

# בניית מערכת ללחיצה אקוטית על צברים של תאי עטין פרה

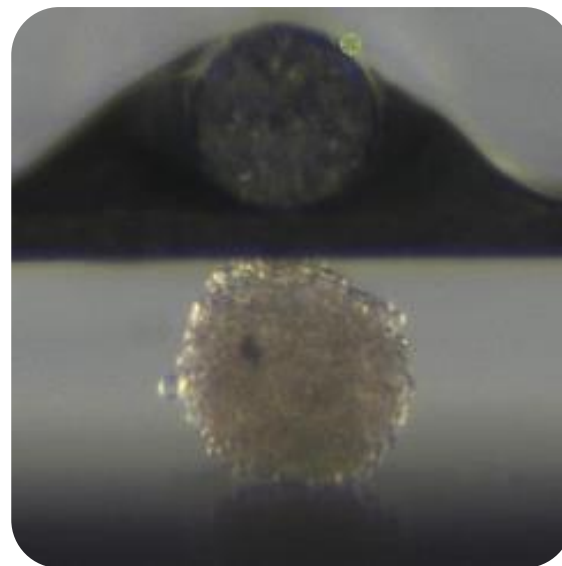
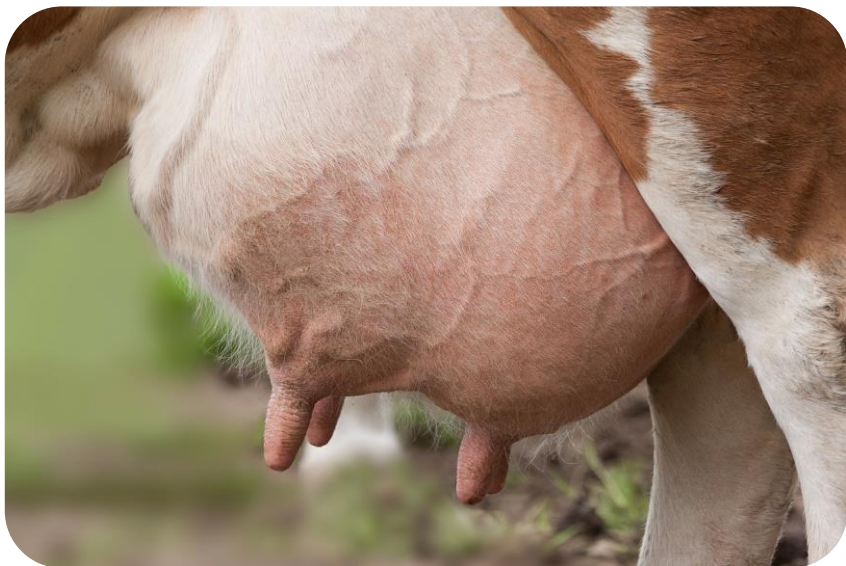
מציג: ניר זהרוני

שיוך: מכון וולקני – מנהל המחקר החקלאי

תאריך: 19.12.24

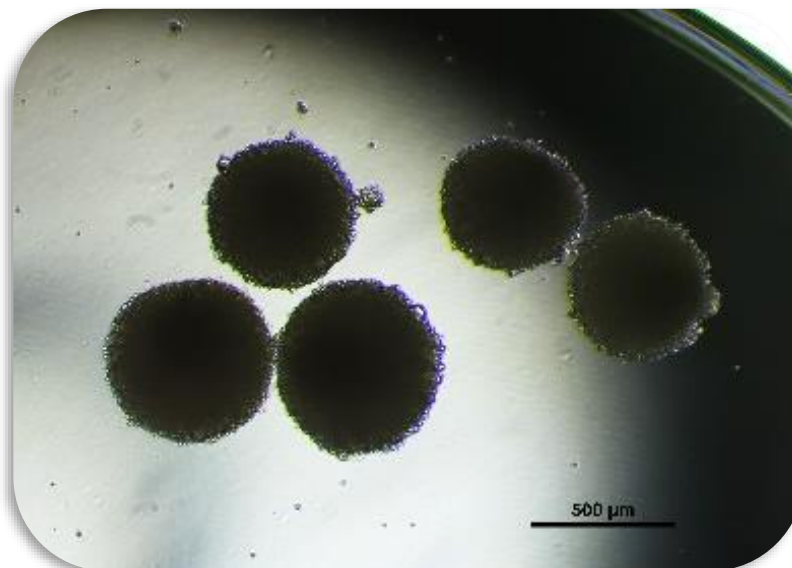


# הדמיית כוחות הלחיצה על הרקמה בעת החליבה



**שימוש בצברי תאים לניסויים:**

- צבר תאים = מבנה תלת-ממדי דמוי כדור
- מורכבים מאלפי תאים הצמודים זה לזה
- גודלם נע בין 100-500 מיקרון



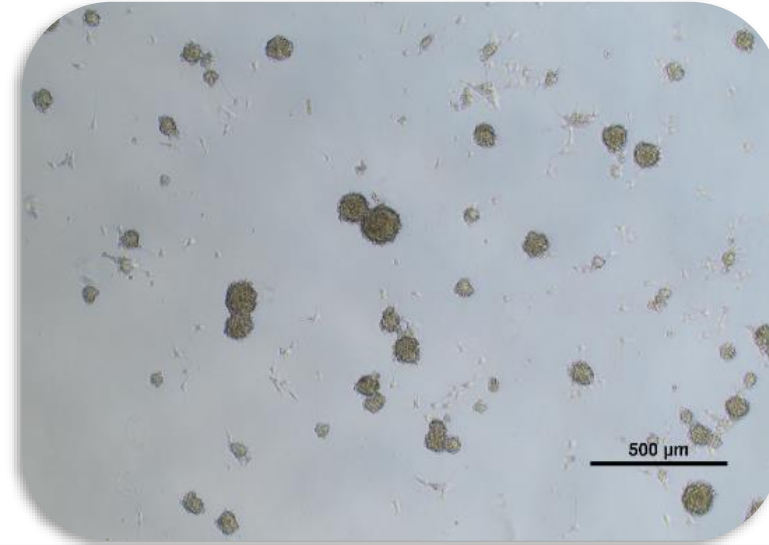
**יתרונות השימוש בצברי תאים:**

- מאפשר ביצוע מספר רב של ניסויים בזמן קצר
- עלויות נמוכות יותר
- מונע פגיעה בבעלי חיים

# מה מייחד את תאי בלוטת החלב?

## אופטימיזציה:

הבנת התכונות המכניות יכולה להועיל לנו בהגברת ייצור רכיבי החלב תוך שמירה על בריאות העטין.

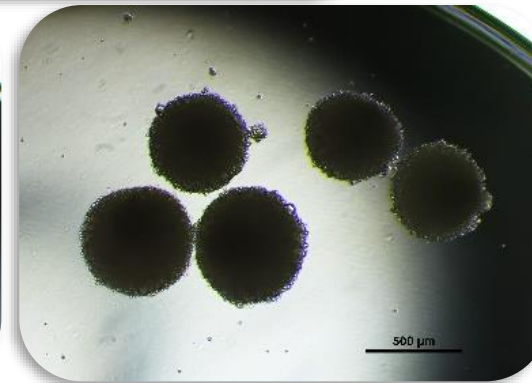
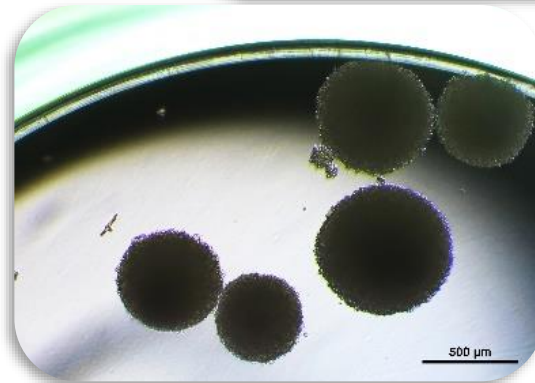


## יכולת הסתגלות:

התאים בעלי יכולת ייחודית להסתגל למגוון רחב של סביבות מכניות משתנות.

## חדשנות:

חקירת תגובות התאים תועיל לנו עבור יישומים חדשניים בחקלאות.



## פונקציונליות:

חיוני לחלוקת תאים, ארגון מרחבי והפרשת חלב יעילה.

# תכנון הניסוי

חזרה על  
התהליך עם  
התרבות  
המטופלת

איסוף המידע  
וניתוחו

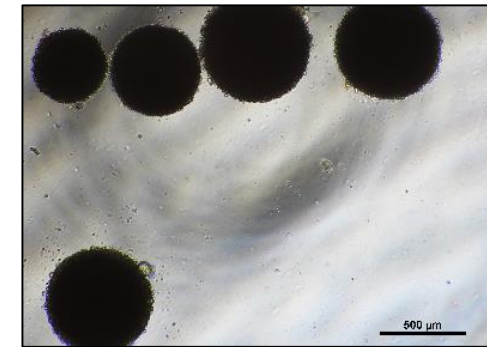
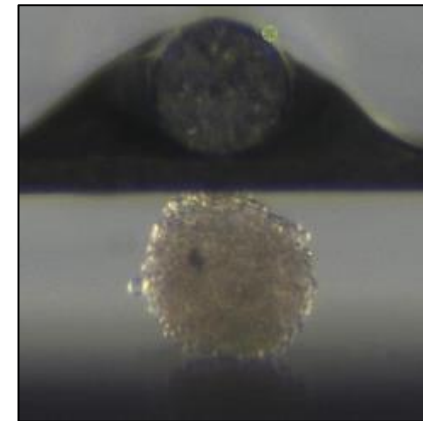
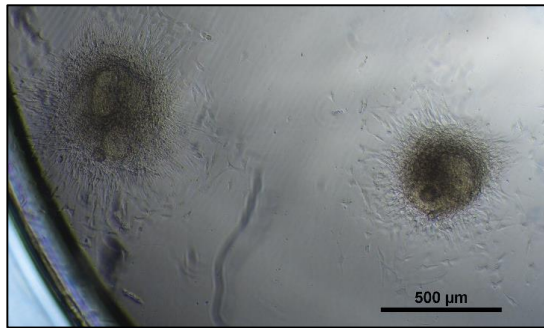
איסוף  
הצברים  
לצלחת  
חדשה  
והמשך ביצוע  
ניסויים

לחיצות על  
הצברים

יצירת תרבית  
המכילה צברי  
תאים

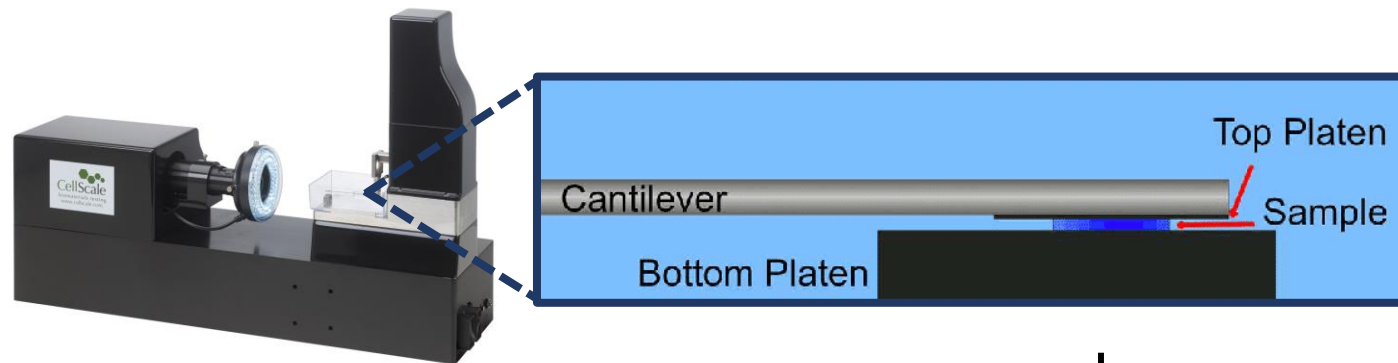
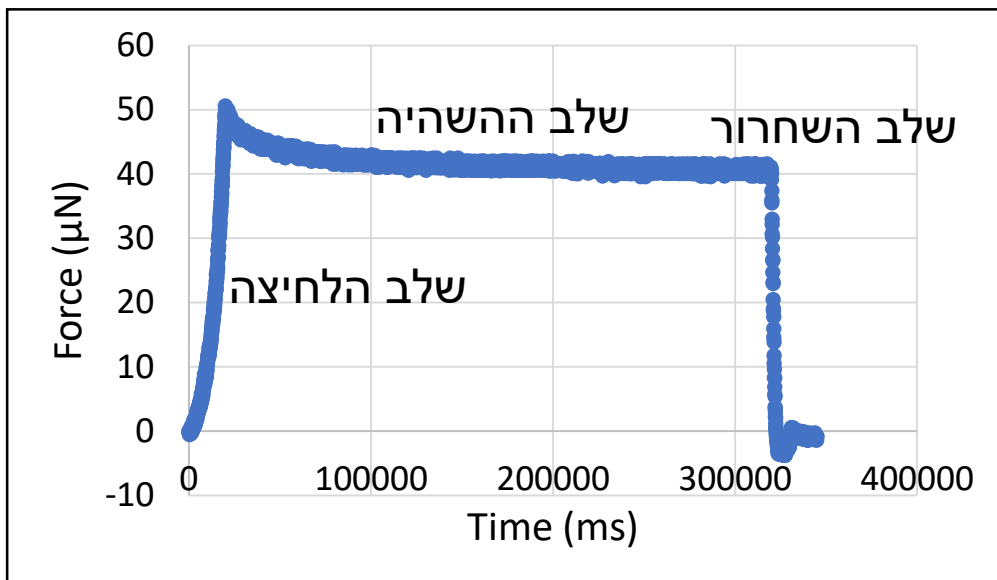


A	B	C	D	E	F	G	H	I	J	K							
Set Name	Cycle	Time(ms)	ZTip	Displ	ZBase	Dis	Current	Sz	ZForce	(uN)	XTip	Displ	XBase	Dis	XForce	(uN)	Tempera
1	70PressS1-Compre	0	0	0	0	274	0	0	0	0	0	0	0	0	0	0	25.2
2	70PressS1-Compre	67	-0.32823	0.7	274.328	0.135452	-0.43764	0	0	0.180603	25.2						
3	70PressS1-Compre	133	0.437641	1.2	273.562	0.108268	0.437641	0	-0.1806	25.2							
4	70PressS1-Compre	200	1.42233	1.5	272.578	-0.09175	-0.43764	0	0	0.180603	25.2						
5	70PressS1-Compre	267	0.437641	1.9	273.562	0.438407	-0.32823	0	0	0.135452	25.2						
6	70PressS1-Compre	333	2.0788	2	271.921	-0.07378	-0.32823	0	0	0.135452	25.2						
7	70PressS1-Compre	400	1.42233	2.4	272.578	0.238387	-0.43764	0	0	0.180603	25.2						
8	70PressS1-Compre	467	2.29762	2.5	271.702	0.042251	-0.43764	0	0	0.180603	25.2						
9	70PressS1-Compre	533	2.95408	3.1	271.046	-0.18739	0.437641	0	-0.1806	25.2							
10	70PressS1-Compre	600	3.06349	3.4	270.937	0.015067	0.437641	0	-0.1806	25.2							
11	70PressS1-Compre	667	3.82936	3.9	270.171	-0.17719	-0.32823	0	0	0.135452	25.2						
12	70PressS1-Compre	733	4.04918	4.2	269.952	-0.06115	1.20351	0	-0.49696	25.2							
13	70PressS1-Compre	800	4.81405	4.7	269.186	-0.2534	-0.43764	0	0	0.180603	25.2						
14	70PressS1-Compre	867	4.81405	5.1	269.186	-0.04707	0.437641	0	-0.1806	25.2							
15	70PressS1-Compre	933	4.92347	5.6	269.077	0.072851	-0.43764	0	0	0.180603	25.2						
16	70PressS1-Compre	1000	5.68934	6.1	268.311	-0.03887	0.328231	0	-0.13545	25.2							
17	70PressS1-Compre	1067	6.45521	6.6	267.545	-0.14659	0.547052	0	-0.22575	25.2							
18	70PressS1-Compre	1133	6.66462	7.1	267.435	0.0146	-0.43764	0	0	0.180603	25.2						
19	70PressS1-Compre	1200	7.33049	7.4	266.67	-0.09512	1.31292	0	-0.54181	25.2							
20	70PressS1-Compre	1267	8.20578	8	265.794	-0.33252	-0.32823	0	0	0.135452	25.2						
21	70PressS1-Compre	1333	8.31519	8.3	265.685	-0.13007	1.31292	0	-0.54181	25.2							
22	70PressS1-Compre	1400	8.31519	8.4	265.685	-0.00627	-0.43764	0	0	0.180603	25.2						
23	70PressS1-Compre	1467	8.4246	8.9	265.575	-0.01015	0.437641	0	-0.1806	25.2							
24	70PressS1-Compre	1533	9.19047	9.4	264.81	-0.11967	0.547052	0	-0.22575	25.2							
25	70PressS1-Compre	1600	9.19047	10	264.81	0.080468	-1.31292	0	0	0.180603	25.2						
26	70PressS1-Compre	1667	10.0658	10.4	264.824	-0.02743	-0.43764	0	-0.180603	25.2							
27	70PressS1-Compre																



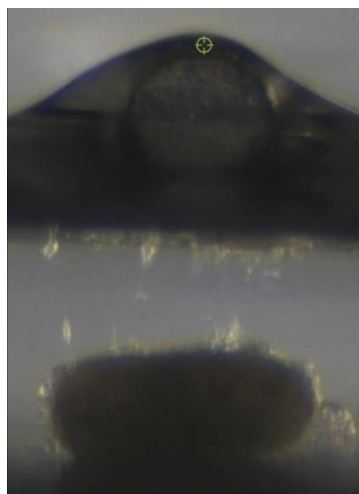
4X

# מכשיר המיקרוטסטר

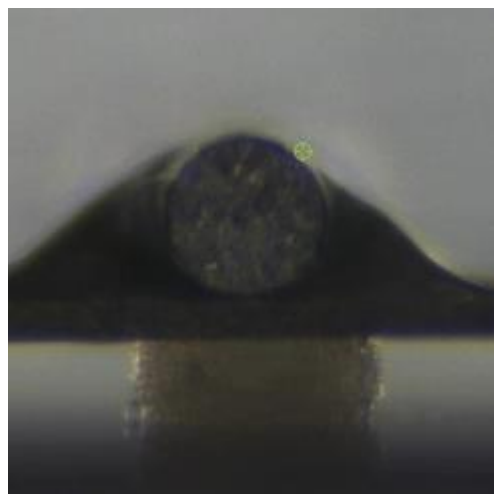


**דיוק:** הפעלת כוח מבוקר ומדויק.  
**שלבים:** לחיצה, השהיה, שחרור ומנוחה.  
**ניתור:** איסוף נתונים בזמן אמת.  
**אנליזה:** מחקר התנהגות מפורט.

