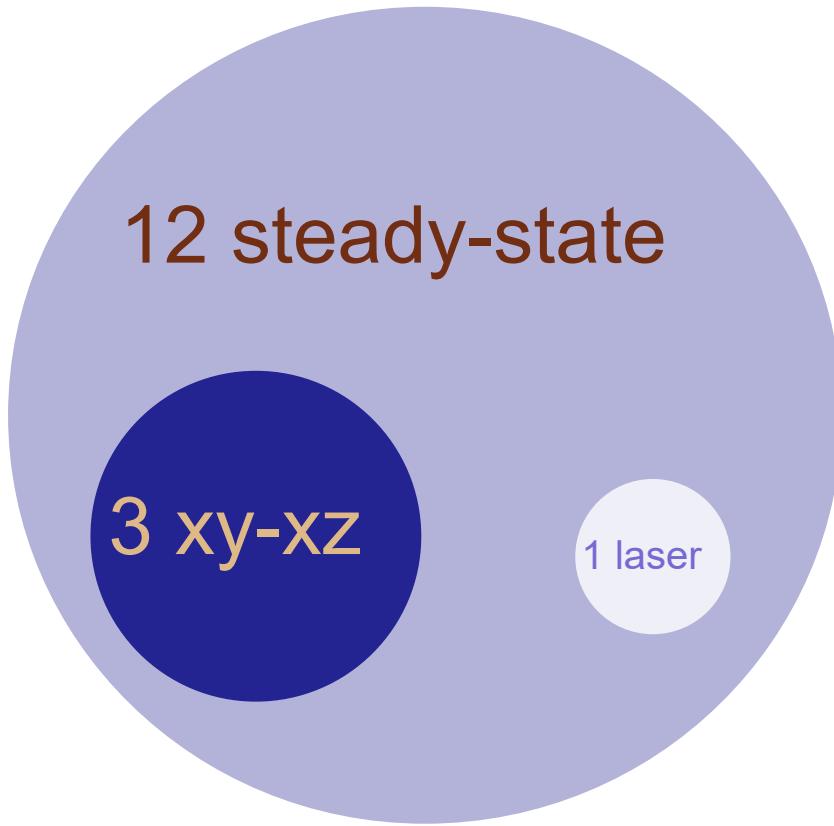


# 0117-0119 Data Analysis

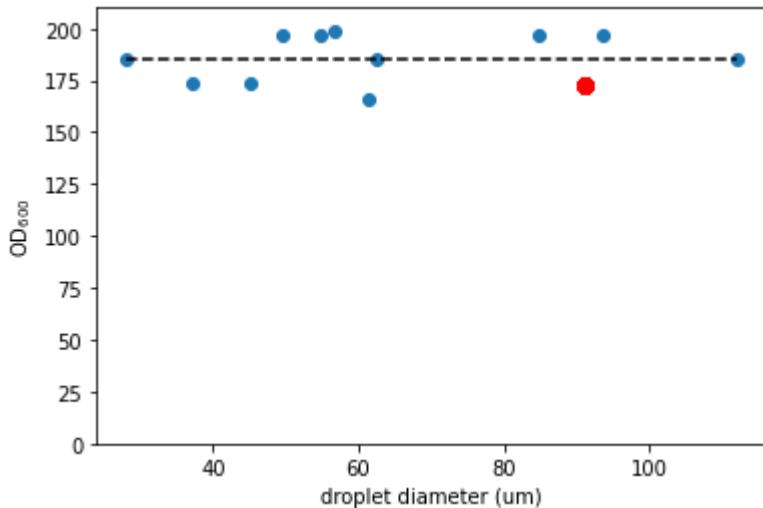
Analysis on the collective motions in droplets data. Since the collective motion is much strong in this data set, I start a new note analyzing the new data.

## I. Overview of the data set

During the 3-day experiment, I have taken 78 videos of 22 different droplets. Most of the droplets contained highly active and dense bacteria (68 videos of 12 droplets), except those for testing percoll and serine effects (10 videos of 10 droplets). Among the 12 droplets, 3 are dedicated to the XY-XZ difference investigation, and all can be used to study the steady-state. [For a complete structured log, click here](#).



The sizes of droplets are carefully chosen, so that they span the range from 28 to 112  $\mu\text{m}$  nicely, providing a good ground for investigating the size dependence. Moreover, the concentrations of bacteria in these droplets center around 185. Strong collective motions are observed in all the droplets in the beginning of each experiment, so the concentration effect can be neglect in the first analysis. The graph below shows the droplet size and OD distribution of this data set.



## II. Experimental subtleties

### A. Serine effect

Bacteria swim significantly faster and last longer with the addition of L-serine. Compare the two videos: [Video: with serine](#) and [Video: without serine](#), difference can be told without further quantification.

### B. Percoll effect

1. Centrifuge with percoll fails. Tried a higher centrifuge speed (5000 g) but still no pellet. Got a very dilute suspension.
2. Adding percoll at the final step makes bacteria less active.

### C. Laser effect

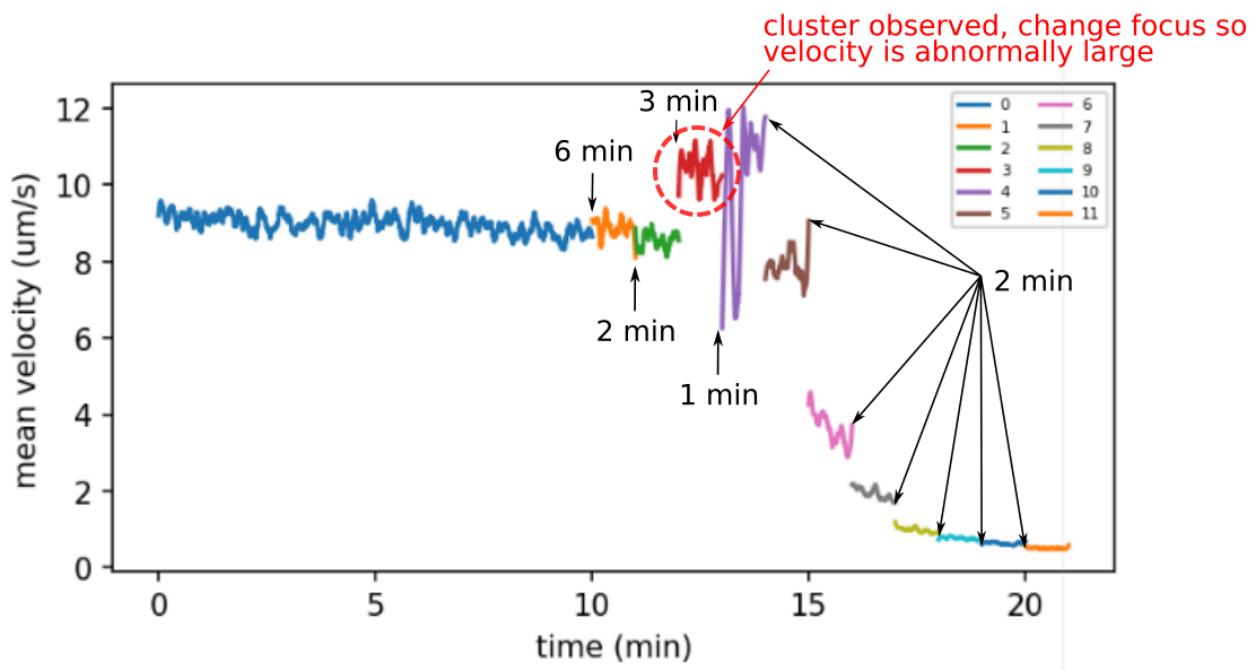
The only bright field video in this data set is a droplet of which  $D = 91 \text{ um}$  diameter and  $OD = 173$  (0117/0-11). We know that activity is a strong function of droplet size, so we compare this droplet with another droplet that has similar size ( $D = 93 \text{ um}$  and  $OD = 197$ , 0118/9-14).

