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TITLE:

Automation of Bio-Atomic Force Microscope Measurements on Hundreds of *C. albicans* Cells

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KEYWORDS:

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SUMMARY:

This protocol aims to automate AFM measurements on hundreds of microbial cells. First, microbes are immobilized into PDMS stamp microstructures and then force spectroscopy measurements are performed automatically on hundreds of immobilized cells.

ABSTRACT:

The method presented in this paper aims to automate Bio-AFM experiments and the recording of force curves. Using this method, it is possible to record forces curves on 1000 cells in 4 hours automatically. To maintain a 4 hour analysis time, the number of force curves per cell is reduced to 9 or 16. The method combines a Jython based program and a strategy for assembling cells on defined patterns. The program, implemented on a commercial Bio-AFM, can center the tip on the first cell of the array and then move, automatically, from cell to cell while recording force curves on each cell. Using this methodology, it is possible to access the biophysical parameters of the cells such as their rigidity, their adhesive properties, etc. With the automation and the large number of cells analyzed, one can access the behavior of the cell population. This is a breakthrough in the Bio-AFM field where data have, so far, been recorded on only a few tens of

cells.

INTRODUCTION:

This work provides a methodology to perform automatic force measurements on hundreds of living cells using an atomic force microscope (AFM). It also provides a method to immobilize microbes on a PDMS microstructured stamp that is compatible with AFM experiments conducted in a liquid environment.

Bio-AFM is a highly specialized technology conceived for applications in biology and then used to study living cells. It requires a trained engineer who can analyze one cell at the time. In these conditions, the number of different cells that can be analyzed is rather small, typical 5 to 10 cells in 4-5 hours. However, the quantity of force measurements recorded on a single cell are usually very high and can easily reach 1000. Thus, the current paradigm of AFM force measurements on living cells is to record hundreds of force curves (FCs) but on a limited number of cells.

Statistically, this approach is questionable, and raises the issue of the representativeness of the sample. Indeed, it is difficult, for example, to evaluate the heterogeneity of a cell population by measuring only a few cells, even if hundreds of measurements are recorded on these few cells. However, it is on the basis of this paradigm that major advances have been made in biophysics, microbiology and nanomedicine[1–3]. Indeed, nanometer analysis at the scale of single cells has provided new information on cellular nanomechanics, on the organization of transmembrane proteins, or the action mechanism of antimicrobial or anticancer drugs[4–7]. Recently however, several high-throughput biomechanical tests conducted on cells have emerged[8], showing the scientific community's interest in changing this paradigm and accessing the cell population heterogeneity. These tests all rely on microfluidic systems to deform cells and optically measure their deformation under stress to obtain an indirect measure of their overall surface elasticity[8]. However, an important issue with these methods is that they are mono-parametric: only cell elasticity can be probed. Moreover, they do not allow the measurement of the mechanical parameters of adherent cells, which can be limiting for the studies of noncirculating mammalian cells or biofilms for example.

Approaches involving AFM have been developed by the teams of S. Scheuring[9] and M. Favre[10]. Scheuring et al. immobilized cells on fibronectin patterns[9], forcing individual cells to take the shape of the pattern[9]. Then this team mapped the mechanical properties of a few cells to define average data, representative of 14 to 18 cells. The development carried out by the Farve et al. aimed at multiplexing the measurements by parallelizing the AFM cantilevers[10]. To our knowledge, this work in the multiplexing direction has not led to measurements on living cells.

An interesting approach proposed by Dujardin's team presents an automated AFM capable of identifying cells and imaging them at the bottom of custom-made wells. Although this method does not allow for the analysis of a large population of cells, it allows the automatic testing of different conditions in each well[11].

Our objective in this work is more ambitious since we wanted to measure at least 1000 cells to

access not an average cell, but, on the contrary, the heterogeneity between cells. The strategy that we developed here to access cell population heterogeneity using AFM is based on the analysis of hundreds of cells on which a limited number of force curves are recorded. Compared to the "classical" approach of recording a large number of force curves on a limited number of cells, this approach should be considered as complementary since it does not provide the same information. Indeed, while the typical method allows one to probe individual cell surface heterogeneity, using our approach, we are able to access the entire cell population heterogeneity. To achieve this objective, we have combined a method that directly immobilizes microbes (here the yeast species *Candida albicans*) into the wells of a PDMS microstructured stamp[12], and develops an original program for moving the AFM tip, automatically, from cell to cell[13] and measuring the mechanical properties of each cell.

PROTOCOL:

1. Microbial cell culture

1.1. Revivify cells from a glycerol stock.

NOTE: *C. albicans* are stored at -80 °C in glycerol stocks, on marbles.

1.1.1. Pick a marble in the -80 °C stock and rub it on yeast peptone dextrose (YPD) agar. Grow the cells for 2 days at 30 °C, before liquid cultivation.

1.2. Prepare liquid cultures.

1.2.1. Fill a culture tube with 5 mL of sterile YPD broth and add a single colony of *C. albicans* cells, grown on the YPD agar plate.

1.2.2. Grow the culture in static conditions at 30 °C for 20 h before harvesting by centrifugation (4000 x *g*, 5 min). Discard the supernatant and eliminate as biohazard waste.

1.2.3. Wash the pellets 2x with 10 mL of acetate buffer (8 mM sodium acetate, 1 mM $CaCl_2$, 1 mM $MnCl_2$, pH 5.2). Centrifuge (4000 x *g*, 5 min) in between washings.

1.2.4. Resuspend the pellet in 2 mL of acetate buffer and use this solution for cell immobilization on the PDMS stamp.

NOTE: This suspension cannot be stored and should be prepared fresh for section 3.

2. PDMS stamp preparation

2.1. Silicon master mold preparation

2.1.1. Draw the desired microstructures using computer assisted design (CAD) software.

2.1.2. If a clean room is available, follow steps 2 to 12 of the previously published protocol[12]. Otherwise, silicon master mold can be acquired from commercial clean room facilities.

2.2. PDMS stamp molding

2.2.1. Prepare 55 g of PDMS prepolymer solution containing a mixture of 10 to 1, mass ratio, of PDMS oligomers and curing agent-(**Table of Materials**).

2.2.2. Mix and degas this solution under vacuum (in the range of 10^{-1}-10^{-2} bars) until all trapped bubbles are removed from the PDMS solution (5−10 min).

2.2.3. Pour 20 g of the degassed solution on the silicon master mold and degas again (in the range of 10^{-1}-10^{-2} bars).

NOTE: The stamp thickness should be around 2−3 mm.

2.2.4. When all bubbles are removed, reticulate the PDMS at 80 °C during 1 h.

2.2.5. Cut the PDMS microstructured stamp with a scalpel (0.5 x 1.5 cm^2) in a direction parallel to the visible microstructure arrays.

2.2.6. Peel the stamp from the silicon master mold.

2.2.7. Return the stamp to exhibit the microstructures on its upper side and deposit it on a glass slide. Make sure to have the microstructures facing up away from the glass slide. Align the microstructures that can be seen on the stamp with the side of the glass slide, which will later serve as a reference for the AFM automation procedure.

NOTE: At this stage, the PDMS stamp is ready for cell immobilization. The PDMS stamps can be stored on the silicon master mold for several months. When all the PDMS is removed from the master mold, a new PDMS stamp can be casted again on the master mold (to keep the master mold safe, it is possible to replicate it in polyurethane)[14].

3. Sample preparation

3.1. Cell immobilization

3.1.1. Centrifuge (500 x *g*, 5 min) 600 µL of the resuspended cell solution to separate the buffer from the cells.

3.1.2. Pipet 200 µL of the supernatant from step 3.1.1 onto the PDMS stamp, and degas under vacuum (in the range of 10^{-1}-10^{-2} bars) for about 40 min.

