Nanotechnologies and Nanoelectronics's FINAL PROJECT

CantiLab

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1 Business and Market

`CantiLab` is a cantilever-based diagnostic platform designed for rapid and precise antibiotic testing. It provides real-time insights into bacterial responses to antibiotics, enabling pharmaceutical companies to evaluate drug efficacy **within minutes**, significantly reducing reliance on traditional time-consuming methods which take more than a day.

The overuse and misuse of antibiotics are contributing to the rise of antibiotic resistance. The World Health Organization projects that by 2050 there will be 10 million infections annually caused by resistant bacteria. Traditional diagnostic methods take about 24 hours to deliver results, in some cases even longer. These delays often lead to empirical prescribing, which can worsen resistance and delay effective treatment.

This global rise in antimicrobial resistance creates an urgent need for faster and more reliable diagnostic tools. Pharmaceutical companies spend billions on R&D annually; our product addresses critical bottlenecks in antibiotic testing, offering substantial time and cost savings. Our business model consists on selling `CantiLab` units to pharmaceutical labs and offering maintenance and software upgrades as part of long-term service agreements.

1.1 How it works

Highly sensitive silicon nitride cantilevers detect bacterial binding, metabolic activity and response to antibiotics. The system incorporates a microfluidic chamber to deliver precise fluid control, enabling efficient bacterial immobilization and media exchange. A laser-photodetector system captures nanomechanical deflections or frequency shifts caused by bacterial activity, providing real-time data.

1.2 Market comparison

Time-to-result performance is tracked against standard culture-based assays to demonstrate the advantages of the nanomechanical platform in terms of speed and precision.

Our platform delivers results in under an hour, compared to 24–48 hours for traditional methods. Detects bacterial metabolic fluctuations at extremely low concentrations. Identifies bacterial susceptibility or resistance and reduces the need for complex laboratory setups as one can test antibiotic resistance with our machine alone.

2 Theoretical background

Atomic Force Microscopy, or AFM, is a high-resolution technique to characterize a sample's topography. Its working principle is based on its atomic-sized tip traversing the surface of the sample and a laser directed at it to detect its movement.

The AFM is the base inspiration for this work. The metabolic activities of bacteria generate oscillations that result from cellular processes that maintain bacterial viability and growth.

By leveraging the cantilever's ability to detect nanoscale oscillations and the sensitivity of the photodetector to these signals, it becomes possible to monitor fluctuations driven by metabolic activity. Analyzing the variations in oscillation patterns of the bacteria allows for an examination of their responses to antibiotics since more oscillations are expected coming from a higher metabolic rate, which should disappear when the bacteria die.

The use of nanomechanical sensors to study non-stimulated dynamics of bacteria and their metabolism-correlated fluctuations was first completed by (1). In this paper, they show that it is possible to observe these fluctuations and use this method to detect the death of bacteria. They introduce a growth medium (Lysogeny Broth) and observe an increase in fluctuations, and later with the introduction of an antibiotic (Ampicillin) the oscillations decrease and remain with further introduction of the growth medium proving the death of the bacteria. They further made experiments with chemically fixed cells, which provoked no oscillations, to prove that it was not the presence of the cells that caused the fluctuations. The time required to observe a decrease in the oscillation amplitude was never more than 5 minutes, and in some cases even took less than 1 minute, which proves the potential of this method to radically revolutionize the characterization of antibiotic resistance.

2.1 Modeling the Cantilever

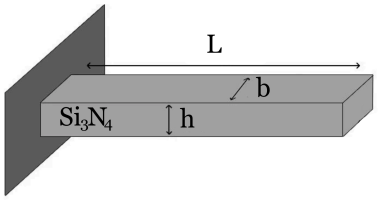

Figure 1: *Cantilever model. Adapted from (1).*

Property	Value
Length (L)	205 µm
Width (b)	40 µm
Height (h)	0.6 µm
Material	Si_3N_4

Table 1: *Cantilever properties.*

The natural frequency of the beam is given by:

$$f_n = \frac{1}{2\pi}\sqrt{\frac{3EI}{\rho AL^3}} \tag{1}$$

where ρ is the density of the material of the cantilever, A is the cross-sectional area, L is the length, E is Young's modulus and I is the moment of inertia.