I will compare two quantities: mean velocity and the time scale that activity decreases.

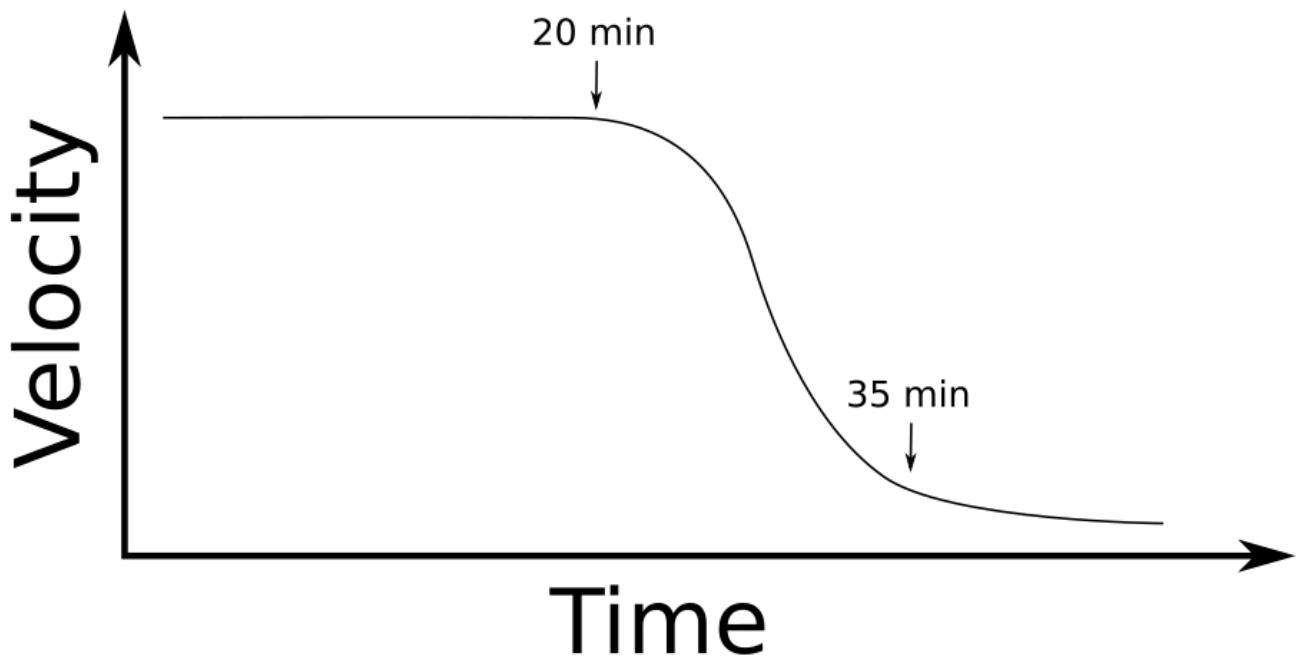
#### 1. Bright field (0117/0-11)

The mean velocity evolution measured by PIV is shown in the following graph. The highest mean velocity captured is around 10 um/s. There are time gaps between the videos, which are not illustrated as lengths in horizontal direction. Instead, they are indicated by text

labels.

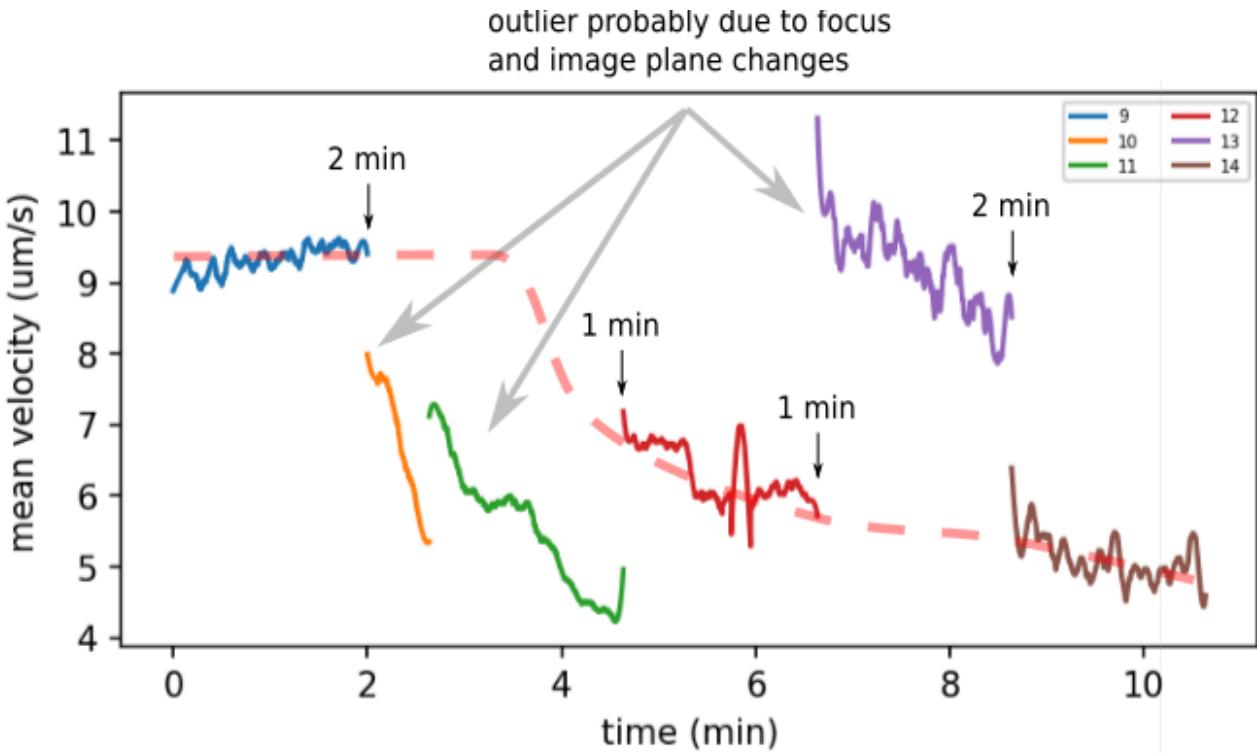


In the first 20 minutes, the mean velocity is roughly constant. Starting from video 3 (red curve), I start to notice the formation of clusters in the middle of the droplet. The activity starts to decrease at roughly this time. So the first 20 minutes can be regarded as "steady state".