NOTE: This step is important to improve the cell immobilization inside the wells. Molecules from the yeast cell wall, present in the supernatant, are probably deposited on the PDMS surface during this pre-wetting step. These molecules, most probably, enhance the adhesion of the cells and contribute to the increase in the stamp filling rate.

3.1.3. After 40 min, with a pipette, remove the buffer from the PDMS surface and deposit, with a pipette, 200 µL of the cell solution from step 1.2.4 for 15 min at room temperature.

3.1.4. Place the cells into the microstructures of the stamp by convective/capillary assembly. For that, manually spread 200 µL of cells suspension across the stamp using a glass slide in both directions with an angle between 30 and 50°. It may be necessary to pass the glass slide several times on the stamp to achieve a high filling rate.

NOTE: A full description of this method is available[13].

3.1.5. Remove the cell suspension with a pipette. Wash the stamp 3x with 1 mL of acetate buffer, pH 5.2 to remove the cells that were not trapped.

3.1.6. Dry the back of the stamp using nitrogen flow, in order to ensure that the stamp will adhere to the dry Petri dish.

3.1.7. Finally deposit the PDMS stamp filled with cells in a Petri dish (**Table of Materials**) and fill it with 2 mL of acetate buffer to maintain the cells in liquid medium.

3.2. Setting the stamp on the AFM stage

3.2.1. Center the stage at 0:0 when starting AFM operations.

3.2.2. Calibrate sensitivity and spring constant of the cantilever on glass and in water as described in Unsay et al.[15]

3.2.3. Take the Petri dish with the stamp and place it in the AFM Petri dish holder.

3.2.4. Align the stamp edge perpendicular to the Petri dish holder Y axis.

NOTE: An acceptable tilt angle is under 5° as illustrated in **Figure 1**.

3.2.5. Place the AFM head onto the stage and be careful that the stepper motors are sufficiently extended to avoid the tip to crash on the stamp.

4. Running the AFM program

NOTE: The AFM program is provided as a **Supplementary Material** (**AutomatipSoftware2019.pdf**). It requires a JPK-Bruker AFM Nanowizard II or III equipped with

a motorized stage and JPK desktop software version 4.3. The program has been developed under Jython (version based on python 2.7)

4.1. Data acquisition

4.1.1. Center the AFM tip on top of the left corner of the 4.5 x 4.5 μm^2 wells (corresponding to the cell size) using the AFM optical microscope. If another well size is needed, center on top left corner of the desired wells.

4.1.2. Perform a 64 x 64 force map (Z range = 4 µm, tip velocity = 90 $\mu m \cdot s^{-1}$, applied force 3 to 5 nN) over a 100 x 100 μm^2 area. Select Force Mapping mode from the Measurement mode drop-down box. In the force control mapping panel input the following parameters: Rel. Setpoint = 3 to 5 nN; z length 4 µm; Z movement: constant duration; extend time: 0.01s; ext. delay:0; Retr delay: 0, Delay mode: Constant Force, Sample rate 2048 Hz; Z closed loop uncheck; Grid: check Square image, Fast 100 µm, slow: 100µm, X offset: 0 µm; Y offset: 0 µm; grid angle: 0 degree; Pixels: 64x64; pixel ratio: 1:1

NOTE: A typical result is shown in **Figure 2**. This image will help measure and verify the pitch between two wells.

4.1.3. Note the coordinates of the center of the top left well (W1) and of the bottom left well (referred as W2 on **Figure 2**). To do so, make a square box around the well. The coordinate of the center of the box appears on the left panel of the AFM software in x,y coordinates boxes.

4.1.4. To open the automation software (**Automatip_scan.py**): in the JPK desktop software click on advance in the top bar menu and select open the script. In the window that opens select the path toward the script file provided in **Supplementary Data** (**Automatip_scan.py**).

4.1.5. Implement W1 and W2 coordinate values in the Inputs box section of the Jython script (Error! Reference source not found.**3**). Input the W1 coordinates in the P1 variable line 239 of the script and the W2 coordinates in the P2 variable line 241.

NOTE: The wells selected as initial coordinates (W1 and W2) should not be too close from the scanning area edge. Otherwise the centering algorithm would not execute correctly because it needs to measure the height on the PDMS surface on each side of the well. For an example, see **Figure 4.**

4.1.6. Attribute the pitch value to the pitch variable line 245 of the script.

4.1.7. Input the well dimension in the *Ws* variable line 248. This is known from the design of the well patterns and can be checked on the same image as the one used to verify the pitch (**Figure 2**).

4.1.8. Write the path to the saving directory in line 251 to save the data at the desired place.

4.1.9. Set the *totalArea* variable line 254 to the desire multiple "*n*" of 100 µm (that is the maximum scan area of the AFM used). The total number of wells that will be probed can be calculated using this value and the pitch: maximum scan area/pitch*n^2.

NOTE: In the example of **Figure 3**, 9 areas of 100 x 100 µm^2 will be analyzed.

4.1.10. Set the force curves matrix, row and column (3, 3 or 4, 4), recorded per well in the *numScans* variable line 257.

NOTE: In the example of Error! Reference source not found.**3**, a matrix of 3 x 3 = 9 FCs will be recorded for each well.

4.1.11. Run the program. Click on the **Start** button.

NOTE: The program first automatically executes a centering algorithm to better determine the center of W1 and W2 wells (step 1). It then automatically acquires the Force Curves (FCs) matrix on each well of the first scanning area (step 2). When all the wells of that area are probed, the script automatically moves the AFM tip to the first well of the next scanning area. The tip is retracted, the microscope stage moves to the next area, the tip is again approached on the stamp and the centering algorithm is executed again to re-center automatically on the first well (1') of that area (step 3). The first area is defined by the user, the second one, is on the right etc. until n is reached. n+1 area is underneath n, n+2 on the left of n+1, etc. until 2n is reached. 2n+1 is underneath 2n, and 2n+2 is on the right on 2n, etc. Globally, the tip serpentines through the total area. Step 2 and 3 are repeated automatically until the total number "n^2" of scanning areas have been probed. **Figure 5** presents the flowchart of the program. It takes ~4 h to complete the program.

4.2. Data analysis

4.2.1. Execute the "Copy files" python script (**Copy_files_L.py**, provided in **Supplementary Data**) to organize the FCs files into one folder. This script was developed with Python 2.7 and the SciPy module. Use Video Studio Code software to open the python script. Input path to the general folder (line 34 of the script provided in supplementary data) and where it will be stored (line 37).

4.2.2. Open the AFM manufacturer data processing software to analyze the force curves. In the top menu **File**, select open '**batch of spectroscopy curves**.

4.2.3. In the batch processing window, select the process provided in **Supplementary Data** (**StiffnessProcess.jpk-proc-force**). Select the last step of the process and click on **Keep and Apply to All**. All force curves will receive the same treatment.

NOTE: The process uses the calibration from the FCs files to convert the deflection curves into force curves calibrated in Newton; a data smoothing algorithm is applied (average of 3

consecutives points); the baseline is translated to rest on the zero axis; the contact point is extrapolated and the FC is offset to place the contact point at coordinate (0,0); the bending of the cantilever is subtracted to the FCs, the retract slope is fitted. At the end of the data treatment, the software generates a file that contains a table giving for each FCs: its name, Young Modulus, contact point, adhesion force, slopes, etc.

4.2.4. Repeat steps 4.2.1 to 4.2.3 for all experiments. Be careful to save the data in different folders (i.e.: "…\TREATED\" and "…\UNTREATED\")

4.2.5. Use the R script provided in **Supplementary Data** to plot histograms and box plots and perform ANOVA statistical treatments.

4.2.5.1. To open the R script (**DataAnalisys.R**), use R studio software and load the files containing the information extracted with the data processing software (.tsv).

4.2.5.2. On the environment window use the **Import Dataset** button, from the list displayed select **from text (readr)** and in the new window select the **Browser** button and find the .tsv file.

4.2.5.3. Once the file has loaded, select the columns (stiffness and adhesion) to be included for the analysis. To run all the code, press **Ctrl+Alt+R**.