The deflection curve $z(x)$ of a beam is described by the Euler-Bernoulli beam theory (2) and satisfies the following differential equation

$$\frac{d^2z(x)}{dx^2} = \frac{M(x)}{EI} \tag{2}$$

where $M(x)$ is the bending moment at position x. For a uniformly distributed load w, this becomes:

$$\frac{d^4z(x)}{dx^4} = \frac{-w}{EI} \tag{3}$$

The cantilever with an uniformly distributed load ω (linear density in N/m) has thus a deflection given by

$$\Delta z(x,\omega) = \frac{\omega x^2}{24EI}\left(x^2 + 6L^2 - 4Lx\right) \tag{4}$$

where L is the length of the cantilever. The moment of inertia is given by

$$I = \frac{bh^3}{12} \tag{5}$$

where the width of the beam is represented by b and h is the height of the beam. Combining the previous results, the deflection at the cantilever's tip ($x = L$) can be written as

$$\Delta z(\omega, L) = \frac{\omega L^4}{8EI} = \frac{3}{2}\frac{L^4}{bh^3}\frac{\omega}{E} \tag{6}$$

The deflection of the cantilever comes mostly from two contributions, the bacteria and thermal noise. The force generated by thermal fluctuations can be modeled as

$$F^{\text{thermal}} = \sqrt{\frac{4k_BT}{6\pi\eta R}}\,\sigma(0,1) \tag{7}$$

where η is the shear viscosity of the fluid used, T is the temperature of the fluid, R is the effective characteristic length of the cantilever ($\sim$L) and $\sigma(0,1)$ is a Gaussian distribution centered at zero, with unit variance which is stretched by the square root factor. The metabolism-induced force can be expressed for each bacteria as

$$F_i^{\text{metabolism}} = A\sin(2\pi\nu_i t + \phi_i) \tag{8}$$

On average, bacteria are assumed to create a force of 4×10^{-11} N, a value that is used for the amplitude. For bacteria i, the frequency ν_i and phase ϕ_i are sampled from a uniform distribution, ranging $[1, 100]$ Hz and $[0, 2\pi]$ respectively.

In this work, the parameters used for the cantilever are adapted from the supplementary materials of (1), corresponding to a size $L \times b \times h$ of $205 \times 40 \times 0.6$ μm^3 corresponding to a moment of inertia of 7.2×10^{-25} m^4. The shear viscosity depends on the temperature, for PBS varying from 0.7 to 1.05 mPa·s at temperatures between 20 and 40 ºC (3), and values not straying far from these for the remaining fluids, so a value of 0.95 mPa·s is used as it corresponds to PBS at 25 ºC, the default temperature in the simulation. The Young Modulus for the cantilever, made mostly of silicon nitride, has values ranging from 170 to 300 GPa depending on the manufacturing process. For the developed model a value of 200 GPa is assumed. At the tip there is also a gold layer (E=80 GPa) which is assumed not to have a significant effect on the cantilever's Young Modulus, since it is only localized at the tip to reflect the laser beam.

3 Simulation

Before any experimental work can be done, a simulation is needed to confirm or fine-tune the parameters of the device. With this in mind, a program was created that allows exactly this, as well as studies the expected response of the cantilever to various situations. The interface of the program can be seen in appendix A.

The effect of the antibiotic was simulated using an exponential decay,

$$F'_{metabolism} = F_{metabolism} \cdot e^{\gamma(t - t_{antibiotic})} \tag{9}$$

where γ is the decay rate and $t_{\text{antibiotic}}$ is the time of injection of the antibiotic. We decided to use $\gamma = 0.9$ min^{-1}, meaning that the metabolism is reduced by half ~ 46 seconds ($\frac{ln(2)}{0.9}$) after its application. This parameter depends both on the effectiveness of the antibiotic and the resistance of the bacteria, and the value used is chosen to match the literature and would be better modeled with an experimental setup.