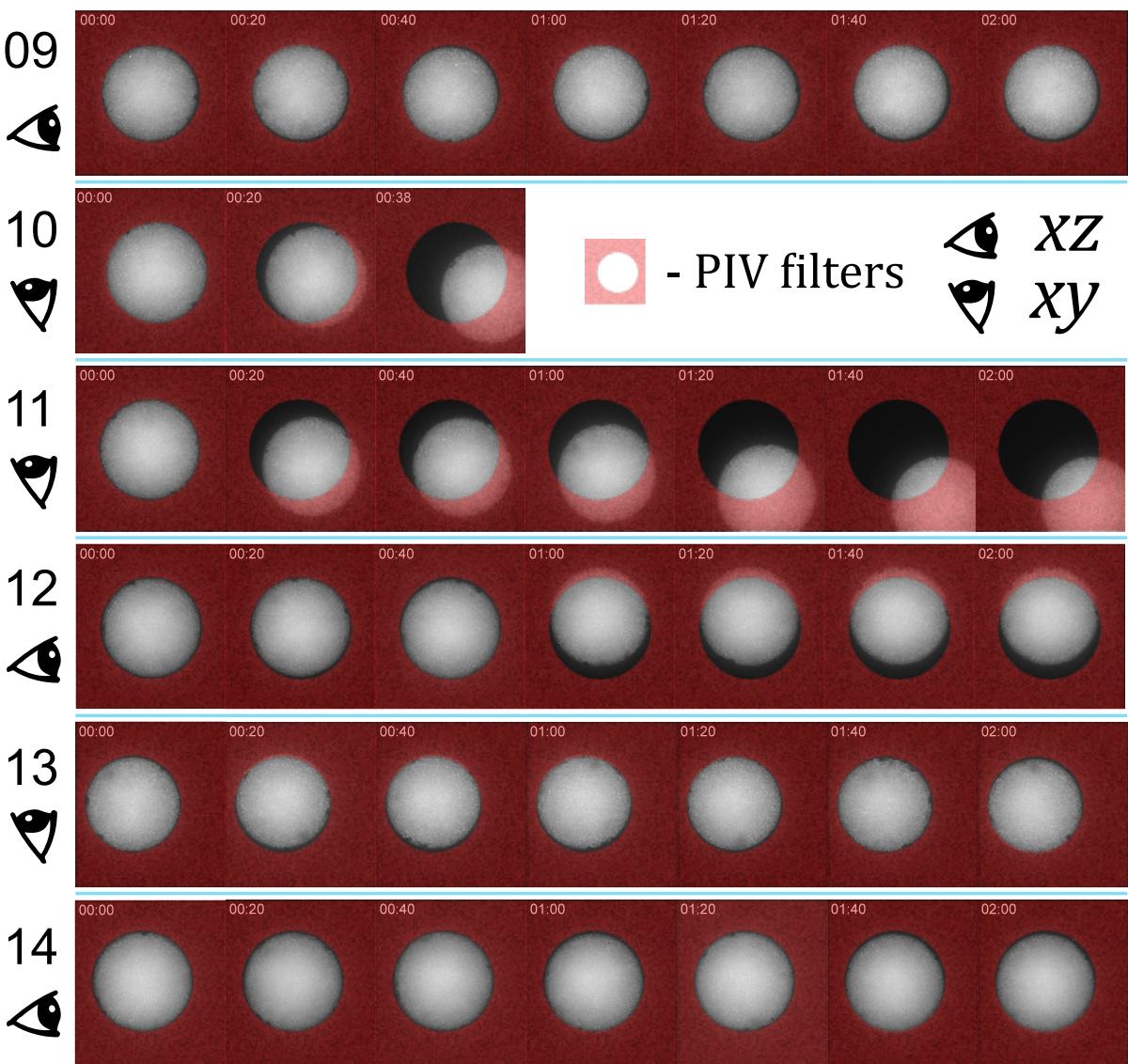


## 2. Confocal, blue laser 11.5% (0118/09-14)

The mean velocity evolution curve is shown below. As the previous one, the time gaps between videos are indicated by text labels.



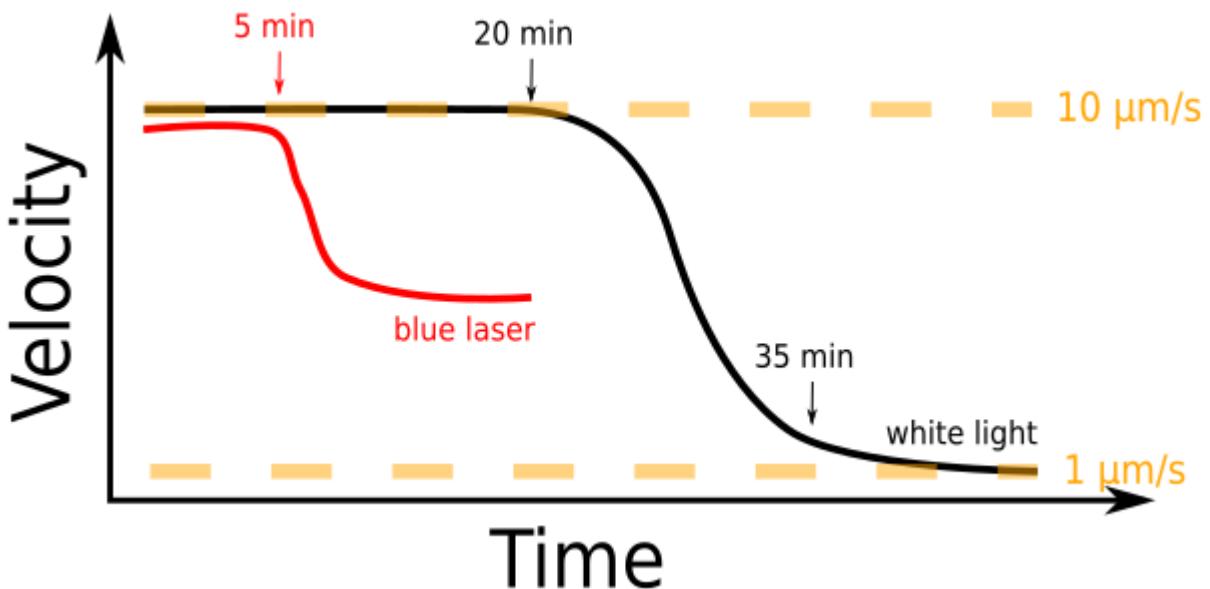
This data set is complicated by some outliers, because I tried to investigate the  $xy$ - $xz$  difference in this experiment, too. Due to the frequent rotations and focus adjustment, the measurements in  $xy$  plane (10, 11, 13) may be inconsistent with the  $xz$  plane (9, 12, 14). Furthermore, the measurement accuracy is affected by the displacement of the droplet during the experiment. In my masked PIV method, a constant mask is provided to each video. If a droplet moves too much, it can escape the mask and thus hinder the velocity detection. Droplets leaving masks in this data set is illustrated in the figure below.