NOTE: The script works with 4 datasets, consider two experiments both having untreated and treated cells. It is possible to execute blocks of the script and see how the variables change according to the functions executed.

REPRESENTATIVE RESULTS:

We used the described protocol to analyze the effect of caspofungin on the biophysical properties of the opportunistic human pathogen *C. albicans* in its yeast form. Caspofungin is a last chance antifungal molecule used when other drugs are ineffective because of the resistance mechanisms cells develop towards antifungals. Its mechanism of action is based on the inhibition of the subunit Fks2 of the complex fks1/Fks2 responsible for the ß glucan synthesis. As ß glucans are a major component of the fungal cell wall[16, 17], we expected modification of the biophysical properties of the cell wall: rigidity and adhesion.

Figure 6 presents typical histograms obtained when all the protocol presented above is applied. The red histogram represents the stiffness repartition recorded on 957 native cells and the blue one on 574 caspofungin treated cells. The first interesting observation is that both histograms demonstrate a bimodal distribution of the values. This observation is possible only because we measured hundreds of cells. On smaller samples, researchers usually observe a single distribution and miss the population heterogeneity.[17, 18]

The second observation concerns the effect of caspofungin. It globally reduces the stiffness of the cells while still two subpopulations exist. In a last step the proposed protocol provides an ANOVA comparison of the native and treated cells as presented in **Figure 7**. It demonstrates that

the two conditions have a different stiffness and that this difference is highly significant (p value < 0.001). This value is reached thanks to the large number of cells analyzed and provides a greater confidence in the obtained results.

The adhesion has also been extracted from the automatically recorded data and we found that the adhesion force between the bare tip and native cells was of 0.64 ± 0.6 nN. In this case also two subpopulations were found: the first one has a mean adhesion force of 0.7 ± 1.4 nN while the second of 4.5 ± 1.5 nN. The treatment with caspofungin had unpredictable effects on the adhesion. In one experiment no effect was observed, but in another experiment, caspofungin induced a decrease in the adhesion to the tip and a reduction of the population adhesion heterogeneity. These results are extracted from Proa et al.[13], where they are presented in totality.

FIGURE LEGENDS:

Figure 1: Acceptable position of the microstructured stamp on the AFM stage. The tilt angle on the left pictures (up to 5°) can be handled by the program but the tilt on the right is important (10°). This figure has been modified from[13].

Figure 2: Typical AFM image of a filled PDMS stamps showing the initial coordinates as W1 and W2, the size of the scanning area (Δ2), the tilt angle (θ). This Figure has been modified from[13].

Figure 3: User input section of the script. P1 and P2 refers to the coordinates of well 1 (W1) and well 2 (W2) of Figure 2. The other parameters are the pitch in µm, the well size in µm (Ws), the directory path for saving the data, the total square area that will be probed by the automated AFM (totalArea is the length in µm of the side of the total square area) and the number of force-curves per wells (numScans). All units are in µm.

Figure 4: Optical image providing an example of valuable (green dots) initial wells. The black square represents the scanning area and the red spots, initial wells that should better be discarded. This figure has been modified from[13].

Figure 5: Program flowchart showing the 5 steps automatically executed by the AFM.

Figure 6: Histograms of the median stiffness values. (**A, B**) Show the median results per cell for native and caspofungin treated cell. This figure has been modified from[13].

Figure 7: Box plots comparing native and treated with caspofungin cells. The 3 stars represent a significance of p<0.001. The box represents 90% of the results, the central line is the median value and the vertical bars represent the range of all the data. This figure has been modified from[13].

Figure 8: Time-position dependency of values. Histograms in the center are the original data which is divided into the different subgroups corresponding to the subpopulations founded (cyan/green). (**A,B**) Show the presence of the two sub-populations at every hour in the

experiment. (**C,D**) show the positions of indentation; on each position it is possible to see the presence of the subpopulations (cyan/green). Subgroup organization was done using the k-means algorithm. This figure has been modified from[13].

Figure 9: The safe area. An area, inside the PDMS well, has been defined as the area where the pyramidal tip does not touche the well edge while reaching the well bottom (in the case of an empty well). This Figure has been modified from[13].

DISCUSSION

The main improvement provided by this methodology is a significant increase in the number of measured cells in a determined amount of time. The counterpart is a reduction of the number of points measured per cell. It means that this method is not designed to provide a detailed analysis of a single cell. The method only applies to cells that can fit in the wells of the PDMS stamp. The stamp is quite versatile, while it contains wells of 1.5 x 1.5 μm^2 up to 6 x 6 μm^2. Still it is impossible to immobilize bacillus or much bigger cells. The stamp and capillary convective deposition cannot be used to immobilize mammalian cells that are much bigger (around 100 µm in length).

In this context, Peric et al.[19] developed a smart microfluidic device to immobilize bacillus like *Escherichia coli* and *bacillus subtilis.* This device makes it possible to immobilize bacillus at defined positions and under physiological conditions. It would be very interesting to adapt the software to the particular size of this device.

Tip contamination can also be a problem in this automated system. In the case of microbial cells, it is not so prevalent, but it is of high importance in the case of mammalian cells. Dujardin et al.[11] addressed this issue by adding a cleaning step in their automated protocol. This step consists of checking the laser sum and activating the cleaning procedure if the sum is too low. The clean step consists of immersing the tip in a well filled with water or ethanol.

A question that systematically arises from this automation work has been: "does the heterogeneity comes from the evolution of the cells during the experiment?". To answer this question, we plotted the stiffness results as a function of time as presented in **Figure 8A,B**. It clearly demonstrates that heterogeneous stiffness values are recorded at any time during the experiment.

In the same context the question of the tip position during the measure emerged. It could be possible that force curves recorded on the edge of a cell would have a different stiffness from FC recorded on the top of the cells. To avoid this inconvenience, we defined what we called the safe area. It is depicted in **Figure 9A,B** and represents an area inside the wells where the tip will not touch the well edges during force measurement. Using this "safe area" we could record FC only on cells and at the top of them. As shown in **Figure 8C,D** the tip position within the safe area is not responsible for the heterogeneity of the results as we found both phenotypes for each position of the tip, in the safe area.

To make sure that the values recorded at each position are homogeneous we plotted the stiffness

values as a function of the position as presented in **Figure 9C,D**. It shows that heterogeneous stiffness values are recorded on each position in the well, which means that the observed heterogeneity is not an artifact due to the tip position in the wells.

The protocol presented in this article represents a conceptual and methodological breakthrough in the field of AFM applied in life science. The large amounts of data generated are compatible with automatic analysis which will undoubtedly allow the classification of cell populations according to new criteria. The application of this protocol to protein or sugar arrays is entirely feasible and requires only a few adaptations to consider the spacing between areas of interest. It is therefore a versatile protocol that is the result of strong interdisciplinary collaboration.

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DISCLOSURES:

The authors have nothing to disclose.

