

# Electronic Supplementary Information: Configurable lipid membrane gradients to quantify diffusion, phase separation and binding density

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## 1 Stamp Specifications

### 1.1 Creating molds by 3D printing

We used OpenSCAD[1] open-source software to procedurally generate the 3D printed molds. The code to generate the molds is included below. The mold is principally a cube that has two negative features that are 1 mm deep and 0.5 mm wide (see Fig. 1). These parallel negative features are nominally spaced 0.75 mm apart and are approximately 6 mm long, though all of these parameters are readily tunable for different applications. These negative features in the mold become ridges in the stamp, that we will later deposit lipids on. Two of the cube's walls have cut-outs large enough to place in a handle for the stamp. Another OpenSCAD script is used to create the cross-shaped handles (see Fig. 2). The mold and handle are printed in acrylonitrile butadiene styrene (ABS) plastic with an Afinia 3D printer. The molds are filled with polydimethylsiloxane (PDMS, Dow Corning Slygard 184), which was 10% by mass curing agent, and the handle is inserted while curing. Molds are put under vacuum in a glass desiccator for 30 minutes before being put in the oven to assist in removing air bubbles. The stamps are baked

for 2 hours at 80 ° Celsius to solidify the PDMS. After baking, the PDMS stamp is allowed to come to room temperature before removal from the mold.

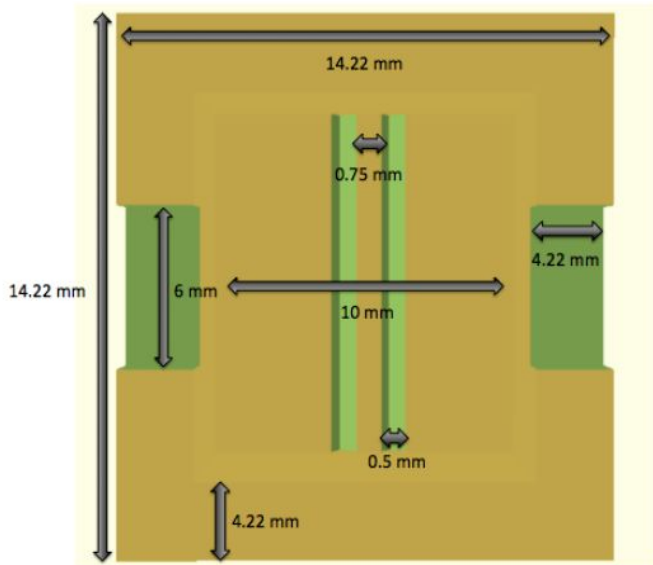


Figure 1: Planar view of 3D printed stamp mold generated with the OpenScad code.

### 1.2 Creating molds by laser cutting

We drew the desired features of the stamp on Raster Engrave, custom software that came with our laser cutter. For the double-lined stamps that are typically used for collisions, the engraved lines are 6 mm long and are spaced 1 mm apart (see Fig. 3A), though these parameters are readily tunable. Two directly adjacent lines were drawn per feature, to add stamp width. This pattern was engraved onto a 12 in. x 12 in. x 1/8 in. piece of clear acrylic. Retina Engrave settings are set to Power 90, Speed 60. Afterwards, we laser cut out the portion of acrylic that we wished to use as the stencil, typically a 2 in. x 1/2 in. x 1/8 in rectangle (see Fig. 4). Vector Cut settings are set to Power 100, Speed 20. We poured a layer of PDMS on top, and allowed it to bake for 2 hours at 80 °Celsius. Next, we peeled off the PDMS layer, which now had raised ridges, and attached it using double sided tape to the flat side of a mini binder clip which served as our stamp handle (see Fig. 5).

