Constructing an *in vitro* system of applying stretching pulses on MECs similar to the milking procedure

Guy Dabby

Volcani Institute Agricultural Research Center

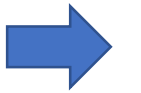
The Hebrew University of Jerusalem

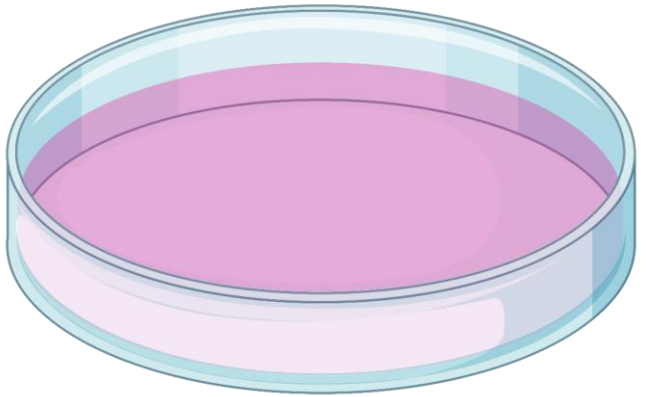
האוניברסיטה העברית בירושלים
THE HEBREW UNIVERSITY OF JERUSALEM

Mechanical Forces During Milking

- Milking applies shear, compression, and cyclic tensile forces on mammary glands.
- Mechanical forces can impair cell function and secretion, but their impact on MECs is unclear.
- To our knowledge, no in vitro models exist to study these forces on bovine MECs.

From Udder to Cell Culture

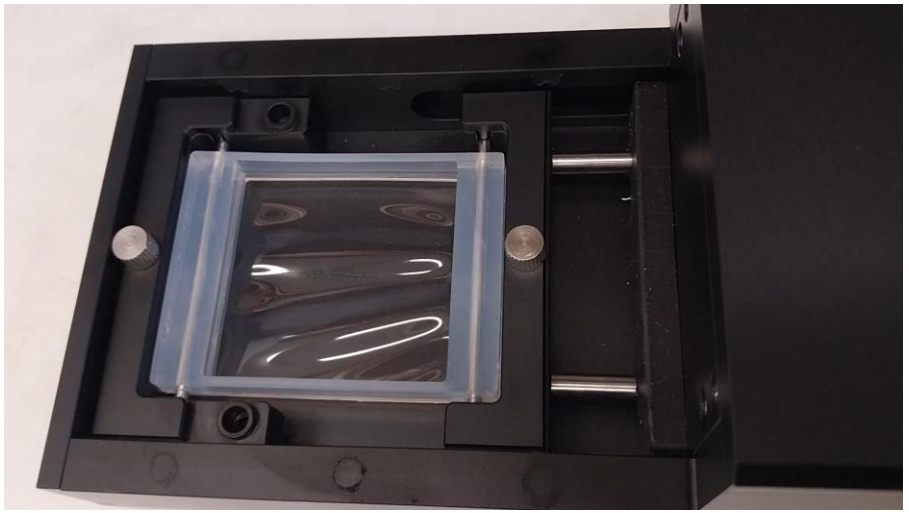




L1 bovine mammary epithelial cells generously provided by Prof. Itamar Barash (Volcani Inst.):
German and Barash, *In Vitro Cell Dev Biol – Animal* 2002

CytoStretcher

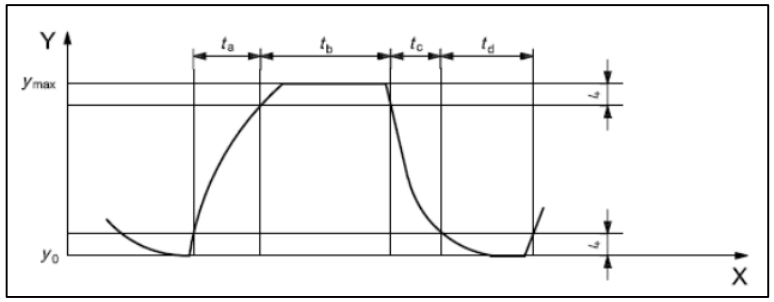
- Fitted for tissue culture systems
- Exert tensile forces on cells
- Control on:
  - % Strain
  - Velocity
  - Number of Cycles



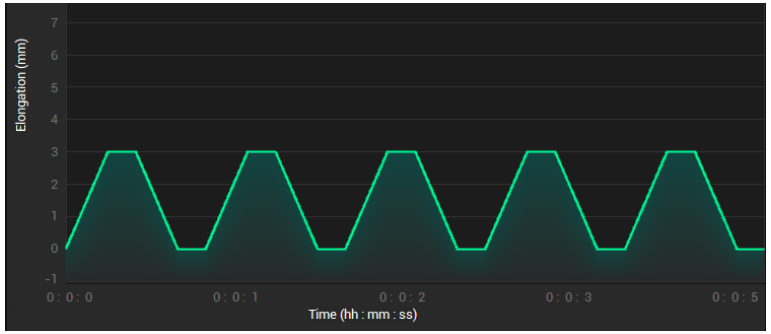
https://www.curibio.com/cytostretcher

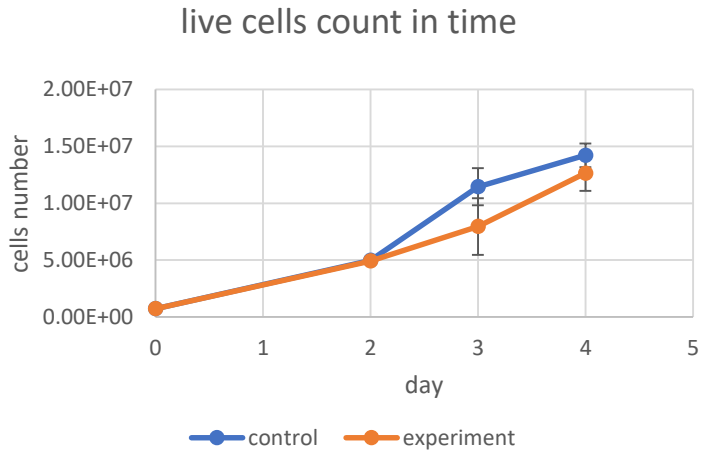
Modelling Milking

- Building a system to mimic milking forces directly on udder cells in a controlled lab environment.
- Testing how mechanical forces like stretching affect cell behavior without relying on live cows.
- Eliminating the high costs and difficulties associated with performing these experiments on animals.
- Cells showed proper proliferation.

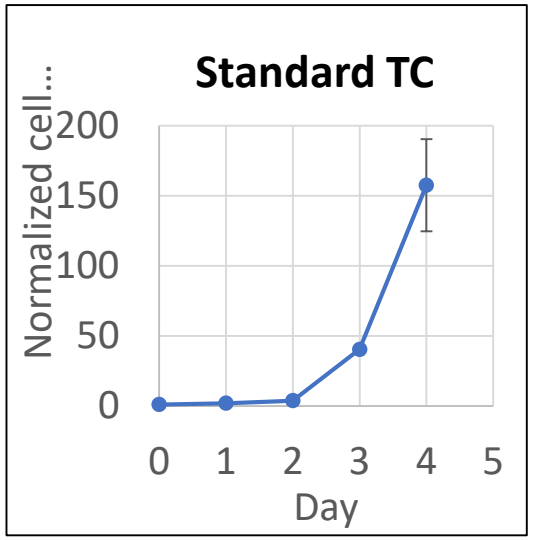


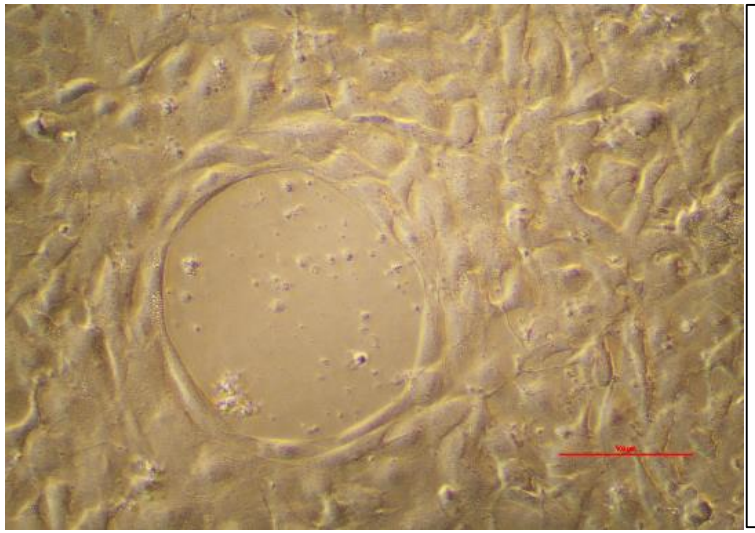
ההמלצות הישראליות למתקני חליבה. הוצא לאור ע"י מאל"ה- מועצת החלב (2007).