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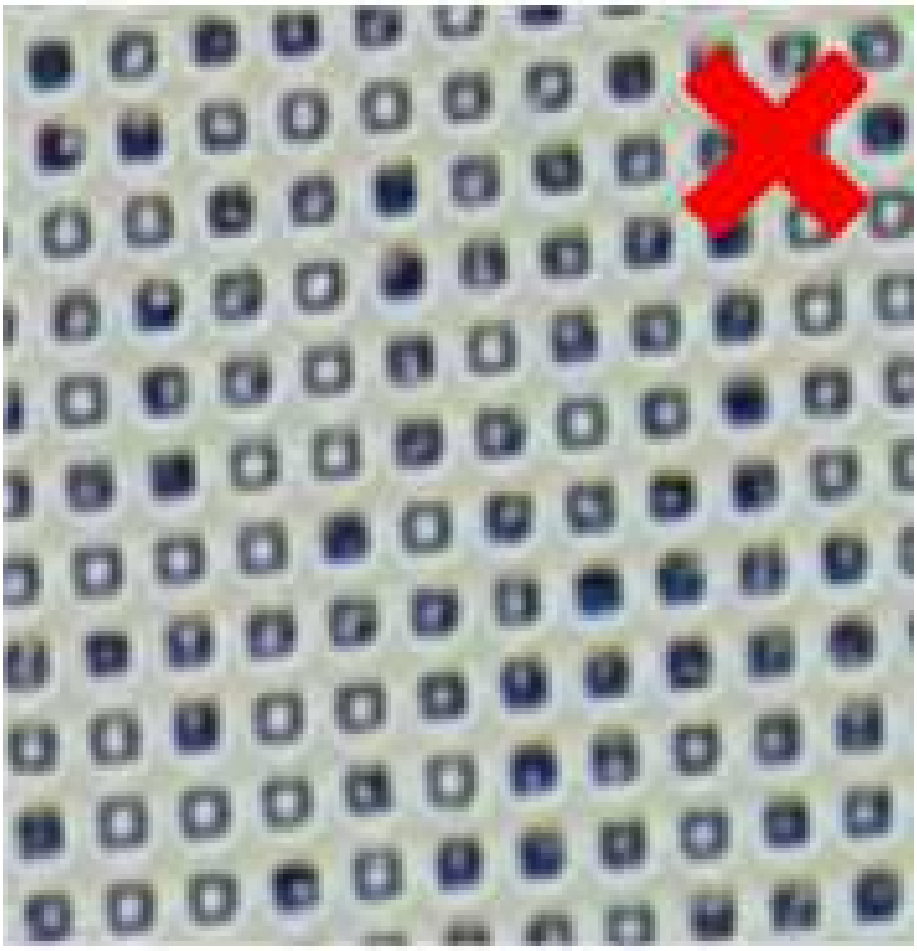
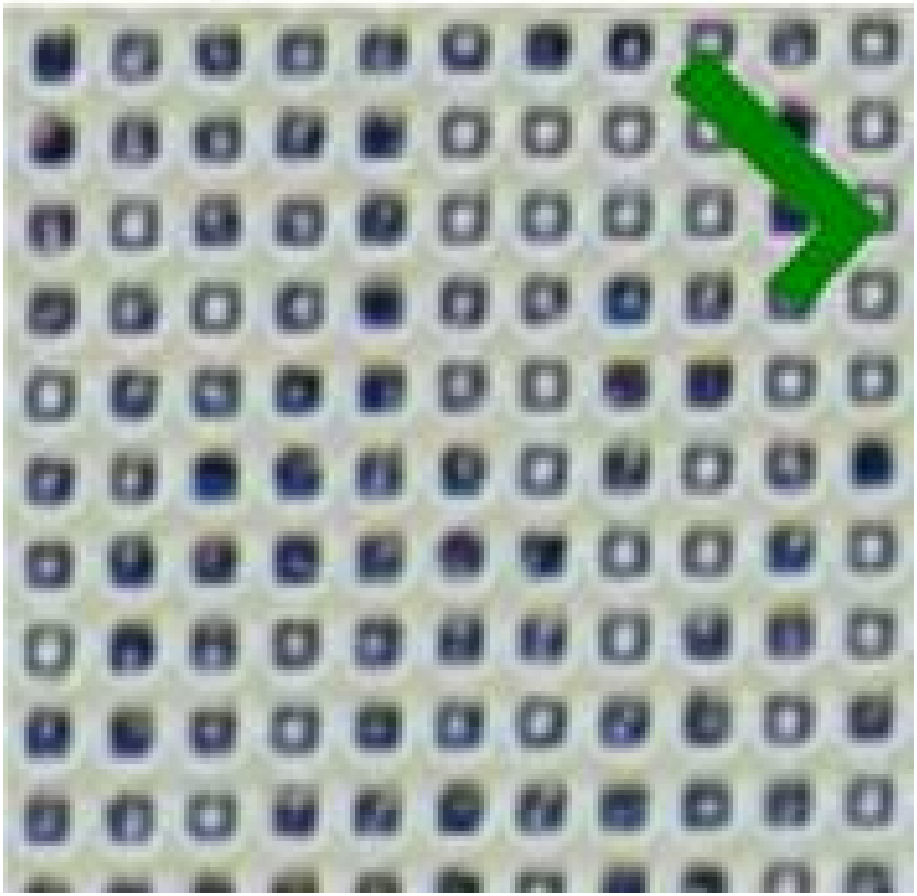
Figure 1





