Submission ID #: 61315
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18701123

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

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Y**

If **Yes**, can you record movies/images using your own microscope camera?

Y

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: All screen capture files, do not film

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length

Number of Shots: **37**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Etienne Dague**: Atomic force microscope automation is a key innovation in the evolution of the technology toward biomedical applications and opens access to the investigation of the biomechanical properties of cell populations **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: take 2 , last one

REQUIRED:

1.2. **Childérick Severac**: Our method makes it possible to record AFM measurements for 1000 cells in 4 hours. Compared to the usual methods, which take 4 hours to measure 10 cells, this really is a significant gain of time **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Etienne Dague**: AFM automation is a prerequisite to bringing this technology into hospital labs. This proof of concept protocol suggests its potential for use in the development of specific medical diagnostic strategies **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can skip for time* NOTE: last one

OPTIONAL:

1.4. **Cécile Formosa-Dague**: This protocol uses a versatile PDMS stamp that can be filled with various microbes, allowing different systems to be explored **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can skip for time*

Protocol

2. Polydimethylsiloxane (PDMS) Stamp Preparation

2.1. To prepare the PDMS (P-D-M-S) stamp, mix 55 grams of PDMS prepolymer solution at a 10:1 mass ratio in PDMS oligomers and curing agent **[1]** and degas the resulting solution under vacuum at 1 x 10^{-1} to 1 x 10^{-2} bars for 5-10 minutes **[2]**.

2.1.1. WIDE: Talent adding PDMS to container, with PDMS and curing agent containers visible in frame NOTE: CU mix at the end

2.1.2. Talent placing solution under vacuum

2.1.2 B CU bubbles(take 2)

2.2. When all of the trapped bubbles have been removed **[1]**, pour 20 grams of the degassed solution onto a silicon master mold to a 2-3-millimeter thickness **[2]** and degas again **[3]**.

2.2.1. Shot of solution without bubbles NOTE: 2nd part

2.2.2. Solution being added to mold

2.2.3. Talent placing solution under vacuum

2.3. When all of the bubbles have been removed, reticulate the PDMS at 80 degrees Celsius for 1 hour **[1]** and use a scalpel to cut the PDMS microstructured stamp parallel to the visible microstructure arrays **[2]**.

2.3.1. PDMS being reticulated

2.3.2. Stamp being cut

2.4. Then peel the stamp from the silicon master mold **[1]** and place the stamp microstructure side-up on a glass slide with the microstructures aligned with the side of the slide **[2]**.

2.4.1. Stamp being peeled NOTE: also with 2.3.2

2.4.2. Stamp being placed onto slide NOTE: also with 2.3.2

3. Cell Immobilization

3.1. To prepare the cells for the device, centrifuge 600 microliters of the cell suspension of interest **[1-TXT]** and add 200 microliters of the supernatant onto the stamp **[2]**.

3.1.1 prepare cell tube

3.1.1. B WIDE: Talent placing tube(s) into centrifuge **TEXT: 5 min, 500 x g, RT**

3.1.1 C transfert supernatant into new tube

3.1.2. Talent adding supernatant to stamp, with cell collection tube visible in frame

3.2. Degas the supernatant under vacuum for about 40 minutes **[1]**.

3.2.1. Talent placing stamp under vacuum NOTE: take 4

3.3. When all of the bubbles have been removed, replace the supernatant from the PDMS surface with 200 microliters of the resuspended cell solution **[1]** for a 15-minute incubation at room temperature **[2]**.

3.3.1. Cells being added to stamp

3.3.2. Talent setting timer, with stamp and tube of cells visible

3.4. To load the cells into the microstructures of the stamp, use a glass slide held at a 30-50-degree angle to spread the cells across the stamp in both directions several times as necessary **[1]**.

3.4.1. Slide spreading cells across stamp *Videographer: Important/difficult step* NOTE: take 1 CU, take 2 ECU

3.4.2. Shot of filled microstructures *Videographer: Important step* NOTE: LAB MEDIA and ECU: 3-4-2t2 From 20 s to the end + Photos: 20201113_x10_2, 20201113_x40_2, 20201113_x60

3.5. When a high filling rate of the microstructures has been achieved **[1]**, use a pipette to remove the cell suspension **[2]** and wash the sample three times with 1 milliliter of acetate buffer per wash to remove any untrapped cells **[3]**.