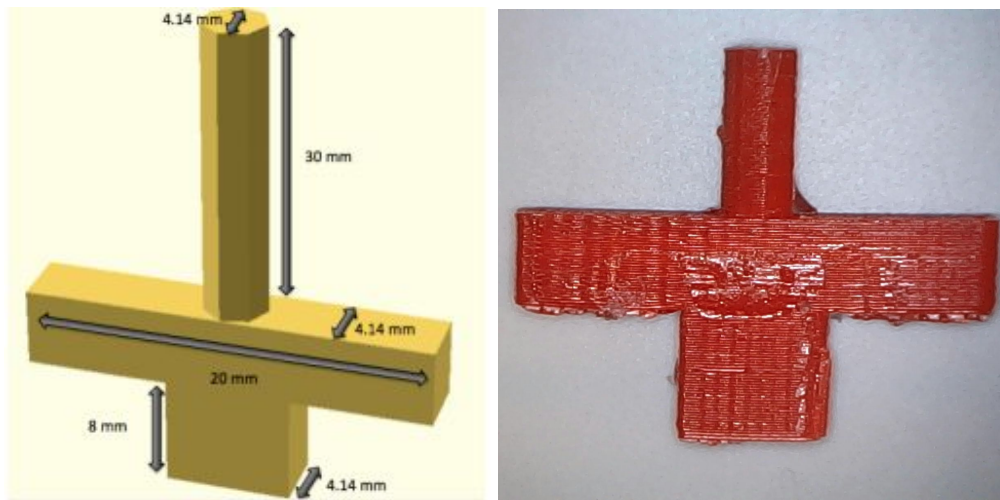


Figure 2: Side view of 3D printed stamp handle.

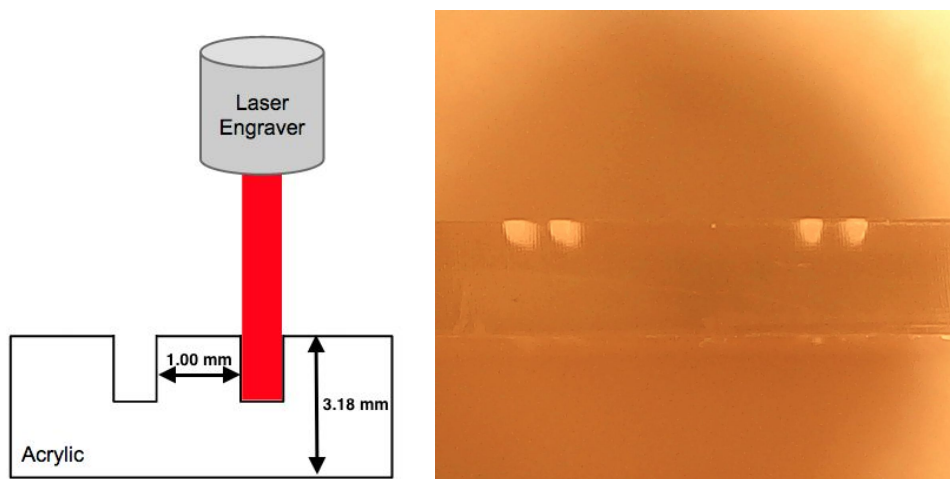


Figure 3: Side view of acrylic stencil created with the laser engraver.

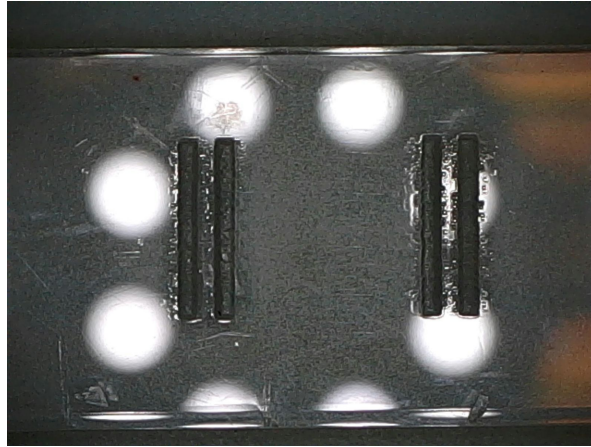


Figure 4: Planar view of laser engraved stamp mold.



Figure 5: Side view of stamp created with the laser cutter.