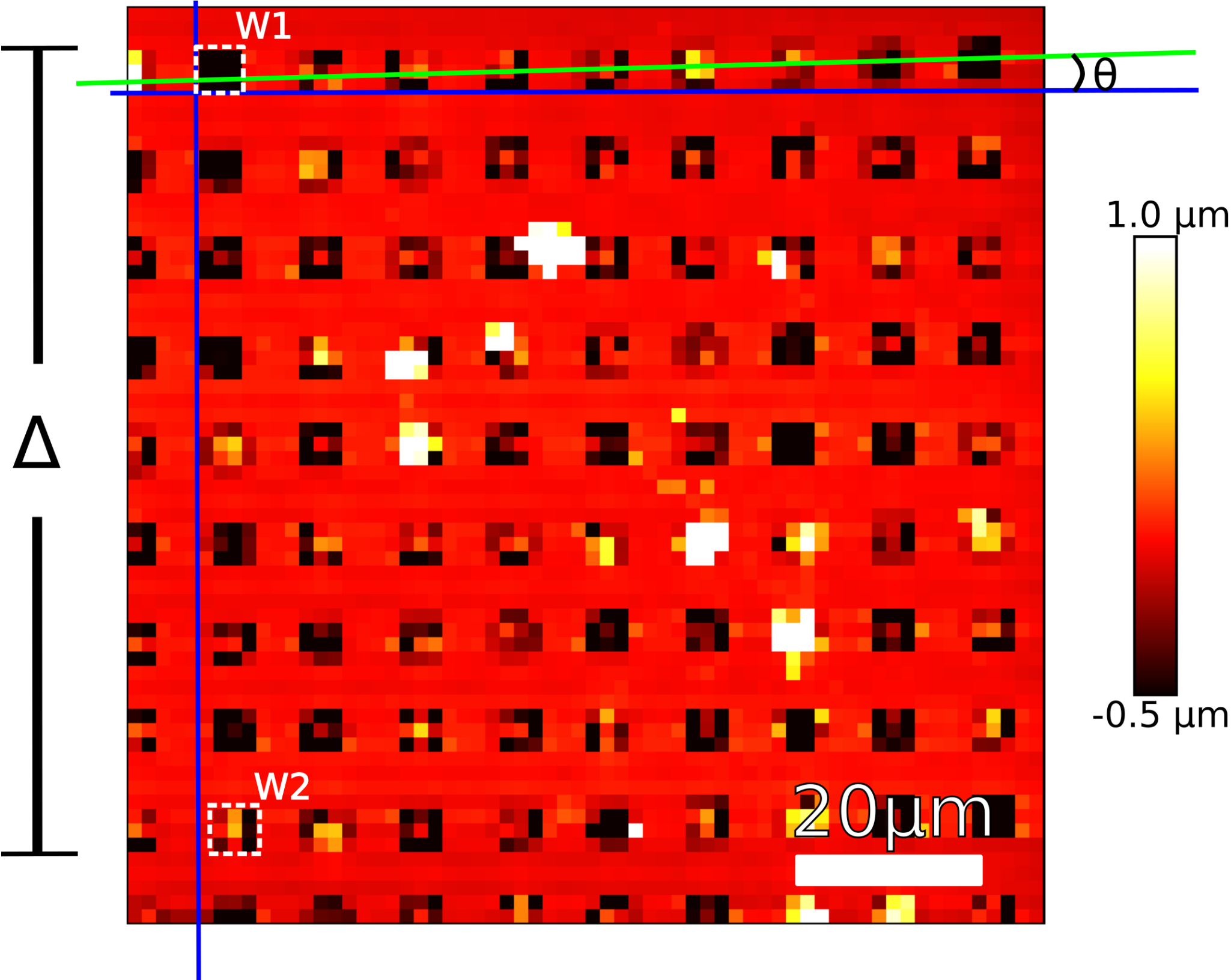


Figure 2

Click here to access/download;Figure;Figure2.eps

Figure 3

Click here to access/download;Figure;Figure3.2.pdf

```
234 #*****************************Inputs block*****************************
235
236 #-----------The working area is defined as 100 x 100 micrometers-----------
237 #Points 1 and 2, take this coordinates from the center of each well, MANUAL INPUT
238
239 P1=[42.893e-6 , 36.031e-6] # these are exemples of W1 coordinates with units in meters
240
241 P2=[44.425e-6 , -36.655e-6]  # these are exemples of W2 coordniates with units in meters
242
243
244 #-----------Pitch-----------
245 pitch= 10.5e-6 #units in meters
246
247 #Well dimensions assuming square wells
248 Ws=4.5e-6 #units in meters
249
250 #Path for saving directory
251 path="~/YourDirectoryForSavingData/"
252
253 # Pattern area
254 totalArea = 300e-6 # units in meters, totalArea represents side length of a square
255
256 # ForceScan matrix for the wells
257 numScans=[3,3] # total number of measurements per wells
258
259 #**********************************************************************
```

