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Waveguide Evanescent Field Fluorescence Microscopy

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There are many powerful microscopy technologies available for the investigation of bulk materials as well as for thin film samples. Nevertheless, for imaging an interface, especially live cells on a substrate only Total Internal Reflection Fluorescence (TIRF) microscopy is available. TIRF microscopy is an evanescent based microscope and allows imaging the interface without interference of the bulk.

Recently, we developed waveguide evanescent field fluorescence (WEFF) microscope, which is an alternative to TIRF and an evanescent field based microscope. In this method, the light is coupled into a waveguide (a glass substrate which supports a layer, waveguide layer, with higher refractive index) via an optical grating. The coupled light propagates as a waveguide mode and exhibits an evanescent field on top of the waveguide Figure 1. This evanescent field can be used as a surface-bound illumination source to excite fluorophores. It serves as an powerful tool for quality control of thin films, to study cell-substrate contacts, and investigating the effect of external agents and drugs on the cell-substrate interaction in real time and in vitro.

The WEFF microscopy set-up is shown in Figure 2. Briefly, a laser beam is coupled into the waveguide by using a grating coupling. The waveguide with the grating were mounted on the sample stage of an inverted microscope. Various modes of the multimode waveguide were excited by changing the angle of incidence between the laser beam and the grating on the waveguide. A long pass filter was used to block the excitation wavelength. The images were taken by a digital camera and with the assistance of the imaging software.

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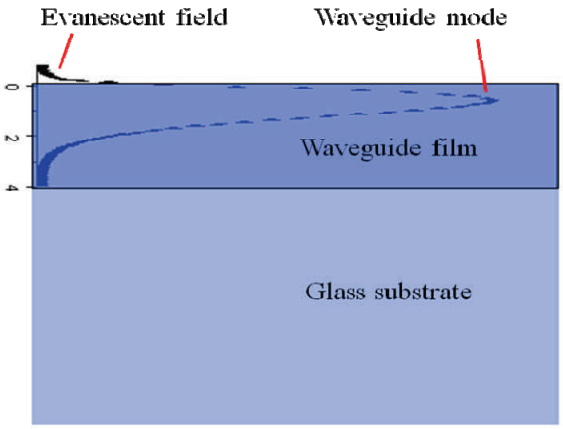

Figure.1 Schematic of a waveguide film on a glass substrate. The evanescent field of the coupled light on the waveguide surface excites the fluorophores on a cell membrane or in solid thin films.

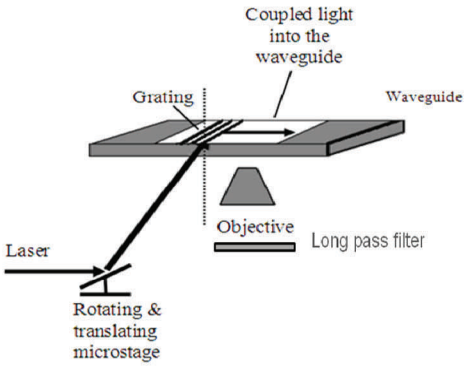

Figure 2. A schematic of WEFF microscopy. A laser beam is directed onto a coupling grating by a mirror mounted on a translating and rotating stage. The light couples into the waveguide and propagates as a waveguide mode parallel to the surface. The coupled light produces an evanescent field on the waveguide surface which can excite fluorophores. The emitted light from the labelled specimen can be collected by an inverted microscope.

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The key element of the technique is a waveguide with a grating on its surface or embedded in it. We used ion-exchange to fabricate the waveguides and Laser Interference Lithography (LIL) (Nanofabrication Facility) Figure 3 as a simple and inexpensive method was used to fabricate the gratings. The principle of LIL is based on two-laser beam interference. A thin layer of photoresist is deposited on a flat glass substrate using the Solitec spin coater in the Nanofabrication Facility. After a soft backing the substrate is placed in the optical setup. The laser beam is incident on a mirror and the substrate which are perpendicular. Both mirror and substrate are mounted on a rotation stage with a 90 degree angle between them. The photoresist is exposed to the interference fringes during a time that is adjusted according to the intensity of light. Figure 4 is a SEM image of a photoresist grating on a glass waveguide surface.