The bacteria are assumed to be uniformly distributed on the cantilever with a density of 46.000 bacteria/mm^2 in accordance with (1). When applying the antibiotic after 5 minutes the results obtained are in figure 2.

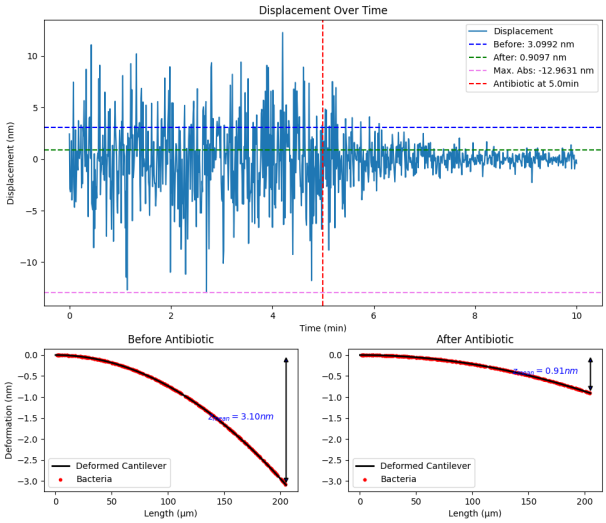

Figure 2: *Simulation window for the default parameters mentioned in sections 2 and 3. The upper plot represents the displacement of the tip with respect to time. The bottom plots represent the average cantilever deformation before and after the antibiotic application. The mean displacement of the tip before antibiotic application obtained is 3.1 nm, after Antibiotic 0.9 nm, and an overall max displacement of 13.0 nm.*

You can see the effect of the antibiotic reducing the metabolic activity of the bacteria, remaining what is only thermal fluctuations as was expected when comparing to (1). When increasing the length of the cantilever to 500 μm we see an increased displacement corresponding to a higher sensitivity but also a less rubost system which can break more easily, and for a small cantilever with length 50 μm the exact opposite, where its vibrations are on the order of 10^{-11} which are very hard to detect experimentally due to its stiffness. The same happens when increasing the height of the cantilever as it is not able to bend as much. Varying the width of the cantilever has a smaller effect on its ability to bend as expected and this size can be more freely chosen.

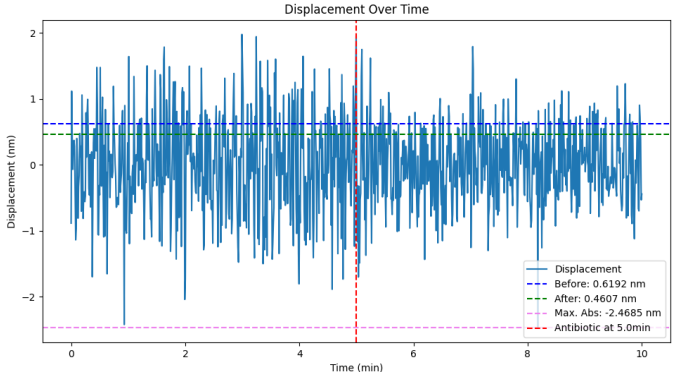

Figure 3: *Plot of the simulation for low bacteria density.*

Decreasing the density of bacteria too much also reduces the oscillation amplitude, and for a density of only 1000 bacteria/mm^2 the displacements go to the hundreds of picometers. Figure 3 shows this effect, and the reduced ability to detect the effect of the antibiotic since thermal noise is of the same order of magnitude as the displacements generated by the bacteria. For a higher temperature, the oscillations after the antibiotic is applied remains larger since the thermal noise is larger, and the opposite happens for low temperatures.

4 Fabrication and Usage

4.1 Cantilever microfabrication

The cantilever in our system is designed for high sensitivity to bacterial metabolic activity within a microfluidic chamber. Based on Eq. 1, the cantilever dimensions detailed in Table 1 were designed to achieve a natural frequency of about 1 kHz, which has been experimentally validated for detecting bacterial activity and distinguishing between live and dead cells through metabolic fluctuations, as demonstrated by Longo et al.(4).

An extended version of the fabrication of 4 and 5 can be found in appendix B.

