Fabrication of "Barcode" Nanowires for Multiplexed Detection in Biological Assays

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Abstract and Introduction:

"Barcode" nanowires, composed of multilayers of gold (Au) and nickel (Ni), suggest a possibility of replacing the fluorophore cell labeling technique used today, which will introduce a "macro" magnetic detection and classification method for multiplexed biological assays. Low cost of fabrication [1] and a huge variety of magnetic signatures give barcode nanowires great advantage over fluorophores, which are costly and limited by the spectrally resolvable wavelengths. The nanowires can be functionalized with antibodies, so that different cell types can be tagged with unique barcode nanowires. The ferromagnetic properties of nickel are employed for nanowire characterization and manipulation, e.g. separation from the supernatant in the experimental procedure. Here, fabrication and characterization of barcode nanowires and their application in biological multiplexing studies were investigated. Two cell lines, A549 (human lung carcinoma) and HFF (human foreskin fibroblast), were incubated with two types of barcode nanowires that were conjugated with antibodies targeting the respective cells. The result demonstrated successful specific targeting of cells by the corresponding barcode nanowires. Together with quantitative magnetic characterization, successful functionalization of our barcode nanowires showed much promise in various cell detection applications.

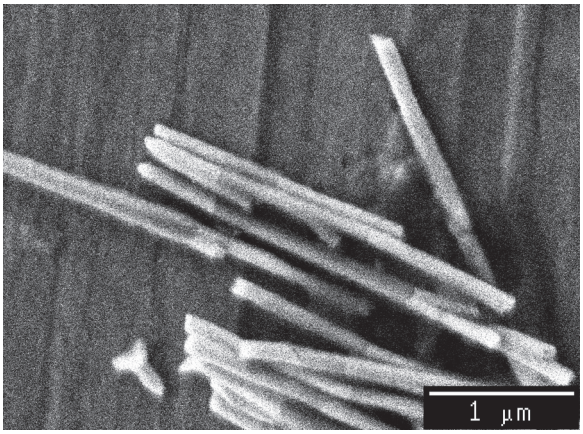

Figure 1: SEM of barcode nanowires (Au-Ni-Au). Minnesota Nano Center.

Experimental Procedure:

Nanowire Fabrication. Nanowires were grown through sequential electrodeposition in gold (HS-434 RTU, Technic, Inc.) and nickel ($NiSO_4$) electroplating solutions. A porous anodized aluminum oxide (AAO) template was sputtered with a copper conductive layer on one side and used as a working electrode. The working electrode, reference electrode, and counter-electrode were all immersed in a plating solution, and electrical charges were applied for metal deposition in the pores. The length of each metal segment was controlled by electrodeposition time. Once the nanowires were grown, the back copper contact was etched by ion-milling process and then freed by dissolving the AAO template in sodium hydroxide. The nanowires were suspended in deionized water until use.

Nanowire Characterization. While still in the template, the nanowires were characterized by vibrating sample magnetometry (VSM) at different angles ranging from 0° to 180°, with 0° being parallel and 90° perpendicular with respect to the magnetic field. Once the nanowires were freed and suspended in an aqueous solution, the nanowires were observed through scanning electron microscopy (SEM) for dimensions (Fig. 1).

Multiplexed Detection. Two types of barcode nanowires, Au-Ni (0.306 μm, 3.104 μm) and Au-Ni-Au (0.126 μm, 1.8 μm, 0.27 μm), were functionalized with primary antibodies, each specifically targeting A549 and HFF cells, respectively. Then, Au-Ni nanowires were conjugated with a green fluorophore, fluorescein isothiocyanate (FITC), to be distinguished from non-fluorescent Au-Ni-Au nanowires. Both A549 and HFF cells were conjugated with blue nuclear stains, Hoechst 33342. Plasma membranes of only HFF cells were tagged with wheat germ agglutinin with green Alexa Fluor 488 conjugate to optically distinguish the two cell types. The nanowires suspended in corresponding cell media solutions were titrated into cell cultures, in which A549 and HFF cells were plated 24 hours in advance, and incubated for six hours at 37°C. The cell media was aspirated, and formalin was added for cell fixation. Finally, formalin was replaced with a phosphate buffered saline (PBS) solution.

Results and Conclusions:

The VSM measurements successfully characterized each nanowire type, providing a specific magnetic signature of coercivity versus angle curves. Coercivity is the magnitude of magnetic field required for any magnetic materials to be demagnetized after reaching the saturation moment, and it can be measured at different angles discussed in the procedure. This way, our characterization methods rely on the nanowires' magnetic properties rather than optical imaging.

Three nanowires with different Ni deposition times, 10, 15, and 20 minutes, gave coercivities of 212.24, 261.17 and 285.37 Oe, respectively, all measured at 0°, and 131.54, 180.09, and 366 Oe, respectively, at 90° (Figure 2).

