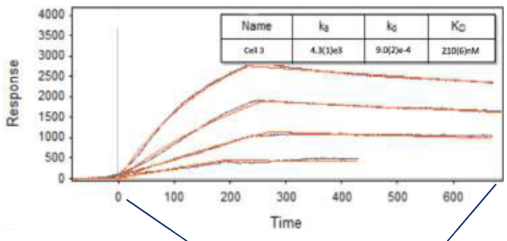
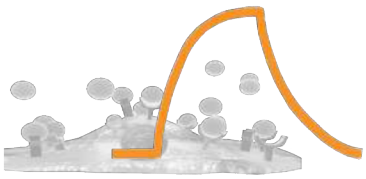
Biosensing Instrument

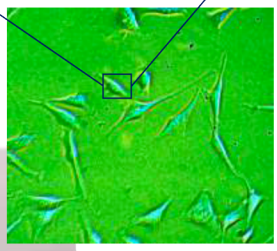
*Surface Plasmon Resonance Microscopy*
*Label-Free Analysis System*

# SPRm 200 Series

*Integrated optical microscopy with SPR*
*A new way to study cell membrane protein interactions*



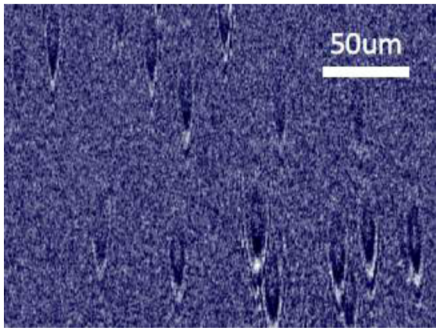





*Multiple SH-P1 cells binding with WGA*

- **Study label-free molecular binding activity on live cells**
- **Quantitatively map binding affinity and kinetics in real time**
- **Simultaneously perform bright field imaging and SPR measurements**
- **Study drug effects on multiple cells and sub-cellular structures**
- **Monitor nanometer scale binding events**

*Binding activities of Nano materials (2um size)*




The SPRm 200 system opens a new frontier in the study of molecular interactions by integrating optical microscopy with Surface Plasmon Resonance (SPR) technology. Designed especially for in-vitro, label-free measurement of binding activity and cell kinetics, SPRm 200 provides a spatial visualization of cellular structures together with local binding activities. Thus, real-time interactions of the drug and membrane protein can be measured in its native state without needing to extract proteins from the cell. With its outstanding sensitivity and stability, SPRm 200 also allows for the measurement of nanometer scale binding activities, the study of bacteria and virus interactions with antimicrobial drugs, and the development of new methods for nanoparticle drug delivery.

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# Integration optical microscopy with SPR

SPRM combines the optical imaging with SPR technology, providing spatial mapping of the binding activity on living cells [2-4]. As shown in FIG 1, the light condenser illuminates the cell grown on sensor surface, and the camera at the bottom captures the bright field image of the live cells. Simultaneously, the SPR light source projects its beam at its resonance angle onto the sensor and the reflected beam is collected by the SPRM detector; the SPR response at each pixel is mapped in a SPR image.

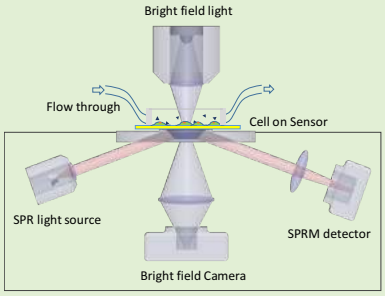



**FIG.1**

The SPRM detector measures the resonance angle changes and records a sensorgram at each pixel of the sensing area, in contrast to conventional SPR, which detects the average resonance angle change of the entire sensing area (often referred as channel). SPRM provides sensorgram data for the entire sensing area as shown in FIG.2. Thus it provides much more localized information and makes it possible to study heterogeneous surface binding and interactions for membrane proteins in their native states.

