# Imaging Live DU145 Cancer Cells Using Scanning Probe Microscope

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## Abstract:

**Different body cells have unique surface proteins that conduct electricity. Cell electrophysiology is the key to differentiating cancerous cells from healthy cells *in vivo*. Silicon carbide (SiC) is a biocompatible, chemically inert, thermally stable material on which we have tested the viability of DU145 cells. Using highly doped SiC, the relative conductivity of cells can be detected via current-sensing atomic force microscopy (CSAFM) with a flow cell to examine the living DU145 cells in media. A parallel part of this experiment is to functionalize the SiC surface to achieve specificity when attaching cells to confirm that the CSAFM method of identification is successful. After cleaning, an oxide layer is formed on the surface, then 3-aminopropyltriethoxysilane (APTES) is attached to result in a surface amenable to protein attachment followed by antibody attachment, which is the key to binding specific cells in specific areas of the substrate.**

## Introduction:

SiC possesses properties that make it an ideal substrate for a new method of detection and treatment. This experimental method relies on the ability to identifying different types of cells based on the electrical signatures of the membranes from a unique combination of surface proteins, ion channels, and membrane potentials [1].

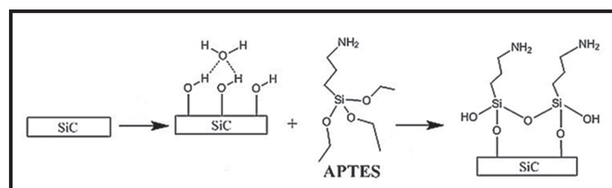


*Figure 1: The APTES reaction with SiC.*

## Experimental Procedure:

All experiments were performed with commercially purchased 6H highly doped SiC. All samples were ultra-sonicated for three minutes each in methylene chloride, acetone, and isopropanol, sequentially. Next they were submerged in a 5:1:1 mixture of deionized (DI) water, $H_2O_2$, and $NH_4OH$ at 80°C for 10 minutes, which is the standard RCA cleaning procedure.

Two methods of oxidation and further cleaning were used to increase the reactivity of the SiC surface. One was oxygen plasma etching using a Plasma-Therm model 790 plasma enhanced system using a 20% oxygen/80% argon gas mixture for one and five minutes. The other was a tube furnace at 1000°C for 10, 30, and 60 minutes, with oxygen flowing for the duration of the heating period and argon as the samples cooled. After a thin oxide layer developed on the SiC, the samples were left exposed to air for three hours to ensure surface chemisorption of water molecules and increase the ability of the SiC to hydrolyze APTES in the next stage.

APTES silonization was performed in a class 100 clean room in an anhydrous $N_2$ environment. The samples were placed in a 1:49 solution of APTES in toluene for a duration of 30 minutes. They were then ultra-sonicated in toluene for 10 minutes and isopropanol for one minute, then dried under a stream of $N_2$ gas to remove any loose APTES molecules [2]. The steps are illustrated in Figure 1.

Additionally, DU145 prostate cancer cells were tested for viability on SiC. Cells were combined with RMPI media and the concentration was adjusted to $5 \times 10^4$ cells/ml —the optimal seeding count determined in previous experiments. SiC was placed in a sterile 6-well culture plate, some carbon-face up, others silicon-face up; 2 ml of the cells were added to each well and incubated overnight at 37°C at 5% $CO_2$. Inverted light microscopy was used to confirm binding of cells to the substrate. Two plates were used for sizing, with the cells on the substrates measured after 24 hours, and another two examined viability of the cells for an extended growing period of eight days, one checked every 24 hours and one left undisturbed.

CSAFM is the method used to evaluate the electrophysiological properties of the DU145 cells once they were bound to a substrate. The goal was to examine

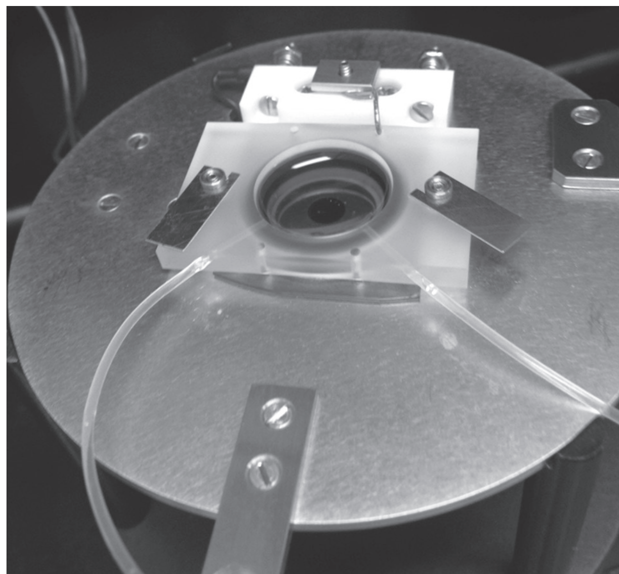

Figure 2: Flow cell and sample in media.