In multiplexing studies, the fluorescent nanowires, which were coated with antibodies targeting A549 cells, were observed to be tagged onto A549 cells only (Figure 3). Equivalently, the non-fluorescent nanowires, which were conjugated with antibodies targeting HFF cells, were found tagged to only HFF cells that were distinguished from A549 cells with their fluorescent plasma membranes (Figure 4). Through this experiment, specific cell-targeting by barcode nanowires was successfully tested and observed. The applications of barcode nanowires in biological assays seem highly viable with our magnetic characterization and antibody-functionalization techniques.

Future Work:

More experiments with various cell lines need to be conducted to ensure standardization of our nanowire-tagging methods. The possibility of nanowires being internalized after the tagging can also be looked into, in comparison to prior research findings of non-specific internalization of barcode nanowires in osteosarcoma cells [2]. The magnetic characteristics of our barcode nanowires suggest more possibilities of research topics, such as manipulation of cell matrix with influence of magnetic field gradient on the internalized nanowires.

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References:

[1] Schwarzacher, W. Interface 15.1 (2006): 32-35.

[2] Sharma, A., et al. Magnetics, IEEE Trans. on 49.1 (2013): 453-456.

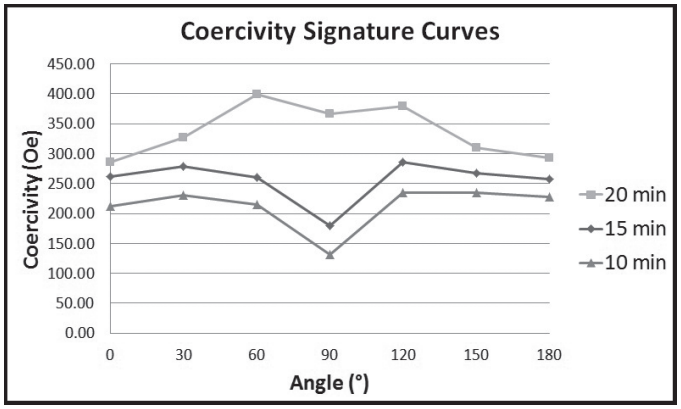

Figure 2: Coercivity vs. Angle curves of nanowires differentiated by deposition time of nickel.

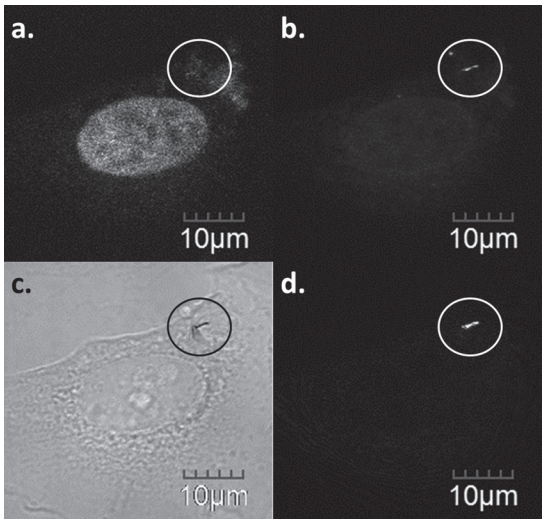

Figure 3: Fluorescence (a. 405 nm excitation laser, b. 488 nm), bright-field (c), and reflectance (d) images of A549 cells tagged with fluorescent nanowires (circled). (a) Fluorescence of nucleus confirming presence of a cell (identified as A549 by non-fluorescent plasma membranes in b); (d) Nanowires are the only metallic objects that can reflect light in the culture, confirming the identity of the dark spot in (c) to be nanowires. University Imaging Center.

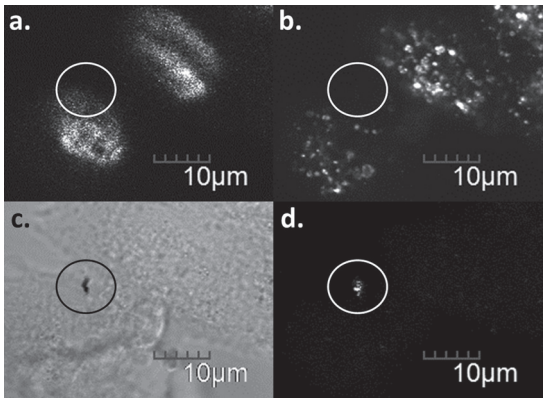

Figure 4: Fluorescence (a. 405 nm, b. 488 nm), bright-field (c), and reflectance (d) images of HFF cells with fluorescent plasma membranes (b) tagged with non-fluorescent nanowires (circled). University Imaging Center.