This problem is most pronounced in video 10 and 11, therefore I don't want to include them in the analysis. Since the magnitude of 13 is also abnormally high, I also exclude it in the current analysis. As a result, we are only going to look at videos 9, 12 and 14, which are all imaged in the  $xz$  plane. The velocity evolution in these three videos follows a "constant the decrease" trend nicely, as indicated by the red dashed line in the velocity curve plot.

### 3. Simplified comparison

Below is a simplified velocity evolution of these two samples. Under bright light, the activity of bacteria shows a longer steady state ( $\sim 20$  min), whereas under blue laser, the steady state is much shorter ( $\sim 5$  min).



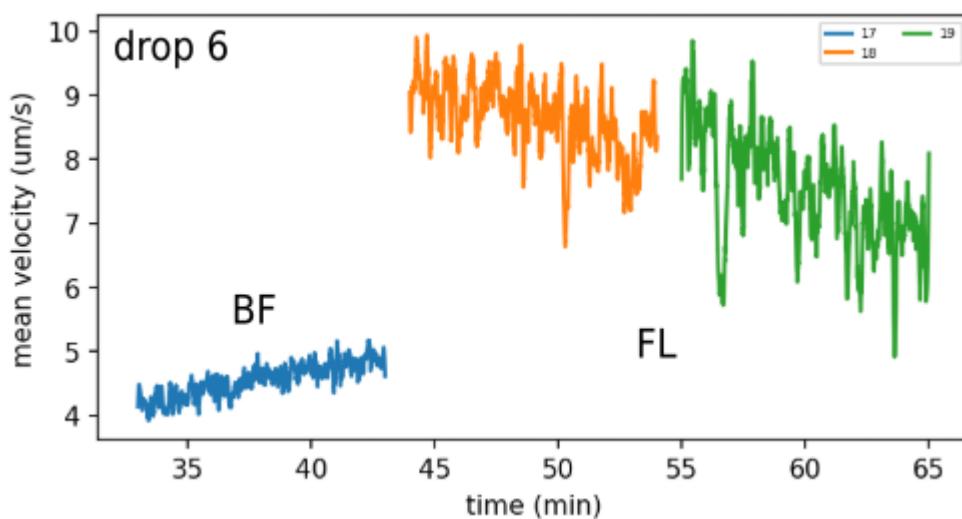
## II. Life time of a droplet

In the previous section, we compared the velocity evolutions of two droplets of similar size under bright light and blue laser. We see a clear effect of blue laser already, which suggests that in order to obtain steady state in the experiment, I should avoid using blue laser. Lasers of other wavelength, or bright field microscopy are the ways to go.

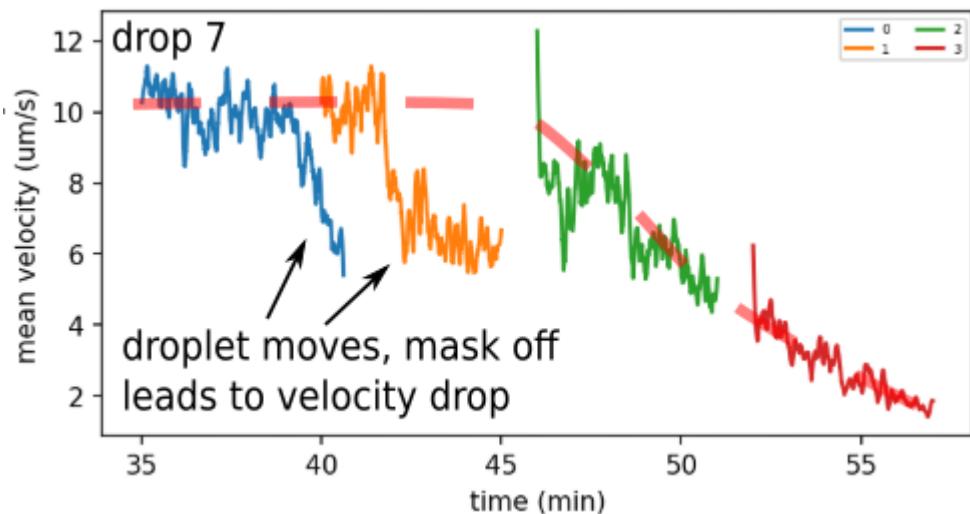
In this section, we summarize the mean velocity evolutions of all other droplets (excluding percoll and MB w/o serine tests). We will see that besides illumination condition, there are other factors that affect the lifetime of a droplet.