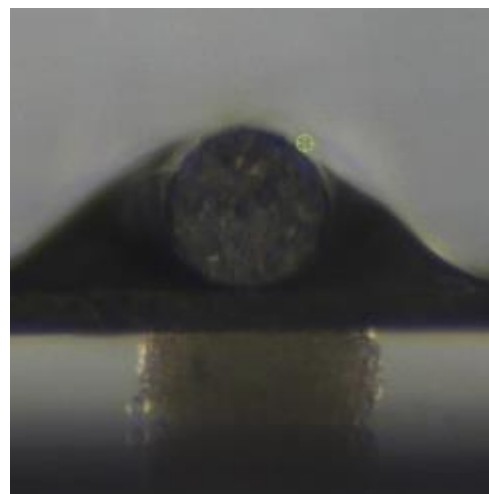
שלב המנוחה



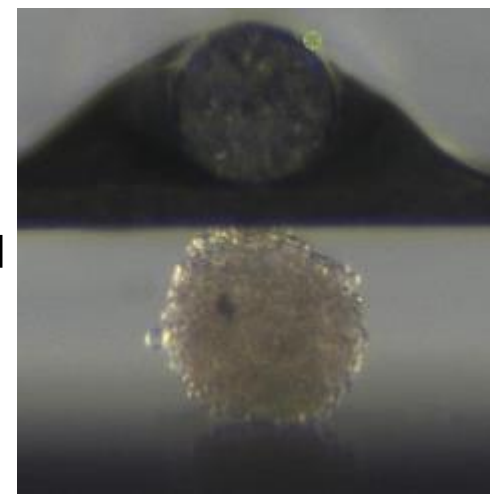
שלב השחרור

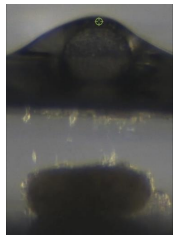


שלב ההשהיה



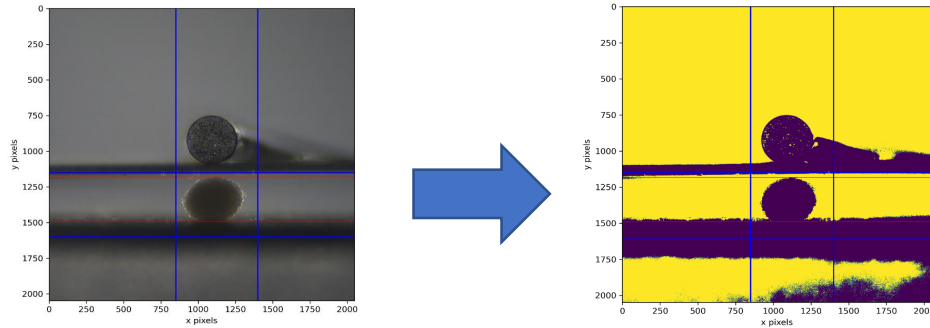
שלב הלחיצה



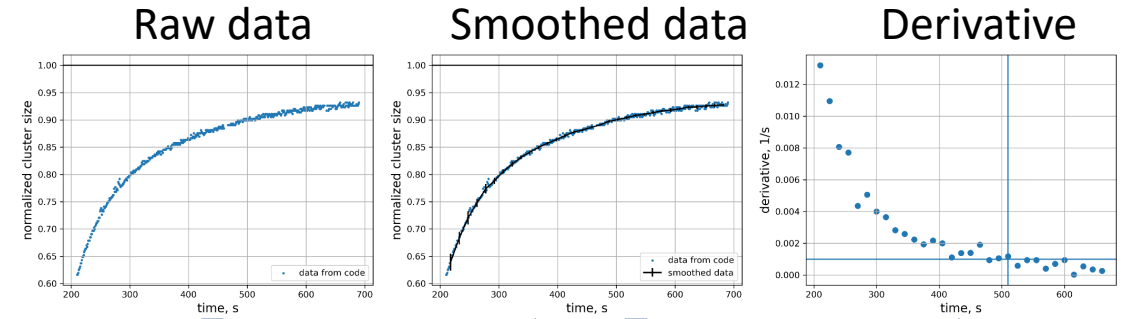


# שלב המנוחה – התאוששות של הצברים

## Determination of the cluster size



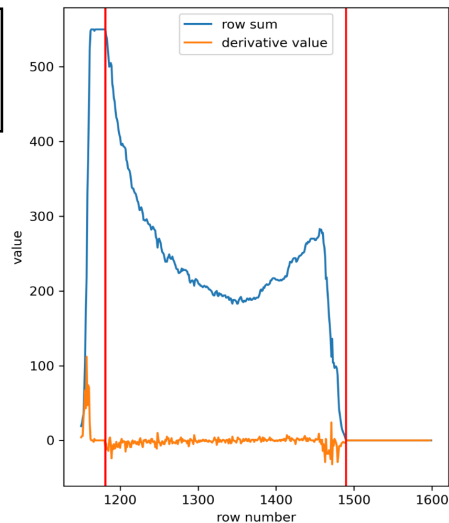
We choose the best threshold and brightness and transform the image to binary image (black & white photo). We focus on the middle blue segment.



Average to every 10 points in the graph.

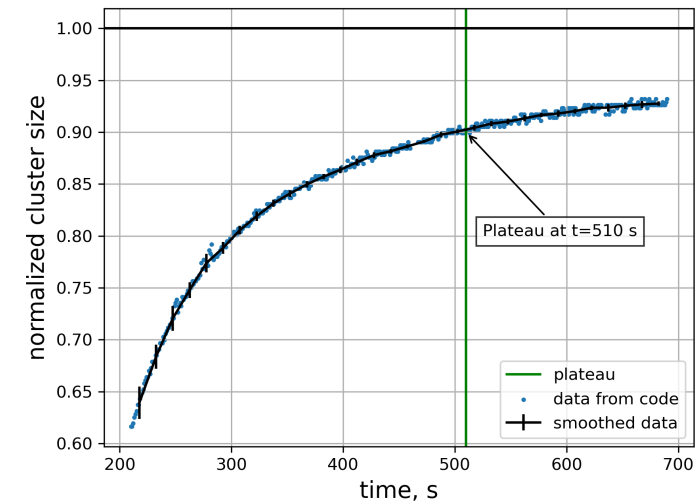
Derivative to the smoothed graph

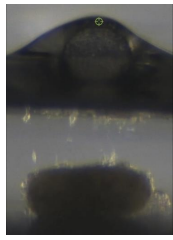
Blue graph –  
Number of white  
pixels in each row



Orange graph –  
derivative of blue graph

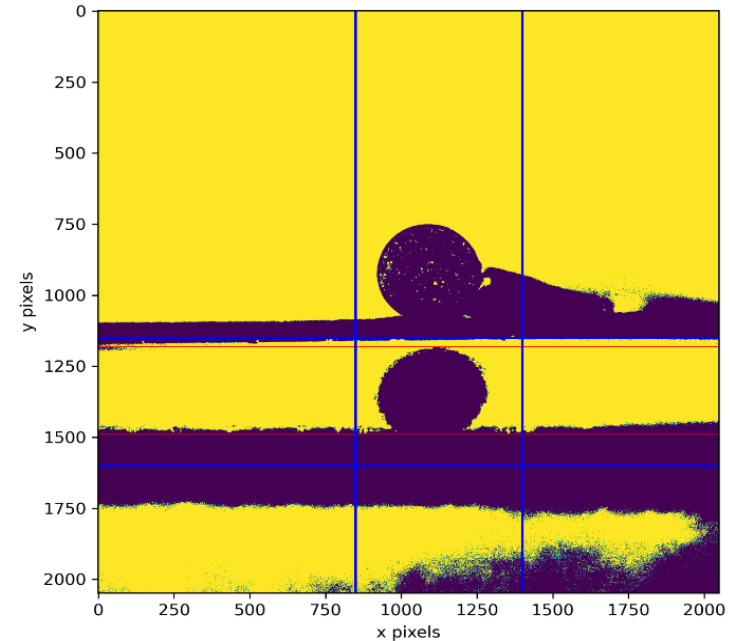
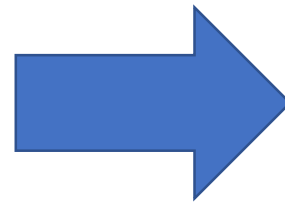
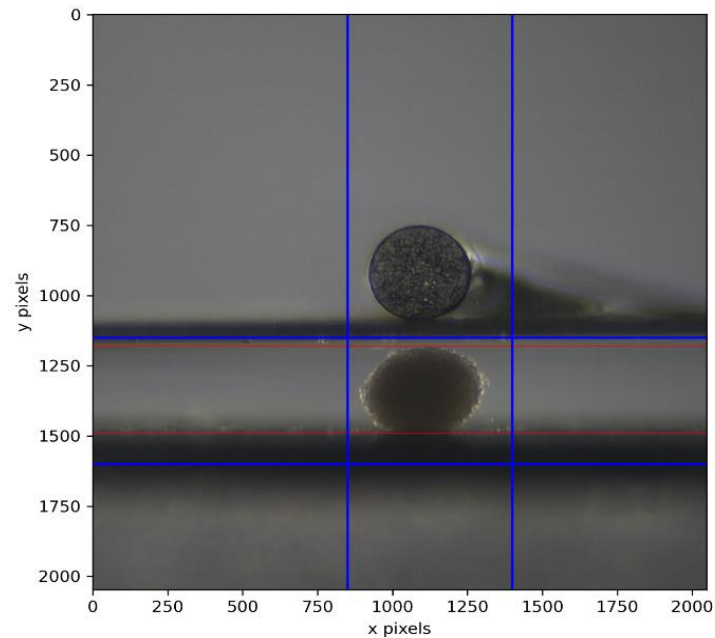
## Final results



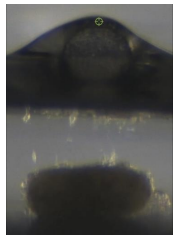


# שלב המנוחה – התאוששות של הצברים

## Determination of the cluster size



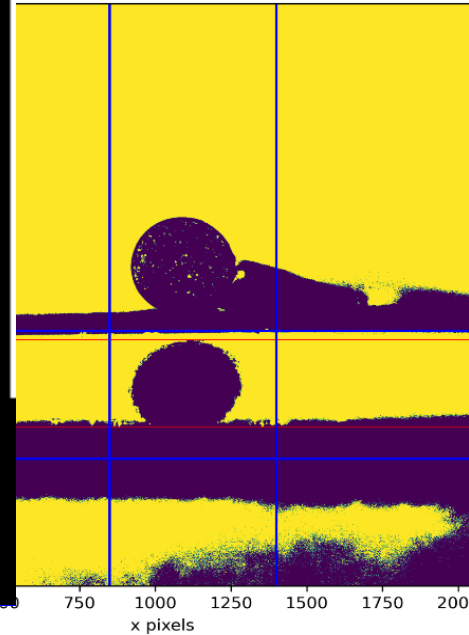
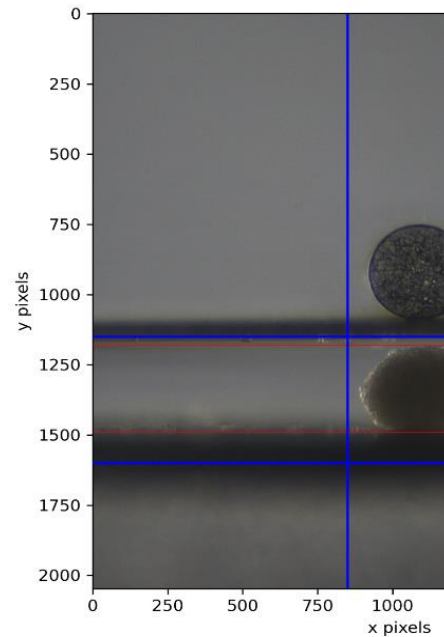
We choose the best threshold and brightness and transform the image to binary image (black & white photo). We focus on the middle blue segment.



# שלב המנוחה – התאוששות של הצברים

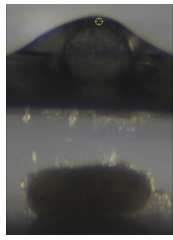
Detect

size



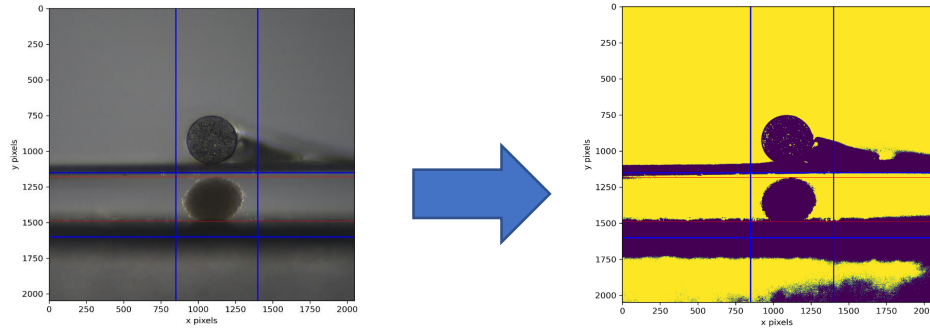
We choose the best threshold and brightness and transform the image to binary image (black & white photo). We focus on the middle blue segment.



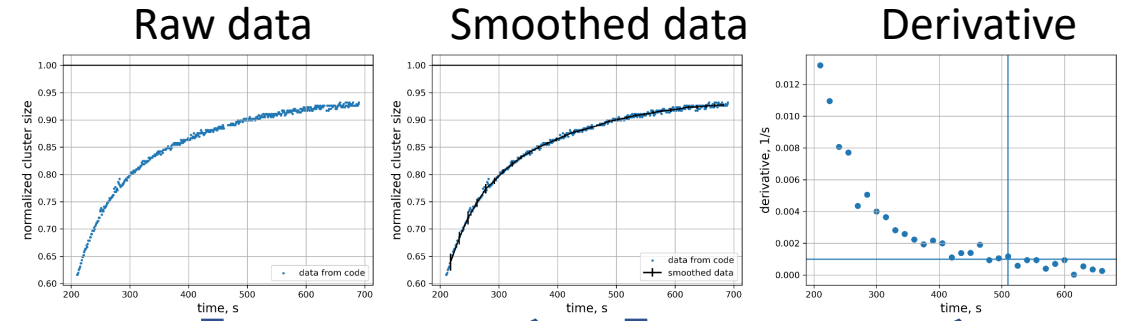


# שלב המנוחה – התאוששות של הצברים

## Determination of the cluster size



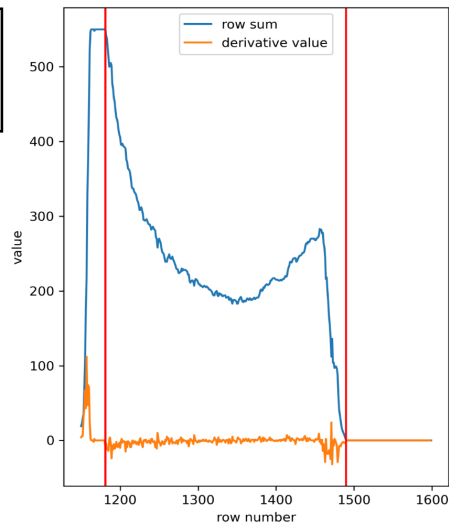
We choose the best threshold and brightness and transform the image to binary image (black & white photo). We focus on the middle blue segment.