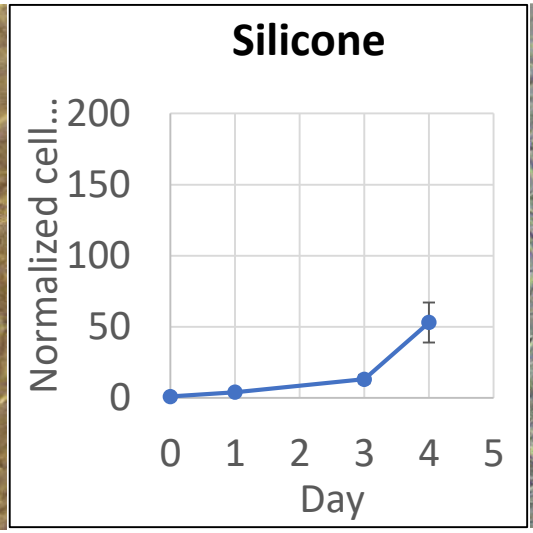


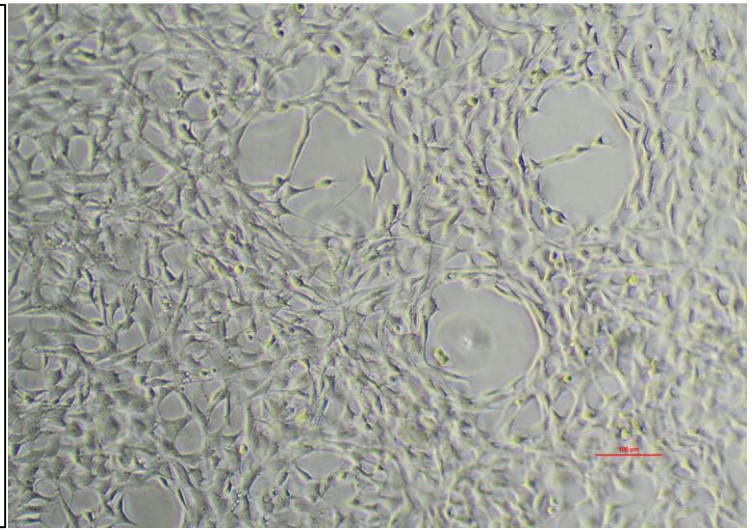


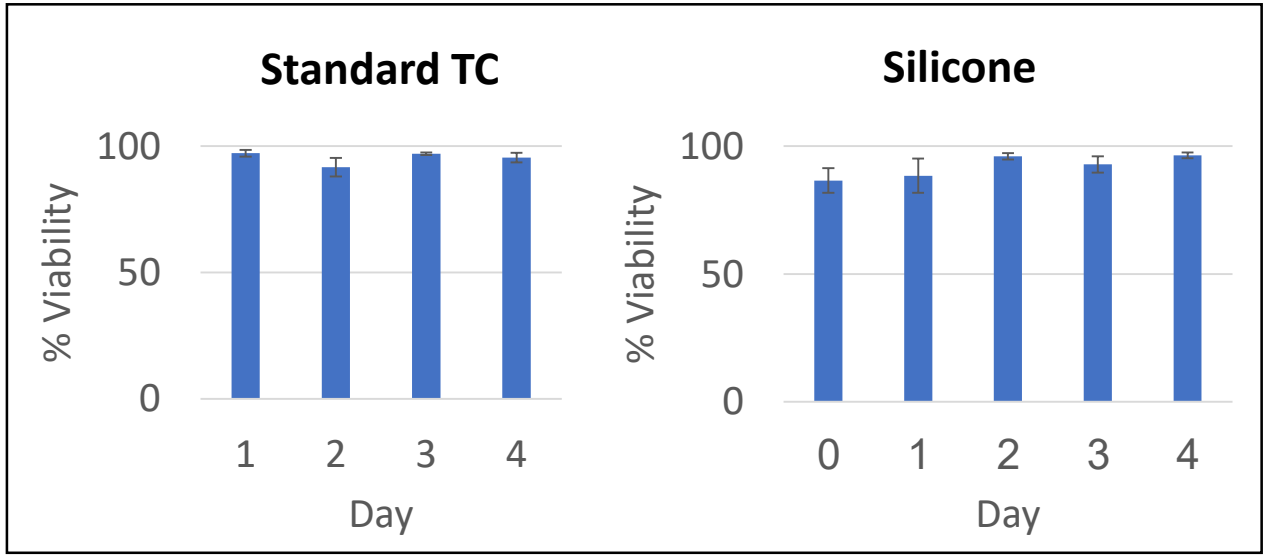
Proliferation



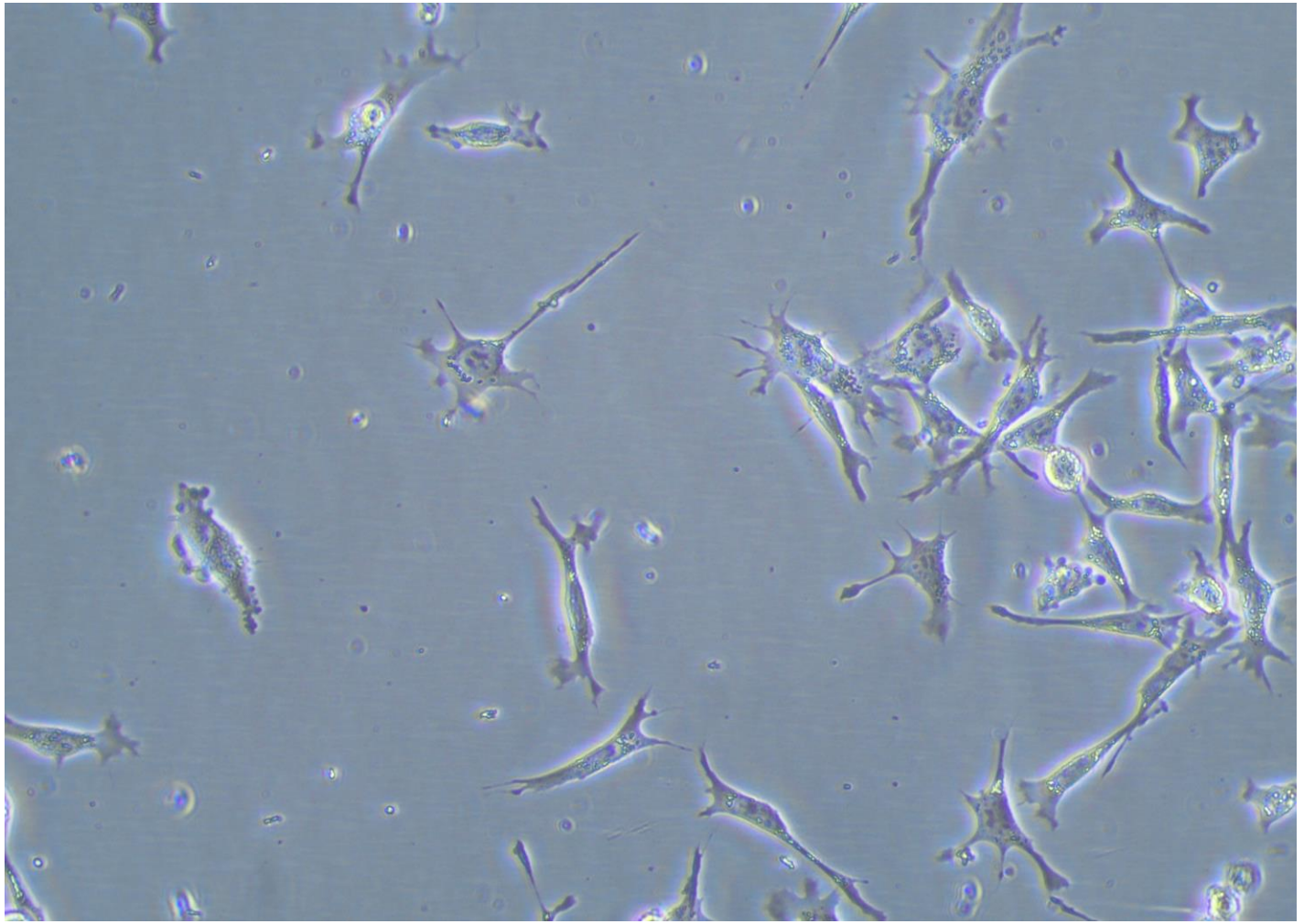




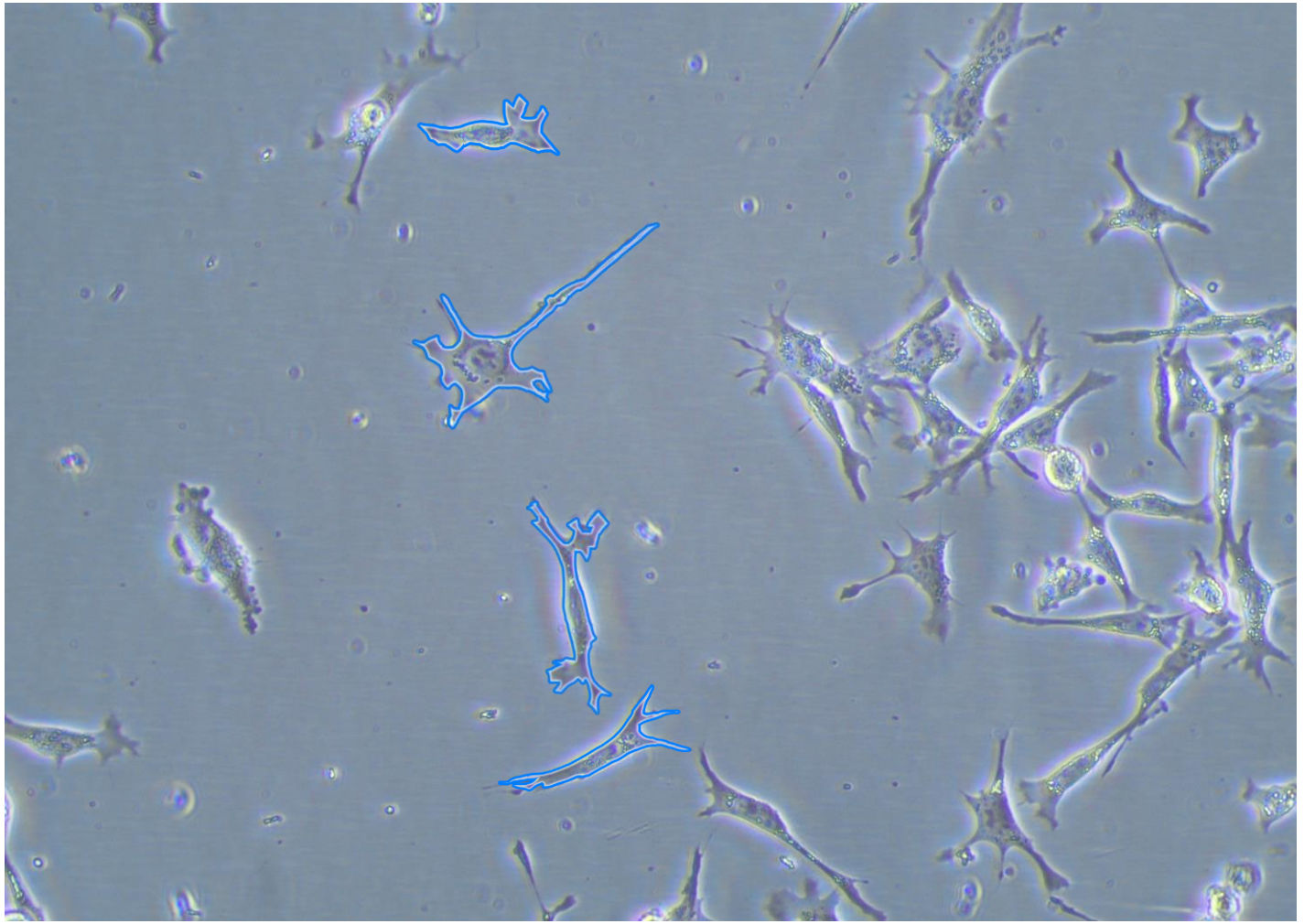