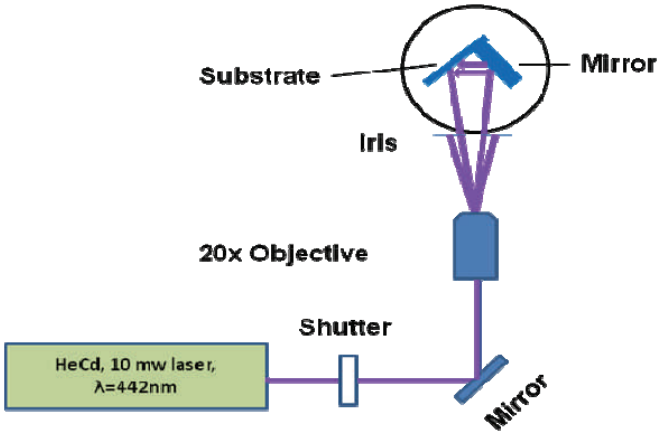


Figure 3. Laser Interference Lithography set-up.

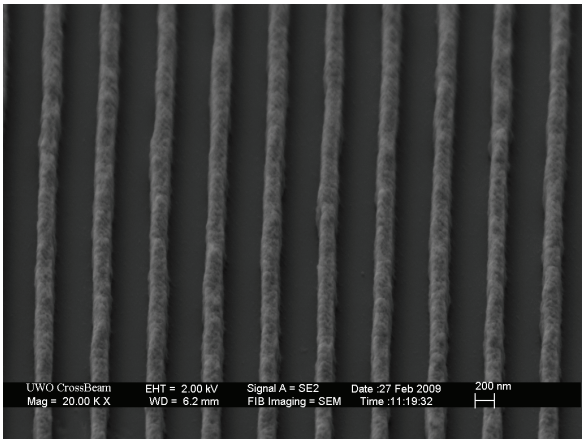


Figure 4. SEM image of a photoresist grating on a glass waveguide surface.

To test the WEFF microscope at the first step an ultra-thin film was prepared by Langmuir-Blodgett trough in the Nanofabrication Facility. Figure 5 shows the WEFF microscopy image of a stained phase separated lipid monolayer deposited on the waveguide surface. Figure 5 (a) is its image when directly deposited on the waveguide. The lipid monolayer deposited onto ten layers of stearic acid (Fig. 5 (b)) looks different, although the deposition characteristics in the LB process behaved very similar and the same amount of material was deposited. The image pattern still exhibits a laterally inhomogeneous appearance, but the bright domains disappeared and are replaced by a more ruptured structure.

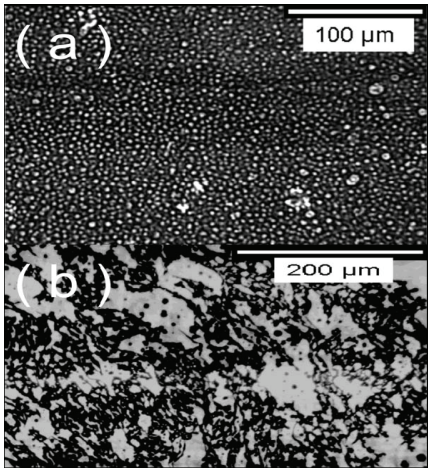


Figure 5 (a) Image of the stained lipid monolayer located directly on the waveguide surface (integration time: 163 s). (b) Image of the stained lipids on ten layers of stearic acid (integration time: 212 s) (excitation wavelength 543 nm, 560 nm long pass filter).

As a first application in cell biology, and in particular to image the cell-substrate contacts, images of MC3T3-E1 cells (osteoblasts) cultured on the waveguide surface and loaded with the carbocyanine membrane probe, (DiI) were taken. Osteoblasts are derived from mesenchymal stem cells and are bone forming cells. The intensity distribution of the illuminated cell can be used to study the cell contact to the waveguide surface Figure 6.

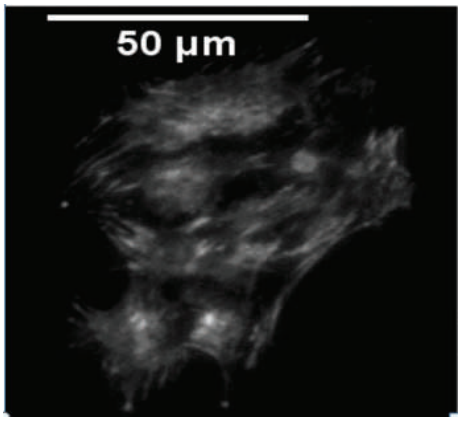


Figure 6. Images of an osteoblast cell on the waveguide surface which was captured by the WEFF microscopy (Objective magnification: 20X, Imaging time: 20s).