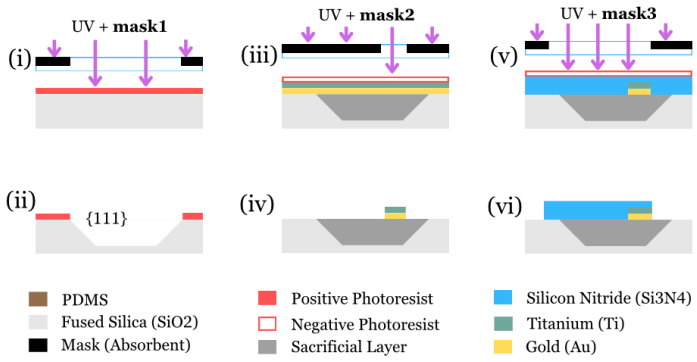

Figure 4: *Cantilever fabrication: (i) Photolithography using PM-1 and (ii) KOH anisotropic wet etching to create the motion cavity. (iii) SiO_2 deposition, (iv) patterning of a gold-titanium reflective patch using PM-2, (v) Si_3N_4 deposition via LPCVD, and (vi) photolithography with PM-3 followed by etching to define the cantilever dimensions.*

We start with a fused silica (SiO_2) wafer, selected for its transparency, enabling us to project the optical system from below. The wafer is cleaned with acetone and isopropanol to remove surface residues, followed by deionized water rinsing and a standard RCA clean to eliminate both organic and inorganic contaminants.

Using photolithography, a photoresist (PR) layer is spin-coated on the wafer and exposed with UV light through the mask **PM-1** (appendix) to define a 220 × 220 μm square cavity. The exposed resist is developed using a sodium hydroxide-based solution. The patterned region is then wet-etched with potassium hydroxide (KOH) to produce a cavity with precise {111} planes for the cantilever's motion. The wafer is cleaned with isopropyl alcohol and deionized water to remove etching residues.

A SiO_2 layer is deposited via chemical vapor deposition (CVD) to fill and planarize the cavity. Excess material is etched back to create a flat surface, providing a base for further processing.

For the laser reflectivity, two thin metal layers are patterned: the gold, below, allows for optical reflectivity for the laser, while the titanium promotes adhesion between gold and the silicon nitride. Photolithography defines the desired reflective patch, while Reactive Ion Etching (RIE) removes excess material, leaving a well-defined 10 × 40 μm reflective area at the cantilever's tip.

Silicon nitride (Si_3N_4) is chosen for its biocompatibility and mechanical strength(5). A 0.6 μm layer is deposited via Low-Pressure Chemical Vapor Deposition (LPCVD), which ensures uniform deposition and low residual stress in the Si_3N_4 layer to prevent cantilever curling in the cure process. Using **PM-2**, we define the cantilever's 205 × 40μm dimensions. The Si_3N_4 is then plasma etched to define the cantilever more precisely, stopping at the sacrificial layer level.

To enable bacterial binding and metabolic activity measurements, the cantilever can be functionalized with biomolecules. A self-assembled monolayer (SAM) of APTES (aminopropyltriethoxysilane) can be applied, introducing amino groups ($-NH_2$) to establish strong covalent bonds with bacterial adhesion molecules. This layer can be further functionalized with specific antibodies (like anti-E. coli or anti-S. aureus).

4.2 Microfluidic chamber microfabrication

To integrate the cantilever into a microfluidic environment, we designed a compact chamber with input and output channels for microfluid exchange that minimizes dead volume to ensure rapid and wasteless delivery of growth media and antibiotics while maintaining laminar flow to reduce noise (and drift) during fluid injection and ejection. This setup allows direct delivery of fluids to immobilized bacteria while providing unobstructed optical access for laser-based measurements.

