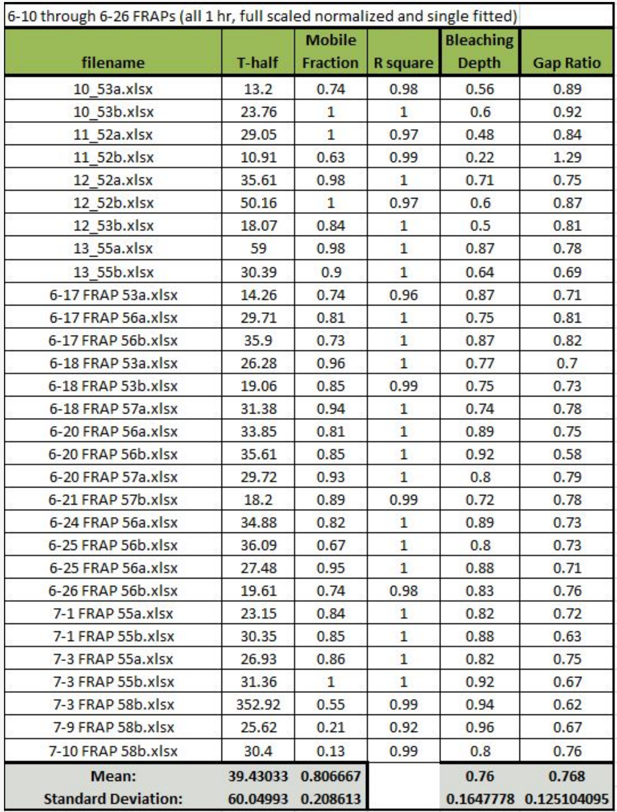


Schaich et al., Biochimica et Biophysica - Biomembranes. 2020

Next Steps

We have many steps to take to improve this analysis

1. Run our simulation on the rest of the available data
2. Automatic boundary and spot drawing
3. A more robust fitting method
4. Streamlining our code

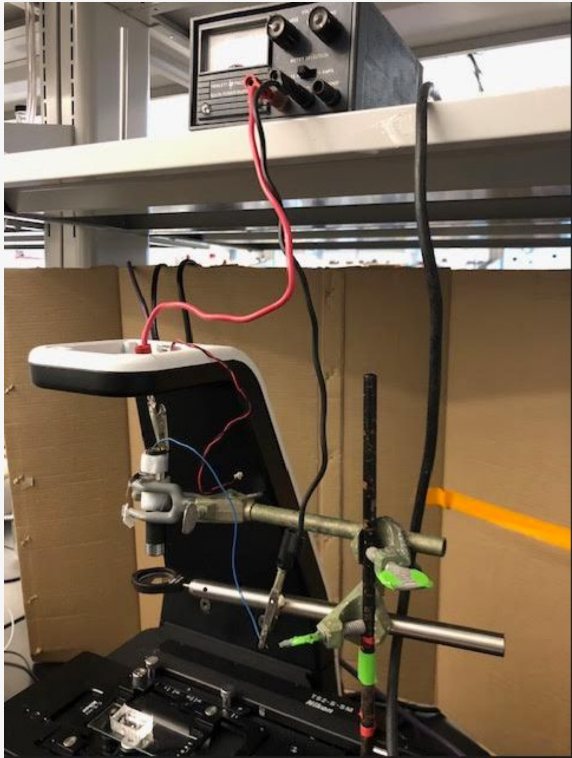


6-10 through 6-26 FRAPs (all 1 hr, full scaled normalized and single fitted)

filename	T-half	Mobile Fraction	R square	Bleaching Depth	Gap Ratio
10_53a.xlsx	13.2	0.74	0.98	0.56	0.89
10_53b.xlsx	23.76	1	1	0.6	0.92
11_52a.xlsx	29.05	1	0.97	0.48	0.84
11_52b.xlsx	10.91	0.63	0.99	0.22	1.29
12_52a.xlsx	35.61	0.98	1	0.71	0.75
12_52b.xlsx	50.16	1	0.97	0.6	0.87
12_53b.xlsx	18.07	0.84	1	0.5	0.81
13_55a.xlsx	59	0.98	1	0.87	0.78
13_55b.xlsx	30.39	0.9	1	0.64	0.69
6-17 FRAP 53a.xlsx	14.26	0.74	0.96	0.87	0.71
6-17 FRAP 56a.xlsx	29.71	0.81	1	0.75	0.81
6-17 FRAP 56b.xlsx	35.9	0.73	1	0.87	0.82
6-18 FRAP 53a.xlsx	26.28	0.96	1	0.77	0.7
6-18 FRAP 53b.xlsx	19.06	0.85	0.99	0.75	0.73
6-18 FRAP 57a.xlsx	31.38	0.94	1	0.74	0.78
6-20 FRAP 56a.xlsx	33.85	0.81	1	0.89	0.75
6-20 FRAP 56b.xlsx	35.61	0.85	1	0.92	0.58
6-20 FRAP 57a.xlsx	29.72	0.93	1	0.8	0.79
6-21 FRAP 57b.xlsx	18.2	0.89	0.99	0.72	0.78
6-24 FRAP 56a.xlsx	34.88	0.82	1	0.89	0.73
6-25 FRAP 56b.xlsx	36.09	0.67	1	0.8	0.73
6-25 FRAP 56a.xlsx	27.48	0.95	1	0.88	0.71
6-26 FRAP 56b.xlsx	19.61	0.74	0.98	0.83	0.76
7-1 FRAP 55a.xlsx	23.15	0.84	1	0.82	0.72
7-1 FRAP 55b.xlsx	30.35	0.85	1	0.88	0.63
7-3 FRAP 55a.xlsx	26.93	0.86	1	0.82	0.75
7-3 FRAP 55b.xlsx	31.36	1	1	0.92	0.67
7-3 FRAP 58b.xlsx	352.92	0.55	0.99	0.94	0.62
7-9 FRAP 58b.xlsx	25.62	0.21	0.92	0.96	0.67
7-10 FRAP 58b.xlsx	30.4	0.13	0.99	0.8	0.76
Mean:	39.43033	0.806667		0.76	0.768
Standard Deviation:	60.04993	0.208613		0.1647778	0.125104095

Conclusion

- FRAP analysis is a powerful method for determining lipid bilayer properties.
- Being able to perform this analysis in microfluidic channels can help users control bilayer fluidity.
- We have shown that our code is able to provide accurate values for the diffusion coefficient



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