### A. Other mean velocity data

Droplet #6 (0117/17-19) | 61 um | 166

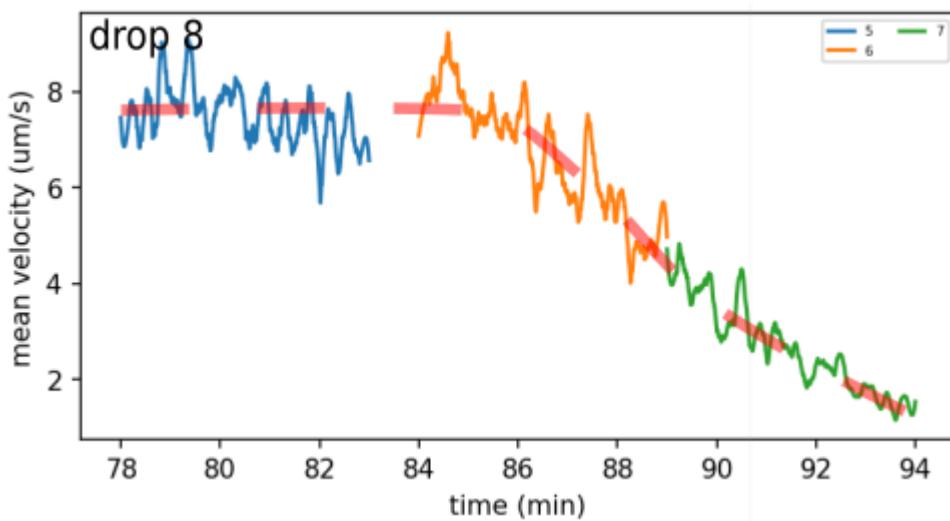


Droplet #7 (0118/00-03) | 55 um | 197

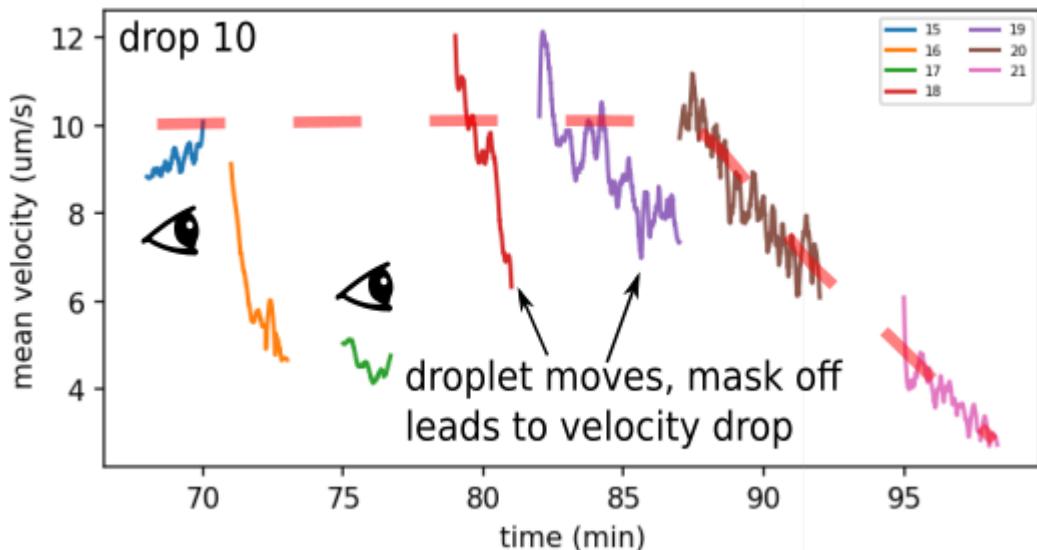


Droplet #8 (0118/05-08) | 50 um | 197

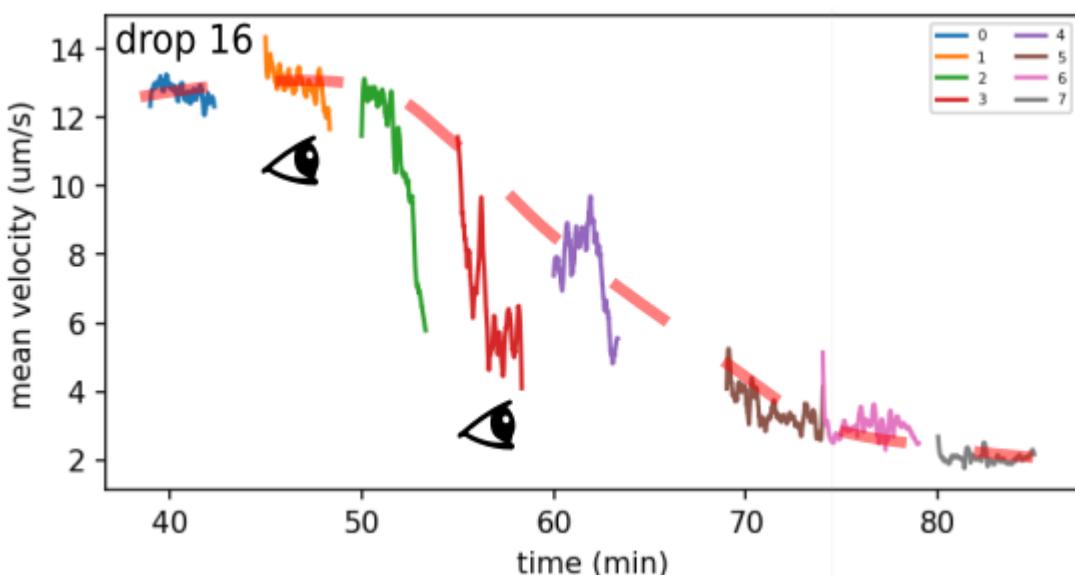
Note: video 08 is a very short video showing the droplet is already frozen, data point is not shown.



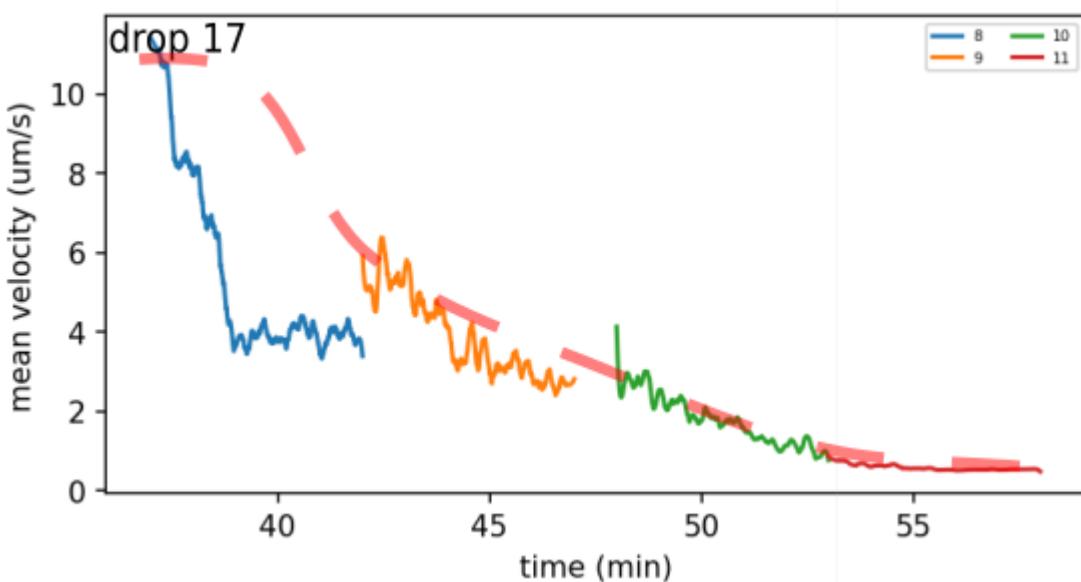
Droplet #10 (0118/15-22) | 85 um | 197 | xyz



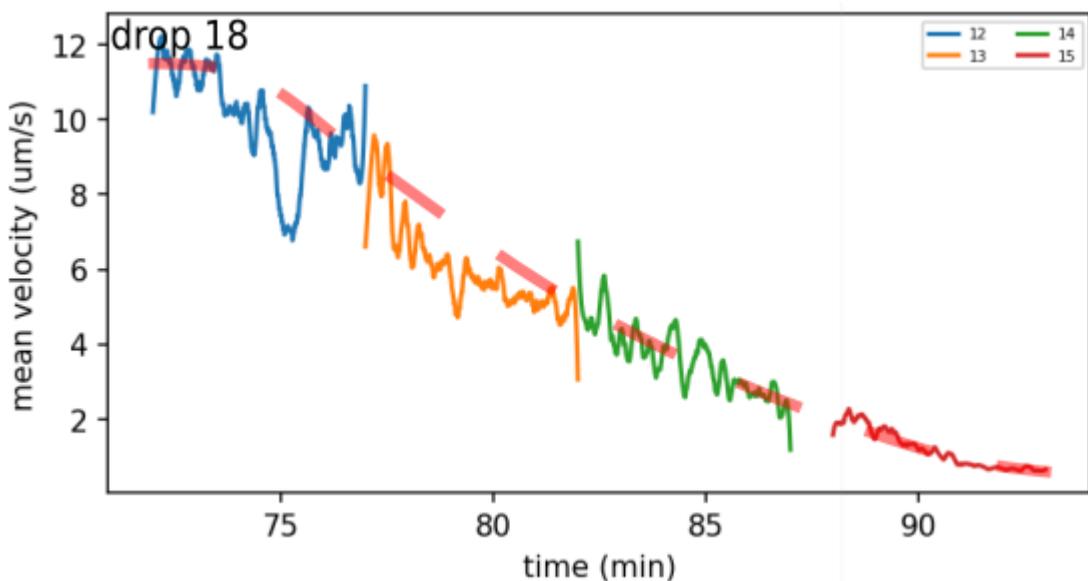
Droplet #16 (0119/00-07) | 62 um | 185 | xyz