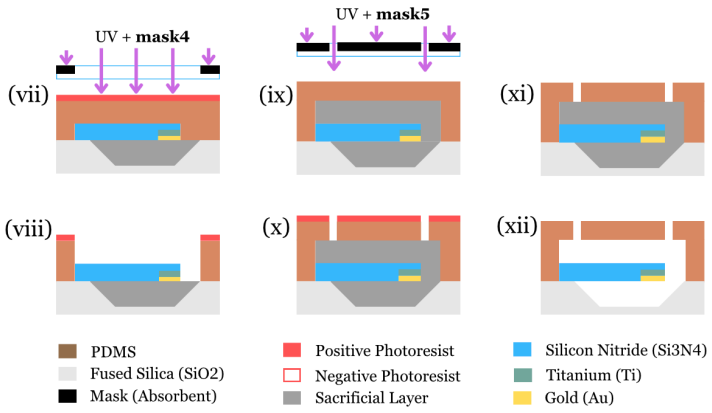

Figure 5: *Microfluidic chamber fabrication: (vii) Photolithography with PM-4 followed by (viii) oxygen plasma etching to create a cavity for cantilever extension. (ix) Photolithography with PM-5 to define inlet/outlet channels, followed by (x) oxygen plasma etching. (xi) Removal of sacrificial layer and (xii) final cleaning with deionized water and CPD.*

The process begins with the creation of a master mold using photolithography on a silicon wafer, which defines the intricate flow channels. Polydimethylsiloxane (PDMS) is chosen for its optical transparency, biocompatibility, and ease of fabrication. The process begins by mixing PDMS in a 10:1 base-to-curing agent ratio, degassing to eliminate air bubbles, and spin-coating a 100 μm layer onto the structure. This layer is baked at 70°C for 1 hour, forming a stable, planar base.

Photolithography is employed to define the chamber geometry: a positive PR layer is applied to the PDMS surface, exposed through **PM-4** to create a 230 × 230 μm chamber center and developed to form the removable pattern. Oxygen plasma etching removes the exposed PDMS, creating a recessed cavity while leaving intact peripheral walls that define the chamber. This cavity

establishes the fluidic space of CantiLab.

The chamber cavity is filled with a fast-curing sacrificial layer, which also merges with the previous one. This step ensures a planarized surface for the top layer that seals the chip. The sacrificial layer is hard-baked or cured for stability. The chamber is then encapsulated by spin-coating a 50 μm PDMS top layer, which is cured at 70°C to ensure a robust chemical seal. This creates a closed environment for fluidic experiments.

Fluid inlet/outlet channels are added to enable the controlled flow of PBS, LB and antibiotics. Photolithography defines 29 μm diameter channels using **PM-5**. Oxygen plasma etching is used to create precise and aligned channels in the PDMS top layer.

The in-outlet channels are utilized to introduce an appropriate etchant (say, hydrofluoric acid) to remove all the sacrificial material within the chamber. The system is thoroughly flushed with deionized water and critical point drying (CPD) is performed to prevent stiction of the cantilever. This leaves a clean, open chamber with a freely oscillating cantilever.

4.2.1 Integration

After individual components have been fabricated, the platform is integrated into a single, cohesive instrument. A mechanical housing of resin is constructed to provide a rigid frame for both the cantilever chip and the microfluidic assembly. Electronic readout modules and signal conditioning circuits are placed in close proximity to the laser detector to minimize noise, while the final data acquisition system streams to our software via USB interface.

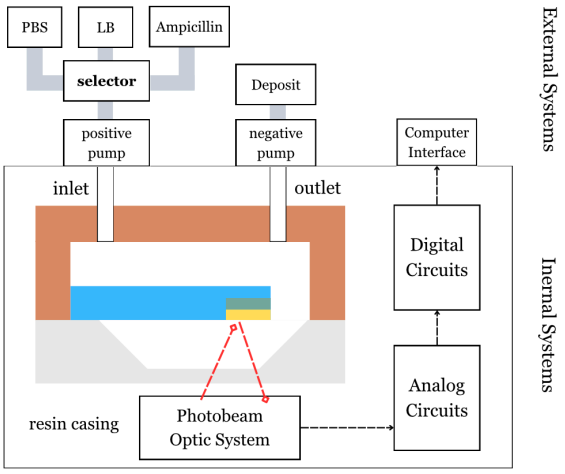

Figure 6: *System integration diagram w/ peripherals and interfaces.*

Ambient temperature should maintain a stable environment near 25ºC for optimal bacterial growth conditions. Fluidic connections allow for automatic or manual injection of growth media and antibiotics into the chamber, although we recommend using a grid for laminal flow. During system assembly, each hardware component is tested to ensure proper alignment and functional integrity, and once the device is sealed, it undergoes sterilization if required. A dedicated software interface allows users to track real-time movement from the cantilever.