### 1.3 OpenScad script to produce stamp mold

---

```
difference() {

//Top Square- Triangles Subtracted
translate([-1,1.01,-5])
rotate(a=[90,0,0])
linear_extrude(height=2, center=true, convexity=10)
square([10,10]);

//Square Base- Positive feature triangles sit on top
translate([-1,-1,-5])
rotate(a=[90,0,0])
linear_extrude(height=2, center=true, convexity=10)
square([10,10]);

//Triangle Indentations of Mold
translate([2.75,0,0])
linear_extrude(height=12, center=true, convexity=10)
polygon(points=[[0.5,1],[0.25,0],[1,0]]);
translate([3.5,0,0])
linear_extrude(height=12, center=true, convexity=10)
polygon(points=[[1.25,1],[1,0],[1.75,0]]);}

//Square Walls of Mold
translate([-1,-12,-6])
rotate(a=[0,0,0])
linear_extrude(height=2, center=true, convexity=10)
square([10,14]);
translate([-1,-12,6])
rotate(a=[0,0,0])
linear_extrude(height=2, center=true, convexity=10)
square([10,14]);
difference() {
translate([-1.5,-12,7])
rotate(a=[0,90,0])
linear_extrude(height=2, center=true, convexity=10)
square([14,14]);

//Center Indentation
linear_extrude(height=4.22, center=true, convexity=10)
translate([13.5,-13,0])
rotate(a=[0,180,0])
square([20,4.22]);}
difference() {
translate([9.5,-12,7])
rotate(a=[0,90,0])
linear_extrude(height=2, center=true, convexity=10)
square([14,14]);

//Center Indentation
linear_extrude(height=4.22, center=true, convexity=10)
translate([13.5,-13,0])
```

```
rotate(a=[0,180,0])
square([20,4.22]);}
```

---

## 1.4 OpenScad script to produce stamp handle

---

```
//Top Handle
cylinder(h=30,r=2.07);
linear_extrude(height=2, center=true, convexity=10)
translate([-4,-2.1,5])
square([8,4.14]);
translate([-2,-2.1,11])
rotate(a=[0,90,0])
linear_extrude(height=4, center=true, convexity=10)
square([10,4.14]);
translate([2,-2.1,1])
rotate(a=[0,-90,0])
linear_extrude(height=4, center=true, convexity=10)
square([10,4.14]);
translate([-13,-2.1,8.5])
linear_extrude(height=5, center=true, convexity=10)
square([10,4.14]);
translate([3,-2.1,8.5])
linear_extrude(height=5, center=true, convexity=10)
square([10,4.14]);
```

---

## 1.5 Lipid Deposition

After the stamps were made, they were cleaned and rinsed with ethanol. 1-2  $\mu\text{L}$  of an ethanol/phospholipid (Avanti Polar Lipids) solution were carefully deposited onto each ridge of the stamp using a glass syringe (Hamilton 1701 RN)(see Fig. 6). The stamps were placed in a dark vacuum for at least 2 hours prior to usage.

## 2 GalCer domain formation

### 2.1 Heating/cooling profiles

The formation of GalCer domain requires heating the sample to the liquid-liquid phase and then slow-cooling to form liquid-ordered and liquid-disordered phases[2]. Stamped lipids were kept at room temperature until the bilayers collided as observed by fluorescence microscopy, at which point the lipids were transferred into a warm PBS bath ( 50 °C, 500 mL). This PBS bath, along with the lipids, was placed at room temperature to slow-cool for three hours. This process yielded a cooling curve (Fig. 7) with a rough average rate of 0.08 °C per minute and maximum rate of 0.13 °C per minute. The timeline of an experiment is included (Table 1).

### 2.2 Control experiment of collisional domain formation

In the main manuscript we describe a gradient of domain-forming solution (63.8% POPC + 27% GalCer + 8% Chol. + 1.2% Texas-Red) to pure POPC (100% POPC). This collision yielded gradients of several

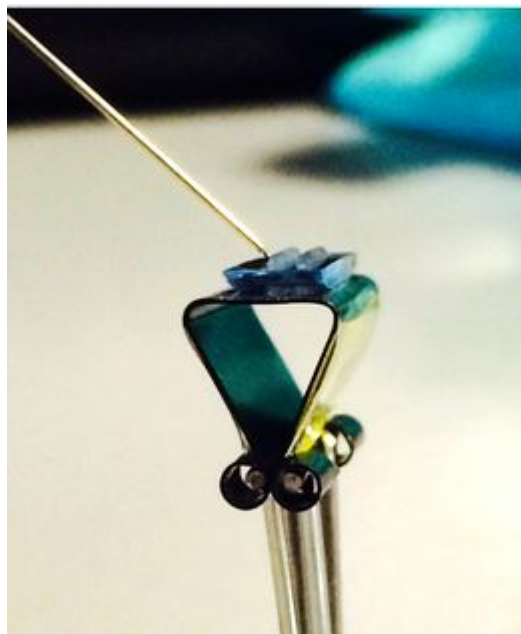


Figure 6: Deposition of lipids onto stamp ridges using a syringe.