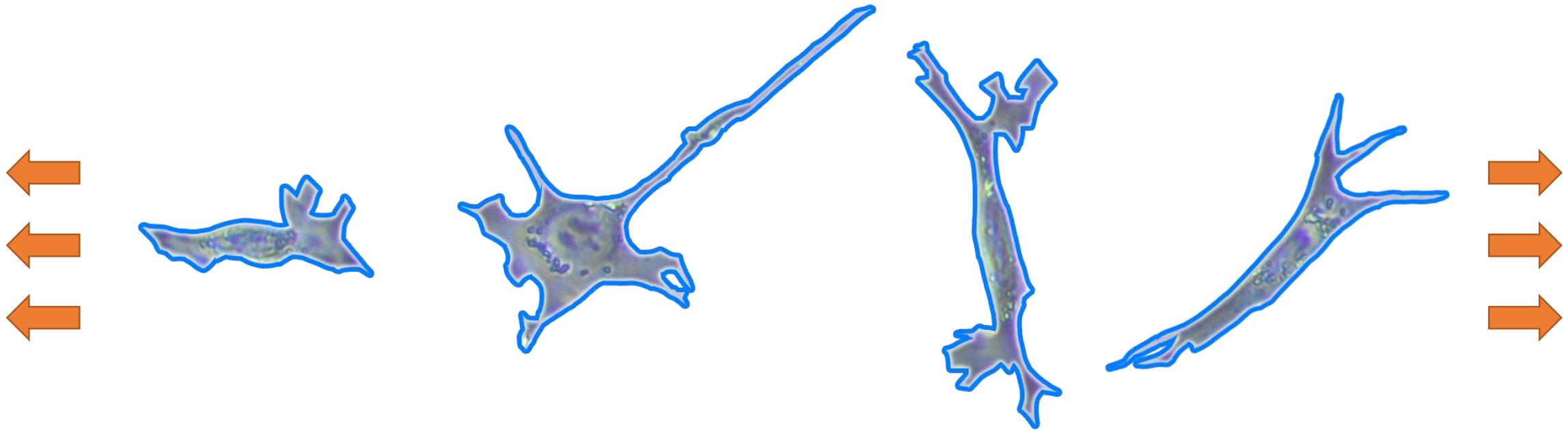
MECs’ Morphology and Uniformity



MECs’ Morphology and Uniformity

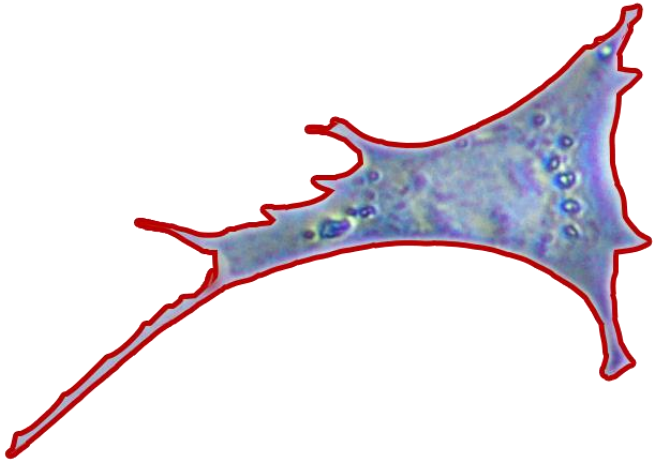


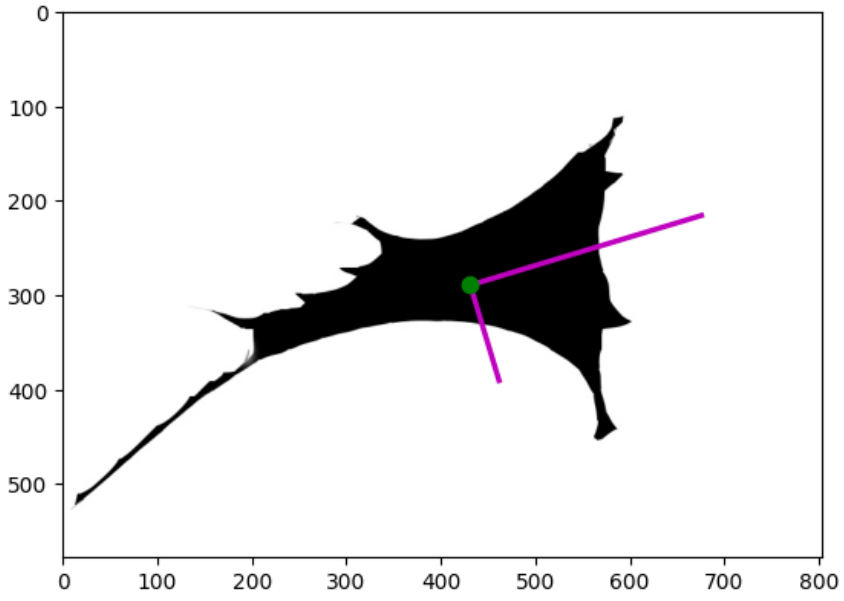
