광집게를 이용한 바이오소재의 표면 탄성계수 측정

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Measurement of Surface Stiffness of Biomaterials Using Optical Tweezers

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ABSTRACT

Since the demonstration of optical trapping phenomenon, the experimental techniques, theories and their applications are escalating continuously. Optical tweezers have been extensively employed to measure different parameters at micro/nano world. In this paper, we demonstrate a new technique to measure the surface stiffness of a biomaterial using optical tweezers. For this purpose, micro particle is trapped and manipulated by a continuous wave (CW) trapping laser and its position detection and monitoring is done by a continuous wave (CW) detection laser. By recording the position detection signal of micro particle on Quadrant Photo Diode (QPD) through detection laser, a new method has been described to measure the stiffness of biomaterials at specific point. This technique can be a milestone to identify and standardize different biomaterials.

Key Words: optical tweezers, surface stiffness

1. Introduction

Optical tweezers are scientific instruments that utilize a highly focused laser beam to apply an attractive or repulsive force (usually on the order of picoNewtons), depending on the refractive index mismatch, to physically hold and move the microscopic dielectric objects. This phenomenon was first reported by Arthur Ashkin in 1970[1]. Years later, Ashkin reported a tightly focused beam of light capable of holding microscopic particles stable in three-dimensions which is now called optical tweezers[2]. After that, optical trapping and manipulation of viruses, bacteria and silica coated particles were also demonstrated[3-4].Trapping and manipulation of micro-particles have been used for three-dimensional imaging[5,6], thickness measurement[7] and nanocoordinate measuring system[8].Furthermore, optical tweezers have also been employed to measure forces on the order of femtonewton[9] which establishes its importance in nanotechnology. Due to the non contact nature of the optical tweezers, it can also be used to measure different parameters related to the properties of the biomaterials and stiffness measurement is one of them. Currently, a lot of research is being done on biomaterials to classify and identify them on the basis of different properties. This classification can help to identify different biomaterials or cells present in the single unit like petridish. After identification of specific cell, it can be treated according to the requirements and the surface stiffness measurement of the biomaterials can help to identify or locate specific biomaterial present in the cluster of other types of biomaterials. In this paper, we are demonstrating a technique by the manipulation of trapped microparticle using optical tweezers to measure the surface stiffness of an important breast cancer cell line named MDAMB231.

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2. Materials and Methods

2.1 Preparation of MDAMB231 Cells

As our work is on MDAMB231 cells, it is imperative to get some samples of these cells and culture them on grid petridish. The cells are grown on the bottom of grid petridish (ibidi, grid size = 50 μm) and mounted on the motorized stage of the optical setup. Figure 1 shows the microscopic image of MDAMB231 cells that are seeded on the grid petridish. Cells were kept in incubator at 37°C, 5% CO2, with 2 mL culture medium prepared by Dulbecco's Modified Eagle Media (DMEM) with 10% v/v Fetal Bovine Serum (FBS) and 1% Antibiotic Solution (Penicillin). Cells were seeded on the glass bottom dish for 48 hours to achieve good adhesion of cells on petridish bottom. Before and after the experiment, cells were washed with Phosphate Buffered Saline (PBS) to remove the contaminations from the petridish.

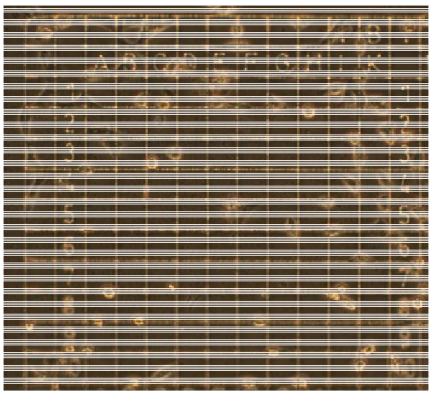

Fig. 1. Microscopic image of MDAMB231 cells grown on the grid petridish

2.2 Microparticle

The technique to measure the stiffness of the cell membrane of MDAMB231 employs the use of microparticle. For this purpose, both metallic and dielectric particles can be used. Since trapping of dielectric microparticles is easier than trapping metallic microparticles, we have used 1.4 μm polystyrene microparticles particles (Spherotech, Cat. No.: AP-10-10). Appropriate amount of microparticle solution is added to the petridish before the experiment. Trapping and manipulation of these types of particle is done in our optical setup which is described in the following.