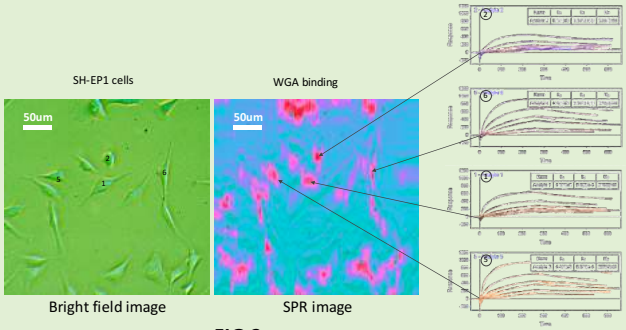
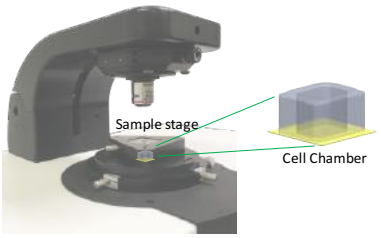



**FIG.2**

*Simultaneous images of bright field and SPR of neuoblastoma cells with WGA binding, Sensorgrams of selected points are ploted on the right.*

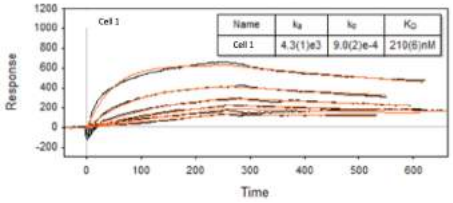
# Lectin-Glycoprotein interaction

Basic cellular and therapeutic processes usually start with the binding of ligands to membrane proteins, and the study of the binding activities of membrane proteins in their native states is critical to the understanding of their biological functions. Here is an example of studying specific interactions between glycoproteins (membrane protein) and lectin (ligand), and the binding activity and spatial distribution of receptors on the membrane of a single cell.




Neuroblastoma cells SHEP1 were incubated in the cell chamber on the sensor surface, then placed onto a SPRM sample stage. PBS buffer was fed into the cell chamber at a rate of 300ul/min at 25 C. The lectin, wheat-germ agglutinin (WGA) 80ug/ml, was injected into the cell chamber, then washed out with PBS buffer. Similar measurements on the cell were repeated with different WGA concentrations (5, 10, 40, 80, 100, 200ug/ml). A 50mM GlcNAc solution was used to regenerate the membrane glycoprotein receptors on the cell surface.

Sensorgrams were recorded at each pixel as shown in FIG.2. By averaging the pixel within the cell image, the binding kinetics for each cell can be derived using first-order binding kinetics theory (see below). The measured result was consistent with previous published data [4].



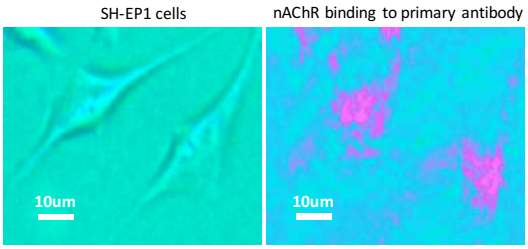

| | $k_a$ ($M^{-1}s^{-1}$) | $k_d$ ($s^{-1}$) | $K_D$ |
|---|---|---|---|
| Reference[4] | 5.2 e3 | 1.2 e-3 | 230 nM |
| SPRm 200 (average) | 5.1 e3 | 1.2 e-3 | 216 nM |