Average to every 10 points in the graph.

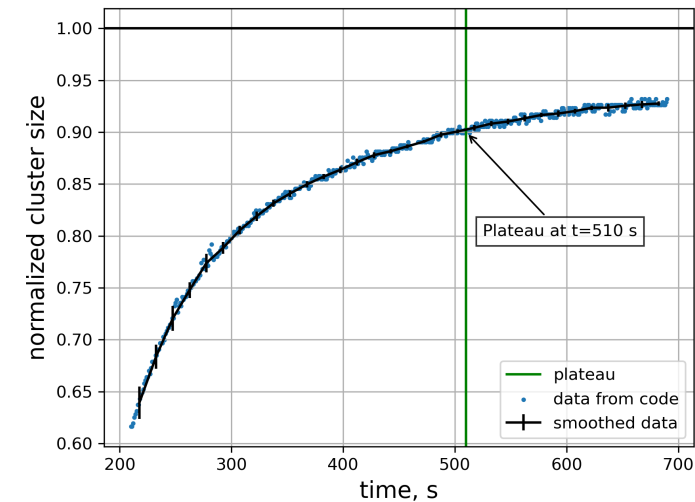
Derivative to the smoothed graph

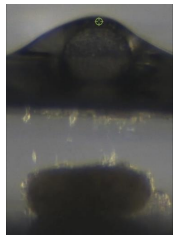
Blue graph –  
Number of white  
pixels in each row



Orange graph –  
derivative of blue graph

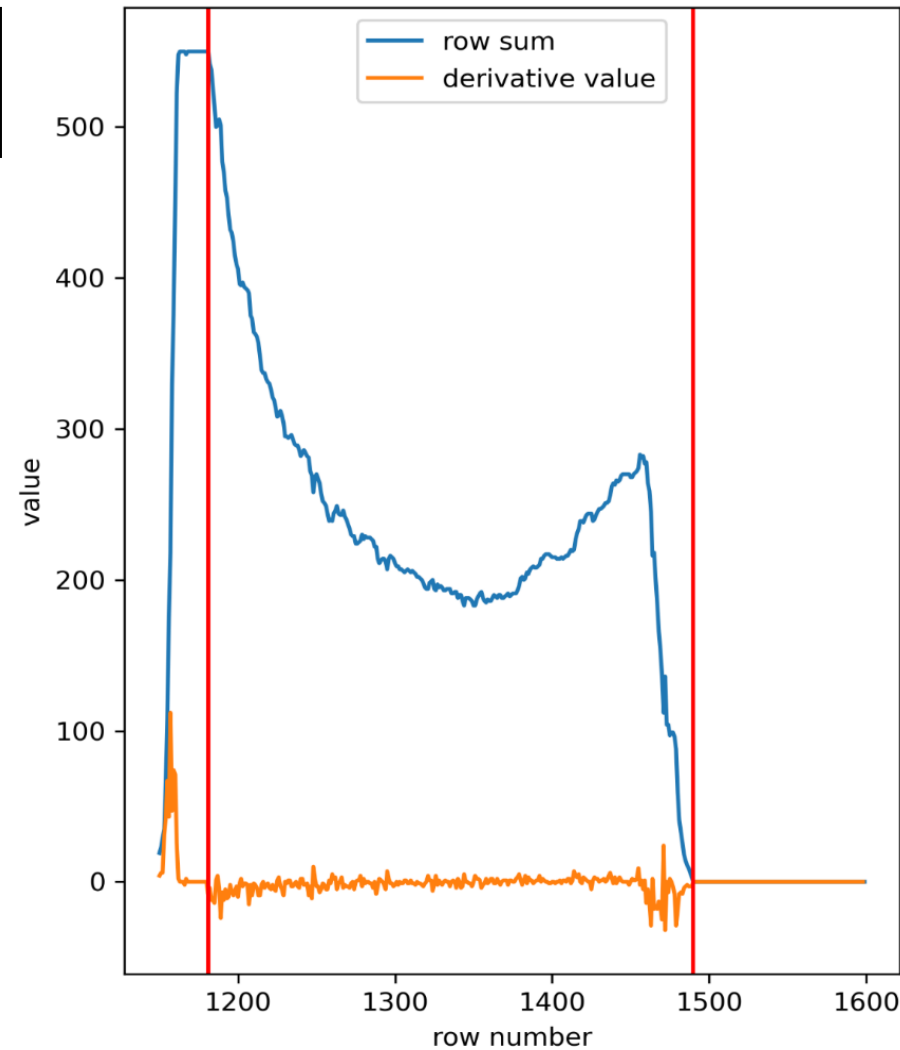
## Final results



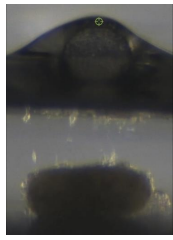


# שלב המנוחה – התאוששות של הצברים

Blue graph – Number of white pixels in each row

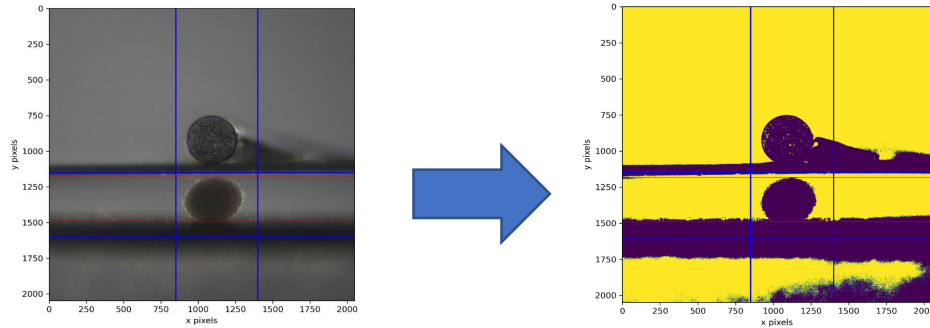


Orange graph – derivative of blue graph

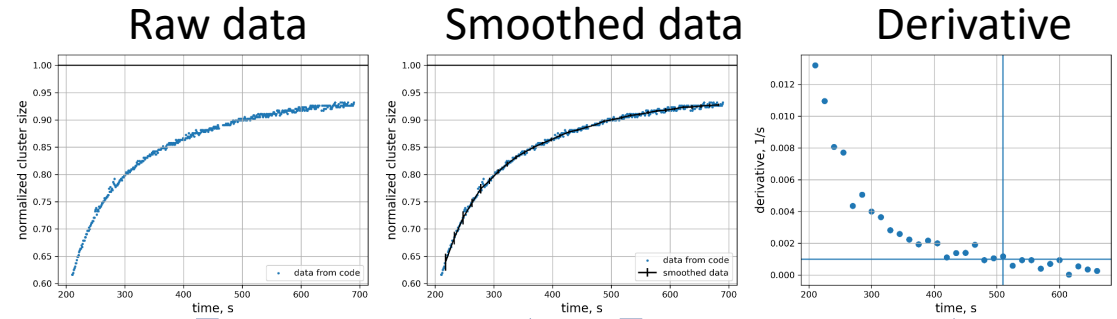


# שלב המנוחה – התאוששות של הצברים

## Determination of the cluster size



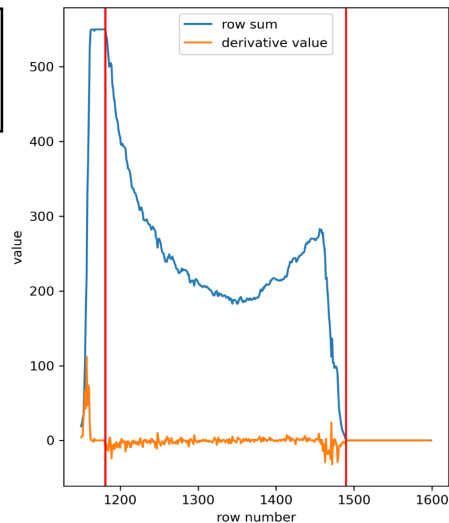
We choose the best threshold and brightness and transform the image to binary image (black & white photo). We focus on the middle blue segment.



Average to every 10 points in the graph.

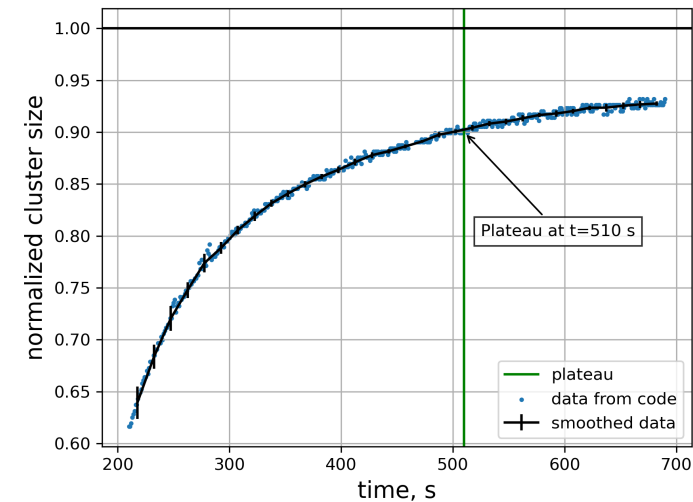
Derivative to the smoothed graph

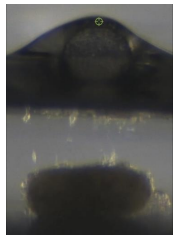
Blue graph –  
Number of white  
pixels in each row



Orange graph –  
derivative of blue graph

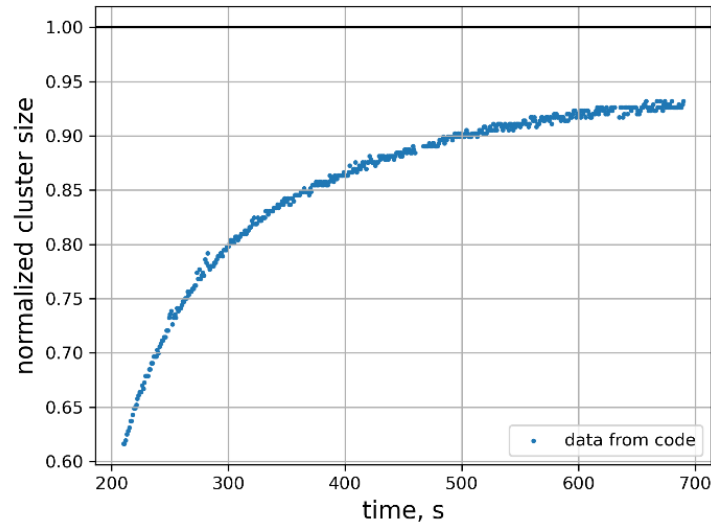
## Final results



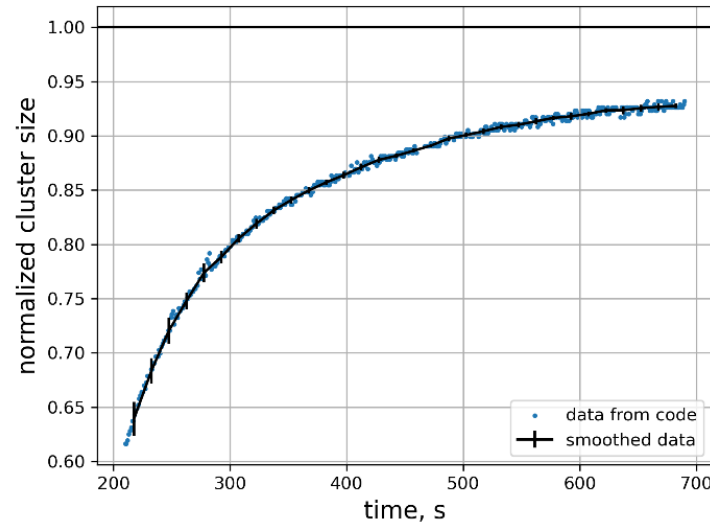


# שלב המנוחה – התאוששות של הצברים

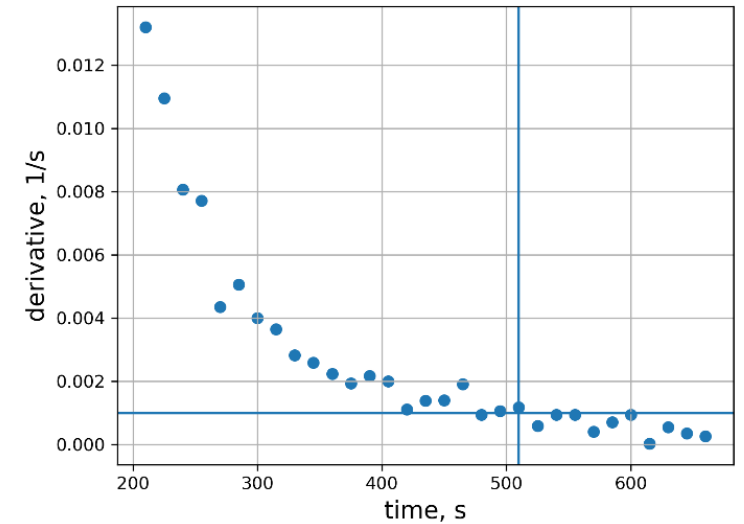
## Raw data



## Smoothed data

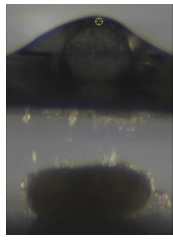


## Derivative



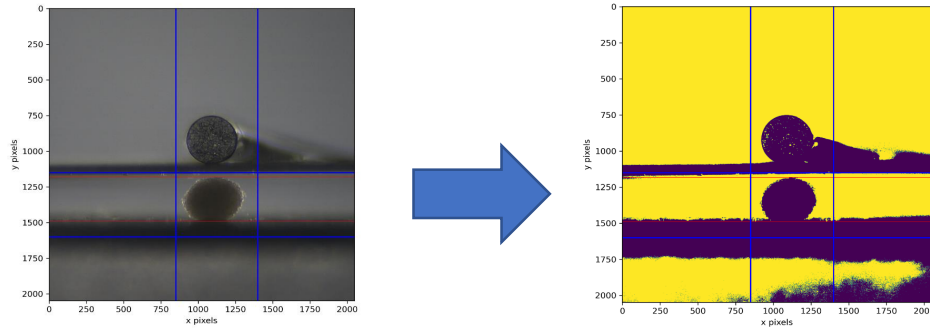
Average to every 10 points in the graph.

Derivative to the smoothed graph

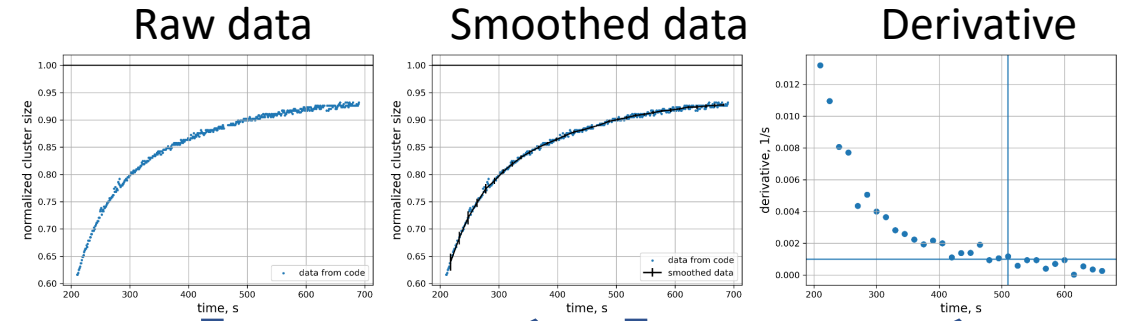


# שלב המנוחה – התאוששות של הצברים

## Determination of the cluster size



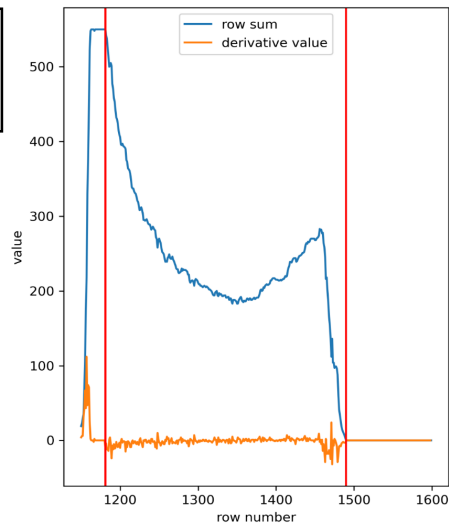
We choose the best threshold and brightness and transform the image to binary image (black & white photo). We focus on the middle blue segment.