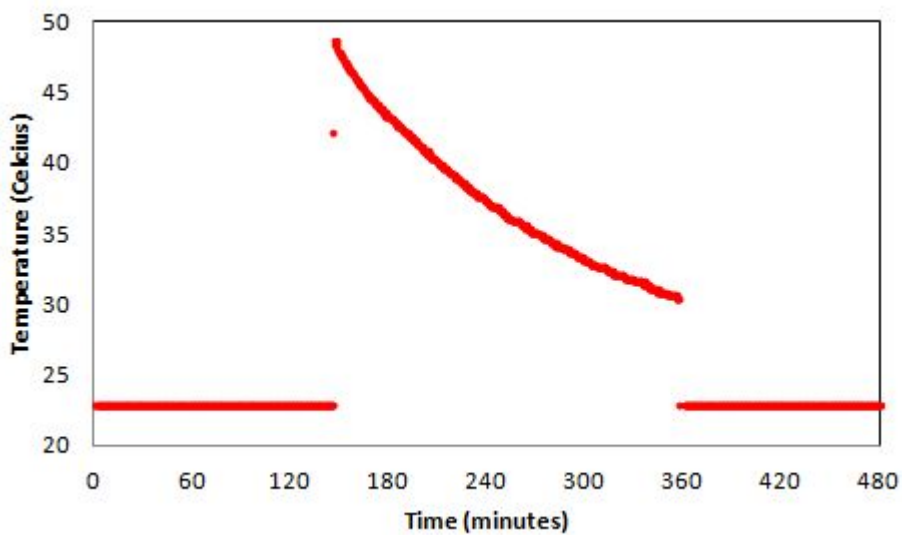


Figure 7: Temperature profile of a sample.

Table 1: Timeline of a GalCer domain gradient experiment

0 - 2.5 hours	Samples spread, collide and mix at room temperature
2.5 hours - 5.5 hours	Samples transferred to a $\approx 50^{\circ}\text{C}$ , 500mL PBS bath to slow cool.
5.5 hours onward	Samples imaged at room temperature

components. We conducted a control experiment by colliding two symmetrical bilayers of the same domain-forming solution, with the exception that only one side was doped with Texas-Red (Fig. 8). This collision yielded a mostly uniform phase behavior, and the fluorescence intensity decreases from the doped side to the non-doped side. There was not a significant gradient in domain characteristics.