We demonstrated a straightforward alternative to TIRF microscopy; waveguide evanescent field fluorescence microscopy. Images were taken from a demixed lipid monolayer fabricated with LB technology. As a first biological sample a plasma membrane of an osteoblast cell was imaged. This inexpensive method also has a high axial resolution in a nanometre regime.

References:

Abdollah Hassanzadeh, Michael Nitsche, Silvia Mittler, Souzan Armstrong, Jeff Dixon, and Uwe Langbein, APPLIED PHYSICS LETTERS 92, 233503 (2008).

Abdollah Hassanzadeh, Souzan Armstrong, Jeff Dixon, Silvia Mittler, APPLIED PHYSICS LETTERS 94, 033503 (2009).

Professor Bernie Kraatz Florence Bucke Prize Winner

New biosensors become lab on a chip

By Mitchell Zimmer

Bernie Kraatz takes a chip from its protective case and balances the centimeter-square biosensor at the end of his index finger.

Chemistry Professor Bernie Kraatz, is director of the Nanofabrication Facility where he is developing chips that detect specific molecules such as those associated with disease or pollutants. Some applications may be worn on clothing with a readout, while others could plug into a computer.

This device, he notes, has 16 separate ports able to detect four different biologically active agents. The surfaces of these chips "can be prepared to detect a number of biological molecules related to disease, cancer and genetic defects."

This work has earned Kraatz this year's Florence Bucke prize. The prize, recognizing some of the best research in the Faculty of Science, is in memory of Florence Bucke (BA'26) who taught school in Fort Erie until 1971.

The idea for the device was developed while Kraaz was working in the Nanofabrication Facility in Salt Lake City. When he moved to Saskatoon, he refined the biosensors so that they could detect genetic mutations. His lab developed a working product now marketed by Adnavance Technologies Inc, a firm that describes itself as a developer of direct detection molecular diagnostic tests.

Now at Western, Kraatz is director of the Nanofab Lab and taking biosensors to the next level. He is modifying the chip to fit directly into a PCI slot of a computer.

"So now you don't have to worry about engineering the interface, it is already done," he says. His lab is also developing a reader interface which interprets signals from the sensor to visualize a simple yes/no answer. The number of samples that can be assessed on a chip is growing, too. Kraatz's lab is developing chips containing an array of 96 probes.

While nanofabrication and microfabrication play key roles in developing these devices Kraatz also thinks of the big picture applications for this technology. In one scenario shipping perishable goods across international borders could take less time if the authorities had these devices on hand.

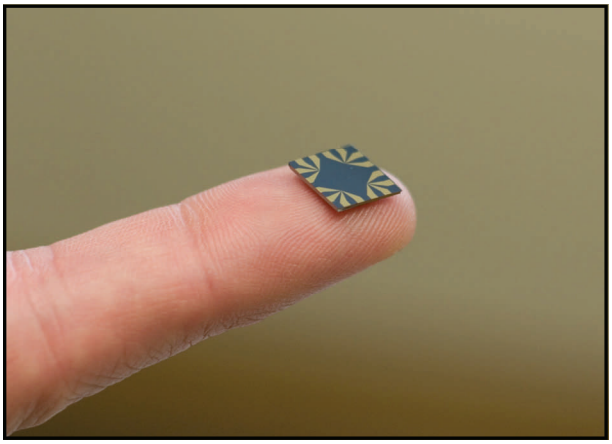
"Canadian industry is losing billions of dollars every year when they ship things down to the USA," he says. If organisms such as flies stow away on a truck, "border guards have the authority to stop the truck at the border and if there is something on there that can be spoiled easily - meat, flowers, any kind of fresh produce- that is lost."

It would be possible to take a DNA sample from the organism and run it through an array on a chip to determine if that fly is a potential threat as a pest. "The idea here is that we are able to detect genetic information that is species specific."

Kraatz is also working on biochemical tools for screening how new drug molecules interact within disease processes such as cancer or AIDS.