Average to every 10 points in the graph.

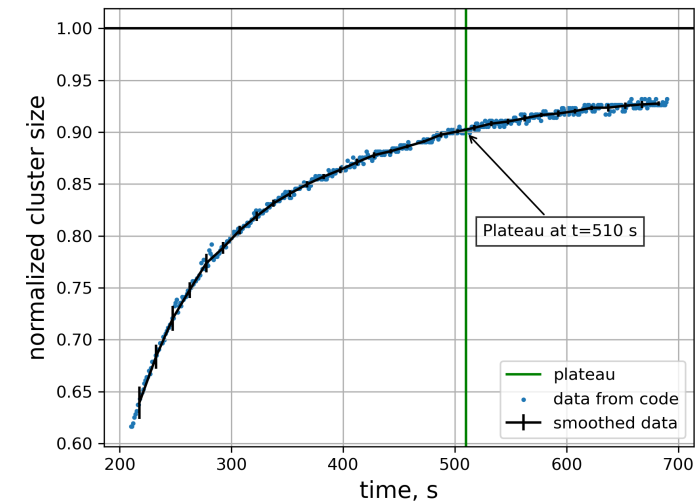
Derivative to the smoothed graph

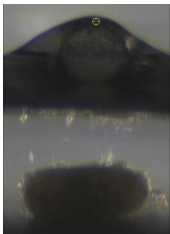
Blue graph –  
Number of white  
pixels in each row



Orange graph –  
derivative of blue graph

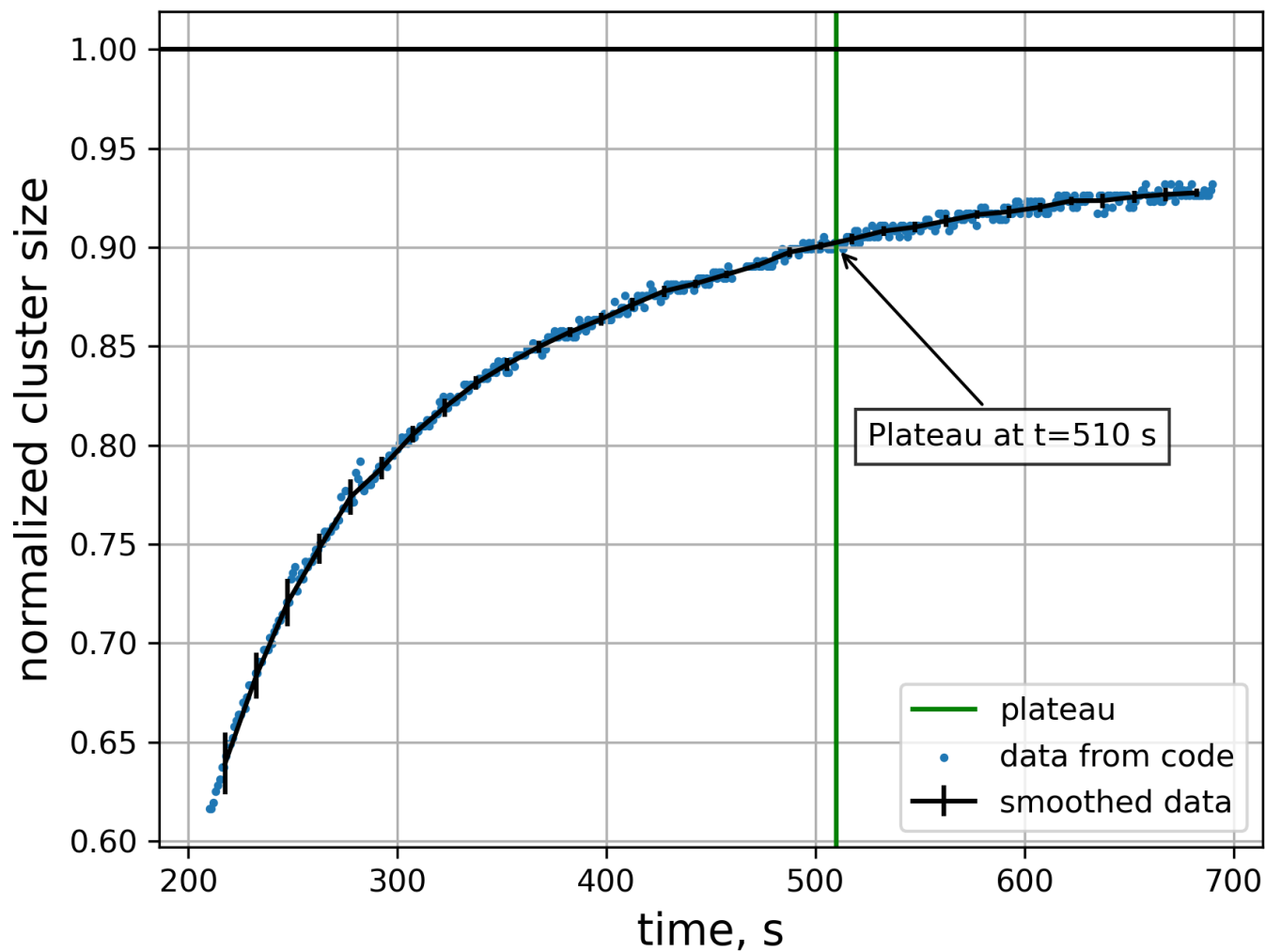
## Final results



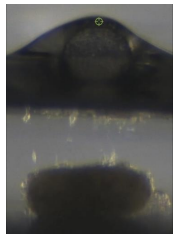


# שלב המנוחה – התאוששות של הצברים

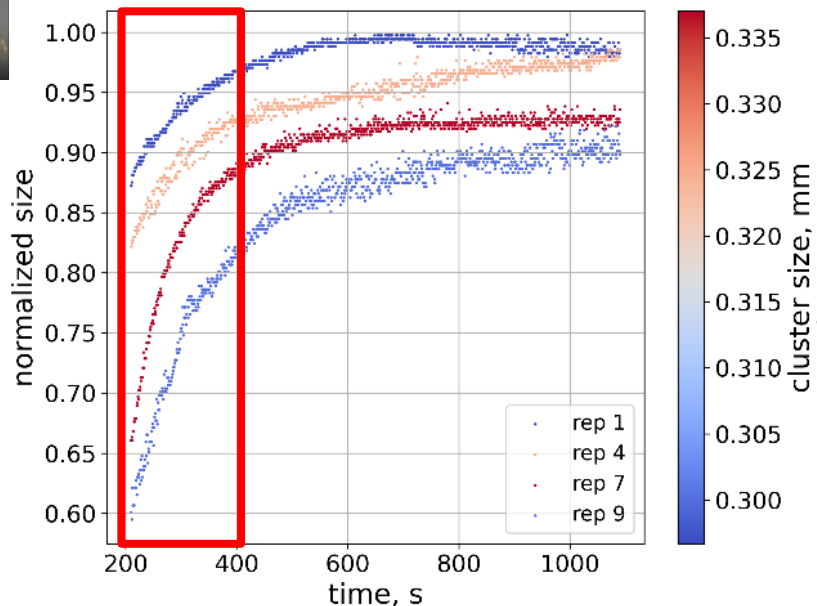
## Final results



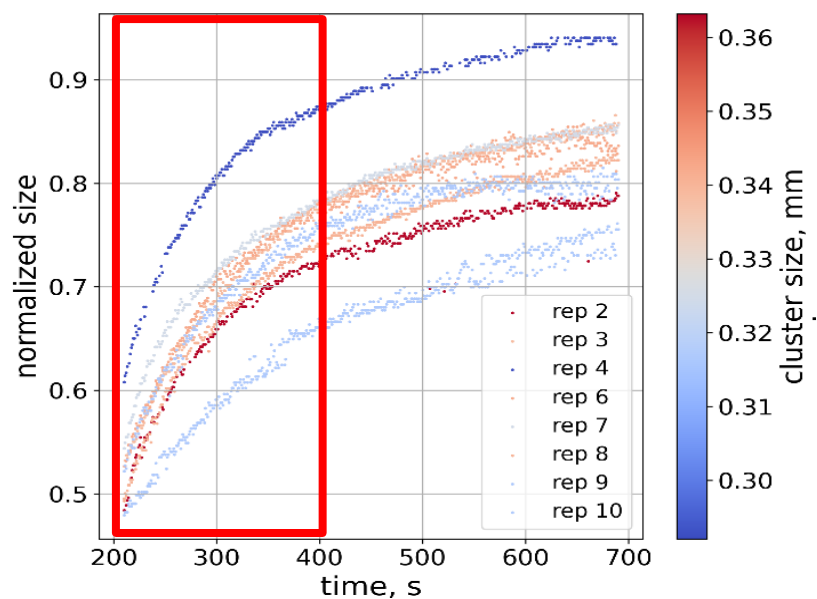
# שלב המנוחה – זמן להתאוששות – סיכום



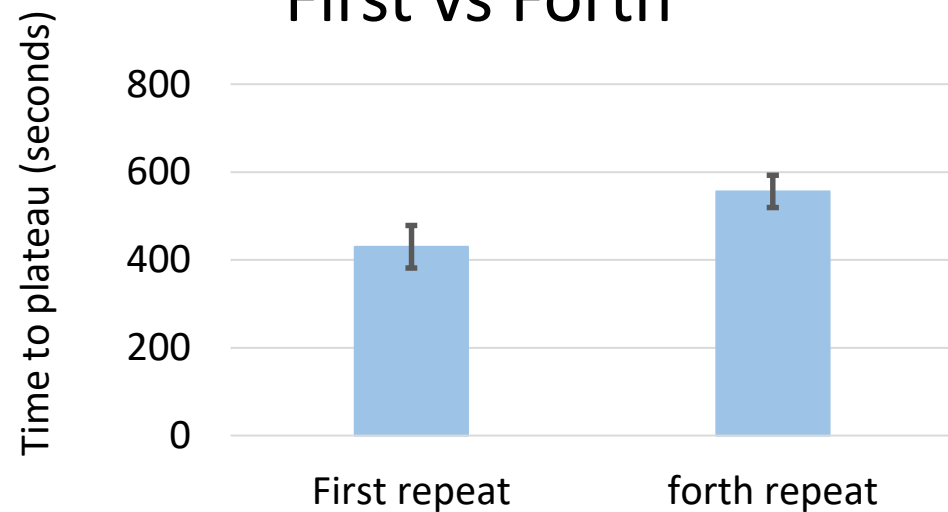
חזרה ראשונה



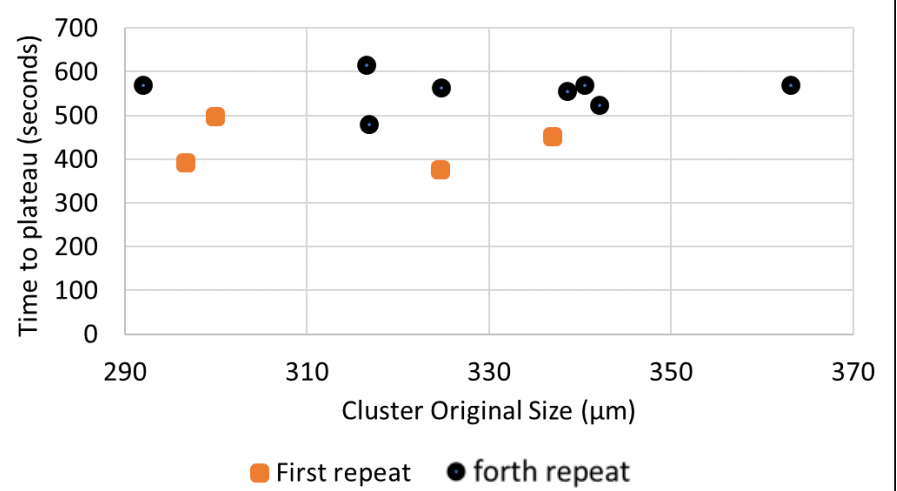
חזרה רביעית



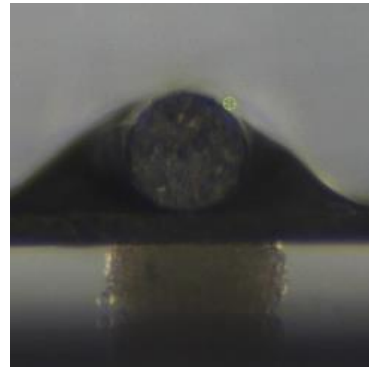
Rest Phase Comparison - First vs Forth



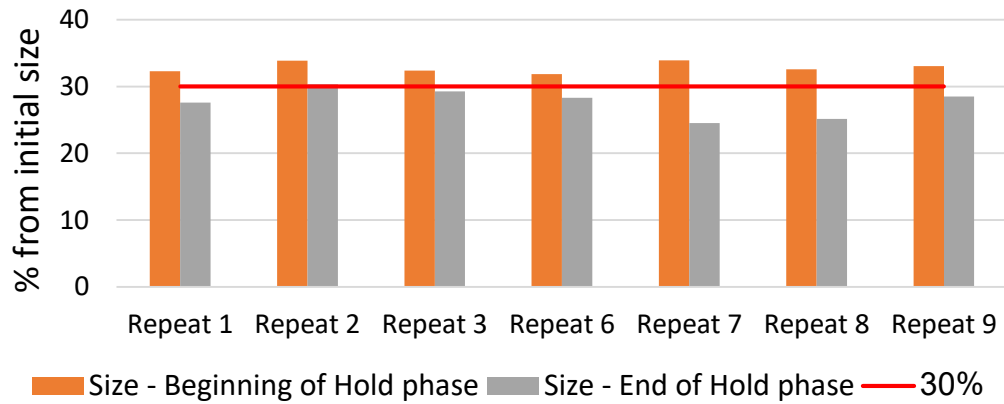
Time to plateau First vs Forth



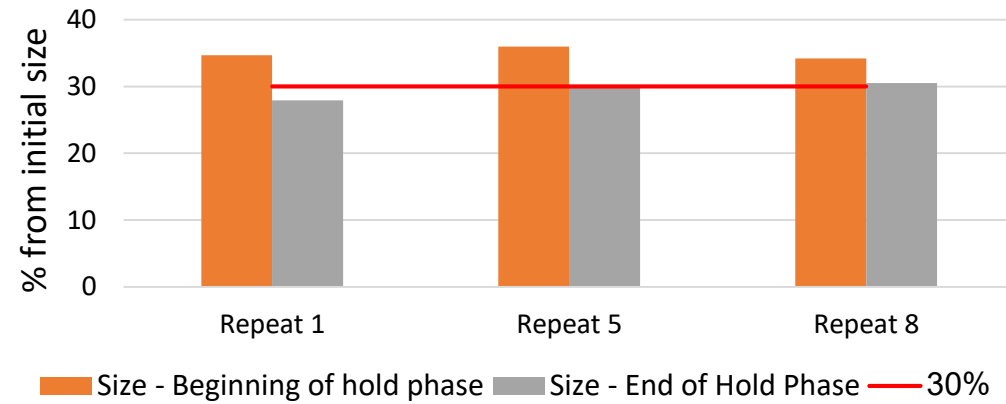
# שלב ההשהיה – לחצנו על הצברים 70% מגודלם המקורי



