

REAL-TIME DETECTION OF BACTERIAL BIOFILM GROWTH USING SURFACE PLASMON RESONANCE IMAGING

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ABSTRACT

Surface Plasmon Resonance imaging (SPRi) was used for real-time detection of bacterial growth inside microfluidic channels. First, 50 micron diameter beads were spotted on a gold coated prism surface and visualized with both SPRi and a stereo microscope to validate images. Then, fluorescent *Escherichia coli* was loaded into microfluidic polydimethylsiloxane (PDMS) channels on the SPRi sensor surface and observed for 6 hours. Afterwards, the sample was imaged with a fluorescence microscope. Both images showed bacterial growth at the same locations in the channels, confirming that bacterial growth can be directly monitored in real-time by SPRi.

KEYWORDS

Surface plasmon resonance imaging (SPRi), Biofilm, Bacterial growth, Chemotaxis, Microfluidics

INTRODUCTION

Surface Plasmon Resonance imaging (SPRi) provides label-free qualitative and quantitative information about events occurring near a surface (~200 nm) over a relatively large area (~1 cm²). [1] It is expected that the combination of SPRi and microfluidic systems will allow increasing complex experiments and events to be monitored at the sensor/channel wall, such as the interaction of lipids and cells with immobilized biospecific markers or the growth of biofilm. [2-4] SPRi works by shining light through a prism onto a gold sensor surface and detecting the intensity of the reflected light that exits the prism using a CCD camera. Cell movement and growth in channels causes local changes in refractive index. [5] When the refractive index at a location on the surface changes, the intensity of light reflected at that point changes and the difference is recorded. [6] In this study, we used SPRi for real-time detection of bacterial growth inside microfluidic channels. To our knowledge, this is the first time that SPRi has been used to study bacterial biofilm formation.

Many environmental molecules and drugs affect bacterial movement, known as chemotaxis. Bacteria also communicate with each other by using signaling molecules called autoinducers. Using autoinducers, bacteria can locate each other, organize to form protective biofilms, and coordinate production of various toxins and antibiotics. [7] While much is known about how signaling molecules regulate intracellular machinery and cellular functions, relatively little is known about the overall physiological response (phenomics) and intercellular relationships. We are interested in studying bacterial movement and biofilm formation in the presence of various chemical gradients. To achieve this inside of an SPRi system, nanochannels that deliver chemicals without allowing microbes to escape are necessary. [8]

EXPERIMENT

A fabrication scheme for creating PDMS microfluidic channels connected by nanofluidic channels is shown in Figure 1. *E. coli* cells in Lysogeny Broth (LB) growth media were loaded into one of the microfluidic channels; and, a second channel, connected to the first via a nanochannel, was filled with only LB growth media. The cells were grown for 24 hr at 37 °C. The results of this experiment are shown in Figure 4.

To calibrate the SPRi-Lab+ system (Horiba), 50 μm beads in distilled (DI) water were placed directly onto a gold coated prism surface, as shown in Figure 2. Images of different locations on the bead covered surface were obtained using both SPRi and a stereo microscope. The results are shown in Figure 5.

Then, we made two separate linear channels in PDMS and attached them onto a gold coated prism. One of the channels was filled only with LB growth media, as a control, while the second channel was filled with a few Green Fluorescent Protein (GFP) labeled *E. coli* cells in LB growth media (Figure 3). We continuously monitored the two channels simultaneously using difference imaging in the SPRi for six hours at room temperature. Afterward, we removed the chip from the imaging system and inspected the channels through the PDMS using a stereo microscope with fluorescence imaging.

RESULTS AND DISCUSSION

The delivery of food to bacteria via a nanochannel is shown in Figure 4. The bacteria consumed all of the food in the main channel first and sensed the additional food as it diffused across the nanochannel. The bacteria accumulated at the entrance of the nanochannel but were unable to cross over to the microchannel containing additional food. In future work, these more complex fluidic geometries will be integrated with SPRi.

To demonstrate the utility of SPRi, 50 μm diameter beads were spotted on the sensor surface of a glass prism. Since the beads are stable, and immobile in the absence of flow, they constitute an exceptional control experiment. The results of this set of experiments are shown in Figure 5. Images obtained with a stereo microscope are shown in the left column (Figure 5 a, c, e, g) and SPRi images taken from the same spot are shown in the right column (Figure 5 b, d, f, h). By comparing the two images from the same location we showed that we can sensitively detect material that is attached to the sensor surface using SPRi.