Droplet #17 (0119/08-11) | 37 um | 174

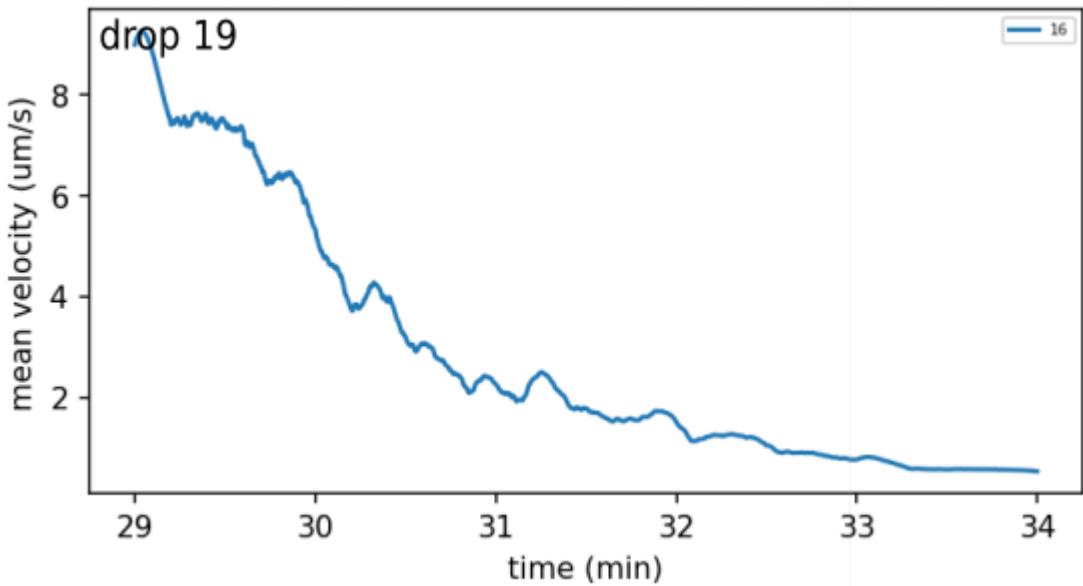


Droplet #18 (0119/12-15) | 45 um | 174

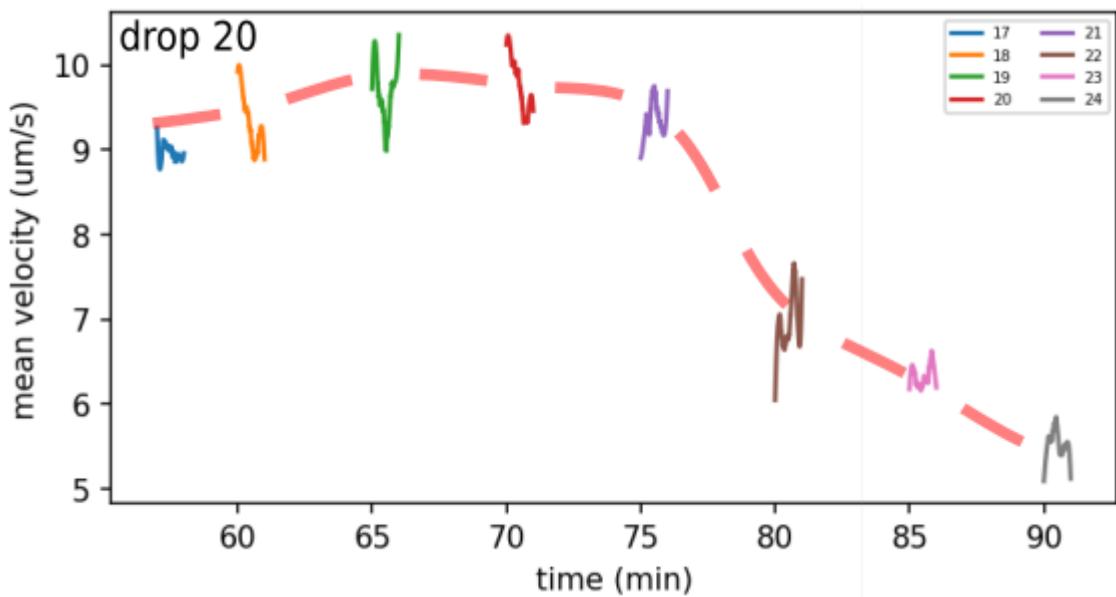


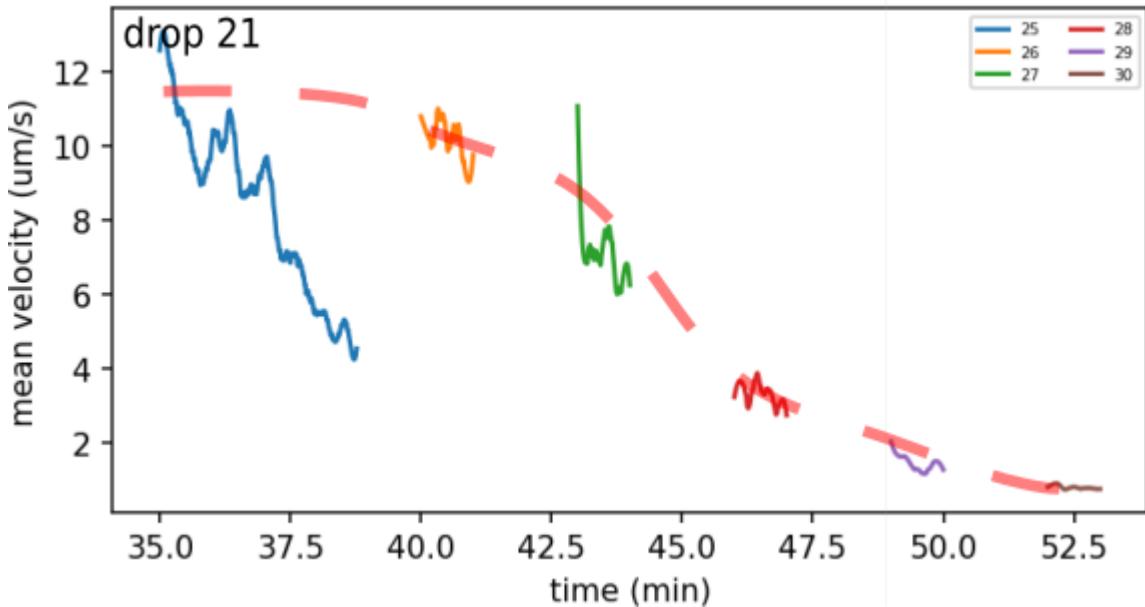
Droplet #19 (0119/16) | 28 um | 185

Note: this is a single video, velocity start to decrease within the first minute.