4.3 Usage and validation

4.3.1 Calibration protocol setup

Align the laser beam onto the cantilever's reflective gold-coated surface and ensure the photodetector captures the reflected beam. Record the baseline noise in the absence of bacteria in the liquid chamber to establish the signal-to-noise ratio. Use our piezoelectric actuator to vibrate the cantilever and measure its natural frequency in air and liquid, confirming it matches the design specification ($\sim 1\,\text{kHz}$).

4.3.2 Functional usage

Follow these steps to verify bacterial responses to antibiotics using the `CantiLab` setup:

1. Inject a bacterial suspension (PBS) into the microfluidic chamber and allow sufficient time (10–15 minutes) for binding to the cantilever surface.
2. Monitor fluctuations caused by bacterial metabolic activity from the laser-photodetector system and record the deflection or frequency shift as the baseline signal for live bacteria.
3. Replace the growth medium with the test antibiotic at a defined concentration (like ampicillin at $10\,\mu g/mL$).
4. After a 5-minute stabilization period, record changes in cantilever deflection or variance over a period of 25–45 minutes.

A decrease in variance or deflection indicates bacterial death or metabolic suppression, while sustained activity suggests resistance. For accuracy, repeat tests and analyze results statistically.

5 Conclusion

The simulation in 3 demonstrated a clear reduction in cantilver oscillation amplitude, following antibiotic application, consistent with findings from (1), linking bacterial death to decreased fluctuations. It also accurately predicted how the cantilever dimensions influence sensitivity and noise.

The fabrication in 4 is viable and aligns with state-of-art pratices. PDMS for the microfluidic chamber further supports the system's feasibility, as its optical transparency, flexibility, and ease of fabrication are industry standards for lab-on-a-chip applications. The integration of gold and titanium layers integrates with the optical system. Meticulous attention is required during fabrication to avoid defects that could compromise functionality.

This way, our system can provide a fast platform for antibiotic susceptibility testing, that can significantly reduce the time-to-result compared to traditional methods, which offers a practical solution to the growing threat of antimicrobial resistance.

References

[1] G. Longo, L. Alonso-Sarduy, L. M. Rio, A. Bizzini, A. Trampuz, J. Notz, G. Dietler, and S. Kasas, "Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors," *Nat. Nanotechnol.*, 2013.

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[3] X. Xu, D. Zhang, S. Tong, F. Liu, W. Wei, and Z. Liu, "Experimental study on shear viscosity and rheopexy of escherichia coli suspensions," *Rheologica Acta*, 2022.

[4] G. Longo, L. Alonso-Sarduy, L. Marques Rio, A. Bizzini, A. Trampuz, J. Notz, G. Dietler, and S. Kasas, "Rapid detection of bacterial resistance to antibiotics using afm cantilevers as nanomechanical sensors," *Nature Nanotechnology*, 2013.

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A Simulation Interface

The simulation is designed to model and analyze the response of a cantilever beam to bacterial activity. As shown in Figure 7 the user interface, allows individuals to manipulate the bacteria population, as well as the dimensions and physical parameters of the cantilever. Additionally, it enables the analysis of simulation outputs and comparison with theoretical predictions in real-time. The top-left panel contains input controls that allow users to adjust the cantilever's length, width, thickness, and Young's modulus of the material. This panel also facilitates the examination of how variations in temperature, bacterial density, and antibiotic exposure time affect cantilever oscillation. On the right side of the interface, the main panel displays the outputs: the cantilever's displacement over time is shown at the top, while the mean deflection of the cantilever before and after the introduction of the antibiotic is visualized at the bottom. Beneath the control panel is the history panel, which allows users to review and manage all previous simulations, including their input parameters. In the bottom left section of the interface, action buttons are available to run simulations, save and export output graphs, delete and clear history, and review the theoretical framework upon which the simulation is based.