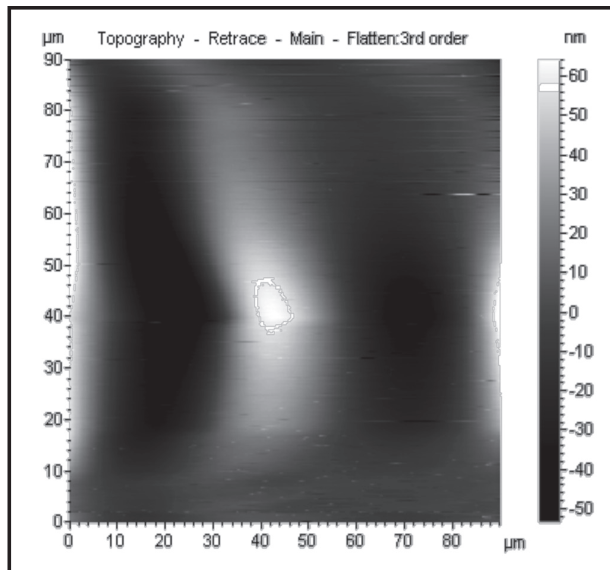


Figure 4: A topographical AFM image of a fibroblast.

living cells, so a method of keeping them in fresh media while being scanned was necessary. We constructed a pump system with a flow cell for Agilent Technologies 5500 AFM shown in Figure 2 to allow media to flow in and overflow to be drawn out of the sealed area containing the sample. This allowed the cells to be scanned while living for about six hours out of the incubator and sterile environment.

## Results and Conclusions:

The carbon-face samples grew the most and the largest cells, though both carbon- and silicon-face samples did as well as or better than the control well in cell attachment after 24 hours. Images of this experiment are found in Figure 3. These findings confirm previous experiments done by this lab.

For CSAFM, it was found that when the cells were left for at least six days in undisturbed incubation, they were attached enough to probe with the tip of the AFM and examine topographically. Before that time, the cells were either not confluent enough or not attached strongly enough to remain in place when probed with the tip of the sensor. In Figure 4 are some of the first topographical images obtained. The current-voltage part of the examination was not completed, as the media was too ionic to obtain accurate electrical profiles of the cells themselves. This is being addressed by our lab's collaborators.

## Future Work:

In the future, the method of functionalization needs to be confirmed and taken further with the attachment of Protein A and IgG antibodies. This will allow for specific attachment of cells in predetermined locations, so the AFM can be confirmed as a valid method of identification. Also, the cells need to be more strongly adhered to the surface of the SiC so the AFM tip does not remove them during scanning. A different media or additive may help with creating more junctions for cell attachment.

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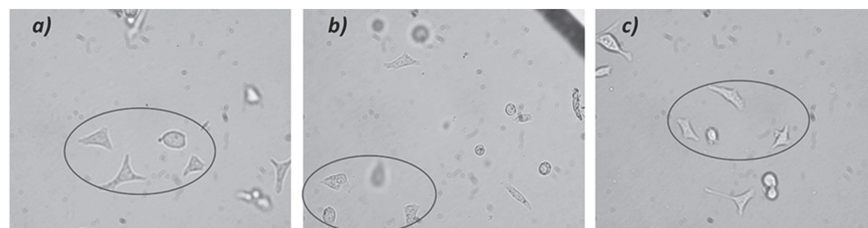


Figure 3: a) Carbon-face. b) Silicon-face. c) Control (empty well). Images taken at 24 hours (20x 1.6x).

## References:


[1] Rzany A, Schaldach M. Progress in Biomedical Research. 2001; 6: 182-194.

[2] Williams, E., et al. Applied Surface Science. 258, (16), 6056-6063 (2012).