MECs’ Morphology and Uniformity



Geometrical Properties

- Shape
- Orientation
- Size

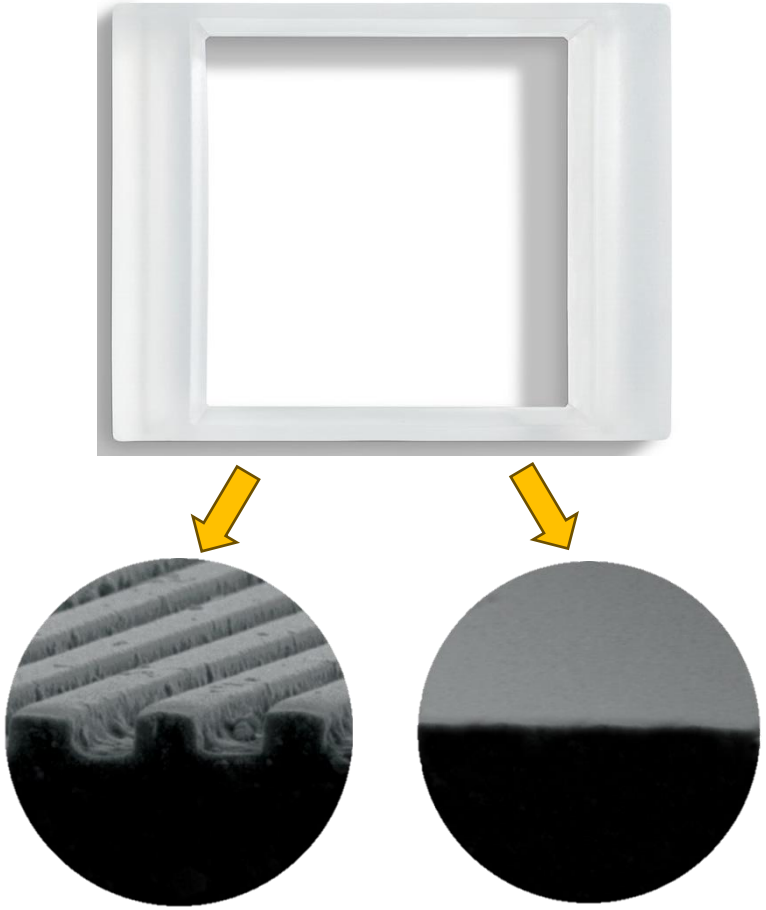




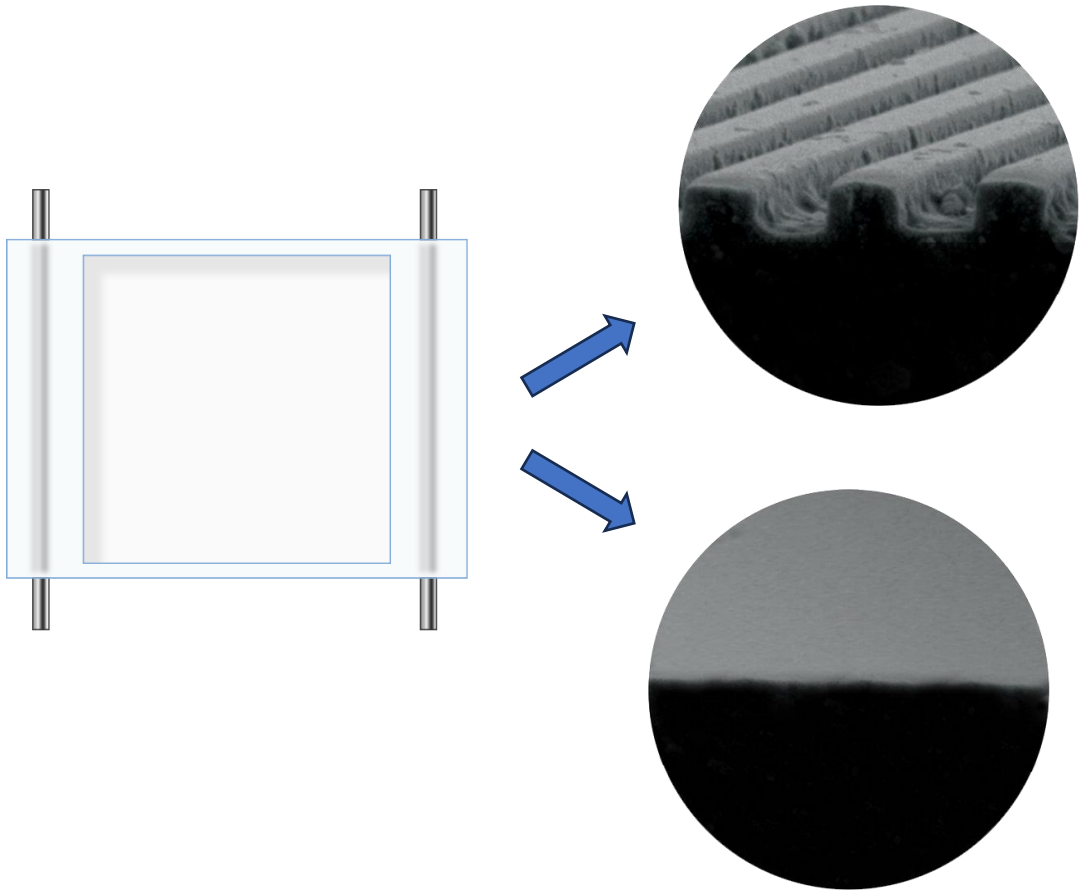
2 Approaches in Order to Create Uniformity