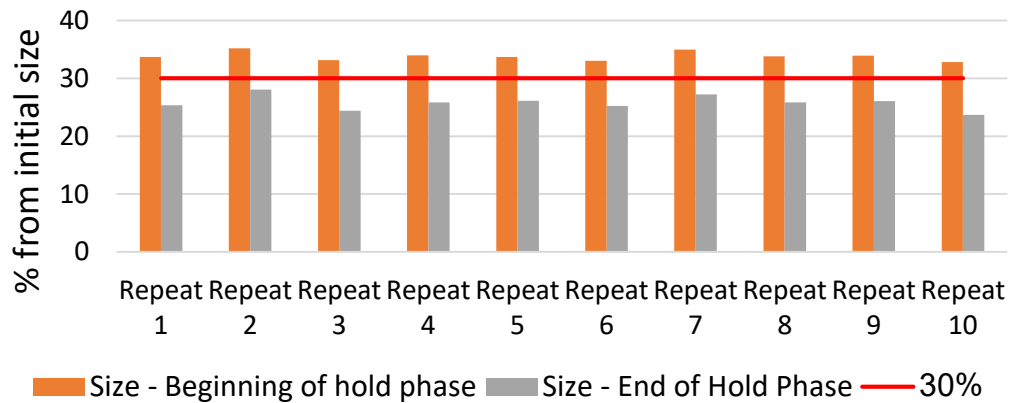
### First Repeat - % Size of Clusters



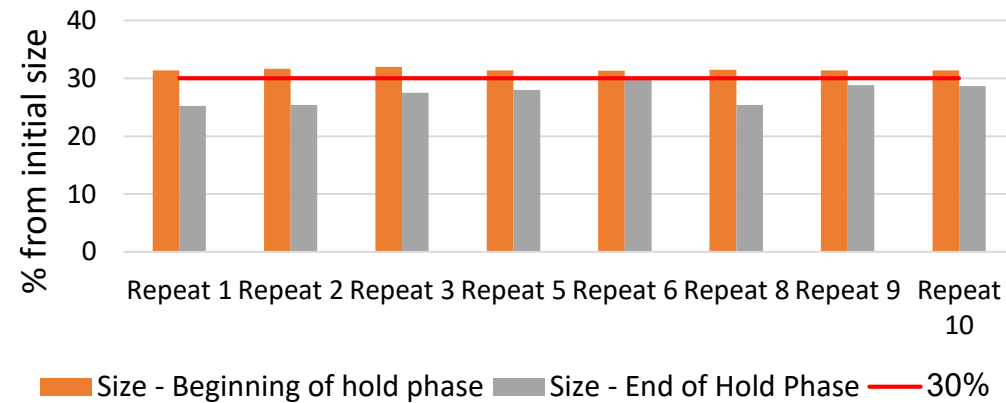
### Second Repeat - % Size of clusters



### Third repeat - % Size of Clusters

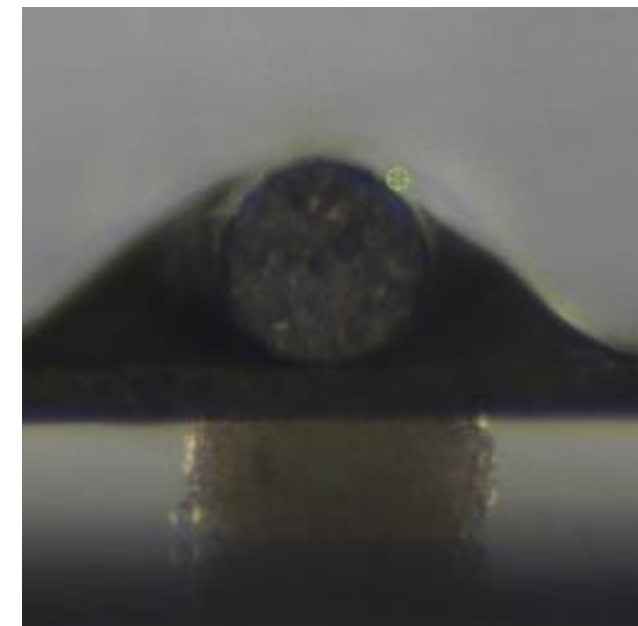
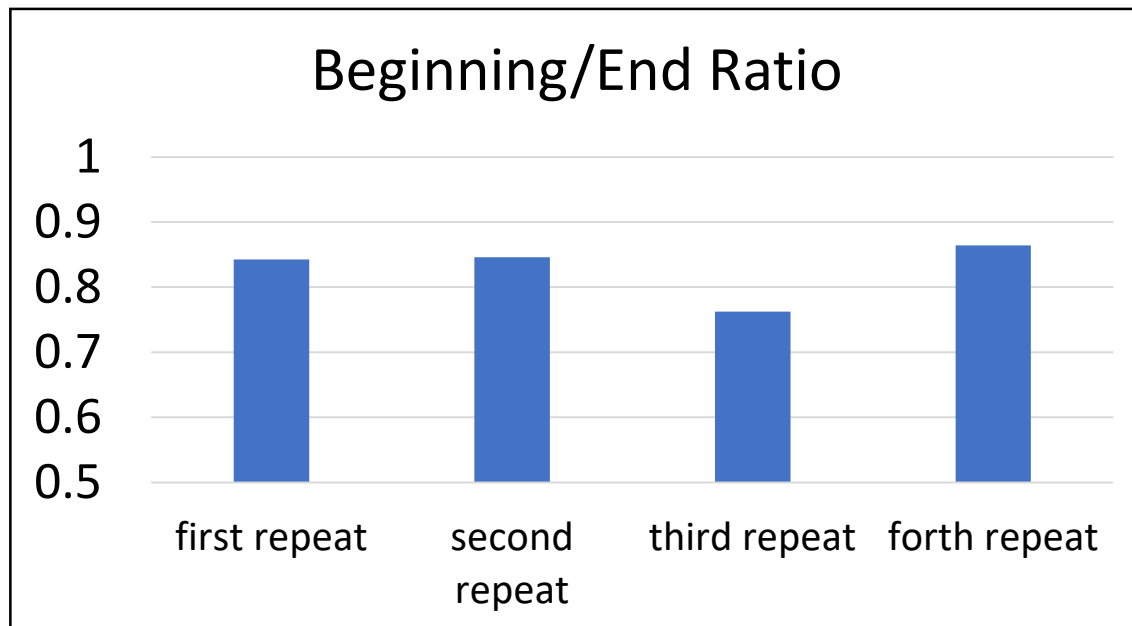
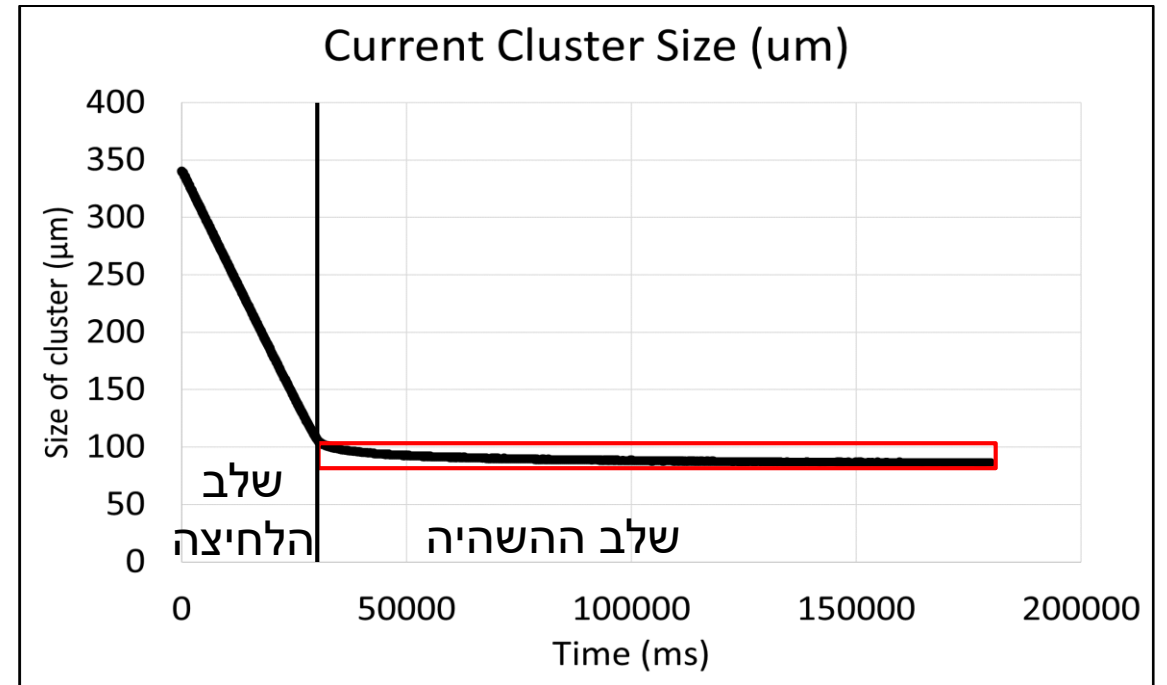
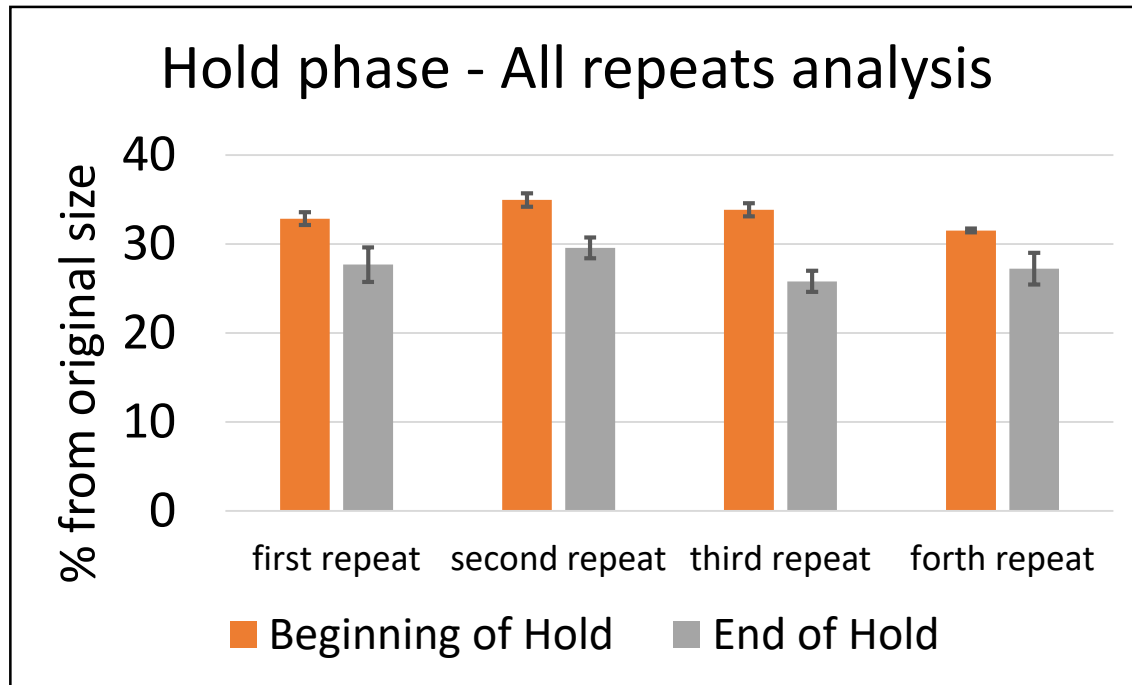


### Forth Repeat - % Size of Clusters

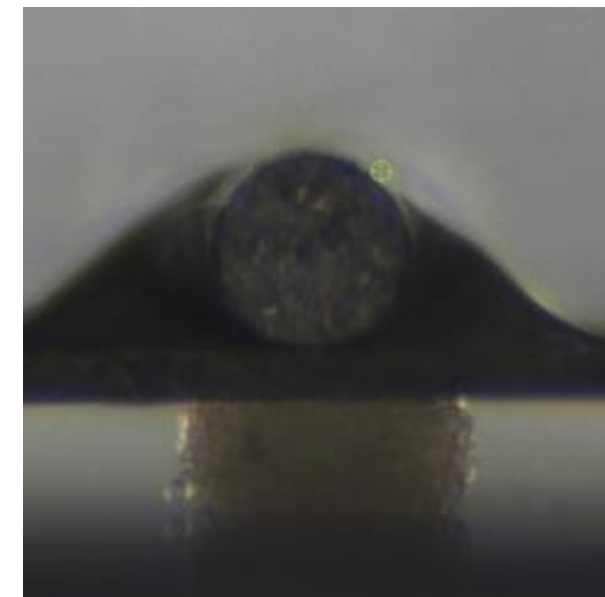
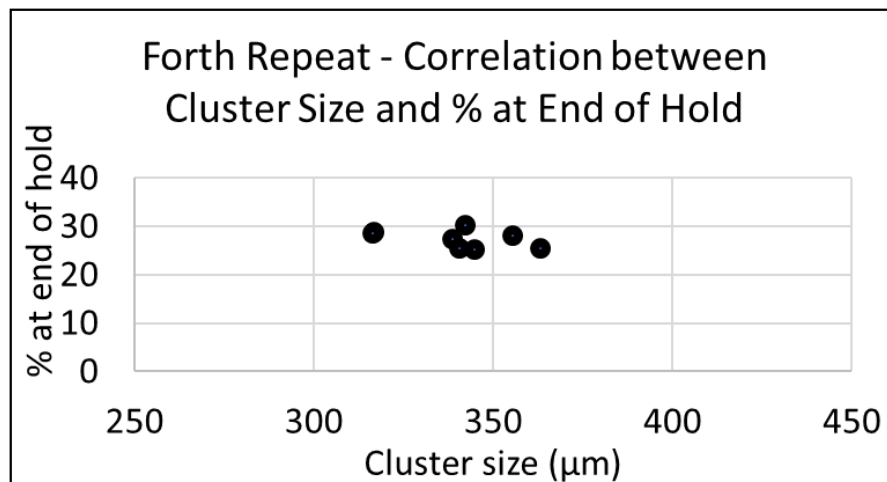
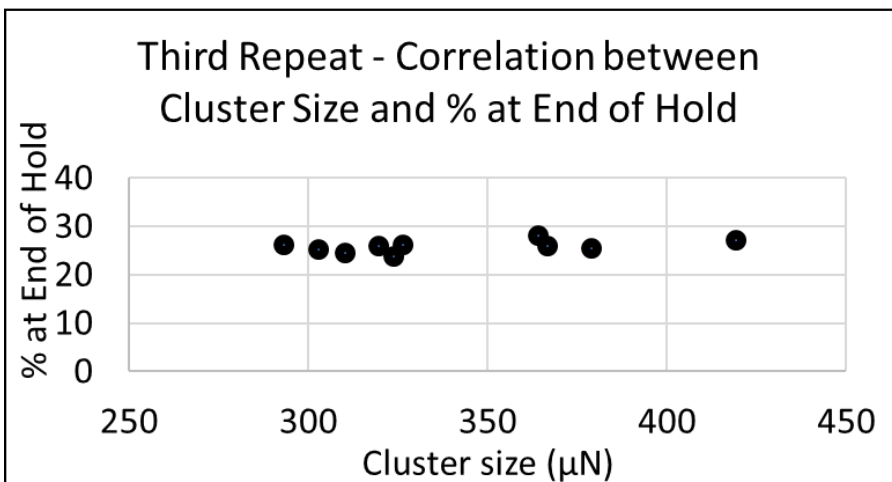
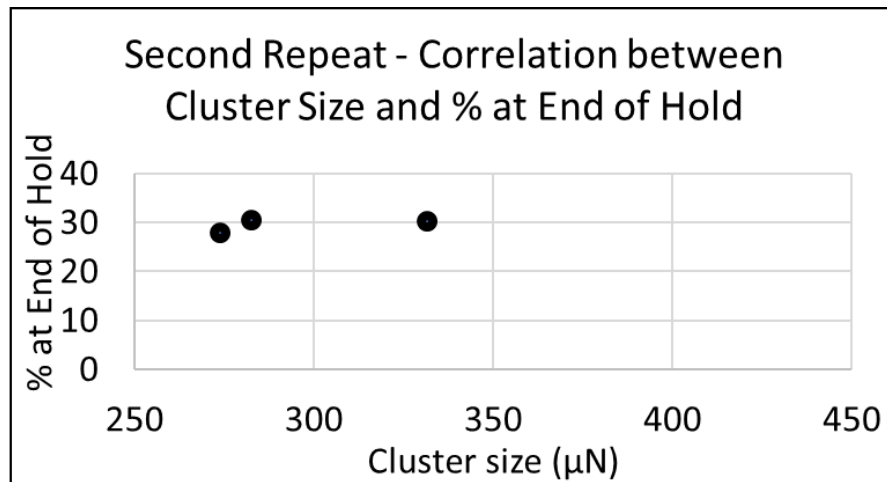
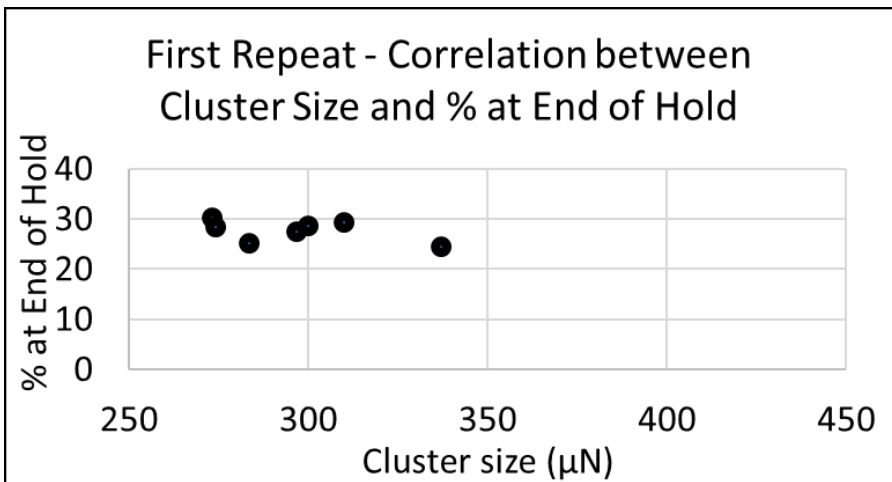