## Selected References


1. K Syal, R Iriya, Y Yang, H Yu, S Wang, S Haydel, HY Chen, NJ Tao, "Antimicrobial Susceptibility Test with Plasmonic Imaging and Tracking of Single Bacterial Motions on Nanometer Scale", ACS Nano, 10, 845-852, 2016
2. F Zhang, S Wang, L Yin, Y Yang, Y Guan, W Wang, H Xu, NJ Tao, “Quantification of Epidermal Growth Factor Receptor Expression Level and Binding Kinetics on Cell Surfaces by Surface Plasmon Resonance Imaging", Analytical Chemistry, 87(19), 9960-9965, 2015
3. W Wang, L Yin, L G-M, S Wang, X Yu, S Eaton, S Zhang, H Chen, J LaBaer, NJ Tao, “In situ drug-receptor binding kinetics in single cells: a quantitative label-free study of anti-tumor drug resistance”, Scientific reports, 4, 1-7, 2014
4. W Wang, Y Yang, S Wang, V Nagaraj, Q Liu, J Wu and NJ Tao, "Label-free measuring and mapping of binding kinetics of membrane proteins in single living cells", Nature Chemistry, 4, 846-853, 2012
5. Wang, Shaopeng, et al. "Label-free imaging, detection, and mass measurement of single viruses by surface plasmon resonance." Proceedings of the National Academy of Sciences 107.37 (2010): 16028-16032.

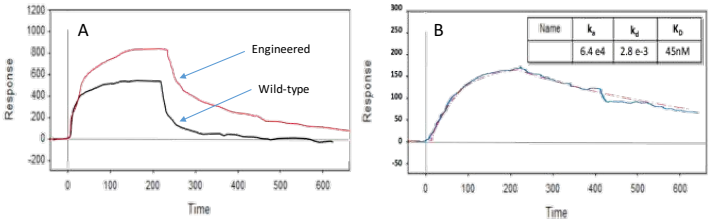
# Mapping binding activites of nAChRs

nAChR membrane receptor plays a critical role in neurotransmission and nicotine addiction. The conventional method to map their distribution in cells is based on fluorescence-labelled secondary antibodies, which does not provide direct kinetics and is prone to false positives. SPRM directly measures the binding of primary antibody to nAChR without the secondary antibody, which is a simpler process that overcomes the drawbacks associated with the use of a labelled secondary antibody [4].



Bright field image SPR image

Engineered SH-EP1 cells express alpha4beta2 nAChR in the cell membrane, which binds to its primary antibody anti-alpha4. Images on the right show the bright field and SPR images of the engineered SH-EP1 cells. The SPR image shows the distribution of nAChR binding to its primary antibody, clearly a heterogeneous surface binding. The wild-type SH-EP1 cells are also used as a control. Sensorgram A shows that the two types of cells have significant differences in their SPR responses. Subtracting the wild-type response (mostly due to bulk refractive index effect) from the engineered SH-EP1 cell gives the binding kinetics of nAChR to its primary antibody with kon=6 x10^4 /Ms, koff=3x10^-3 /s and KD = 45nM, as shown in sensorgram B.




# Nanoparticle detection

At nanometer scale, a SPR response signal generated by a nanoparticle is very unique. Like placing a small rock in the middle of a gentle shallow stream, it produces a wake pattern. The SPR light projects onto the sensor at its resonance angle, creating a propagating surface plasmonic (SP) waves along the metal film surface shown in FIG.3. When a nanoparticle binds to the sensor surface, it acts as a scattering center in the SP waves, creating a wake pattern with a footprint much larger than its actual size (up to 100X). This enlarged footprint enables SPRM to detect particles size smaller than the optical diffraction limit. Nanometer scale binding activities can be monitored and studied by measuring and mapping these large footprints.

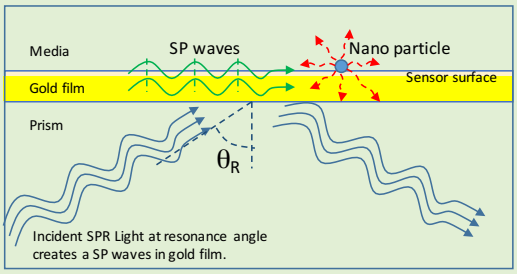
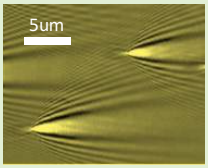