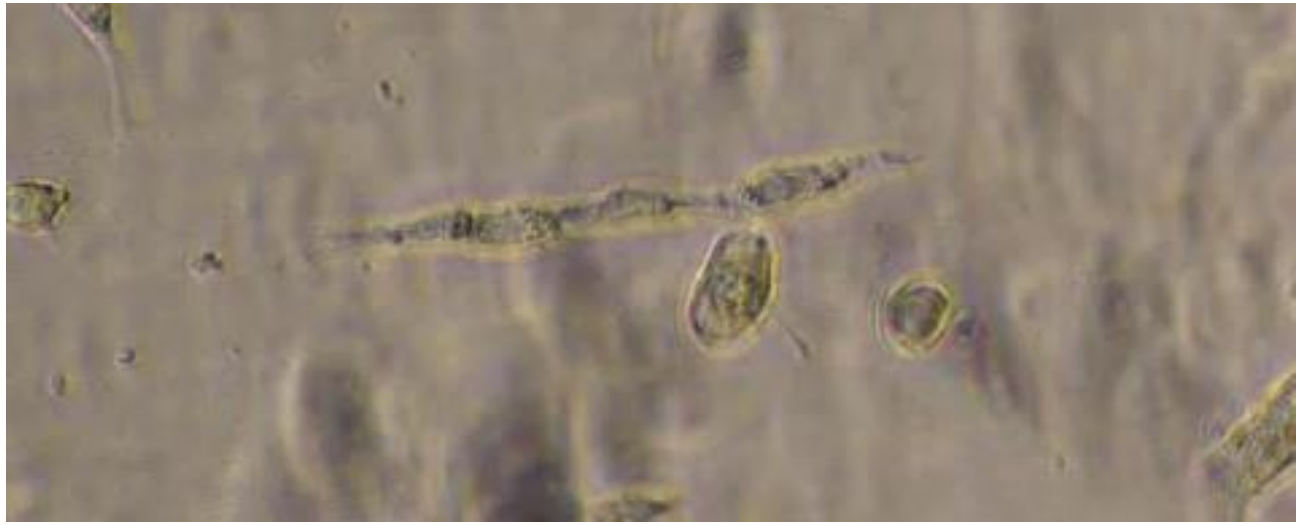
- Topography
- Cyclic Stretching

Topography



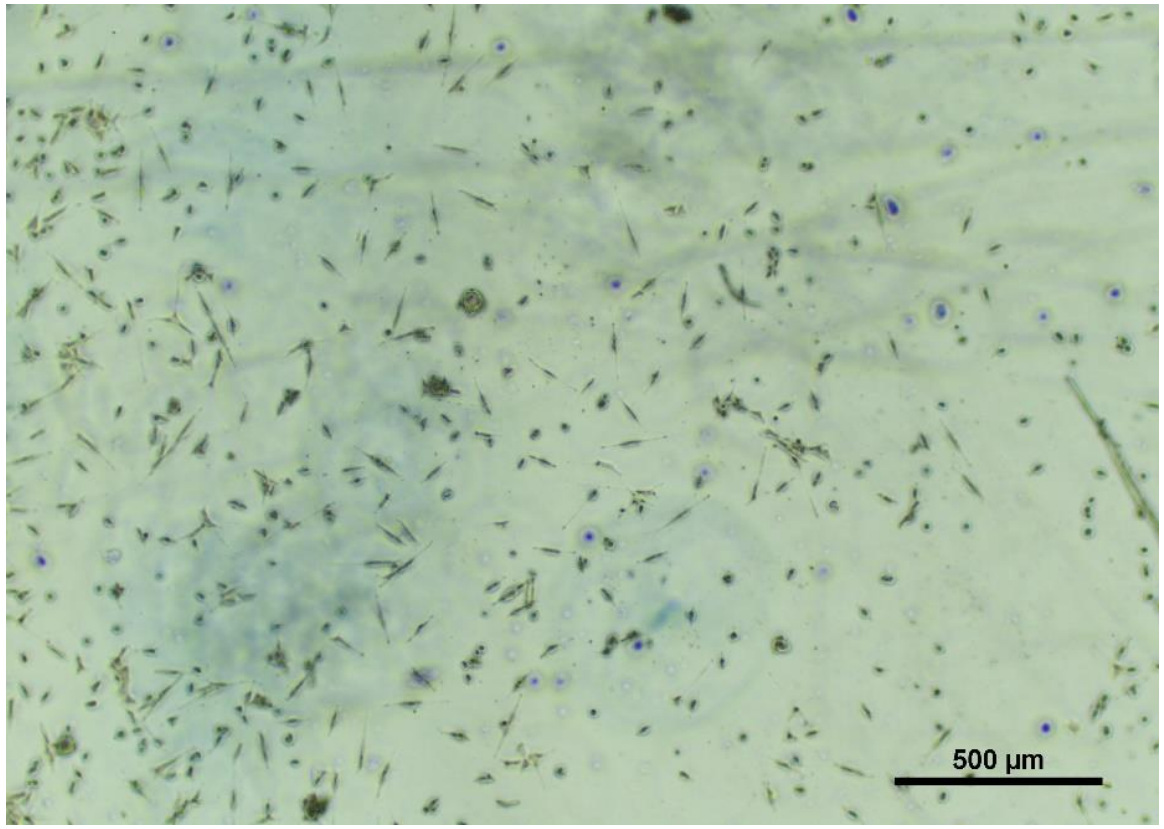
Chamber Topography





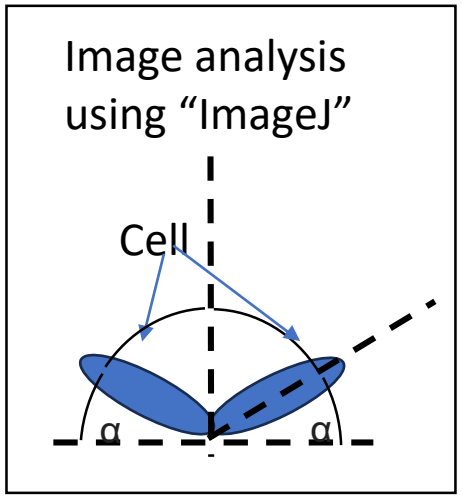
Lice Comb Experiment

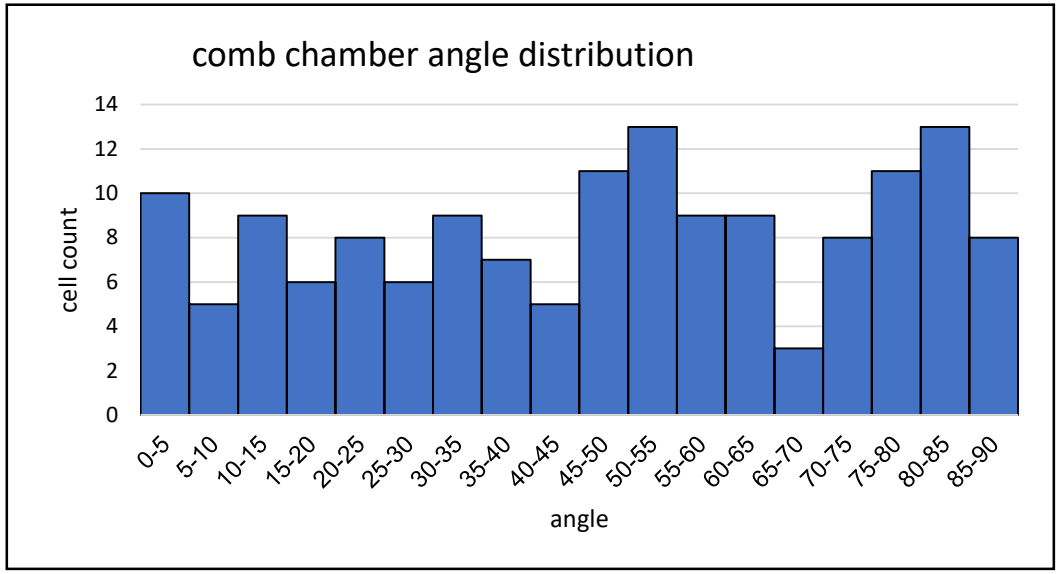
- Cells were incubated for 1 day on a CS chamber that was placed on a lice comb in order to influence the topography of the chamber.

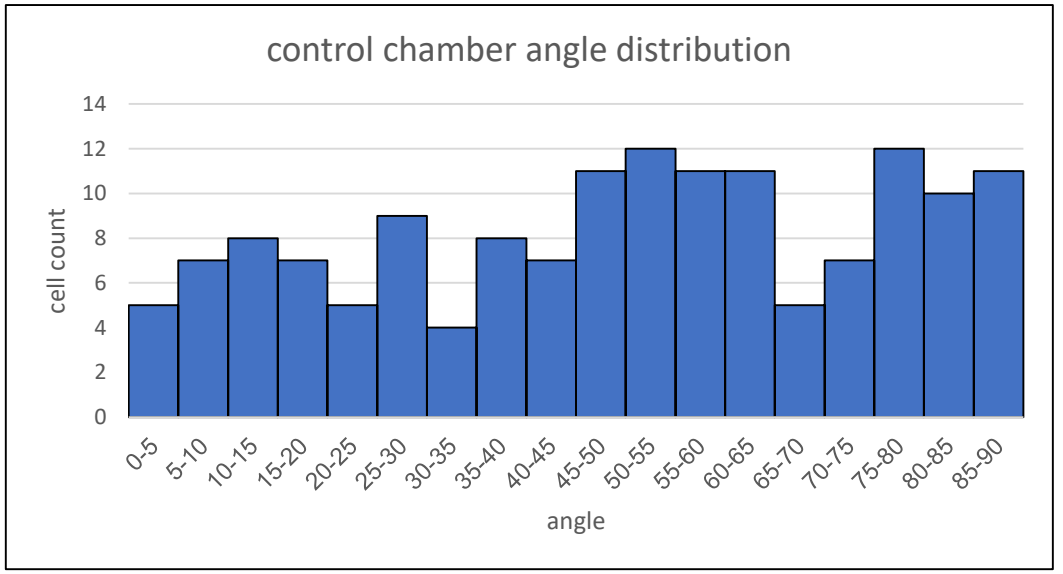


Results Analysis

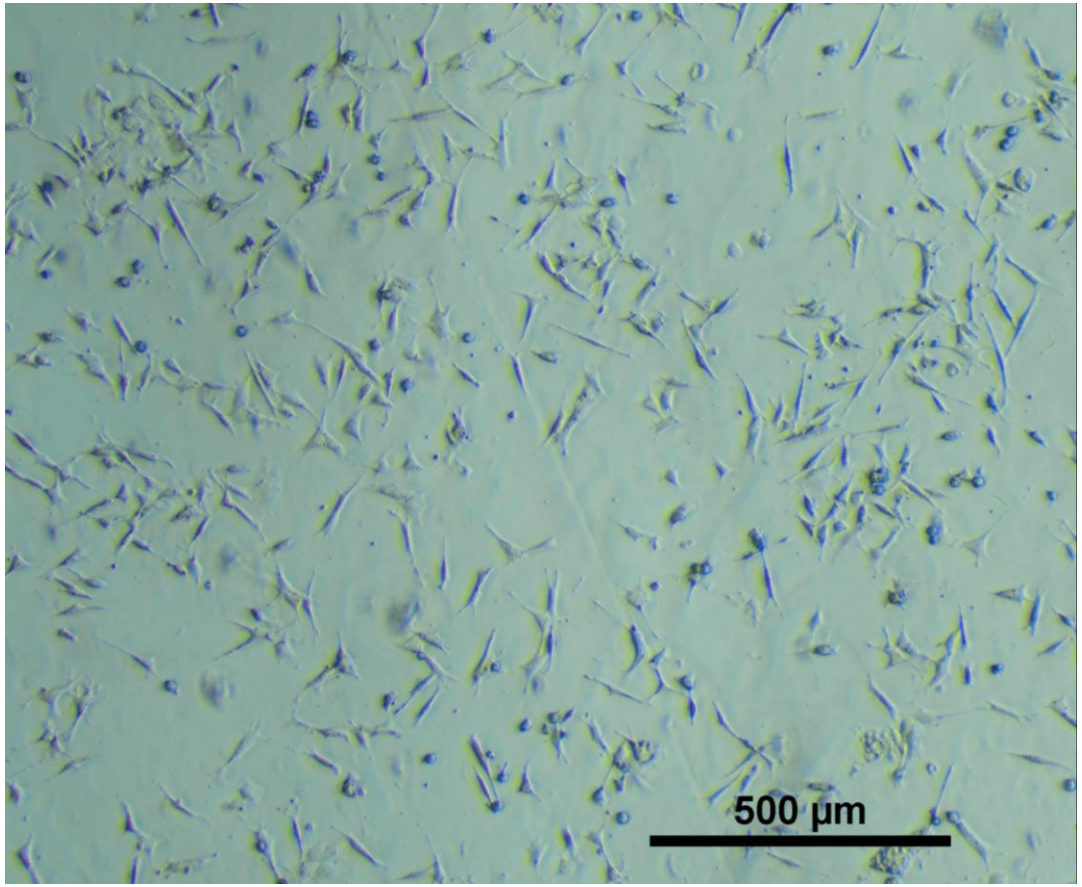
- The lice comb topography did not affect cells alignment.