2.3 Experimental Setup

The setup for this experiment is shown in figure 2. Optical trapping and manipulation of microparticle is done by a continuous-wave, near infrared (NIR) fiber laser (B&W Tech, 1064 nm, 5W, TEM00). This beam reaches the beam expander through dichroic mirror (DM1). Beam expander is installed in the setup to broaden the incident laser beam (3X). This broadening is required to overfill the back aperture of the objective lens to maximize the trapping force at its focus. This broadened laser beam arrives at mirror (M1) which reflects the beam towards lens (L1). This lens focuses the 1064 nm laser beam on dichroic mirror (DM2). For relative position detection of trapped plasmid coated microparticle, a diode laser beam (Power Tech, 685 nm, and 50mW) is extended by lens (L2) and combines with 1064 nm trapping laser beam at dichroic mirror (DM2). Then trapping (1064 nm) and detection (685 nm) laser beams pass through lens (L3) and then directed to the oil immersion objective lens (Olympus, 100X, 1.4 NA), mounted on a piezoelectric objective positioner (OP) through another dichroic mirror (DM3) whose purpose is to reflect the laser beams and transmit the image plane of the microscope. Using the optical alignment techniques, both lasers are made concentric in lateral axis (x and y axes).

For high speed control of piezoelectric device to move the laser foci in axial direction, a DSP controller (nPoint, C.300 series) is used. Finally, trapping and detection laser beams are tightly focused onto the MDAMB231 cell culture dish which is placed at the motorized stage capable of moving in x, y and z axes having 50 nm resolution. The transmitted and scattered light of laser beams at the sample specimen are captured by the condenser lens, and reflected by DM4 and focused by lens L4 on quadrant photodiode (QPD, Thorlabs, PDQ80S1) positioned on manual xyz stage. A band-pass filter (F) blocks all the beams except detection laser beam to reach QPD. A CCD camera (Cooled Retiga EXI, RBRC 03-10) is also installed to get real time bright-field images of the sample, which is imaged through the tube lens (L5). A customized user interface is also developed for remote operation of motorized sample stage, objective positioner and image recording and signal acquisition from QPD.

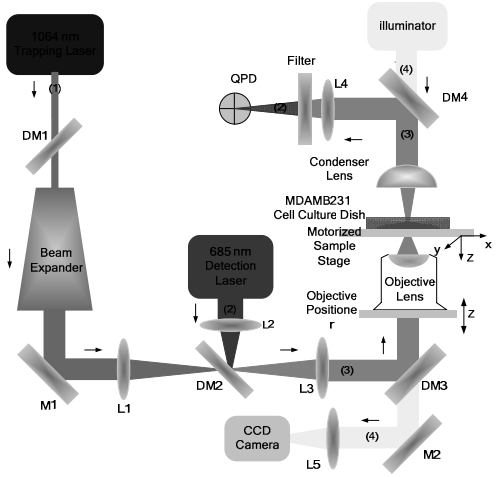

Fig. 2. Experimental setup for stiffness measurement of MDAMB231 cell. (1) is trapping laser, (2) is detection laser, (3) is both trapping and detection

laser, (4) is illumination light

3. Experimental Procedure

MDAMB231 cell petridish is placed at motorized stage and the optical components are turned on. Before the experiment, objective positioner is set at its home position. At first, 1.4 μm polystyrene particle is trapped by trapping laser (1064 nm). Then the trapped particle is manipulated in 50 nm steps of objective positioner towards the MDAMB231 cell membrane and QPD signal from the 685 nm detection laser is monitored continuously. When the trapped microparticle is manipulated towards the cell membrane, QPD signal voltage is almost constant. But when the trapped microparticle touches the cell membrane then a sudden change in the QPD signal is observed and this change is observed as the slope between QPD voltage and objective positioner movement. In the second step, objective positioner is brought back to its home position with the same particle trapped. Now this trapped particle is manipulated towards the petridish bottom where cell is not present. Similarly, when the trapped particle touches the bottom of the petridish, again change in the QPD signal happens which is also observed as a slope between QPD voltage and position of the objective positioner. QPD signal again becomes straight when the microparticle is no longer tapped by the trapping laser beam. It is worth noting that slope for petridish bottom surface is steeper than for cell the membrane.

4. Results

The same type of signal can be obtained for a material whose stiffness is already known and measured by some another method. By comparing the QPD signal values for the stiffness of a know material, stiffness of both petridish and cell membrane or any unknown material can be calculated. Figure 3 shows the QPD signal acquired for the surface of the cell membrane and for petridish surface.

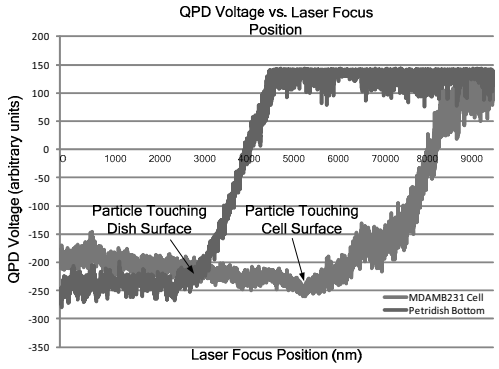

Fig. 3. Graph between QPD voltage and laser focus position. This graph is for both MDAMB231 cell membrane surface and petridish bottom surface.

5. Conclusion

On the basis of above discussion, it can be concluded that trapping and manipulation of microparticle by optical tweezers and QPD signal values can help to measure the surface stiffness of the biomaterials. However, to measure accurate stiffness of the material, it is important to consider the trap stiffness of the incident beam.

Acknowledgements

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