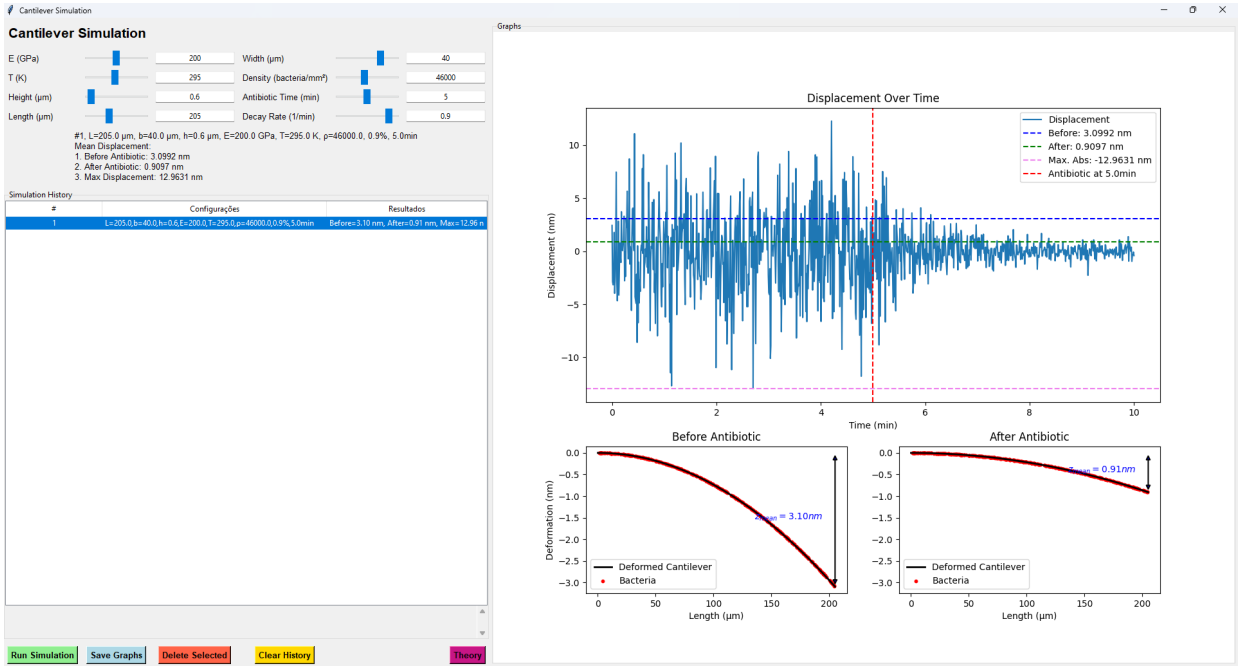

Figure 7: *Interface of the simulation program.*

B Fabrication

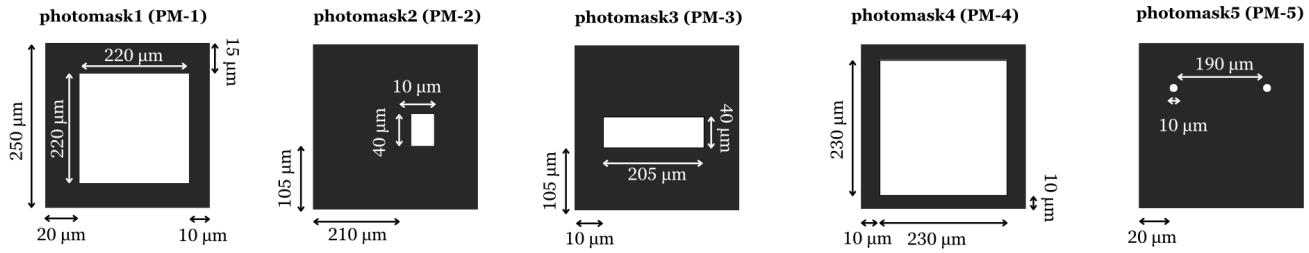

Figure 8: *Photomasks used in our fabrication process to define key structural features of the cantilever and microfluidic chamber. These masks enable precise patterning for creating the motion cavity, reflective patch, cantilever dimensions, microfluidic chamber cavity, and fluidic inlet/outlet channels, ensuring alignment and repeatability throughout the fabrication workflow.*