**FIG.3**

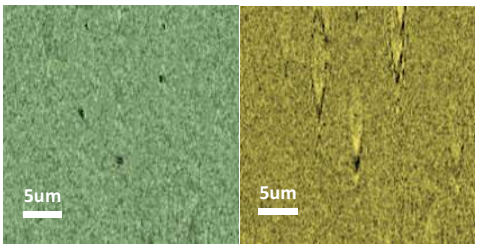


SPR image

The occurrence and intensity changes of these wake patterns in the SPR image provides extremely rich information about binding events between the sensor surface and the nanoparticles, as well as their interactions with other molecules in the media [1, 5].

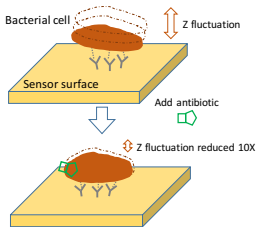
# Bacteria and Antibiotics

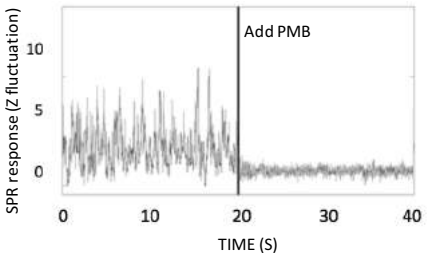
The live *E. coli* O157:H7 cells are tethered on a sensor chip via antibody coupling in a Luria Broth culture medium. They scatter the propagating SP waves creating wake patterns in the SPR image as shown below (right).



Bright field image SPR image

While the bright field image of the bacterial cell (black dots) appears to be constant, the contrast of wake pattern in the SPR image fluctuates significantly. See plot below(right). Monitoring this fluctuation, caused by the bacterial cell nanomotion, provides great insight into its metabolism. When 500 ug/ mL of polymyxin B (PMB), a bactericidal antibiotic, is added into the cell chamber, the fluctuation of the bacterial cell reduces drastically, thus revealing lethality.




This result promises a label-free detection of antibiotic activity in real time and offers a quick, simple, and low cost comparison to the current clinical microbiology approaches [1].

## System Specifications

| | | |
|---|---|---|
| Base Station | Light source | 690 nm |
| | Incident angles | 40-76 Deg (continuous) |
| | Baseline noise | < 0.6 RU RMS (0.1 mDeg RMS) |
| | Baseline drift | 3 RU/hr (0.5 mDeg/hr) (when ambient drifts < 1°C/hr) |
| | Temperature Control Range | 15°C to 40°C (10°C below ambient temperature max) |
| | Field of view | Bright Field: 1200 x 900 um<br>SPR: 600 x 450 um |
| | Magnification | Bright Field: x10<br>SPR: x20 |
| | Resolution | Bright Field & SPR: 1 µm |
| | Stage translation / rotation | 3mm x 3mm / 360 deg |
| | Outer dimension | 690 (w) x 330 (h) x 340 (d) mm |
| | Weight | 23 kg |
| | Power supply | 110-230 V 50/60 Hz |
| Fluid Handling | Sample volume | 1 to 1500 µL (application dependent) |
| | Kinetic constant | $k_a$ <1 X $10^7$ $M^{-1}$ $s^{-1}$<br>$k_d$ >1 X $10^{-5}$ $s^{-1}$ |
| | Dissociation constant | $K_D$ = $10^{-3}$ M (1 mM) to $10^{-12}$ M (1 pM) |
| | Molecular weight cutoff | 200 Da |
| Control System | Computer | Windows operating system |
| | Software | ImageSPR™ software including Data Analysis and Kinetics Analysis packages |

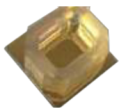
## Sensors and consumables

### Gold Sensor Chip

Highly uniform gold film for reproducible SPR research.

### Cell chamber kit

Gold sensor chip with a silicone well for growing cells; includes chemicals and other accessories for treating sensor surfaces.



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