Finally, we wanted to observe the growth of bacteria using SPRi. Bacterial growth in the channel containing *E. coli* is shown at different time points in Figure 6 b, d, f, whereas no growth was detected in the control channel in Figure 6 a, c, e. The uniform increase in brightness in Figure 6 e and f is most likely caused by a shift in the intensity peak position of the SPR. The distinct difference in surface roughness between the two images demonstrates the growth and attachment of biofilm. Visual inspection showed that the channels remained filled after 6 hours. When viewed with a fluorescence microscope, the control channel was dark (Figure 6 g) while cells expressing GFP in biofilms were observed in the second channel (Figure 6 h), validating the results obtained with SPRi.

CONCLUSION

SPRi can be used to obtain high resolution images of material attached to a surface. This was demonstrated by placing 50 μm diameter beads on a gold coated prism sensor chip and visualizing the location of the beads using both SPRi and optical microscopy. The results also show that SPRi can be used to detect bacterial growth in PDMS channels. Imaging with a fluorescence stereo microscope verified that the bacteria indeed grew in the locations that were observed with SPRi.

ACKNOWLEDGEMENTS

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FIGURES

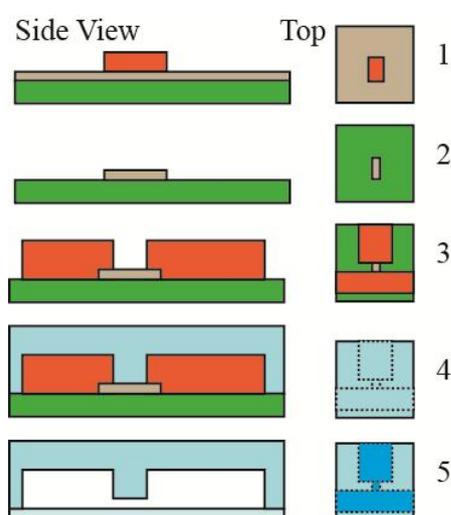


Figure 1. Fabrication schematic for PDMS micro/nanochannels. 1) Positive photoresist developed on a thin Cr film. 2) Cr is over etched and resist is removed. 3) Thick photoresist patterned over nanoscale Cr features. 4) PDMS mold is made. 5) PDMS is bonded to glass cover slip, allowing for high resolution imaging.

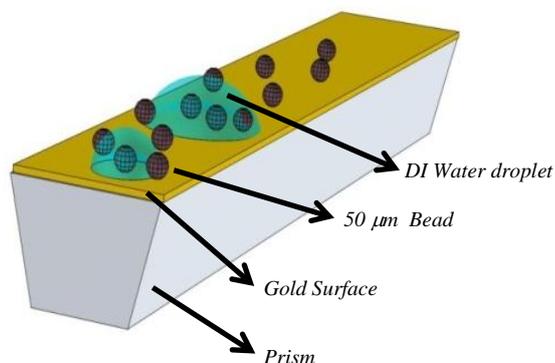


Figure 2. Setup for Surface Plasmon Resonance imaging (SPRi) experiments. 50 μm beads in DI water are placed onto prism coated with 50 nm of gold. The setup is placed inside of a SPR imaging system.

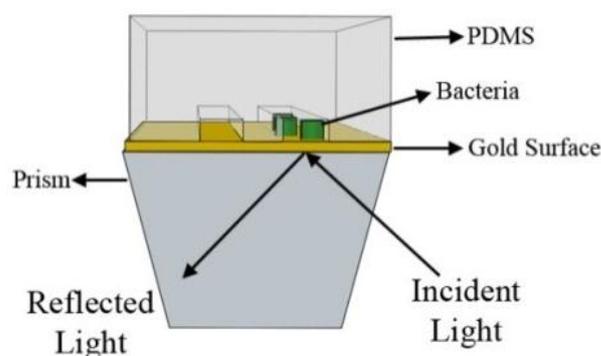


Figure 3. Setup for bacteria growth SPRi experiments. PDMS is reversibly sealed against a high refractive index glass prism coated with 50 nm of gold. One channel is filled with LB growth media while the other is filled with both LB growth media and fluorescently labeled *E. coli* cells. After 6 hours in the SPRi instrument, the setup is removed and images of the channels are obtained through the PDMS using a fluorescence stereo microscope.