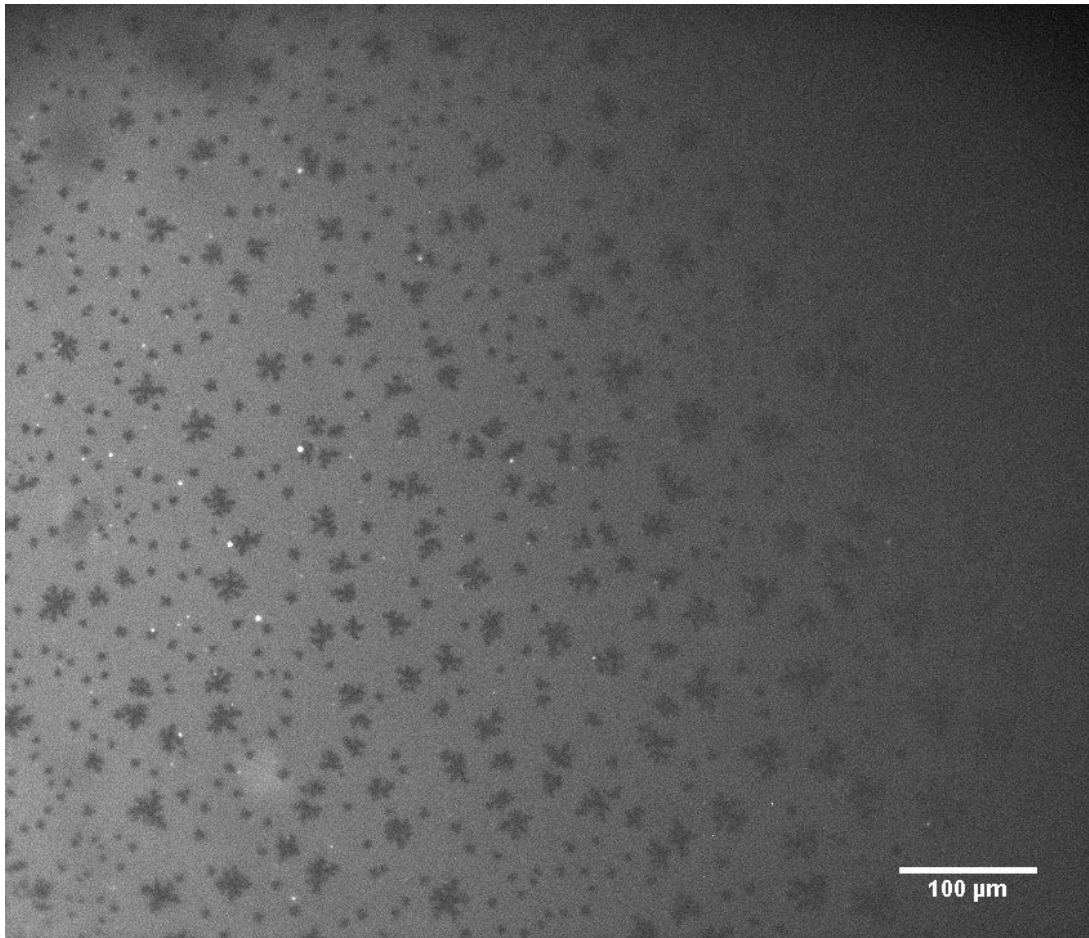


Figure 8: Both the left and right stacks contained similar concentrations (65% POPC + 27% GalCer + 8% Chol.) of components that formed domains, except the left stack was doped with 1% Texas-Red DHPE while the right stack was not. Domains formed in the entirety of the area, and their characteristics do not appear significantly affected by the Texas-Red-DHPE gradient.

### 3 Diffusion coefficient measurement sensitivity to profile placement

Using the POPC collision as a representative dataset, we find the orientation of the profile in the image to be relatively robust to a range of angles. Variations  $\pm 25^\circ$  with respect to the perpendicular of the collision yields a relative standard deviation of 10%. Varying the profile's position from the center to the edge of the image where intensity vignetting is greatest reveals a 13% relative standard deviation in diffusion coefficients (see Figure 9). For comparison, the diffusion coefficients of bilayer placed on glass cleaned by detergent/baking/etching vary by approximately 50%[3].