Cyclic Stretches



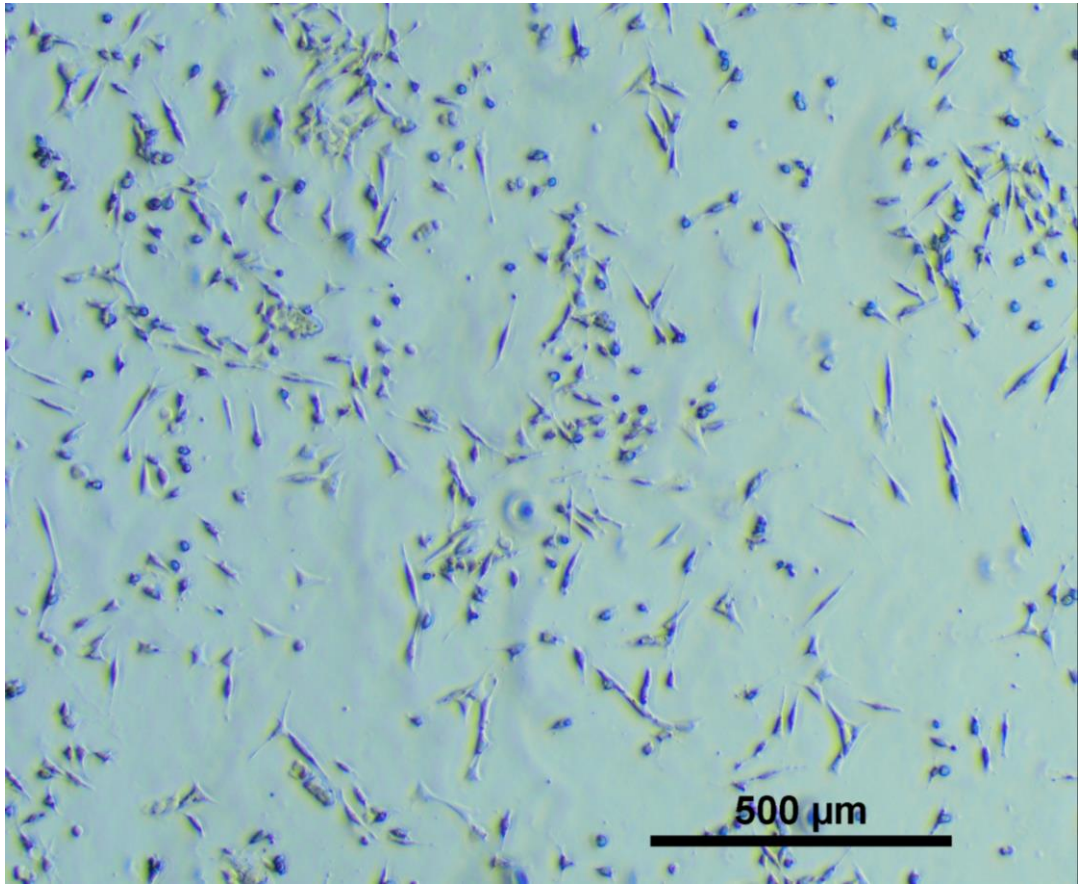
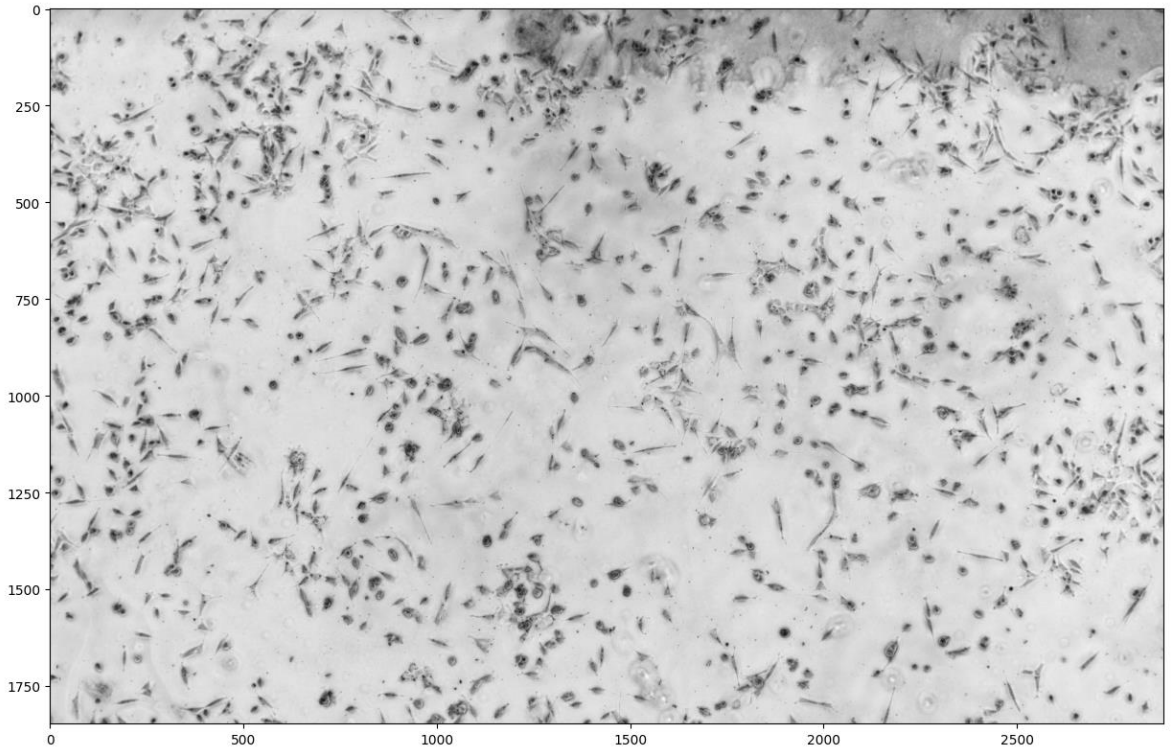
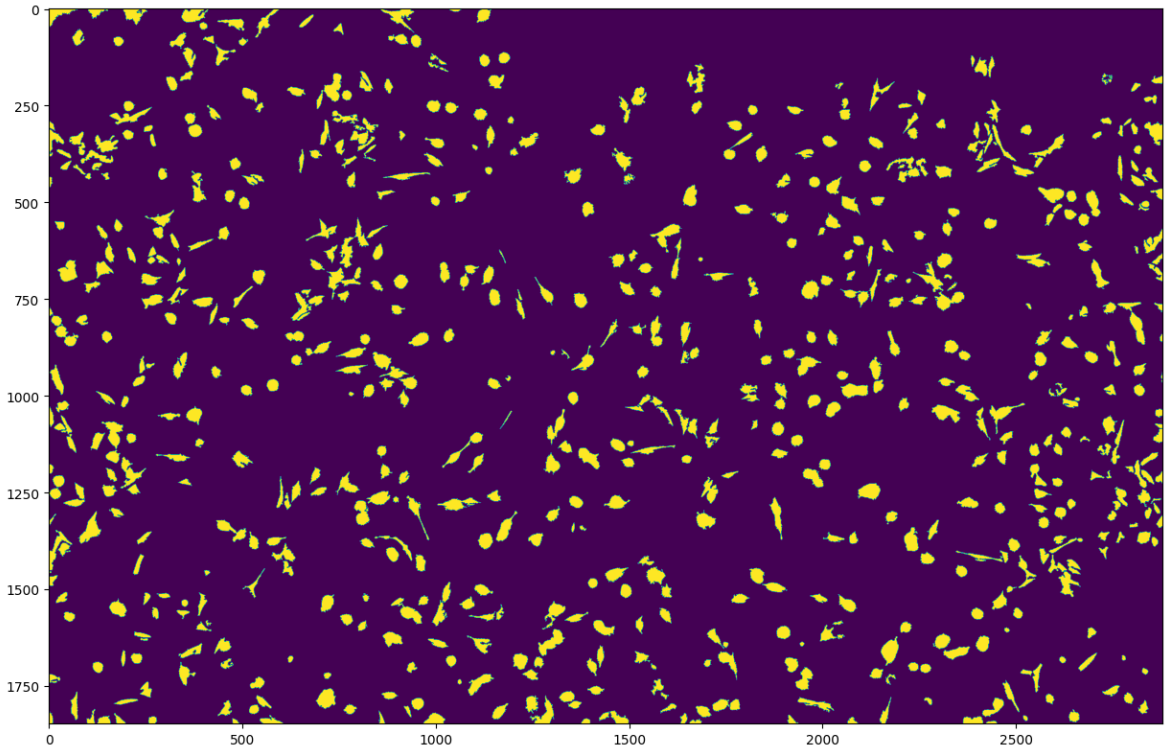