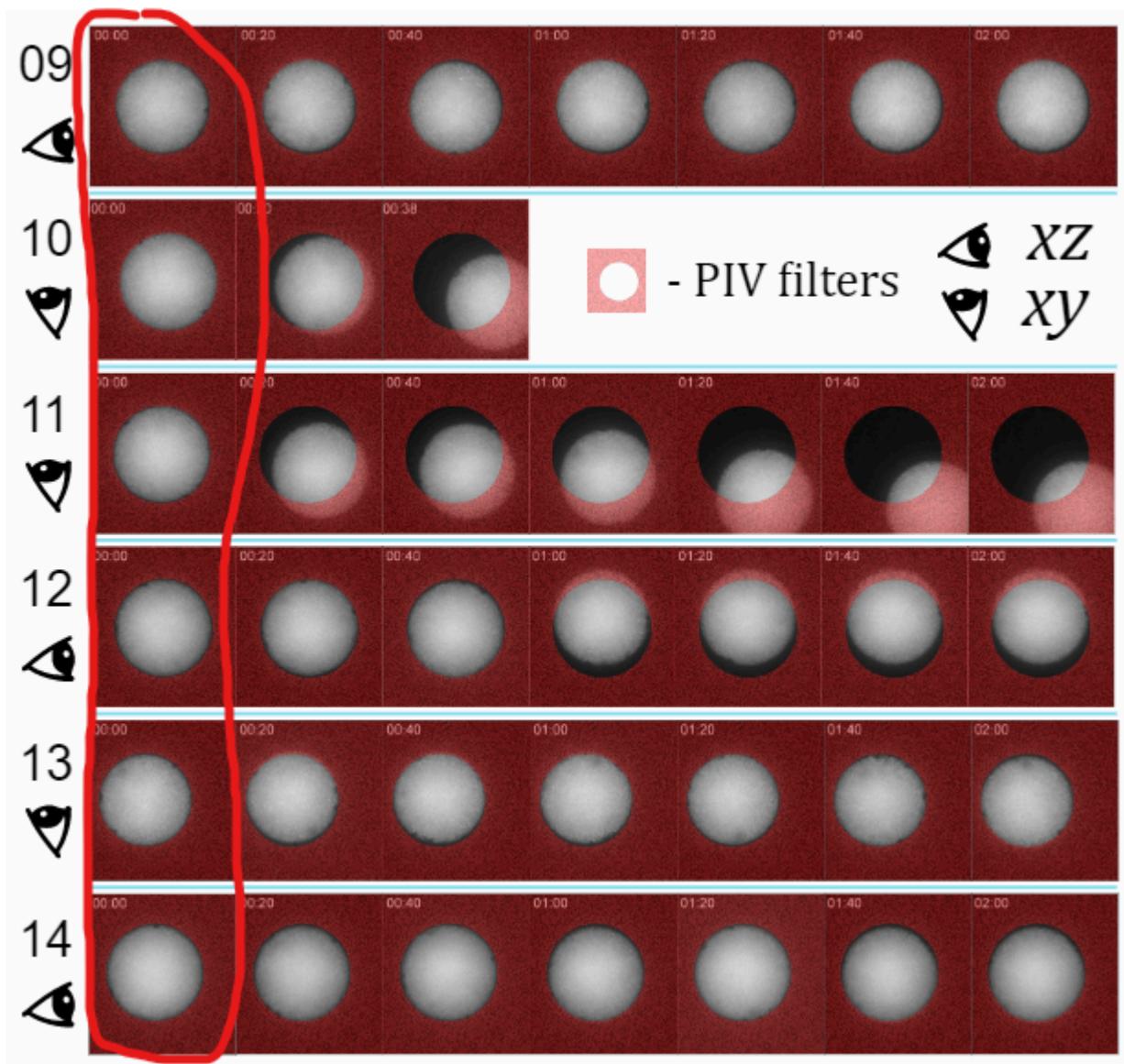
Droplet #20 (0119/17-24) | 112 um | 185



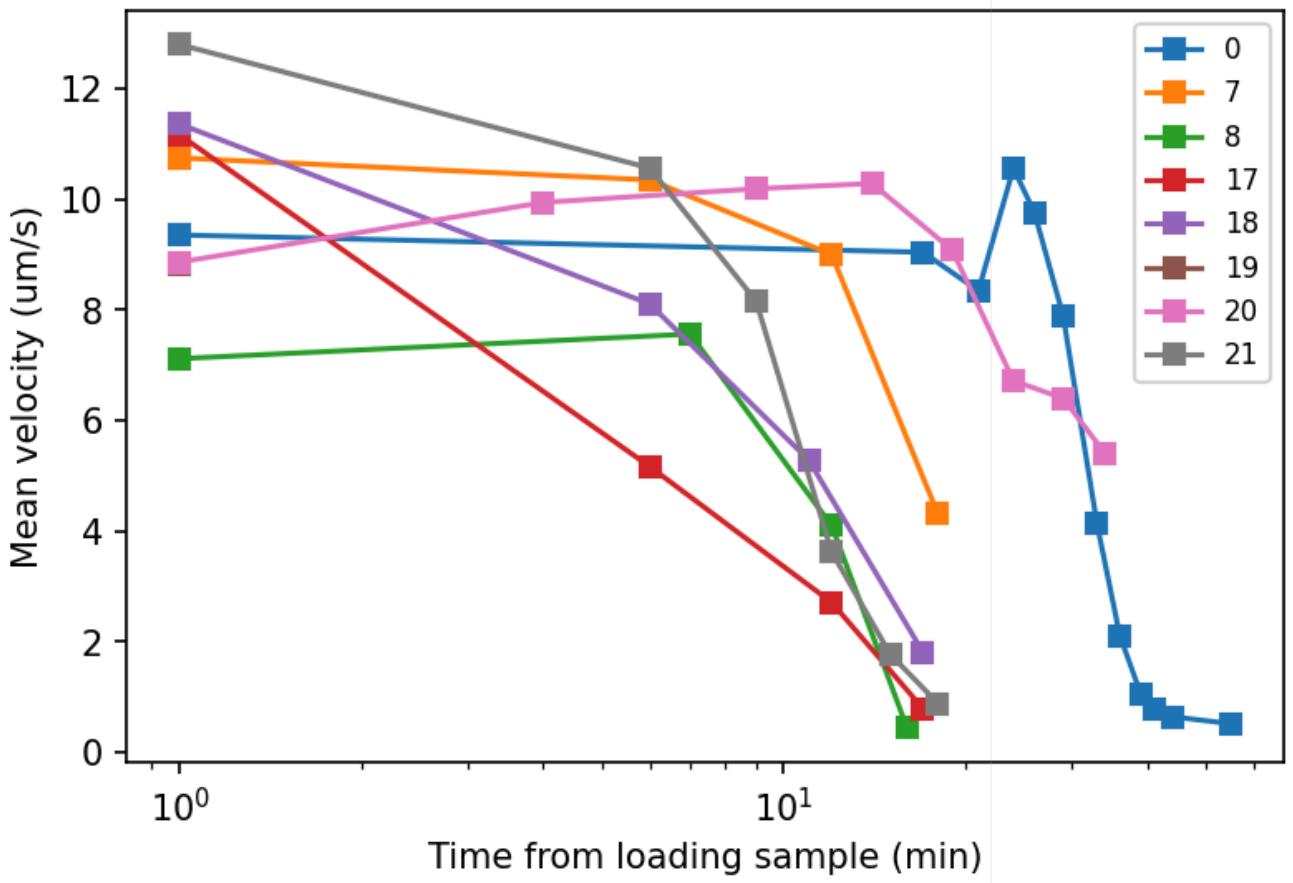


## B. Initial mean velocity evolution

We discussed the issue previously where droplets escape from PIV masks and lead to erroneous velocity measurement. To avoid this error, for each video, we only look at the first 10 s, where the PIV masks are generally more accurate.



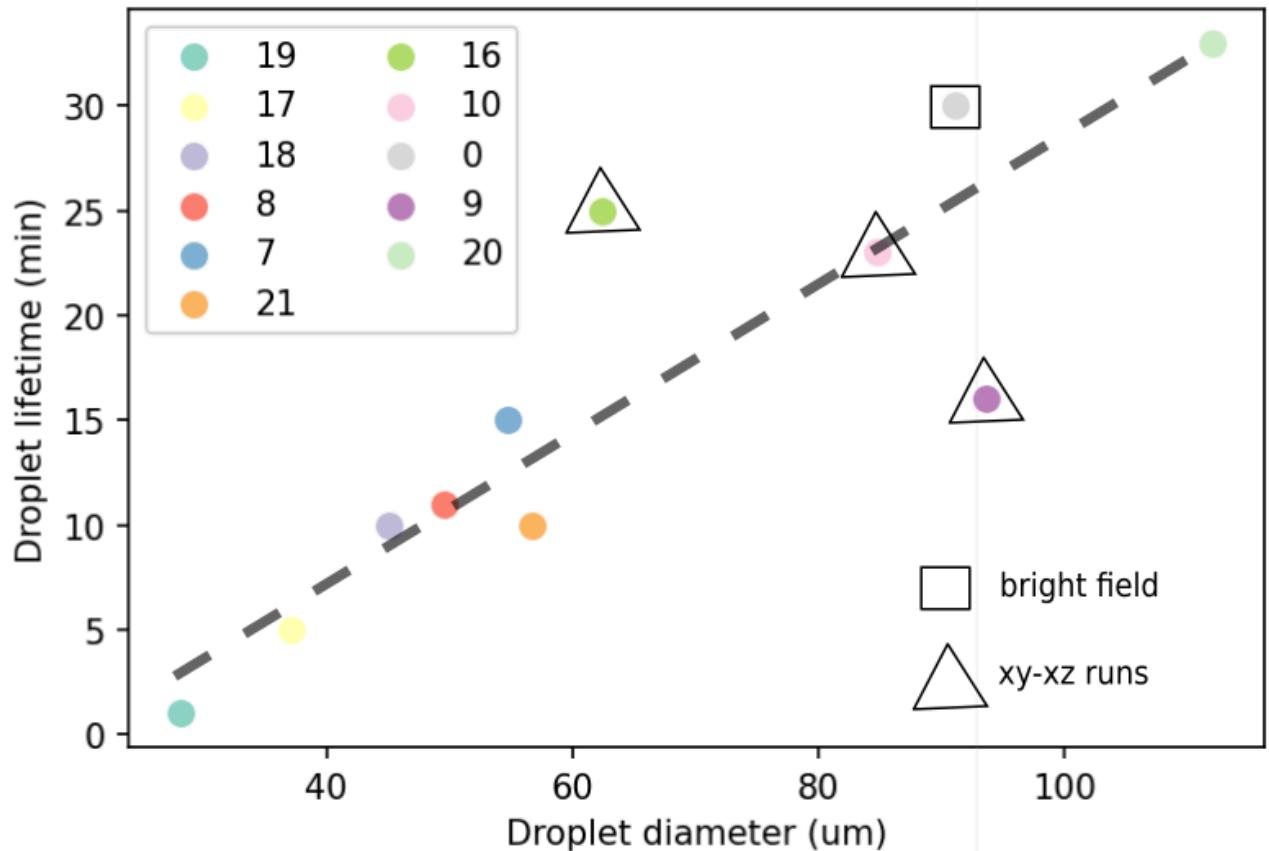
Now, plot initial mean velocity against the time after sample loading.



Note that the  $x$ -axis is in log scale to make the steady-state regimes of all the curves more obvious.