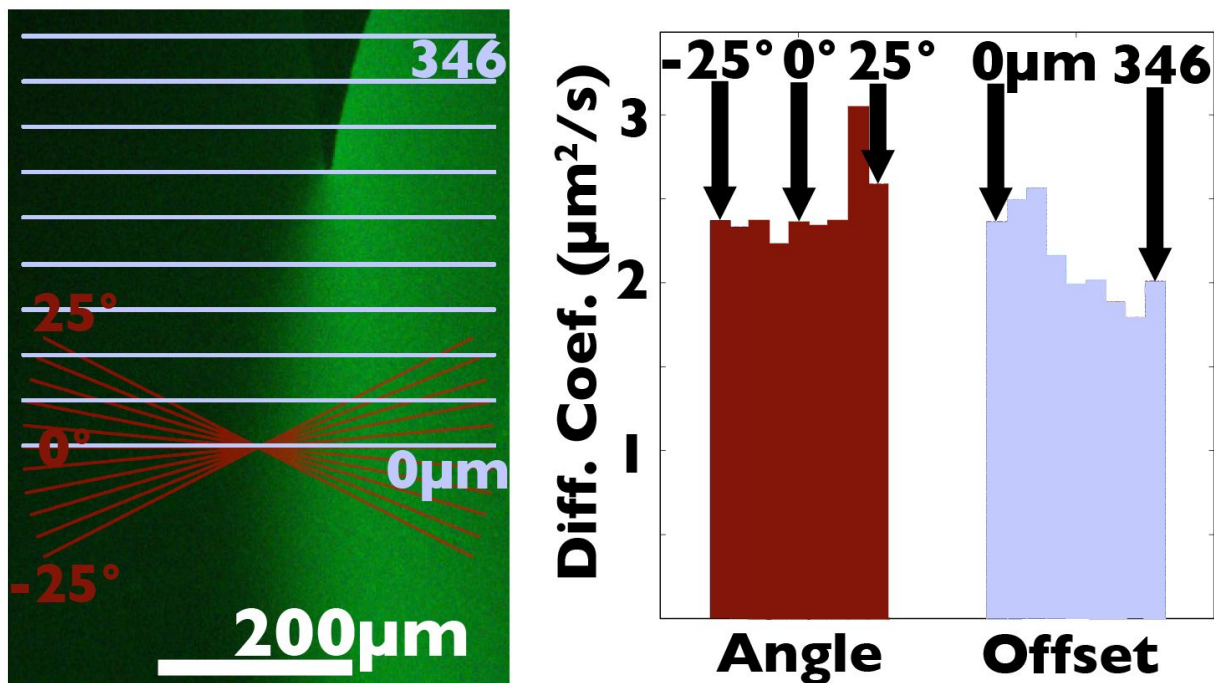


Figure 9: Left: The angles (red) and placements (blue) of profiles analyzed to measure diffusion coefficients. Right: The measured diffusion coefficients for the placement variations.

#### 4 Inhomogeneous spreading and (Eqn. 1)

The analysis that leads to equation 1 of the main text assumes a homogenous distribution of lipids in each spreading membrane at the moment of collision. This approximation is less accurate for some lipids, notably Texas-Red DHPE has been shown to partition strongly to the spreading-front [4], likely due to the membrane's rolling action[5] effectively filtering out Texas-Red DHPE's bulkier headgroup. We tested the effect of this spreading-partitioning on the approximation by colliding a Texas-Red-DHPE doped membrane with an unlabelled membrane, and attempted to fit the fluorescence intensity profile to equation 1. Even with such strong partitioning the system relaxed to a good fit of the equation shortly after the collision (see Fig. 10). Once the profile approximates an erf function we can assume that it will continue to behave per equation 1. This can be shown by re-deriving equation 1 from Fick's second law, using the erf function as an initial condition instead of a step function.

#### 5 Included video

Video is of a collision between POPC membranes, both doped with 3% NBD-DHPE. Images are 2 minutes between frames. Collision scale is shown in figure 1C of the main text.

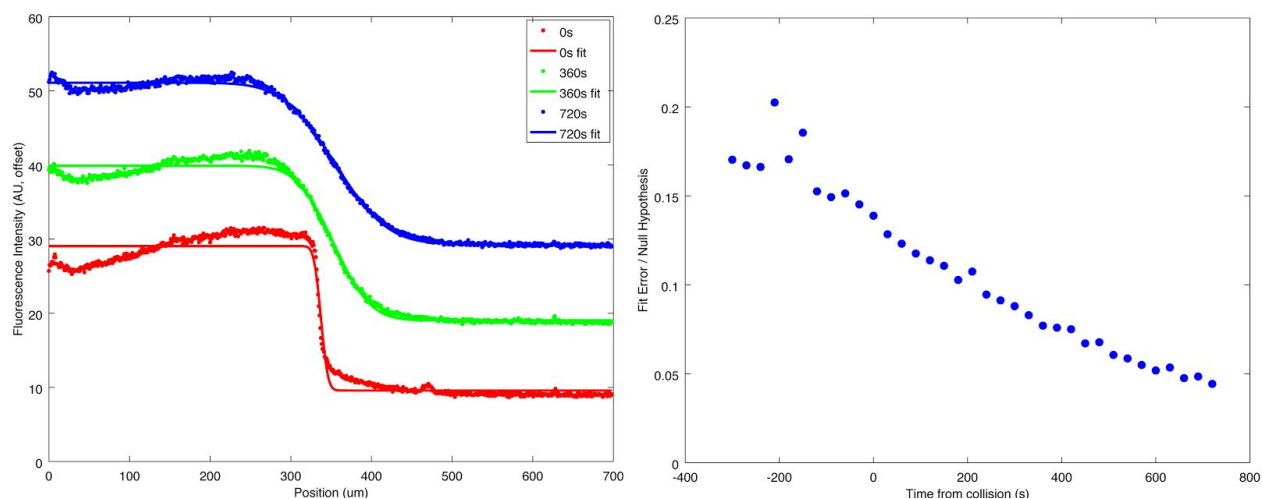


Figure 10: Left: Fluorescent intensity profiles of 1% Texas-Red DHPE in 99% POPC colliding with unlabelled POPC. Red trace is at the moment of collision, where dye partitioning to the spreading front is strongest. Traces are offset vertically for clarity. Right: A measure of how well the profile fits equation 1 as a function of time. Error is defined as (Fit - Data) divided (mean line - Data).

## References

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