Image Analyzing

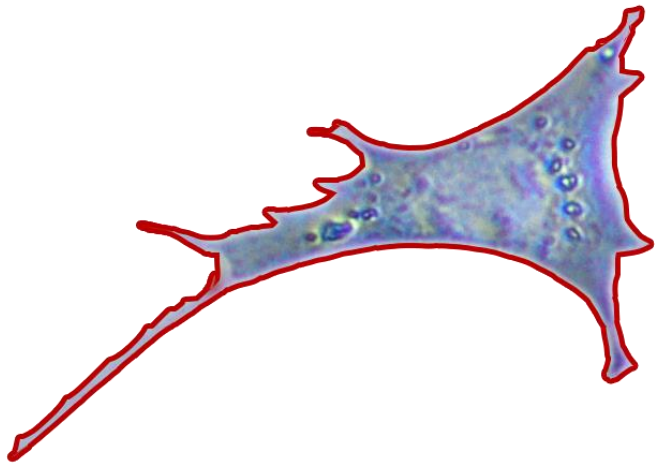




The Data Set

	A	B	C	D	E	F	G	H	I	J
1	label	area	centroid-0	centroid-1	major_axis_leng	minor_axis_leng	perimeter	orientation_degr	aspect_ratio	compactness
2	1	32	2.375	2.03125	8.074909559	5.387969062	19.65685425	35.92730948	0.667248224	0.9608778731
3	2	5	0.4	27.2	3.098386677	1.788854382	5.207106781	71.56505118	0.5773502692	0.4315320925
4	3	11	0.3636363636	40.54545455	7.5438892	1.767142775	10	84.04116205	0.2342482409	0.7234315595
5	4	120	5.458333333	231.8833333	18.20556538	11.36273883	52.59188309	16.19388393	0.6241354552	1.834198494
6	5	37	1.486486486	256.1891892	12.0066578	4.721397356	24.65685425	77.26549261	0.3932316083	1.307566387
7	6	10	0.2	499.3	8.405726635	1.569636817	8	87.8470448	0.1867342213	0.5092958179
8	7	297	7.676767677	610.2255892	26.17290325	24.54285654	118.6335137	83.15241175	0.9377200654	3.770930024
9	8	194	7.025773196	643.1907216	28.78449664	9.45822749	68.49137803	53.40977249	0.3285875591	1.924244325
10	9	23	0.9130434783	656	11.59856821	2.695417579	17.62132034	78.41143295	0.2323922685	1.074333685
11	10	8	1.125	664	5.376509904	2.200713804	7.207106781	42.91478174	0.4093201433	0.5166804894
12	11	4	0.25	669.25	3.464101615	1.414213562	3.207106781	71.56505118	0.4082482905	0.2046241954
13	12	21	3.285714286	852.0952381	9.509270376	3.845672426	16.86396103	12.15146665	0.4044129858	1.077680491
14	13	2	0	944.5	2	0	0	90	0	0
15	14	24	1.041666667	953.9583333	9.428997375	3.517355012	18.03553391	78.70926316	0.373035952	1.0785416
16	15	1	0	961	0	0	0	45	0	0
17	16	8	1.5	1137.5	4.472135955	2	8	0	0.4472135955	0.6366197724
18	17	6960	49.22485632	1179.93046	120.2611629	89.77202752	772.0620126	24.85633099	0.7464756313	6.815304518
19	18	677	9.019202363	1247.103397	60.58487376	22.75777889	201.6101731	75.6460872	0.3756346672	4.777782244
20	19	7	0.1428571429	1261.714286	6.67912053	1.325404044	6.414213562	86.05843034	0.1984399051	0.4677124467
21	20	1	0	1336	0	0	0	45	0	0

Compactness

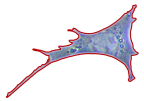


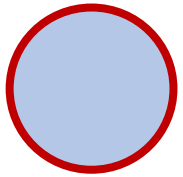
— Perimeter

Area

$$Compactness = \frac{Perimeter^2}{Area \times 4 \times \pi}$$

Compactness





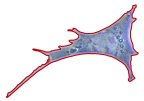
Perimeter

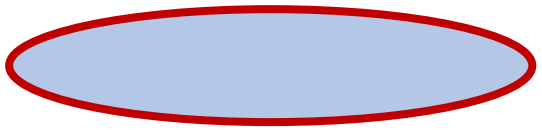
Area

Compactness = 1

$$Compactness = \frac{Perimeter^2}{Area \times 4 \times \pi}$$

Compactness





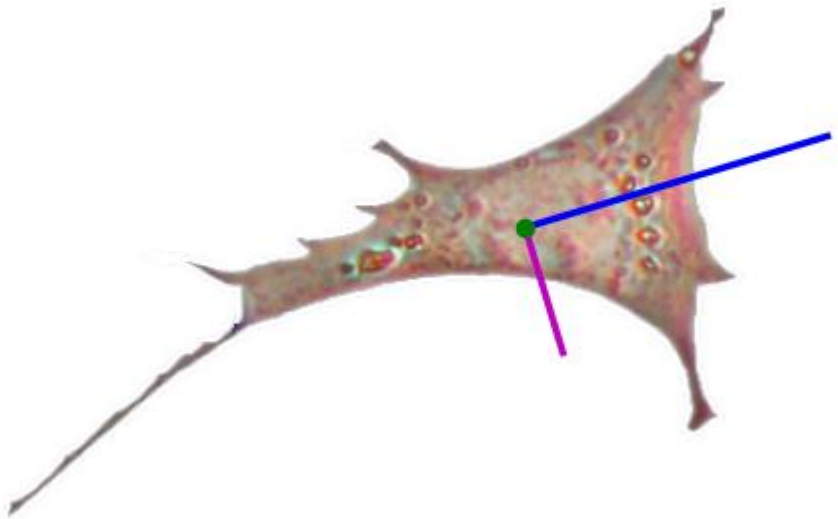
Perimeter

Area

Compactness > 1

$$Compactness = \frac{Perimeter^2}{Area \times 4 \times \pi}$$

Aspect Ratio (A.R.)

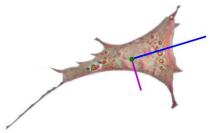


Major Axis

Minor Axis

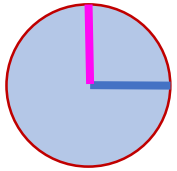
$$A.R. = \frac{Minor\ Axis}{Major\ Axis}$$

Aspect Ratio (A.R.)



Major Axis

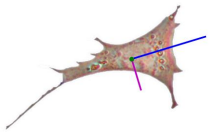
Minor Axis

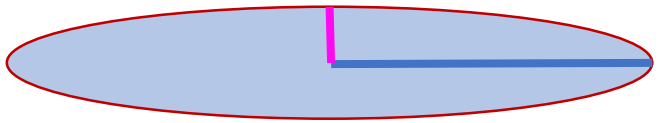


Aspect Ratio = 1

$$A.R. = \frac{Minor\ Axis}{Major\ Axis}$$

Aspect Ratio (A.R.)





Major Axis

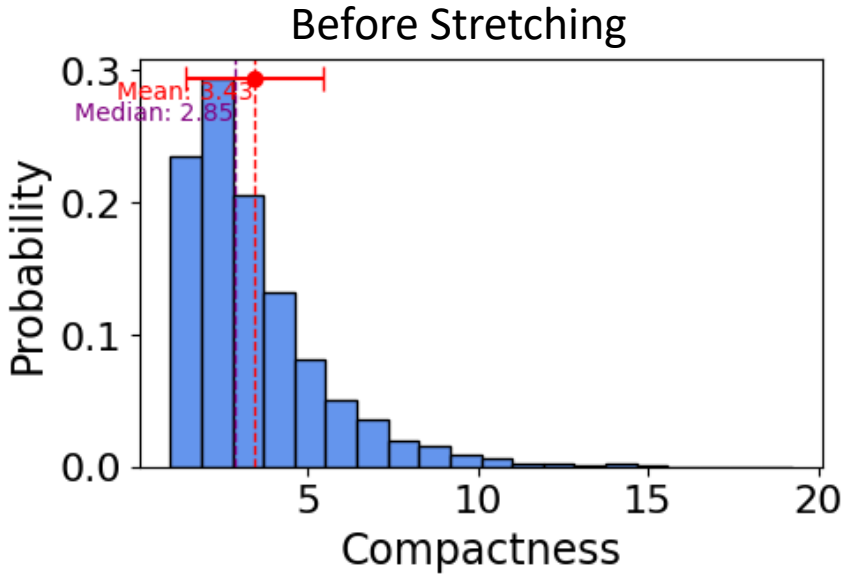
Minor Axis

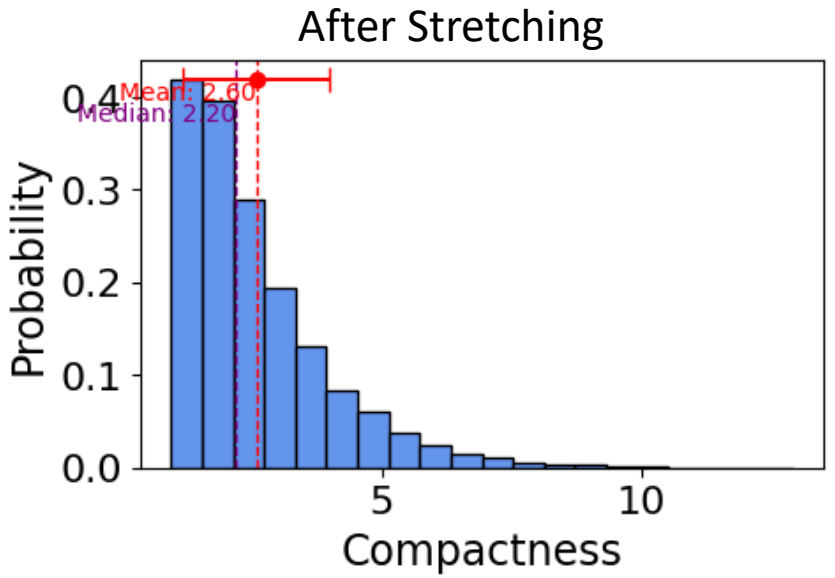
$$A.R. = \frac{Minor\ Axis}{Major\ Axis}$$