Figure 4

Click here to access/download;Figure;Figure4.pdf

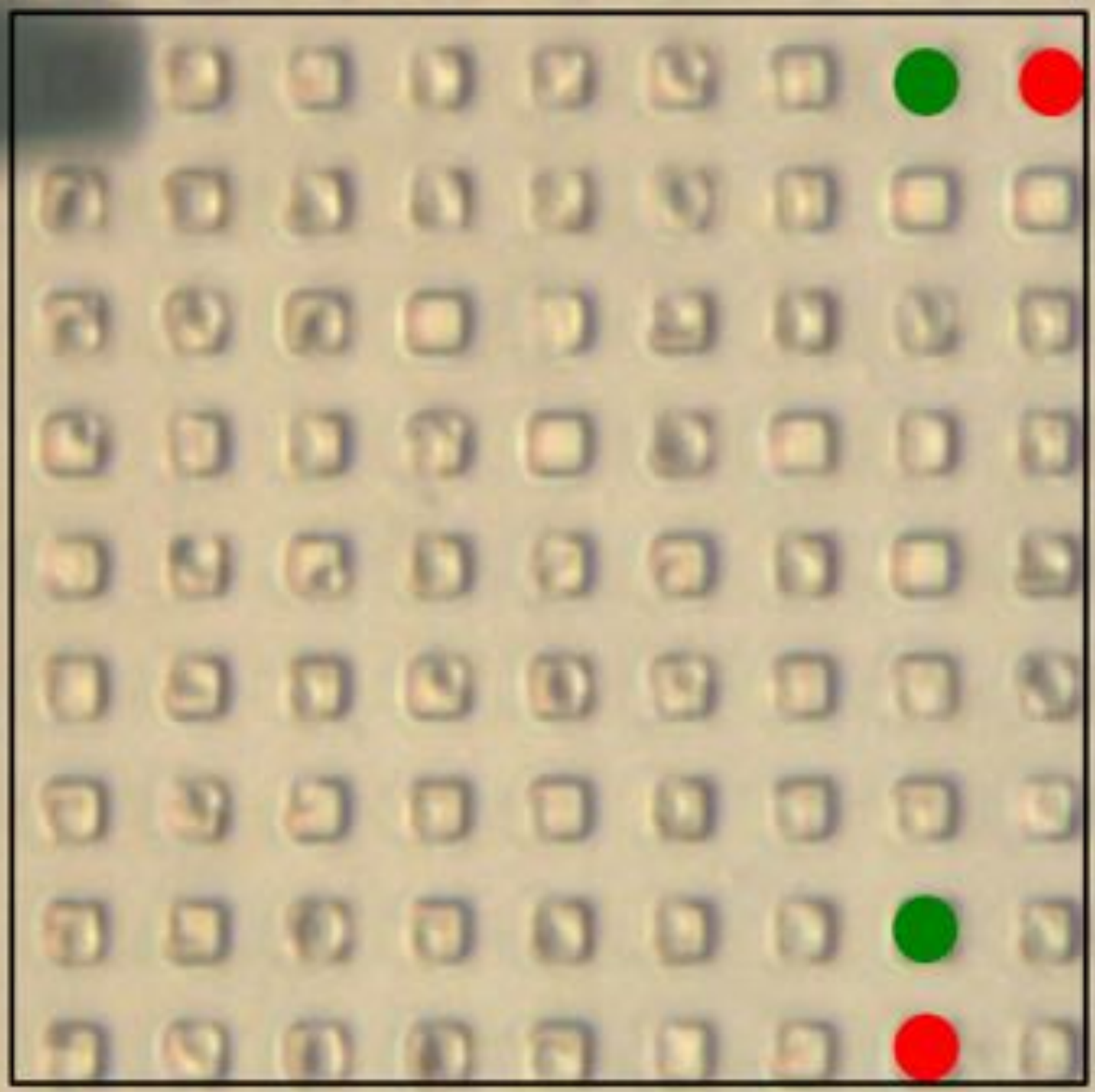


Figure 5

Click here to access/download;Figure;figure5.eps

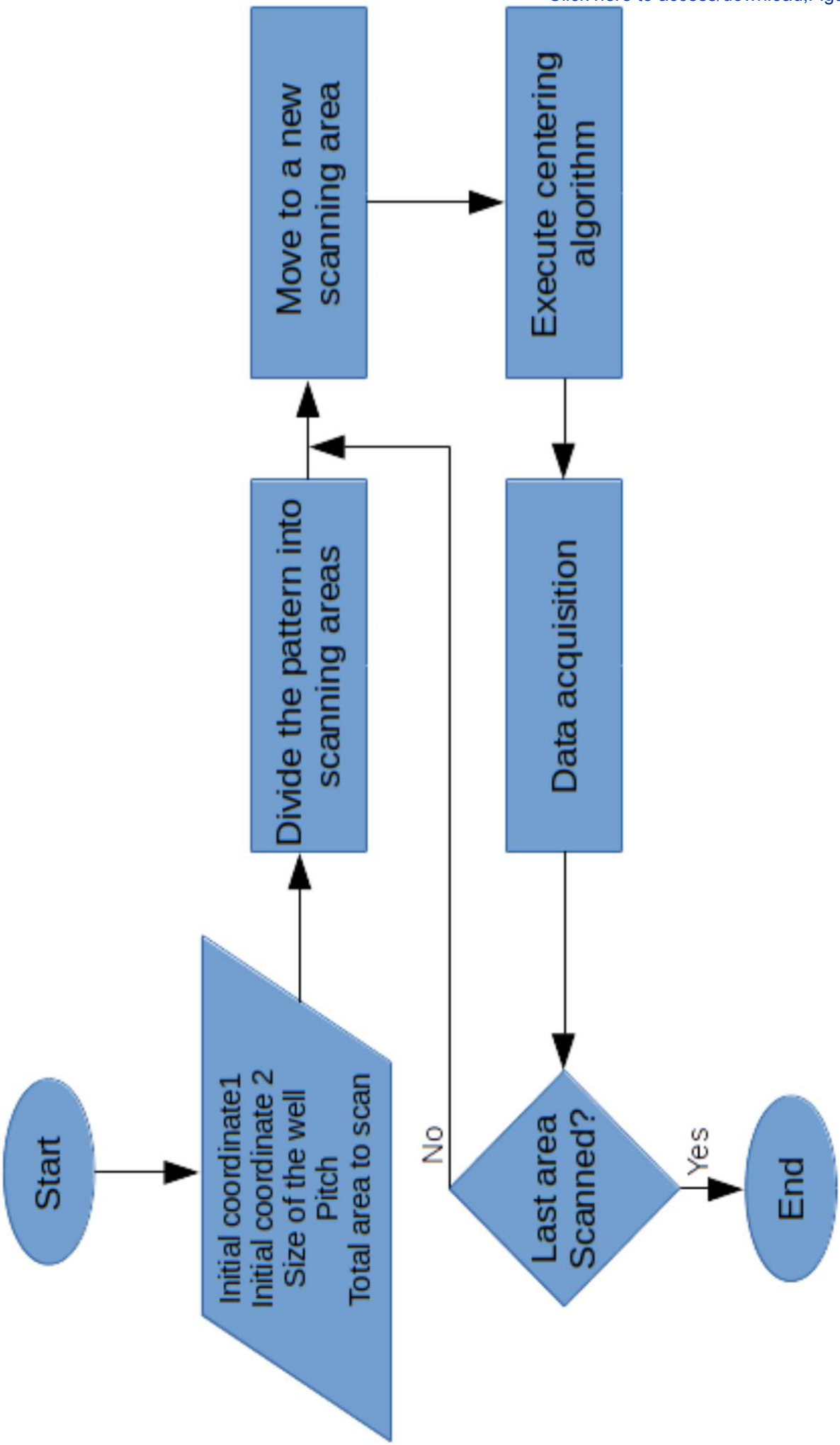


Figure 6