# סיכום ניתוח שלב ההשהיה

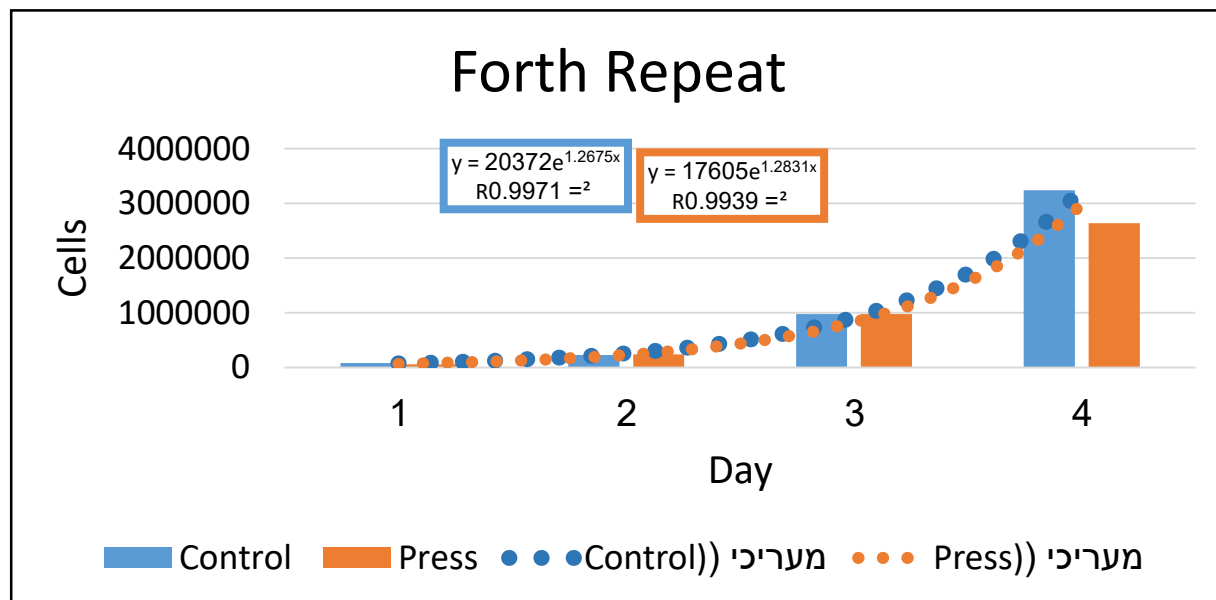
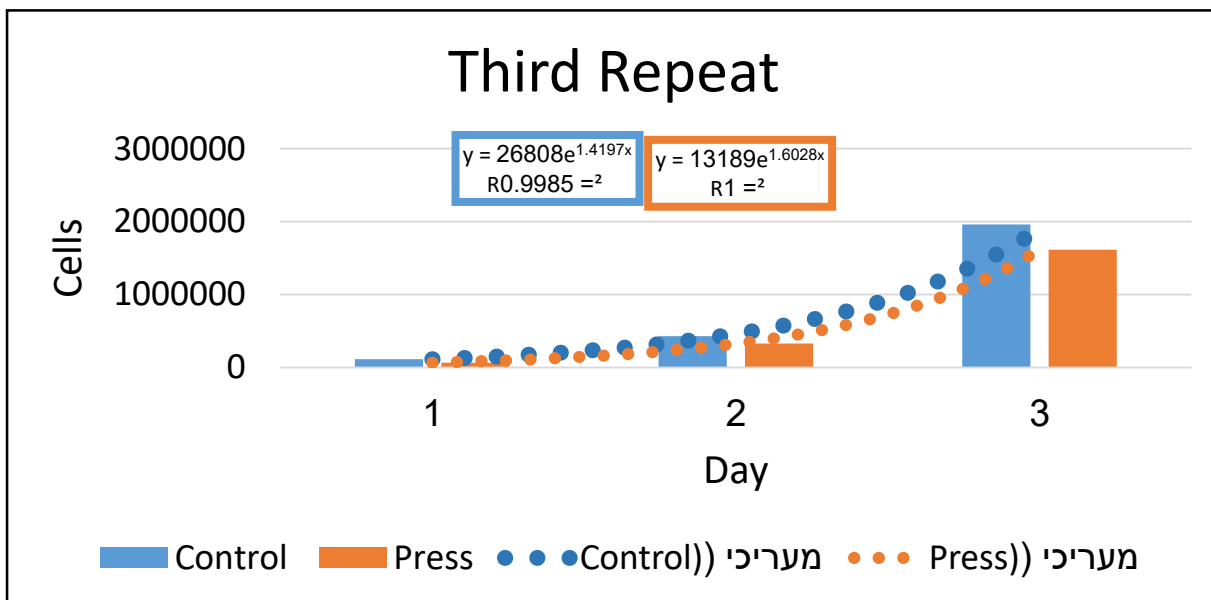
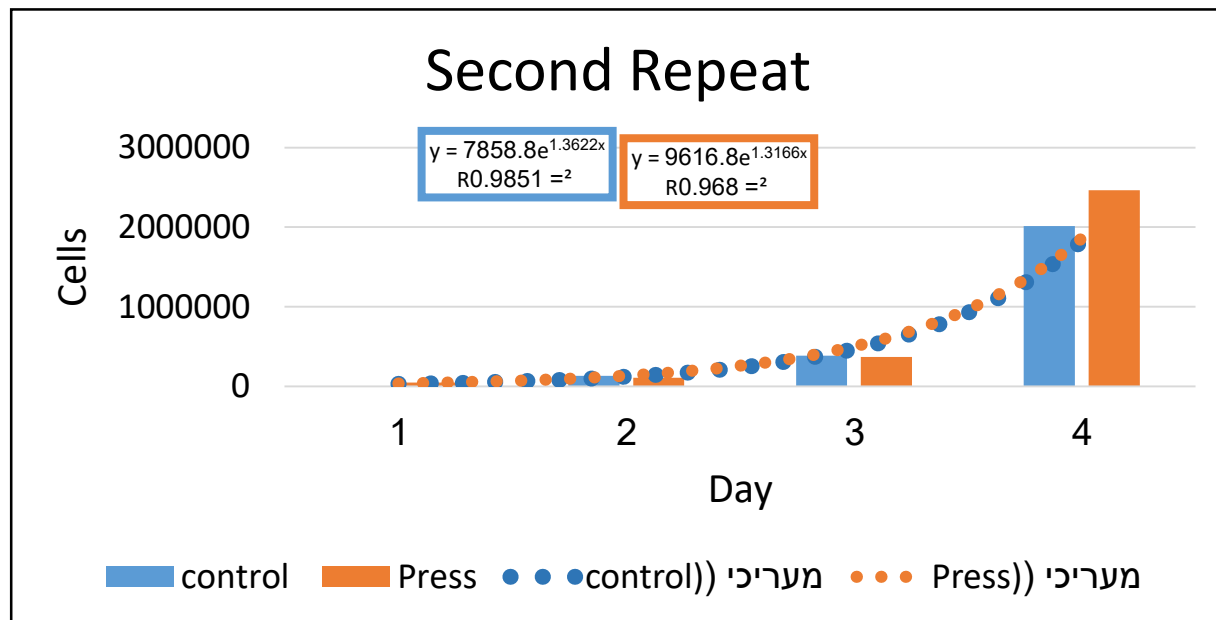
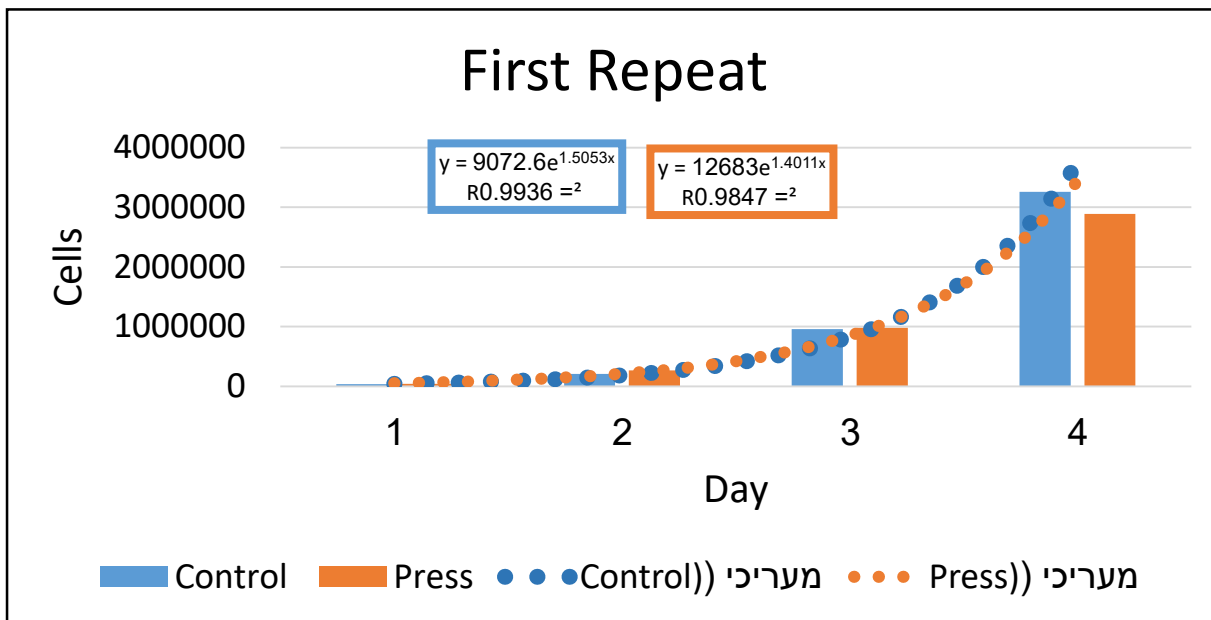