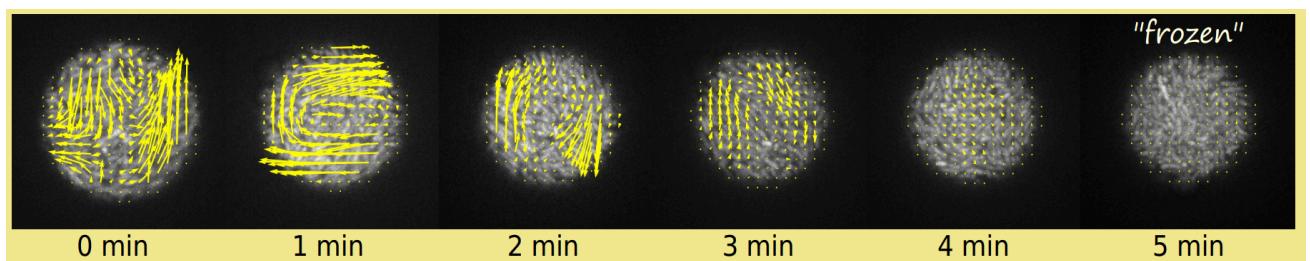
## C. Define an unambiguous lifetime

The first thing come to my mind is to measure the time it takes for the velocity to decrease to a half of the initial velocity. Below, I plot the droplet lifetime against its size, and a linear-like relation is revealed.



### III. Jamming and reactivation

Now we see that bacterial droplets inevitably freeze after some time.



Is this due to "bacteria losing their swimming capability" or "dynamical jamming"? The following experiment, where a "frozen droplet" is reactivated by an active droplet, may provide some insights.