Click here to access/download;Figure;Figure6(1).eps

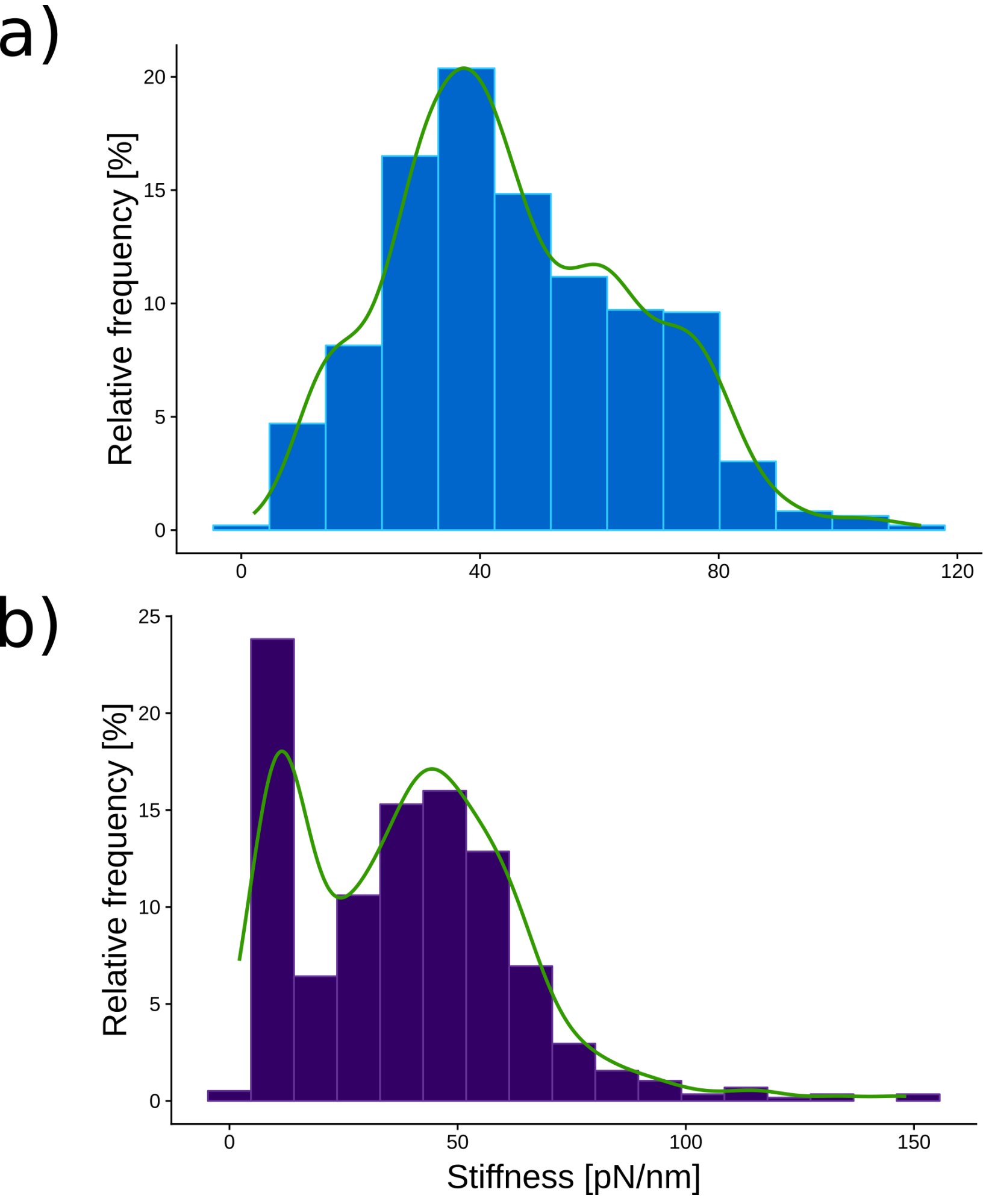
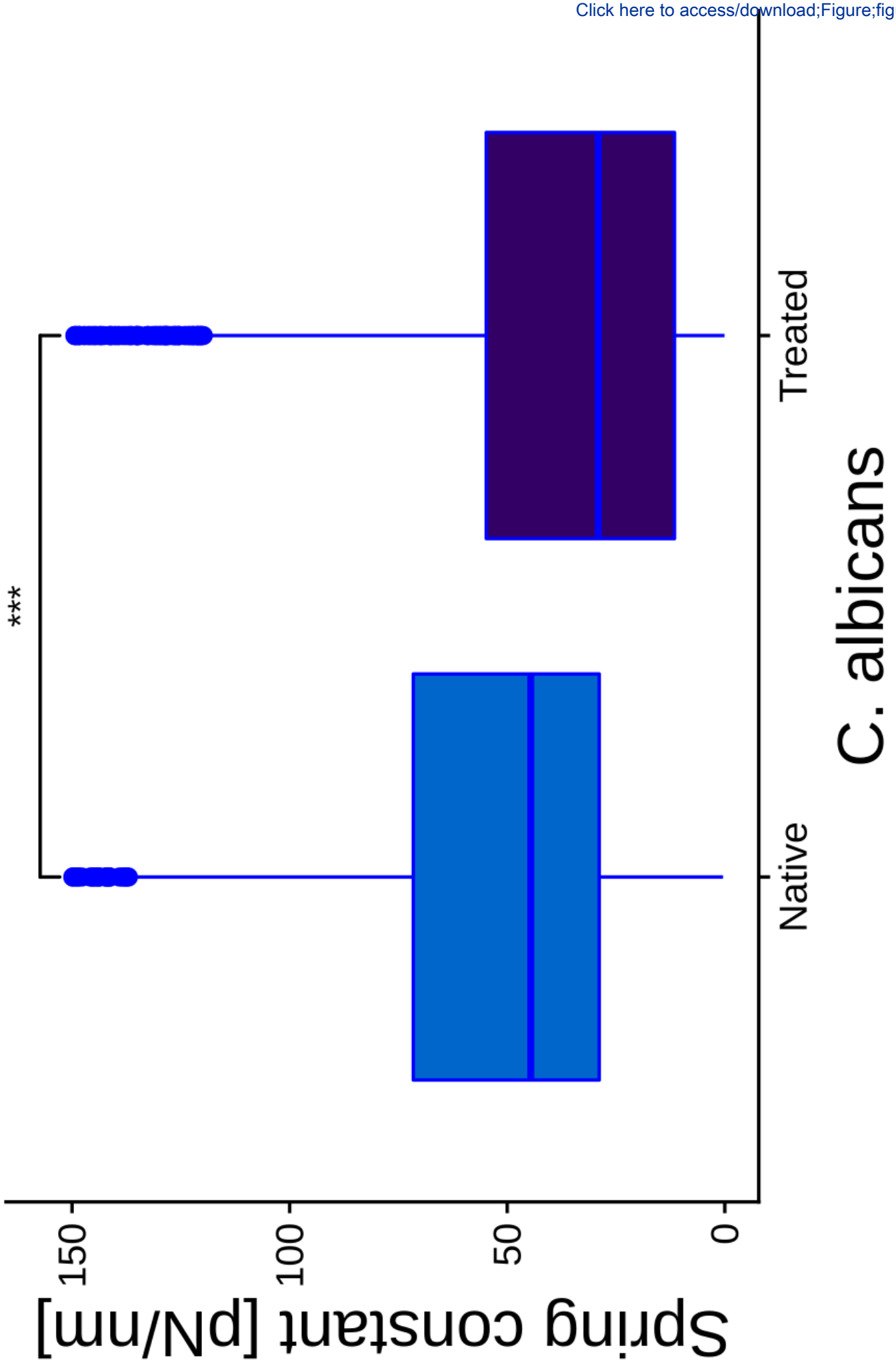
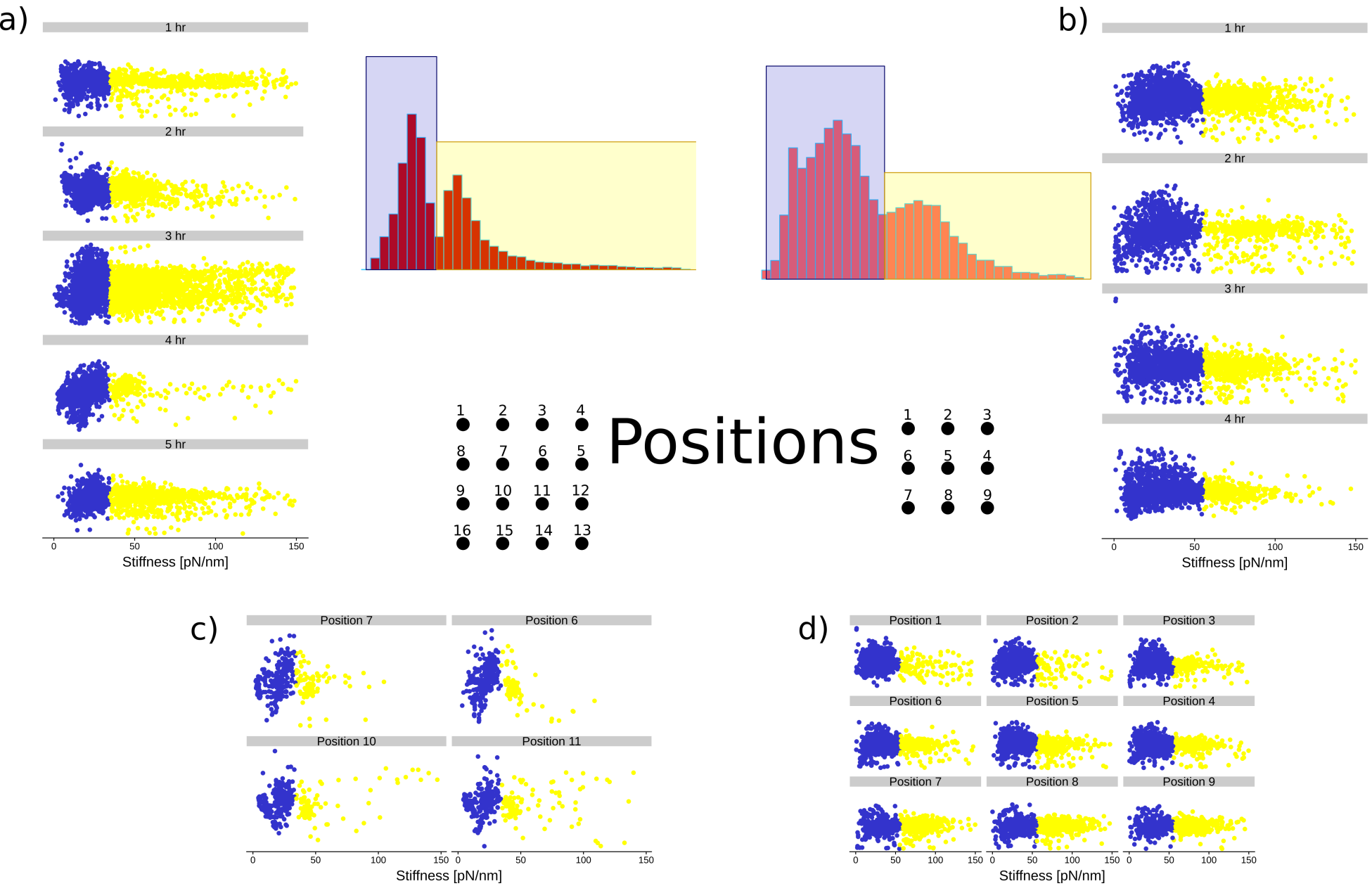
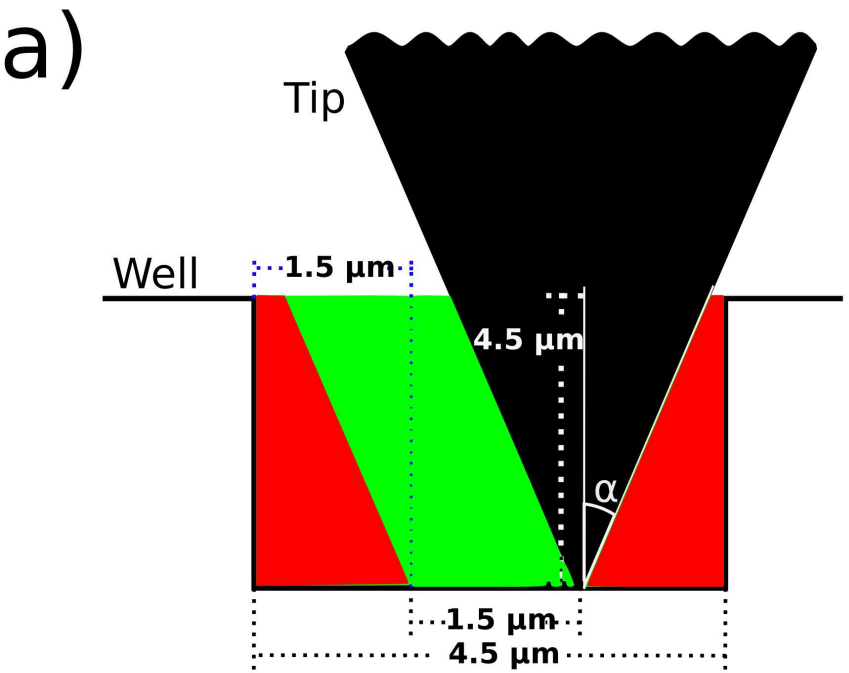
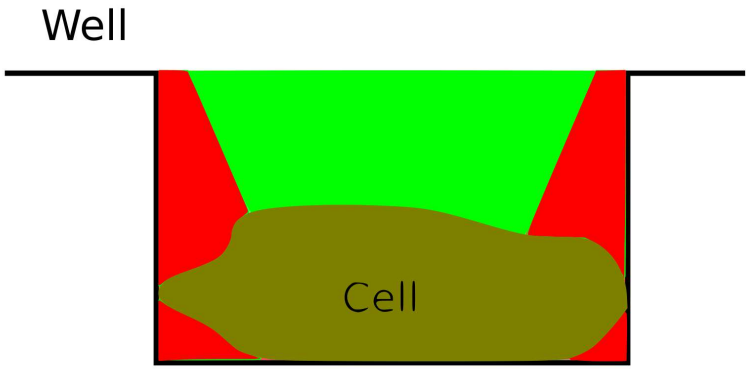