# קורלציה בין גודל הצברים בסוף ההשהיה לגודלם המקורי

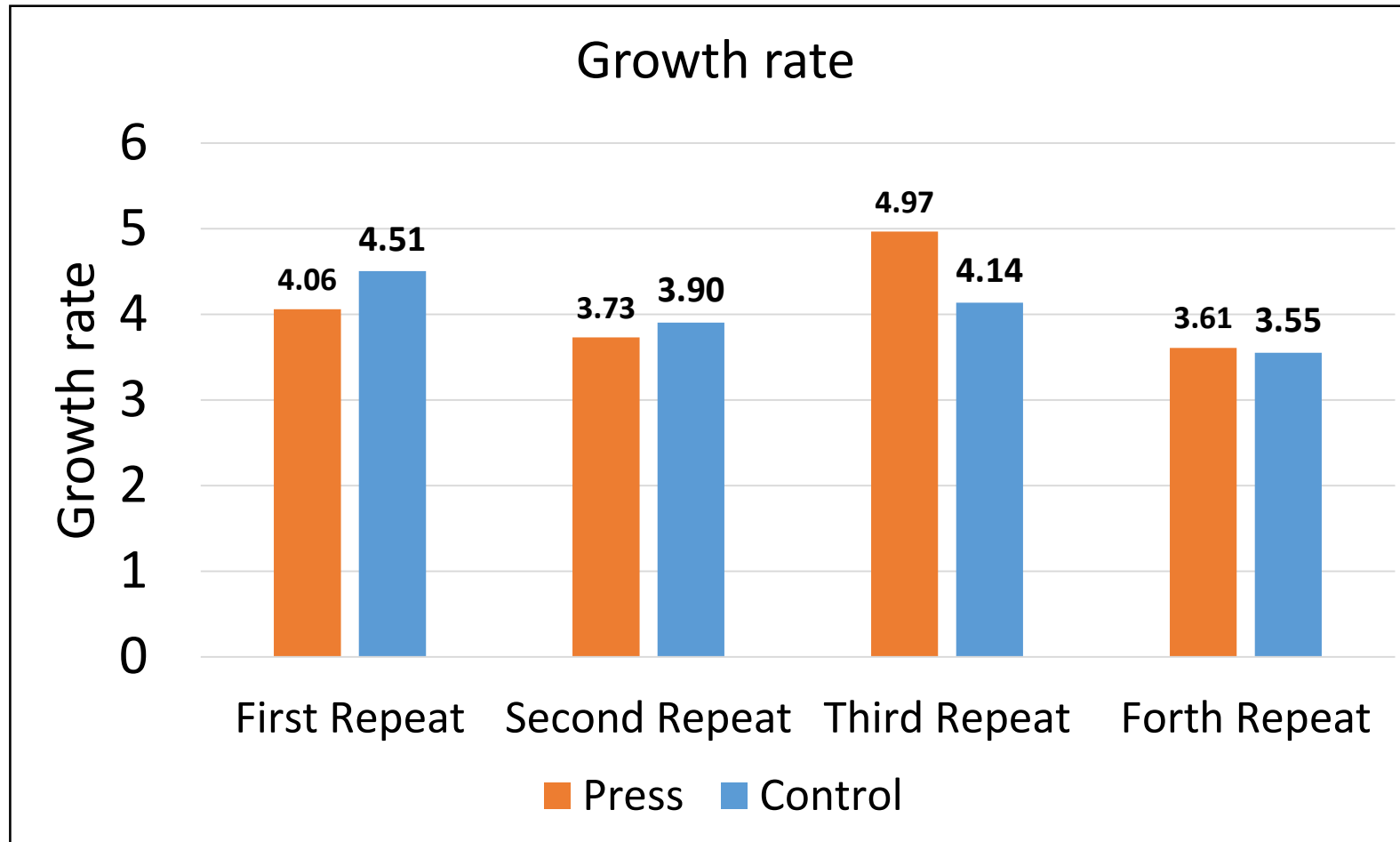


לא נמצא קורלציה ביניהם – ניתן להשתמש בנתון זה כמדד יחיד לבדיקות עתידיות

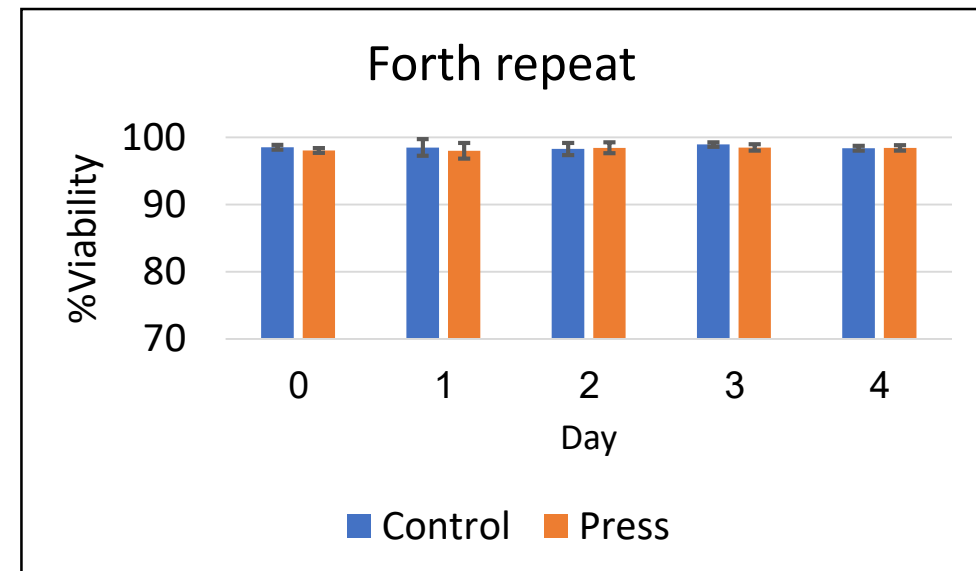
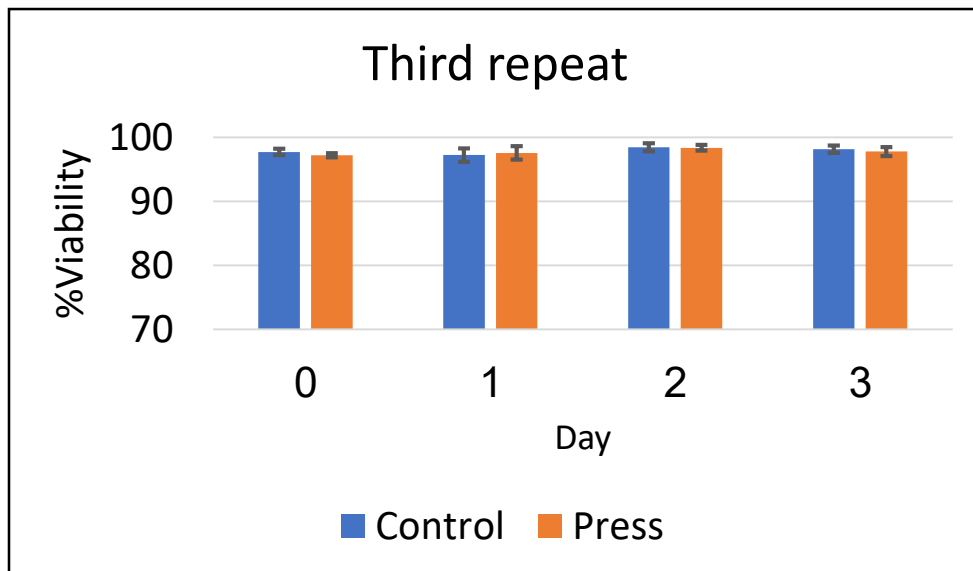
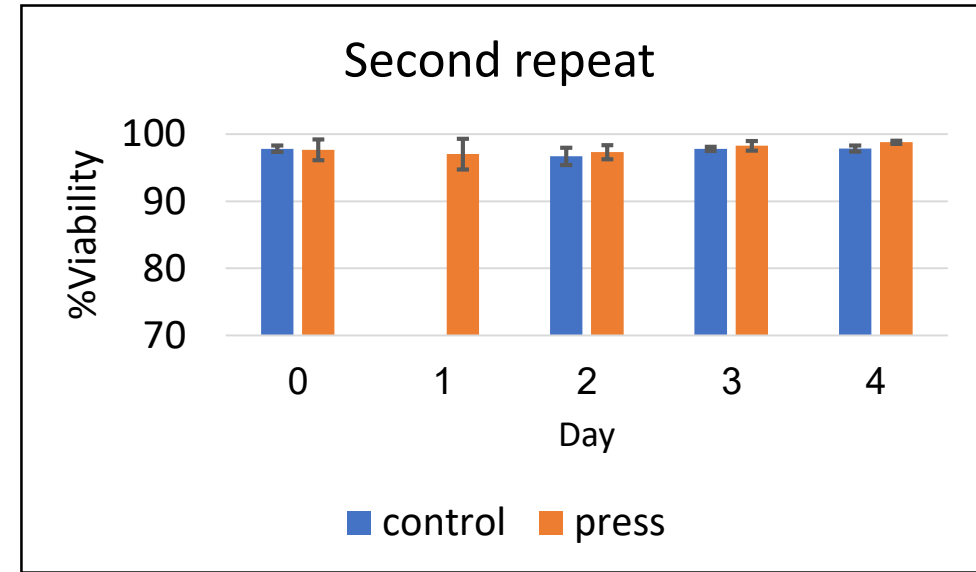
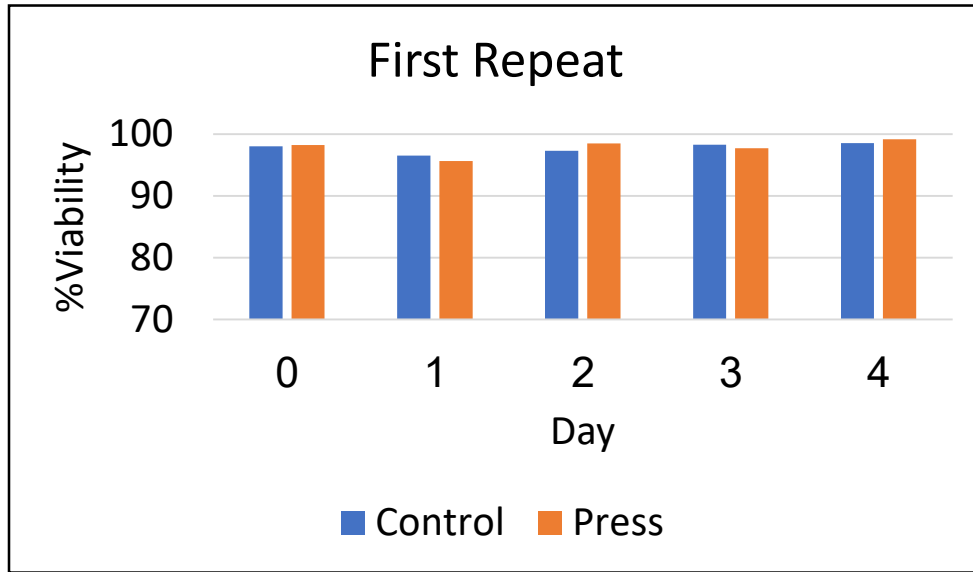
# ניסוי פרוליפריציה השוואתי



