Elisha Moses

Stephan Thiberge
Nava Levit-Binnun
David Biron
Ofer Feinerman
Ilan Breskin

Enhanced imaging and detection using the electron microscope

Department of Physics of Complex Systems

Tel. 972 8 934 3139 **Fax.** 972 8 934 4109
E-mail: elisha.moses@weizmann.ac.il

We present here two of the projects preformed in our lab:

1. Electron Microscopy Of Cells In A Wet Environment: A New High Resolution Imaging Technique (Stephan Thiberge)

We present a new technique of scanning electron microscopy that is adapted to the study of cells and more generally to wet samples. Our system is based on the isolation of the wet environment from the vacuum by the introduction of a thin membrane that is practically transparent to energetic electrons, yet is tough enough to withstand high difference of pressure.

The imaged volume is the close proximity of the membrane, typically a few micrometers into the fluid. This is ideal for the inspection of objects that are stuck to the surface such as adherent biological cells.

The experimental conditions are similar to those under which fluorescent microscopy experiments are performed. The cells are kept wet during the experiment, and no denaturing treatments of traditional electron microscopy are required. Markers are used to label the cells: gold particles are specifically attached to the cell using immunological methods.

One of the main successes of this method is the resolution: 10nm achieved on gold beads. Furthermore it is easily adaptable to all existing Scanning Electron Microscopes, enabling measurements at room temperature and at atmospheric pressure.

We believe that further developments will allow us to follow molecular transport and interactions in cells.

Shown in figure 1: NIH 3T3 cells labelled with concanavilin A observed by Scanning Electron Microscopy in a wet environment with the membranous partition apparatus

2. Enhanced Dynamic Range and Sensitivity of Protein Arrays Using Gold Probes and Scanning Electron Microscopy (Nava Levit-Binnun in collaboration with Ari B. Lindner from the lab of Prof. Zelig Eshhar)

Detection of low-abundance proteins, especially with techniques that can be applied to protein chips, is an unanswered challenge. These proteins are often the ones that are of most interest, as they often serve as key mediators in response to various physiological stimuli. Examples for such proteins are proteins hormones, cytokines, small G-proteins or DNA binding proteins. All current approaches that enable the detection of these proteins at the single (or close to single) molecule level require painstaking techniques with tedious sample preparation and imaging, and are hard to apply with high throughput methods.

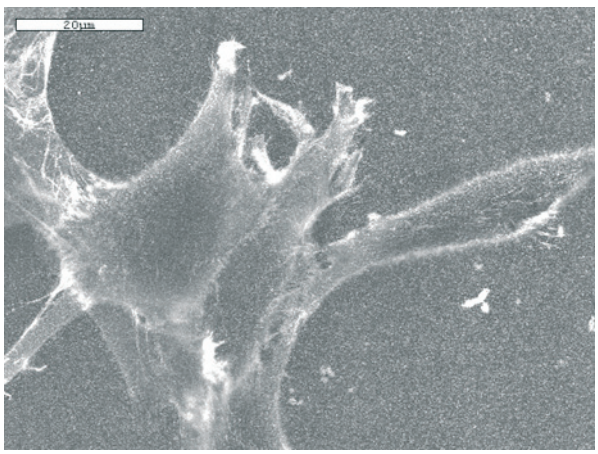

Fig.1 *NIH 3T3 cells labelled with concanavilin A observed by Scanning Electron Microscopy in a wet environment with the membranous partition*

Here we present a rigorous, quantitative method that reaches single molecule detection levels, gives high signal to noise ratios, and demonstrates a broad dynamic range, while retaining easy sample preparation and potential for high throughput abilities. Our method is based upon target-coated gold particles followed by scanning electron microscopy to probe proteins or their ligands, arrayed on a microscope glass slide. Single molecule detection is naturally obtained in this way, and the ability to count singles molecules in a sample can be easily automated.

As model systems we quantified the interactions of biotin-streptavidin and that of an anti-hapten antibody with its cognate antigen. Our results demonstrate an increased sensitivity and a highly enhanced dynamic range over other detection methods such as fluorescence. In addition, our

technique offers several other important advantages such as non-bleaching of the signal, high reproducibility and a reduction of non specific binding.

Shown in Fig. 2: Graph presents the difference between fluorescence probes and gold probes in the detection of different concentration of BSA-biotin molecules. An almost 100-fold increase in sensitivity and enhanced dynamic range are here demonstrated. Inner image: Single molecule detection using 20nm gold probes as they are seen in the EM. Each gold probe is conjugated to Streptavidin that interacts with biotin (attached via BSA to the surface of the glass).

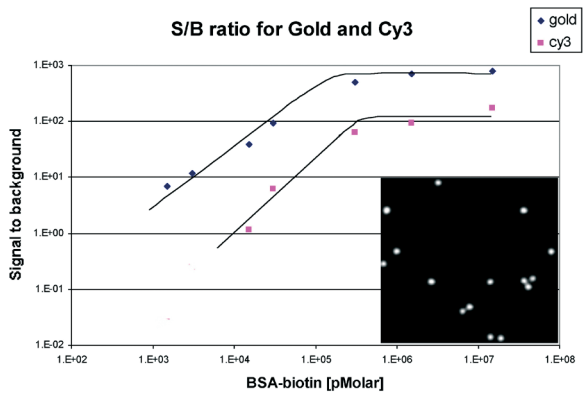

Fig. 2 *Detection of different concentrations of BSA-biotin with either fluorescence (Cy3) or gold labeled Streptavidin probes. Detection abilities are presented in terms of signal to background ratios [defined as (S-B)/B].*