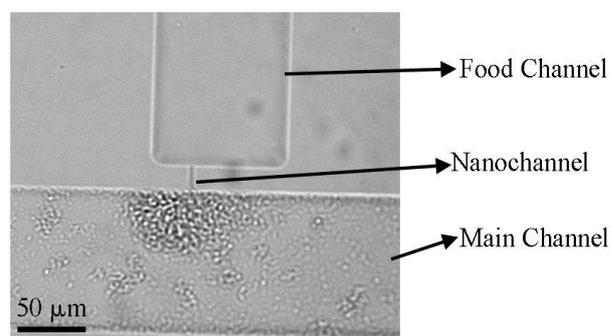


Figure 4. Optical micrograph of bacteria forming a biofilm at the entrance of a nanochannel connecting two microchannels made from PDMS. The bacteria are attracted to the fresh growth media diffusing through the nanochannel, but cannot squeeze through to get to the food source.

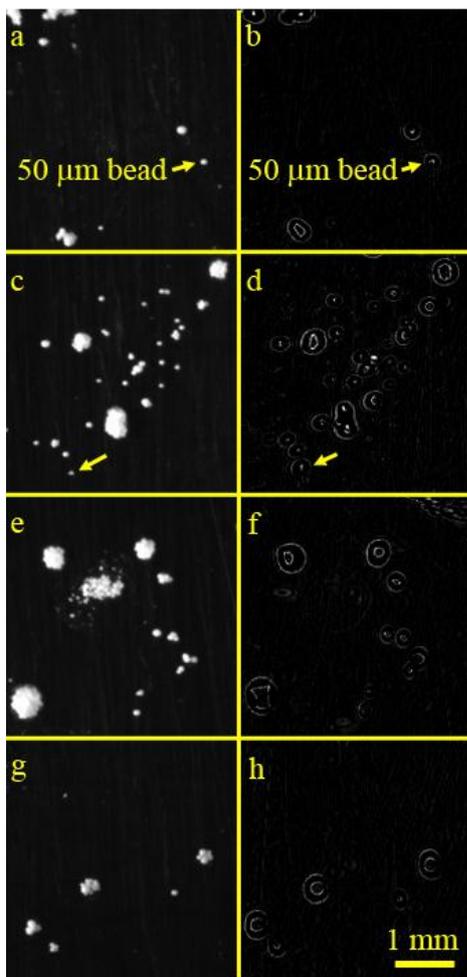


Figure 5. (a, c, e, g) Stereo microscope images of 50 μm diameter beads spread on the sensor surface. (b, d, f, h) SPRi images of the same locations on the chip. Clumping of the beads is seen during drying.

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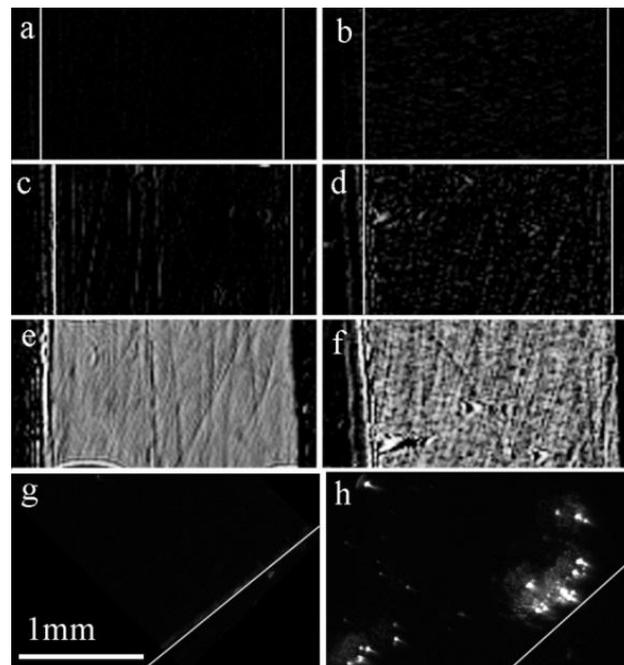


Figure 6. SPR images of blank LB filled channels at (a) 6 min, (c) 1 hr, (e) 6 hr, and channels containing GFP *E. coli* at (b) 6 min, (d) 1 hr, (f) 6 hr. Florescent images of (g) channel filled only with LB, and (h) GFP *E. coli* filled channel after 6 hours in the SPRi. Each image is at the same magnification. White lines have been added to the images to highlight the location of channel side walls.