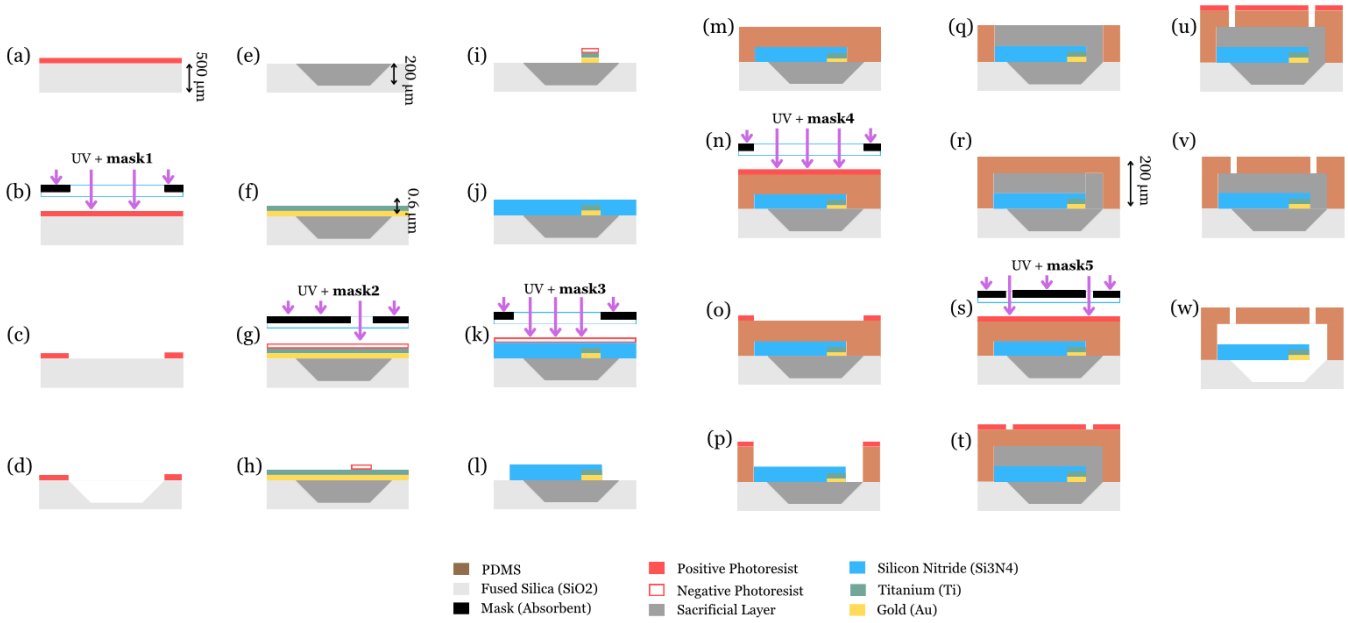

Figure 9: *Our step-by-step schematic of the combined fabrication process for the cantilever and microfluidic chamber. (a)-(d) Initial photolithography (PM-1) and KOH anisotropic etching to create the motion cavity on the fused silica wafer. (e)-(h) Deposition of SiO_2, photolithography (PM-2), and patterning of a reflective patch using gold (Au) for reflectivity and titanium (Ti) for adhesion. (i)-(l) Low-Pressure Chemical Vapor Deposition (LPCVD) of Si_3N_4, photolithography (PM-3), and etching to define cantilever dimensions. (m)-(p) PDMS spin-coating and planarization, followed by photolithography (PM-4) and oxygen plasma etching to form the chamber cavity. (q)-(t) Deposition and planarization of sacrificial material, encapsulation with a PDMS top layer, and photolithography (PM-5) to define fluidic inlet and outlet channels. (u)-(w) Removal of sacrificial material and final cleaning via deionized water flushing and critical point drying (CPD) to complete the functional microfluidic chamber.*