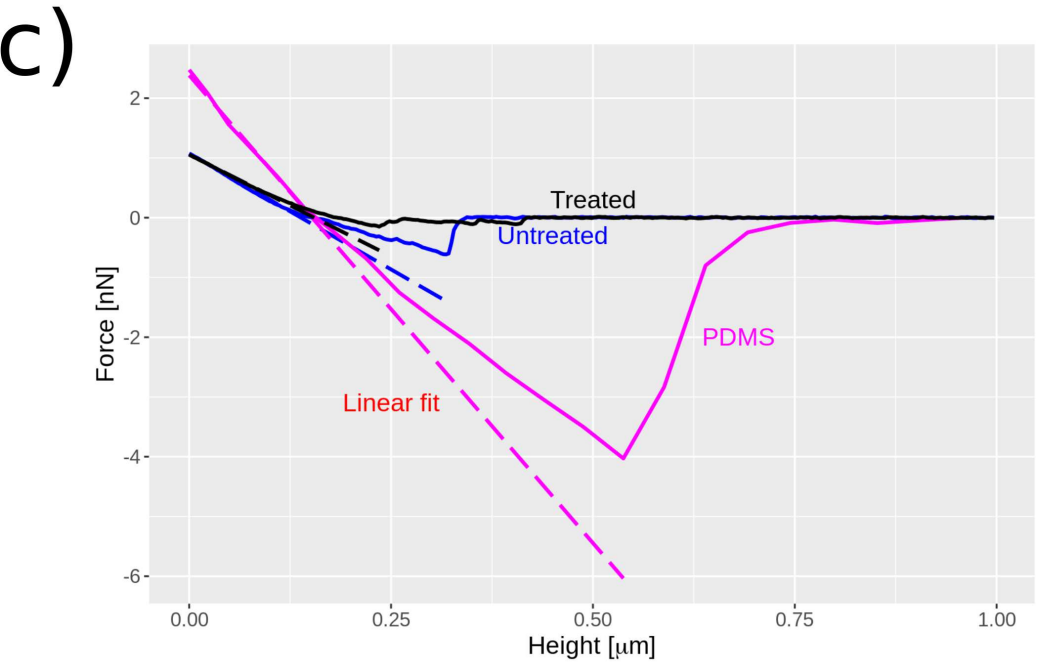
Figure 7











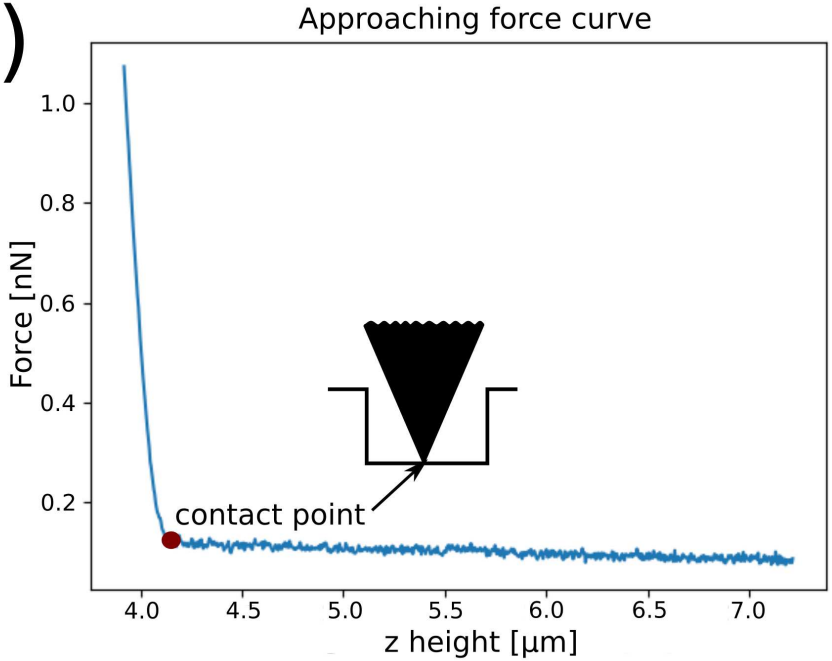


Table of Materials

Name of Material/Equipment	Company	Catalog Number	Comments/Description
AFM cantilever	Bruker AFM probes	MLCT	The cantilevers used were the labeled “C” with resonant frequency of 7 to 10 kHz and k: 0.01 N/m
AFM data analysis	JPK-Bruker	JPK Data processing version minimum 5.1.8	Can be downloaded from a JPK-Bruker user acount
AFM Petri dishes	WPI	FluoroDish FD35-100	The heater was used to monitor the temperature changes during the experiment
Atomic force Microscope (AFM)	JPK-Bruker	Nanowizard II or III	the AFM should be mounted on an inverted optical microscope with a motorized stage
Caspofungin	Sigma-Aldrich	SML0425-5MG	Caspofungin was used with a concentration of 4 MIC (Minimum Inhibitor Concentration)
Code editor	Microsoft	Visual Studio Code version 1.40.1	https://code.visualstudio.com/
Cryobeads	IFU	CB12	
Dessicator/Degassing chamber	Fisherbrand	15594635	The equipment is used to degassing the PDMS stamps for about 50 minutes any dessicator coupled with a vaccum pump will do.
Petri dish heater	JPK-Bruker	PetriDishHeater	This is an add-on to the JPK/Bruker AFM. The heater was used to monitor the temperature changes during the experiment
Sodium acetate buffer pH 5.2	Sigma-Aldrich	S7899	The solution contains 18 mM sodium acetate, 1 mM $CaCl_2$, and 1 mM $MnCl_2$. Adjust the pH with glacial acetic acid. The solution can be stored at 4 °C for 2 months

Statistical analysis language	https://www.r-project.org	R version 3.6.1	R is a language and environment for statistical computing and graphics. It is a GNU project which is similar to the S language and environment
Statistical analysis software	https://rstudio.com	R studio version 1.1.463	collaboration between the R Foundation, RStudio, Microsoft, TIBCO, Google, Oracle, HP and others. RStudio and Shiny are affiliated projects of the Foundation for Open Access Statistics
Sylgard 184	Sigma-Aldrich	761028	Polydimethylsiloxane (PDMS) and curing agent in one set
Yeast Peptone D Broth	Difco	242820	
YPD Agar	Difco	DF0427-17-6	

Dear Dr. Dague,

Your manuscript, JoVE61315R1 "Automation of Bio-AFM measures on hundreds of C. albicans cells.," has been editorially reviewed and the following comments need to be addressed. Please track the changes to identify all of the manuscript edits. After revising the submission, please also upload a separate document that addresses each of the editorial comments individually with the revised manuscript.

Your revision is due by **Mar 23, 2020**.

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision". Please note that the corresponding author in Editorial Manager refers to the point of contact during the review and production of the video article.

Best,

Xiaoyan Cao, Ph.D.
Review Editor
JoVE
617.674.1888
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Editorial comments:

1. Please note that the editor has formatted the manuscript and updated the numbering of protocol steps according to JoVE guidelines. Please review for accuracy. The updated manuscript is attached and please use this version to incorporate the changes that are requested.
2. For each protocol step, please ensure you answer the “how” question, i.e., how is the step performed? Please address specific comments marked in the attached manuscript.

Dear Dr. Cao

Thank you for the edition of our manuscript. We addressed your comments in this revised version. We appreciate your comments and thank you for the improvement of the manuscript.

You will find a clean version and a marked version tracking the changes.

I hope you will appreciate our modifications and answers. We addressed the specific comments that you sent.
With my best regards

Etienne DAGUE