In the case of HIV, "we've developed a detection device that allows us to monitor various HIV proteins that are involved in different stages of the virus....The way this works is that you essentially have something sticking to a [biosensor] surface and the protein docks on. It's a simple handshake or recognition event that allows us to monitor that electrochemically. We can quantify the binding, we can monitor the drug molecules that interfere with this binding. It's like a biochemistry lab on a chip."

Currently, Kraatz is creating a toolset that recognizes biological or chemical warfare agents. His lab is developing this technology along with the Department of National Defense. He sees the final product as a little wearable plaque that displays a simple readout indicating whether the air is good or bad.



The above biosensor chip was manufactured at the Western Nanofabrication Facility

This article was originally printed in The Western News

Reservations for equipment use at the Nanofab can be made online. Nanousers can go to uwo.ca/fab and click on the Online Reservations tab. Please contact Tim Goldhawk at nanofab@uwo.ca for login information and how to reserve equipment time.

NANOFABRICATION FACILITY

Research Western

Western's Nanofabrication Facility is a professionally staffed cleanroom designed to support education, research and industrial collaboration in the fabrication and characterization of structures and devices of sub-micron scale.

The Nanofab is user-fee supported facility. It is open to academic, government and industrial users. The Nanofab is a "hands-on" facility where users are trained and supervised on the use of equipment and processes. Analytical and processing services are also available.

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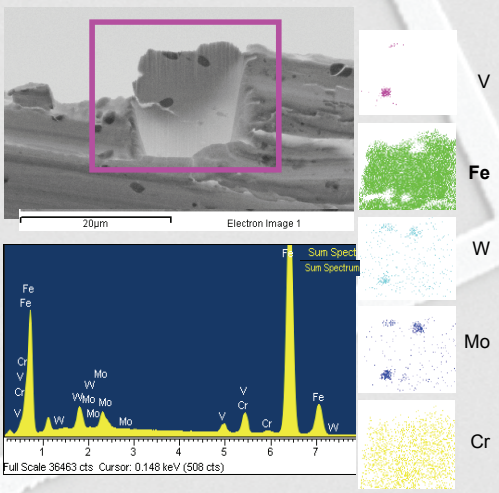
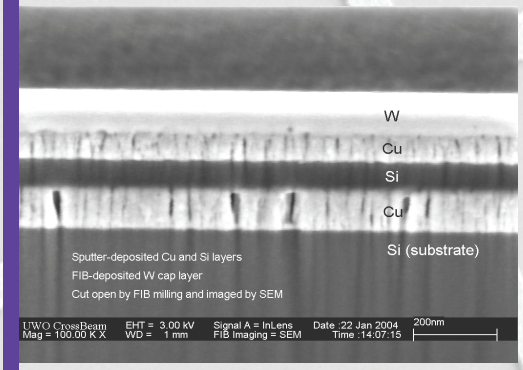
CHARACTERIZATION

Optical Inspection, Electron Microscopy, X-Ray Elemental Analysis

- optical properties: refractive index and thickness mapping
- micrographs: geometric dimensions
- elemental composition
- topographic measurements -*height 100 nm to 65 µm along 2 mm*
- TEM sample preparation by FIB



DEPOSITION



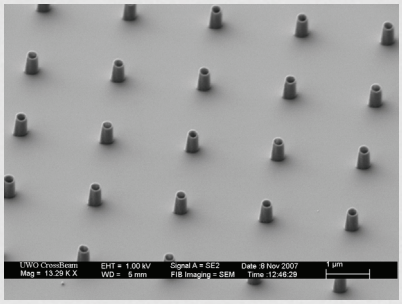
Coat samples with a variety of materials and thicknesses.

- metals (Au, Al, Cr, ..) *1 nm to 1µm*
- silicon, its oxides and nitrides -*1 nm to several µm*
- polymers (photoresist, PMMA) - *100 nm to several µm*
- *ITO—1 nm to 1 µm*
- amphiphilic molecules - *1 - several monolayers*

PATTERNING

Fabricate patterns on samples that are periodic or random using a variety of technologies.

- patterning by light, electrons and ions on virtually any solid flat surface
- structure dimensions :
  - 2D lateral - *50 nm to several µm*
  - depth (3D) - *nm - µm*



ROUTINE ANAYLYTICAL & PROCESSING SERVICES

Scanning Electron Microscopy ♦ Elemental composition by EDX ♦ electron beam lithography TEM Sample Preparation with FIB ♦ thin film deposition ♦ annealing ♦ cleaning ♦ dicing