3.5.1. Shot of filled microstructures NOTE: LAB MEDIA

3.5.2. Cells being removed

3.5.3. Sample being washed, with buffer container visible in frame NOTE: CU at the end

3.6. After the last wash, use nitrogen flow to dry the back of the sample **[1]** and place the stamp in a Petri dish **[2]**.

3.6.1. Stamp being dried

3.6.2. Talent placing stamp into dish

3.7. Then add 2 milliliters of fresh acetate buffer to the dish **[1]**.

3.7.1. Buffer being added to dish, with buffer container visible in frame

4. Atomic Force Microscopy (AFM) Setup

4.1. For atomic force microscopy of the cells, place the dish onto the atomic force microscope stage **[1]** and center the stage at 0:0 **[2]**.

4.1.1. WIDE: Talent placing stamp onto stage *Videographer: Important step*

4.1.2. SCREEN: *412_centering stage.ogv* Talent centering stage *Videographer: Important step*

4.2. When the stage has been centered, move the dish into the microscope Petri dish holder **[1]** and align the edge of the stamp so that it is perpendicular to the y-axis of the Petri dish holder **[2]**.

4.2.1. Dish being placed into holder

4.2.2. Edge being aligned NOTE: CU at the end

4.3. Then place the atomic microscope force head onto the stage **[1]**, taking care that the stepper motors are sufficiently extended to avoid the tip crashing onto the stamp **[2]**.

4.3.1. Talent placing head onto stage

4.3.2. Shot of extended motors

5. AFM Imaging

5.1. For imaging, use the microscope knobs to center the atomic force microscope tip over the left corner of the 4.5- x 4.5-squared micrometer wells **[1]** and select the **Force Mapping** mode in the microscope software **[2]**.

5.1.1. WIDE: Talent centering tip NOTE: take 2 , 2nd part

5.1.2. Talent selecting mode, with monitor visible in frame

5.2. To set a 64 x 64 force map over a 100- x 100-micrometer area, set the **Relative Setpoint** to 3-5 nanonewtons, the **z length** to 4 micrometers, the **Z movement** to constant duration, the **extend time** to 0.01 seconds, the **extension delay** to 0, the **Retraction delay** to 0, the **Delay mode** to Constant Force, the **Sample rate** to 2048 hertz. Uncheck **Z closed loop** and check Square image. Set the **Fast Axis** to 100 micrometers, the **Slow Axis** to 100 micrometers, the **X offset** to 0 micrometer, the **Y offset** to 0 micrometers, the **Grid angle** to 0 degrees, the **Pixels** to 64 x 64, and the **Pixel ratio** to 1:1 **[1]**.

5.2.1. SCREEN: 521: 00:25-02:04 *Video Editor: please speed up*

5.3. Next, open the automation software. In the popup window, select the path toward the script file **[1-TXT]**.

5.3.1. SCREEN: 531: 00:03-00:36 **TEXT: Script (Automatip_scan.py) provided in Supplementary Data**

5.4. Enter the W1 coordinate in the P1 variable line 239 of the Inputs box section of the Jython script **[1]** and the W2 coordinate value in the P2 variable line 241 **[1]**.

5.4.1. SCREEN: 541: 00:20-00:56 *Video Editor: please speed up*

5.4.2. SCREEN: 541: 00:58-01:45 *Video Editor: please speed up*

5.5. Attribute the pitch value to the pitch variable line 245 of the script **[1]** and input the well dimension, which can be determined from the design of the well patterns, into the Ws (W-S) variable line 248 **[2]**.

5.5.1. SCREEN: 551: 00:06-00:50 *Video Editor: please speed up*

5.5.2. SCREEN: 552: 00:08-00:34 *Video Editor: please speed up*

5.6. Write the path to the saving directory in line 251 to save the data at the desired place and set the totalArea variable line 254 to the desire multiple "n" of 100 micrometers **[1-TXT]**.

5.6.1. SCREEN: 561: 00:08-00:58 *Video Editor: please speed up* **TEXT: Total number probed wells to be calculated = maximum scan area/pitch x n^2**

5.7. Then set the force curves matrix, row, and column recorded per well in the Number Scans variable line 257 **[1]** and click **Run** to start the program **[2]**.

5.7.1. SCREEN: 571: 00:07-00:16

5.7.2. SCREEN: 572: 00:00-00:30 *Video Editor: please speed up*

6. Data Analysis

6.1. To analyze the data, use the Video Studio Code software to open the python script **[1]** and execute the "Copy files" python script to organize the force curve files into one folder **[2-TXT]**.