Aspect Ratio < 1

Compactness

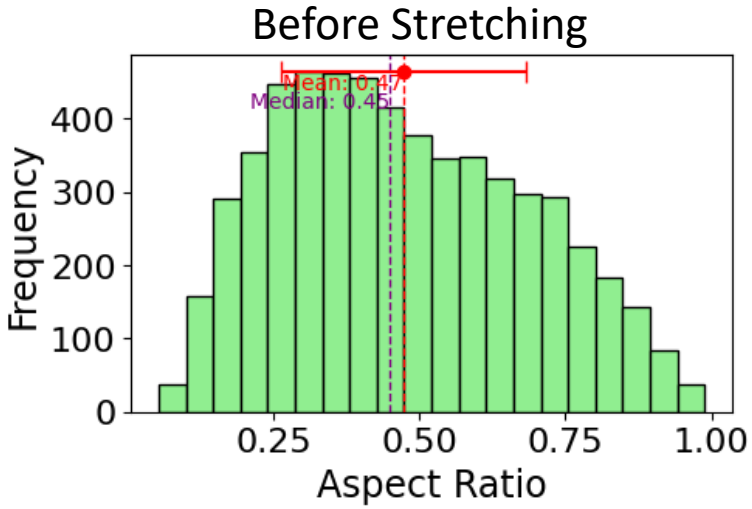
- Cells were incubated for 2 days
- Cyclic stretching in different frequencies
- Decrease of 0.65 in compactness's median on 0.22Hz pulses

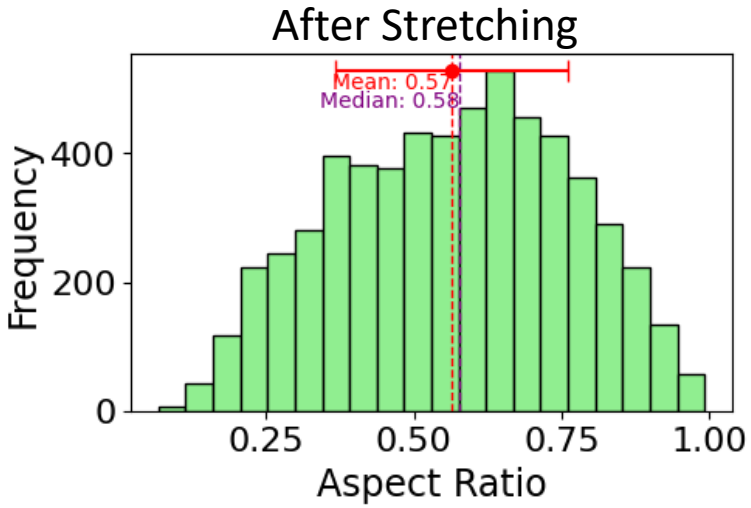




Aspect Ratio

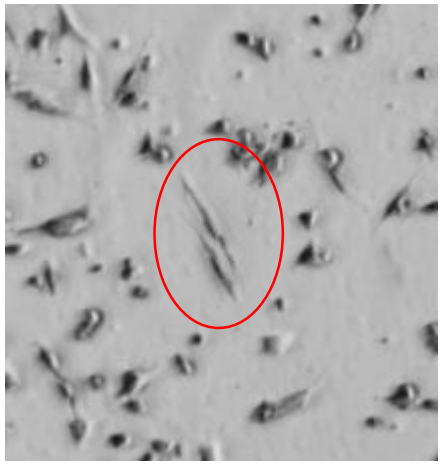
- A.R. median increased after cyclic stretching

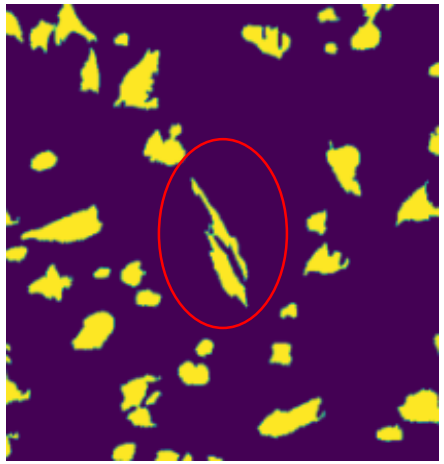




Compactness vs. Area

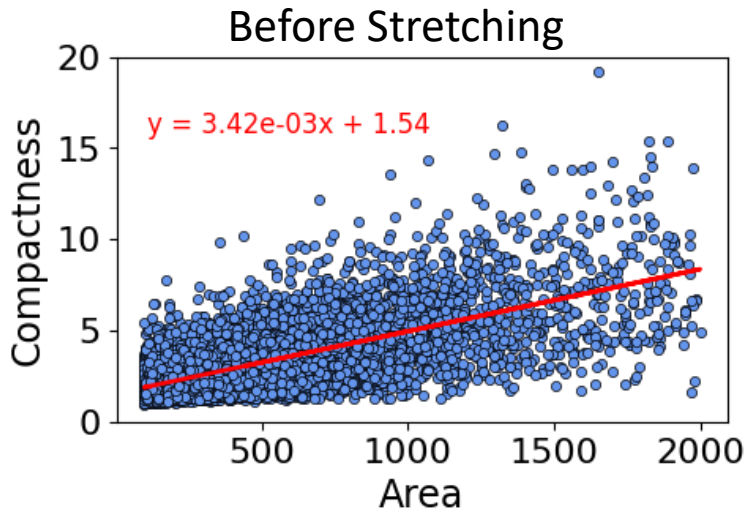
- Connected cells might be segmented as one label
- Area = 1814 pixels
- Compactness value = 10.14

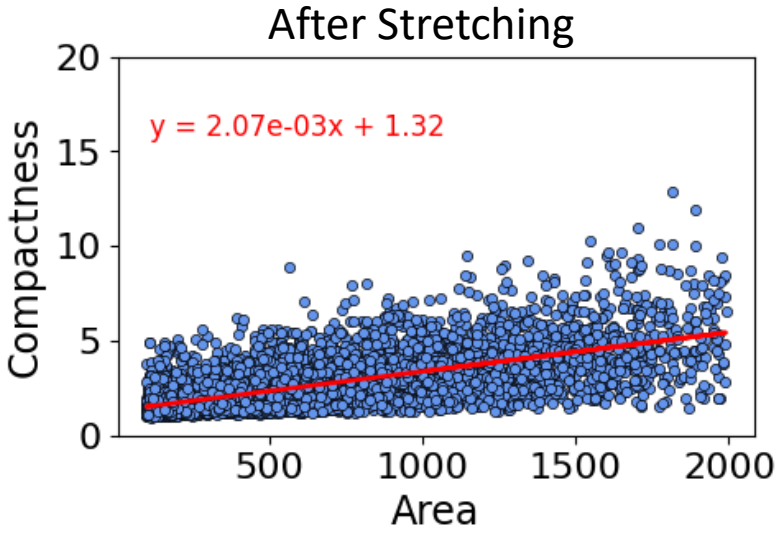




Compactness vs. Area

- Higher compactness values with higher area labels
- After stretching we see a decrease with the slope





Conclusions

- An *in vitro* system that does not harm cell viability
- Reliable way to measure cell morphology
- Cell uniformity effect in lab conditions is possible

Thanks

Dr. Yifat Brill-Karniely (Volcani Inst.)

Dr. Enav Bar-Shira (Volcani Inst.)

Nir Zaharoni (HUJI & Volcani Inst.)

Liron Toledano (HUJI & Volcani Inst.)

Prof. Itamar Barash (Volcani Inst.)

Prof. Berta Lavavi-Sivan (HUJI)

L1 bovine mammary epithelial cells generously provided by Prof. Itamar Barash (Volcani Inst.):
German and Barash, *In Vitro Cell Dev Biol – Animal* 2002