# סיכום ניסוי פרוליפרציה

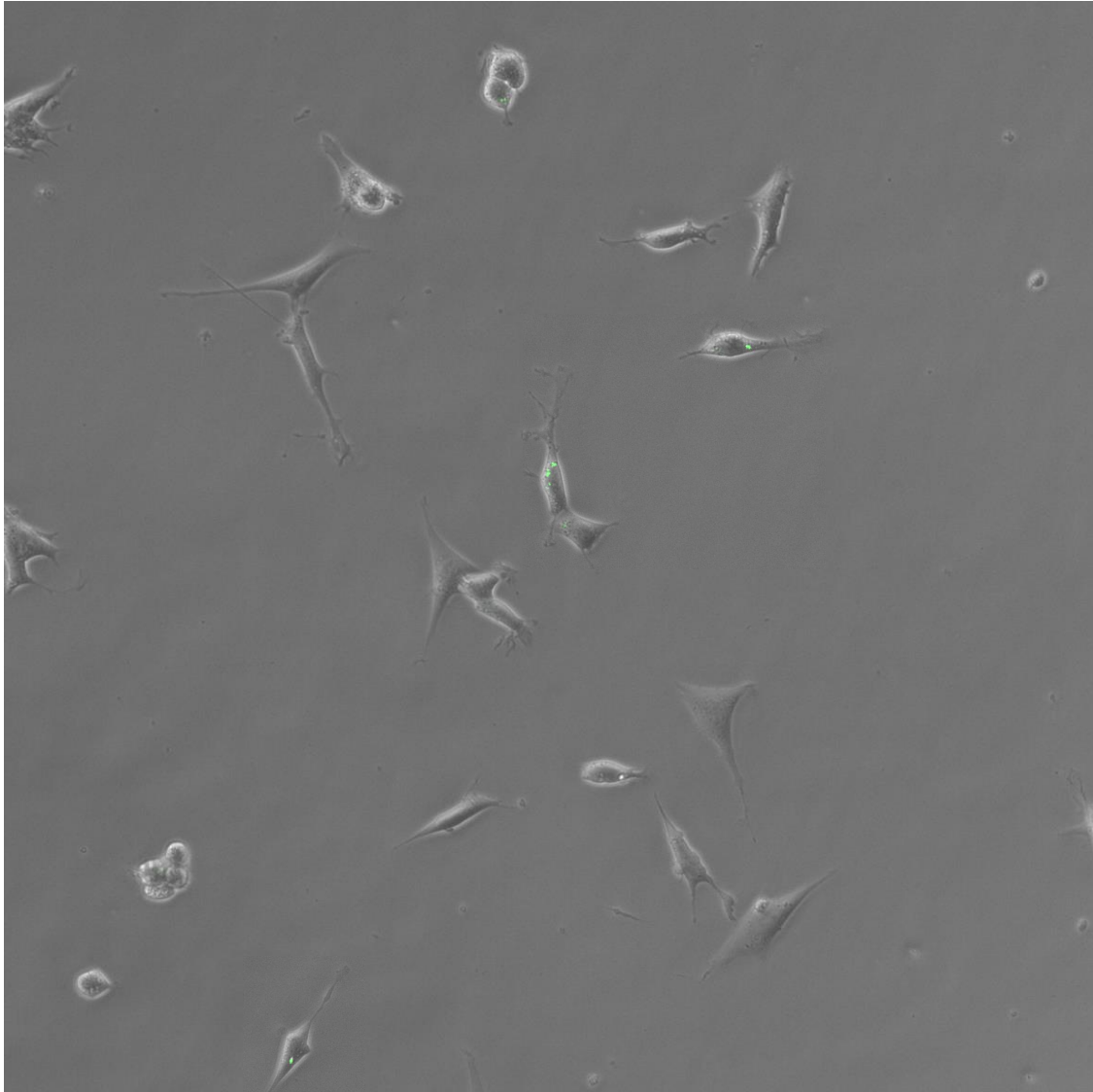


# ניסוי פרוליפרציה – חיות התאים

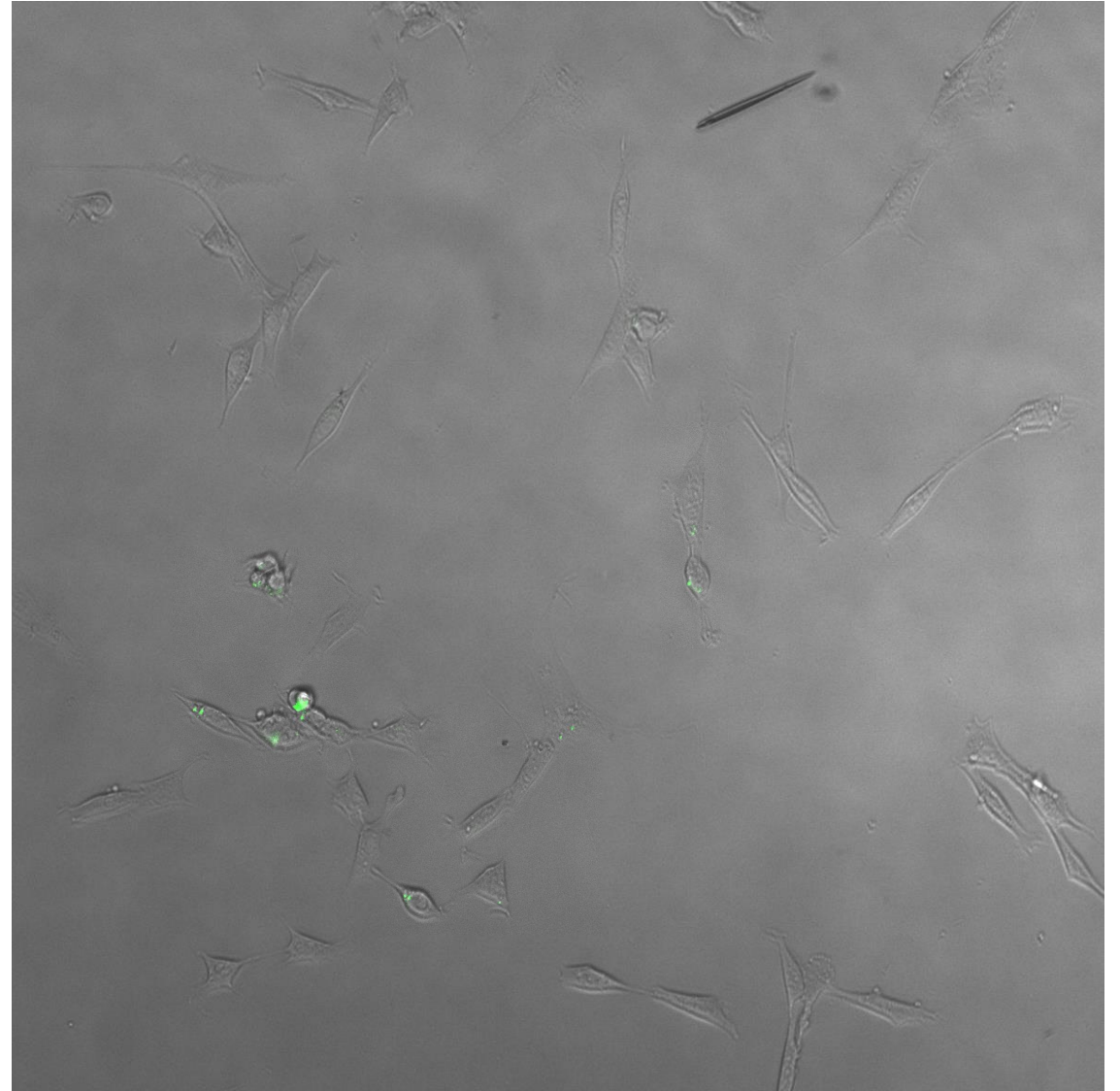


# זיהוי בועיות השומן

Control

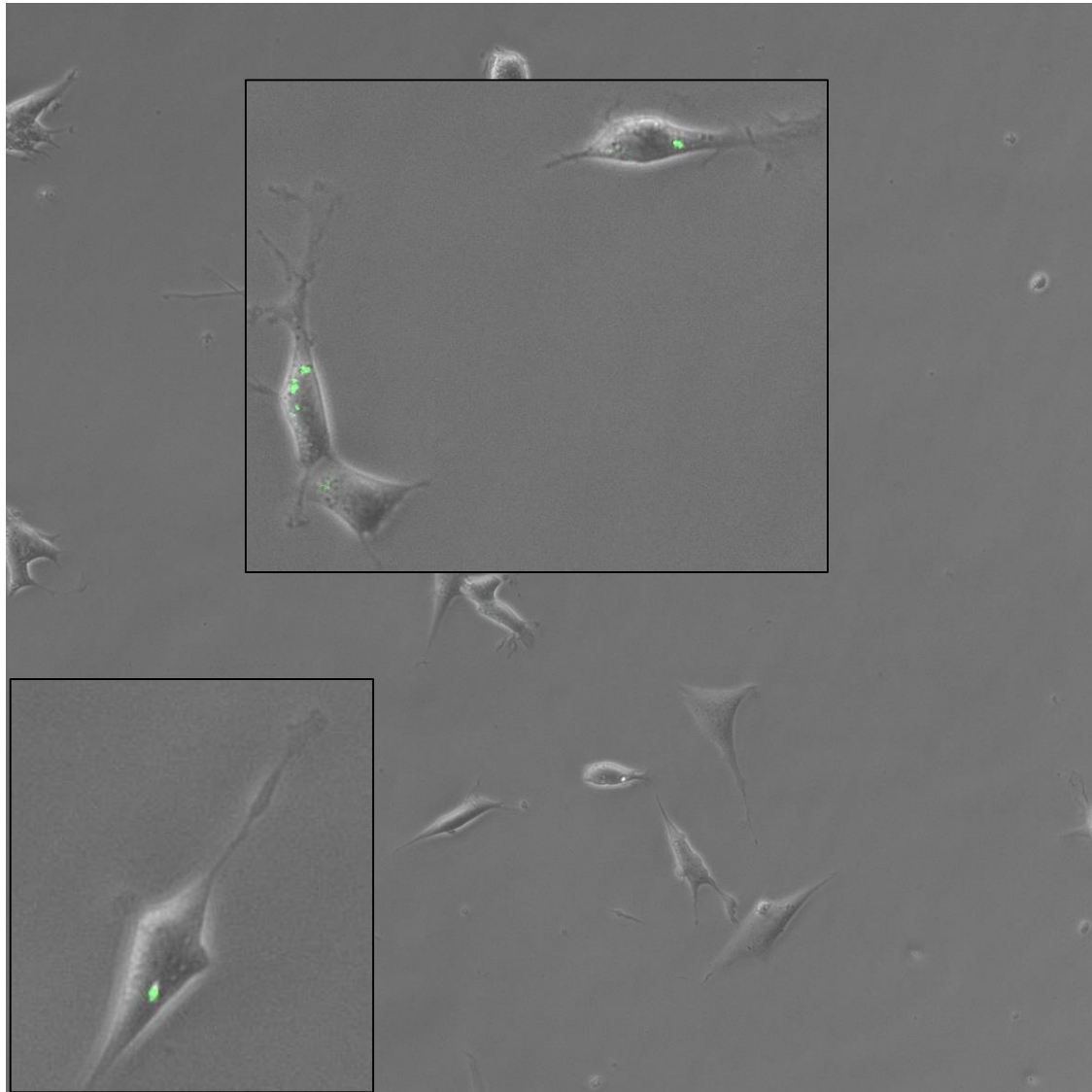


Press

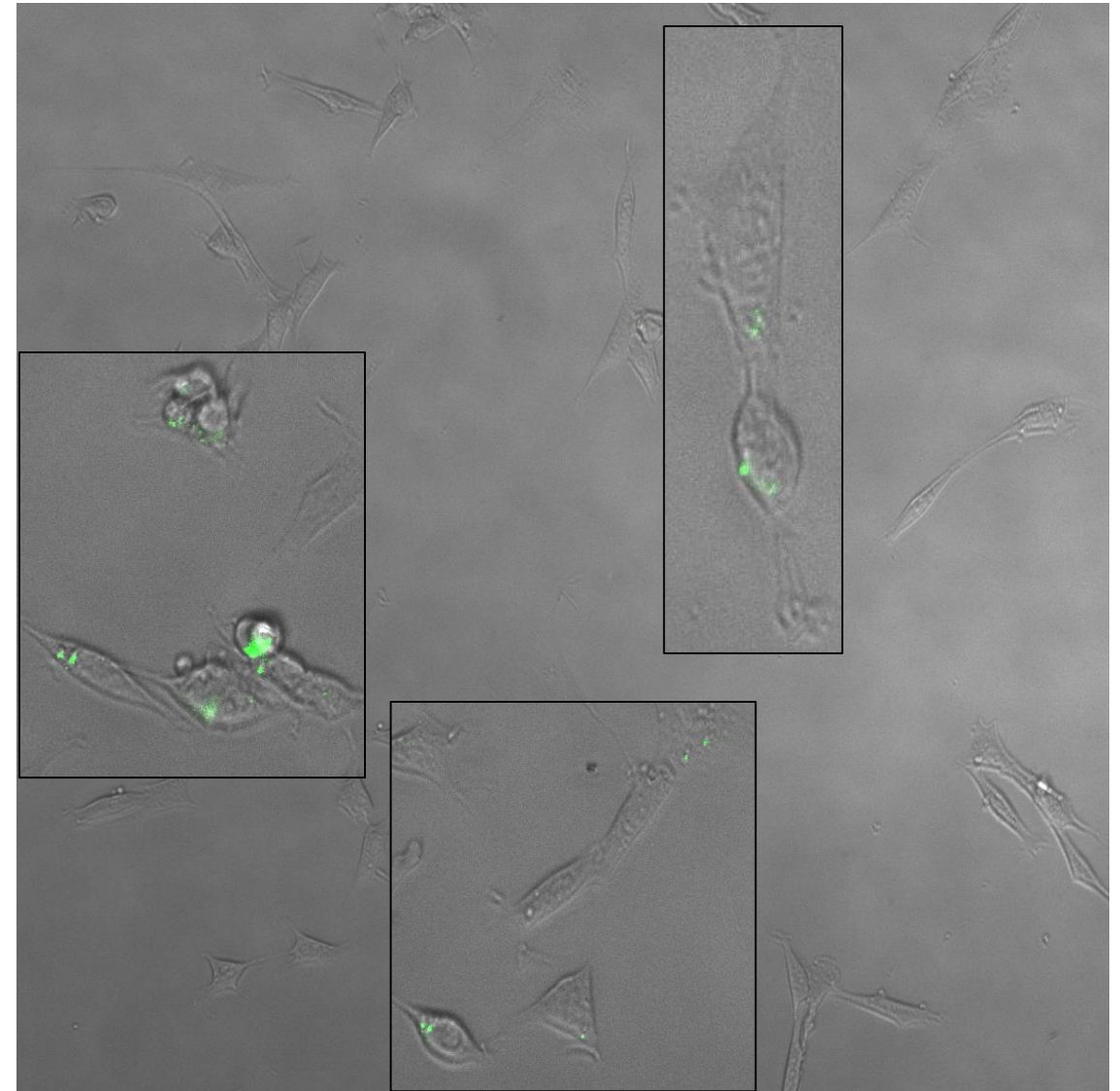


# זיהוי בוועיות השומן

Control



Press



## סיכום:

- בנינו מערכת שבעזרתה אנחנו יכולים למדוד איך כוחות לחיצה משפיעים על תאים מעטין פרה.
- מצאנו פרמטרים שנוכל להשתמש בהם בעתיד להשוואה בין צברים בגדלים שונים.
- מצאנו שהתאים שומרים על חיות גבוהה גם לאחר לחיצה ממושכת בסך 70% מגודלם ההתחלתי.
- גילינו תהליכים שלא ציפינו לראות בזמן הלחיצה, אנחנו נבצע עליהם ניסויים נוספים כדי לנסות להסביר מדוע זה קורה.



# Thanks

**Dr. Yifat Brill-Karniely (Volcani Inst.)**

**Dr. Enav Bar-Shira (Volcani Inst.)**

**Guy Dabby (HUJI & Volcani Inst.)**

**Liron Toledano (HUJI & Volcani Inst.)**

**Prof. Itamar Barash (Volcani Inst.)**

**Prof. Dalit Sela-Donenfeld (HUJI)**

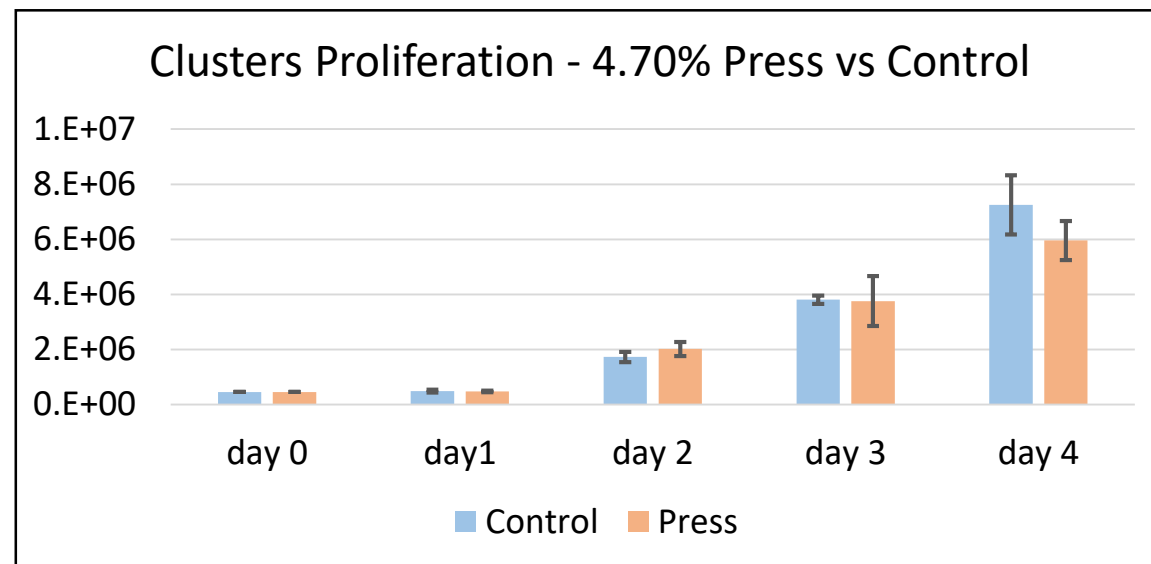


L1 bovine mammary epithelial cells generously provided by Prof. Itamar Barash (Volcani Inst.):

German and Barash, *In Vitro Cell Dev Biol – Animal* 2002

# פרוליפרציה של אגרטים – חזרה רביעית אל מול ביקורת

- All viability was higher than 98%
- If you plate 100mm-NTC plate with cell concentration of 460,000 cells, 4 days after you will get grown clusters.



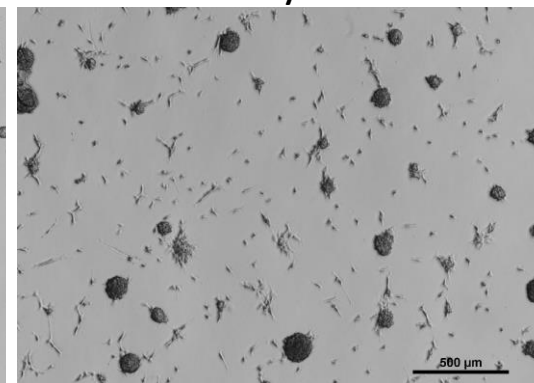
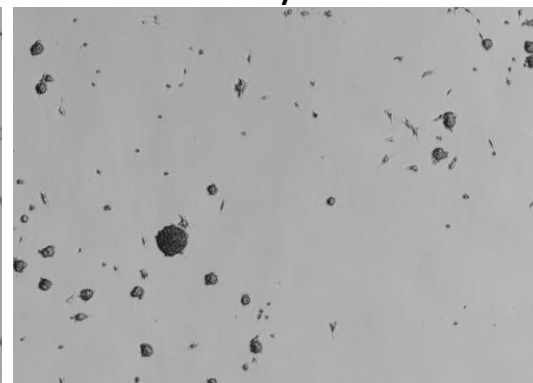
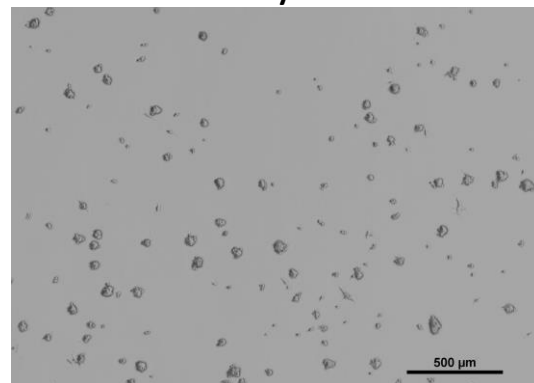
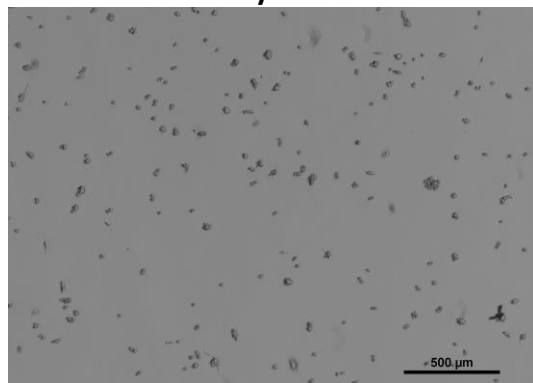
Day 1

Day 2

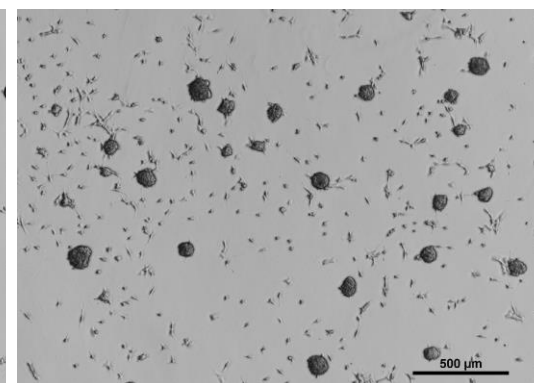
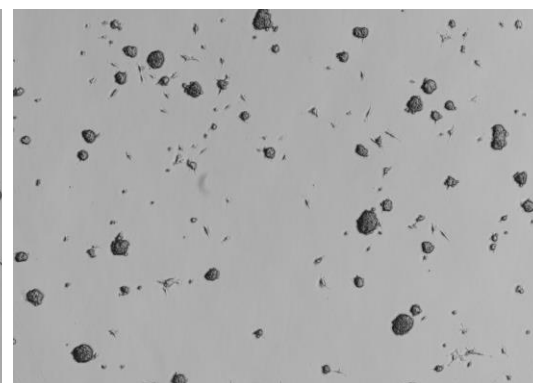
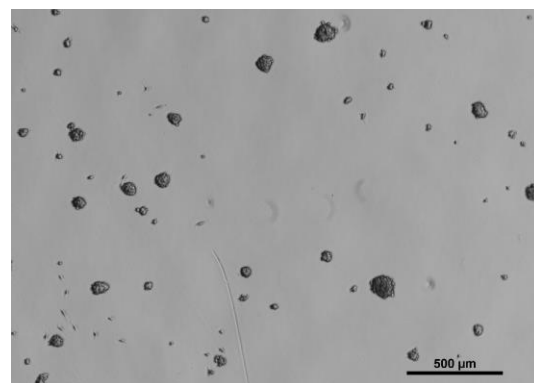
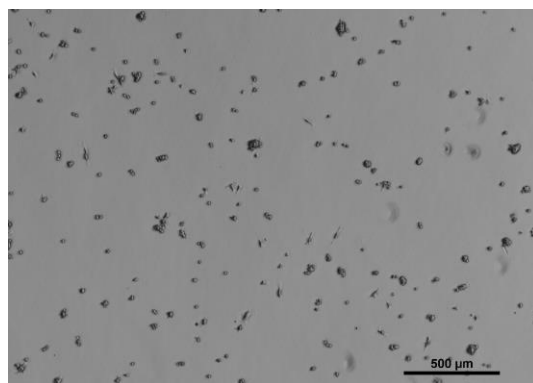
Day 3

Day 4

Control

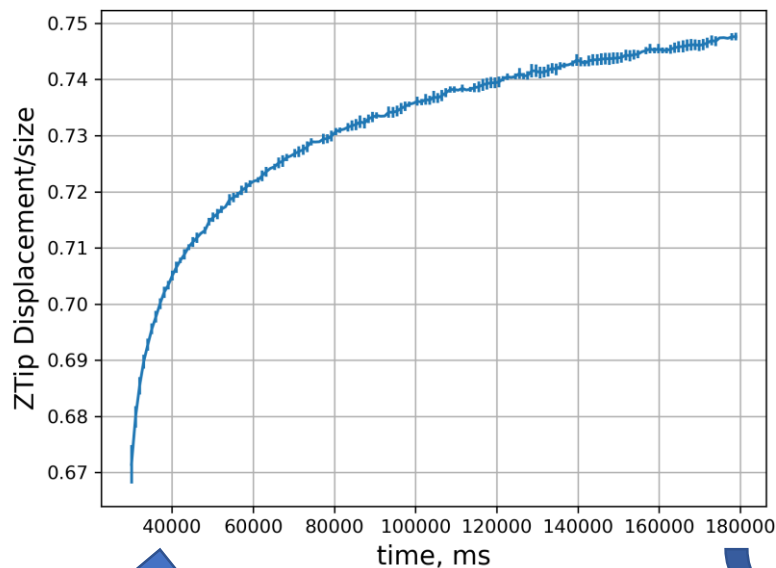
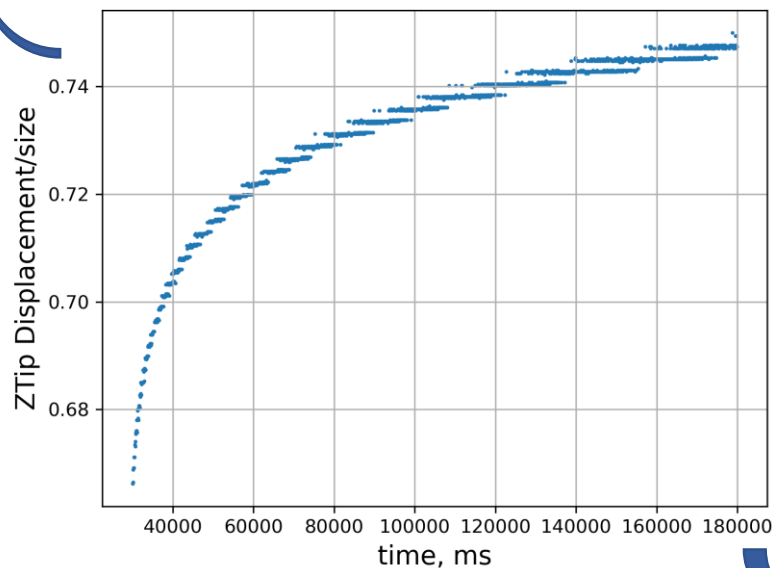


4.70%Press

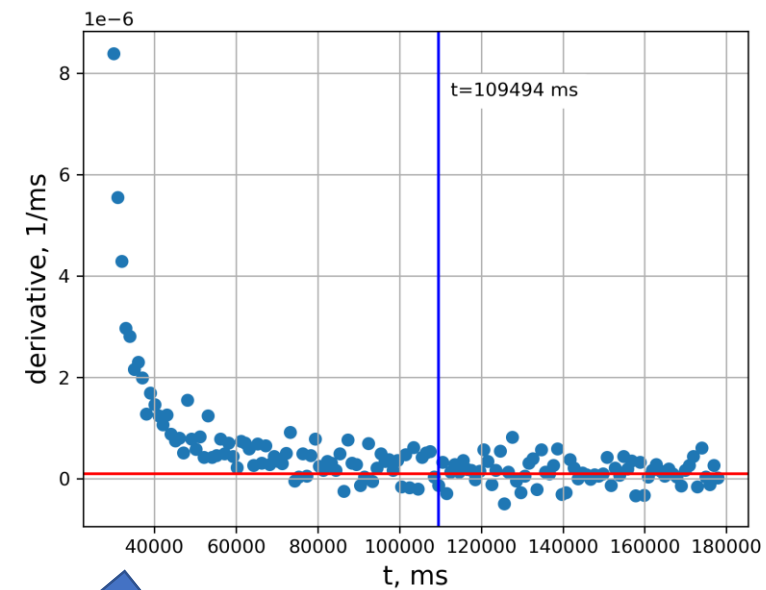


# שלב ההשהיה – זמן הגעה למנוחה

Y-axis units – Ztip Displacement/size This is meant to normalize the height of the press so that it is not dependent on the cluster's size.



Averaging every 2500 points on the X-axis to smooth out the noise.



Derivative to graph B.  
Red line – An arbitrary line that we have set at  $(10^{-7})$ .  
Blue line – Averaging every 5 points. When the average crosses the red line, the midpoint of the five points is marked.

# שלב ההשהיה – סיכום תוצאות