6.1.1. WIDE: Talent opening script

6.1.2. SCREEN: 611_612: 00:20-00:27 **TEXT: Copy_files_L.py provided in Supplementary Data**

6.2. Input the path to the general folder where the data will be stored **[1]**.

6.2.1. SCREEN: 612: 00:14-01:00 *Video Editor: please speed up*

6.3. To analyze the force curves, open the atomic force microscope manufacturer data processing software **[1]**. In the **File** menu, select **batch of spectroscopy curves [2]**.

6.3.1. SCREEN: 631_632: 00:08-00:17

6.3.2. SCREEN: 631_632: 00:22-00:54 *Video Editor: please speed up*

6.4. Next, select **File** and **Load Process** and select the **Stiffness** process **[1-TXT]**. Select the last step of the process **[2]** and click **Keep and Apply to All** so that all of the force curves will receive the same treatment **[3-TXT]**.

6.4.1. SCREEN: 641_642: 00:02-00:08 **TEXT: StiffnessProcess.jpk-proc-force provided in Supplementary Data**

6.4.2. SCREEN: 641_642: 00:15-00:19

6.4.3. SCREEN: 651: 00:03-00:15 **TEXT: Repeat for each experiment**

6.5. To open the R script, load the files containing the information extracted with the data processing software into the R studio software **[1-TXT]**.

6.5.1. SCREEN: 661: 00:00-00:20 *Video Editor: please speed up* **TEXT: DataAnalisys.R provided in Supplementary Data**

6.6. In the environment window, click **Import Dataset** and **From Text reader**. In the popup window, click **Browse** to locate the .tsv file **[1]**.

6.6.1. SCREEN: 671: 00:00-00:16 *Video Editor: please speed up*

6.7. Once the file has loaded, select **Code**, **Run Region**, and **Run All** to run all of the code **[1]**.

6.7.1. SCREEN: 681: 00:02-00:52 *Video Editor: please speed up*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

3.4., 4.1.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.4: is a key step. To ensure a high number of measured cells, the stamp filling rate needs to be as high as possible. The convective capillary assembly is repeated as often as required to achieve a high filling rate that is checked by optical microscope

Results

7. Results: Representative Median Stiffness Analyses

7.1. Here typical histograms obtained using the protocol as demonstrated are shown **[1]**.

7.1.1. LAB MEDIA: Figure 6

7.2. In this analysis, the stiffness repartition was recorded on 957 native cells **[1]**, while in this analysis, the stiffness of 574 caspofungin treated cells were measured **[2]**.

7.2.1. LAB MEDIA: Figure 6 *Video Editor: please emphasize Figure 6A data bars*
7.2.2. LAB MEDIA: Figure 6 *Video Editor: please emphasize Figure 6B data bars*

7.3. Note that using hundreds of cells allows a bimodal distribution of the values to be observed **[1]**. In smaller samples, a single distribution is typically observed and can result in lack of observation of the population heterogeneity **[2]**.

7.3.1. LAB MEDIA: Figure 8 histograms
7.3.2. LAB MEDIA: Figure 8 histograms *Video Editor: please emphasize left histogram*

7.4. A comparison of the two conditions **[1]** highlights the effects of caspofungin on reducing the cell stiffness **[2]**.

7.4.1. LAB MEDIA: Figure 7
7.4.2. LAB MEDIA: Figure 7 *Video Editor: please emphasize treated data box*

7.5. As revealed by ANOVA comparison of the native and treated cells **[1]**, the two conditions exhibit highly significantly different stiffnesses **[2]**. This value was reached due to the large number of analyzed cells, providing a greater confidence in the obtained results **[3]**.

7.5.1. LAB MEDIA: Figure 7
7.5.2. LAB MEDIA: Figure 7 *Video Editor: please add/emphasize bracket and asterisks*
7.5.3. LAB MEDIA: Figure 7

Conclusion

8. Conclusion Interview Statements

8.1. **Childérick Severac**: Adding the supernatant to the PDMS stamp before repeatedly flushing the cells into the wells until the stamp is full is critical to the success of the analysis **[1]**.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.4.) NOTE: last one

8.2. **Etienne Dague**: In this proof of concept, *C. albicans* cells were analyzed, but the protocol can be applied to any group of patterned cells, molecules, or materials **[1]**.

8.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: last one