# Characterizing the Interfacial Topology of Cells Attached to Liquid Crystals

## A study on the interfacial interactions of cells with the liquid crystal using widefield surface plasmon microscopy

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*Abstract*— **Widefield surface plasmon resonance (WSPR) microscope provides high resolution imaging of the cell-substrate interface. We report the application of our WSPR imaging system to study the interaction of keratinocytes with the liquid crystals (LC). Imaging of the fixed cell-LC-gold and fixed cell-gold interfaces were performed in air using a 1.45 NA objective based system. In the WSPR microscopy technique, keratinocytes were found forming multiple narrow concentric rings with the presence of liquid crystals but these cells expressed wide band of contact area when they are directly cultured on the glass surface. Our work showed the feasibility of using WSPR microscopy system in imaging cells interaction with liquid crystals.**

***Keywords- Widefield surface plasmons microscopy, adhesion of cells, keratinocytes, cell-liquid crystal interface***

## I. INTRODUCTION

Surface plasmon waves are excited when an incident p—polarized light strikes a metal-dielectric interface at a specific angle [1]. Due to this characteristic, surface plasmon fields can be used to study the interfacial topography of cells via plasmon resonance (SPR) microscopy [2]. Surface Plasmon microscopy has been shown to be useful in imaging interfacial interactions of living cells and biological molecules [3, 4]. For the system reported in [3,4], the SPR microscopy used was based around a prism which was established by Rothenhausler and Knoll (1988). However, in the prism based system, imaging of the detailed biological structures is of poor resolution which could be due to the large surface plasmon propagation length travelling parallel to the incident plane or low sensitivity of the optic apparatus used. A higher resolution surface plasmon imaging system can be obtained by using widefield surface plasmon microscopy (WSPR) which was developed with a different optical system configuration [5, 6]. In the WSPR microscopy system, adhesion of human keratinocyte (HaCaT) at the interface was clearly enhanced at a sub-micron level resolution. The Kohler optical system setup of the WSPR microscope involves the use of a high numerical aperture oil immersion lens (Numerical Aperture = 1.45), substituting the prism in the usual SPR system [4]. This microscopy system can be applied to study the interaction of cells with a soft substrate.

Cholesteryl ester liquid crystals were found to support cell adhesion without pre-coating with ligands [7] and functioned to be a linear viscoelastic cell traction force transducer [8]. However, liquid crystal with Young's modulus close to the epidermis layer [8] is a soft substrate that could influence the characteristic of the cell adhesion. Hence, it was not well understood how liquid crystals would affect the adhesion patterns of cells in close relation to the morphology of cells. In this paper, we report the application of WSPR microscopy in characterizing the interfacial topology of cells attached to glass cover slip and liquid crystals.

## II. MATERIALS AND METHODS

### *A. Cell Culture and Preparation of Lliquid Crystal Substrate*

Cells for this experiment were obtained from sub-cultured HaCaT cells, a human keratinocyte cell lines, in RPMI-1640 culture media as described in [1]. The cells were maintained in media supplemented with fetal calf serum (PromoCell, UK), L-glutamine (2 mM, Sigma Aldrich, UK), fungizone (2.5 mg/l, Sigma Aldrich, UK), penicillin (100 units/ml, Sigma Aldrich, UK) and streptomycin (100 mg/ml, Sigma Aldrich, UK). Harvested cells from the 25 $cm^2$ culture flasks were trypsinized and re-suspended in 5 ml of supplemented RPMI-1640 culture media ready for the following experiments. Cholesteryl ester liquid crystals were spread at a thickness of ~100 µm on a gold cover slip using a cell scraper (Corning, International) and immersed in the RPMI-1640 culture medium prior to cell plating (Figure 1a). Another gold

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substrate was prepared using similar procedure without the liquid crystal coatings (Figure 1b).

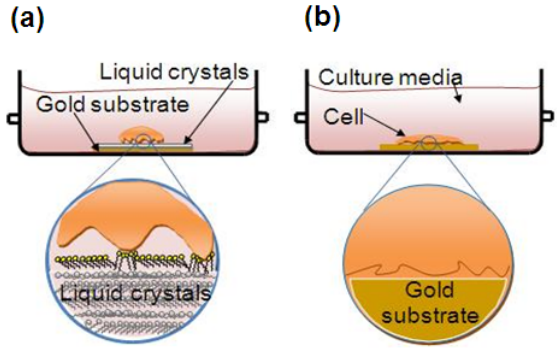


Figure 1. Cell culture in the presence (left) and absence (right) of liquid crystals on gold substrates.

### *B. Widefield Surface Plasmons Imaging of Cell Liquid Crystal Interface*

Two round glass substrates with a diameter of 22 mm and coated with a 50 nm thick gold layer were used in this study. These substrates were coated with cholesteryl ester liquid crystals as described previously. Cells at a density of 1.0 x $10^4$ cells/$cm^2$ were plated in the two Petri dishes, each containing one gold cover slip coated with and without liquid crystals (Figure 1). Subsequently, the two Petri dishes were incubated at 37°C for 24 hours. After incubation, the cells were washed in HBSS twice and fixed in 1 % Formaldehyde for 6 minutes. The fixation was followed by a 5 minute wash and dehydration by a series of 5 minute washes in serial dilutions of ethanol in distilled water from 10 % through to 100 %. After dehydration, each gold cover slip was transferred to a widefield surface plasmon resonance (WSPR) microscope to be imaged (Figure 2a). The WSPR microscope was fitted with a Zeiss Plan Fluar of 1.45 Numerical Aperture (NA) 100x magnification oil immersion objective (Figure 2b).

In the WSPR system used, an incident p-polarised light from a He-Ne laser source with a light wavelength of 633 nm was used to excite surface plasmons at the gold layer at an excitation angle of 46°. The light wave interacts with the interface medium of different reflective index and generates different levels of reflectivity in the surface plasmon. The reflected light contained information associating with the interfacial interactions, and was captured by a monochromatic charged coupled device (CCD) camera linked to Scion software. Similar experiments were repeated three times. A detailed description of the optical system used can be found in [6]. Similar samples were studied in a GX-XDS2 phase contrast microscope equipped with a GT VisionCX digital camera. A long distance flat field achromatic objective lens (20x magnification) with a numerical aperture (NA) of 0.65 in the phase contrast microscope was used to capture images of gold substrates cultured with cells.

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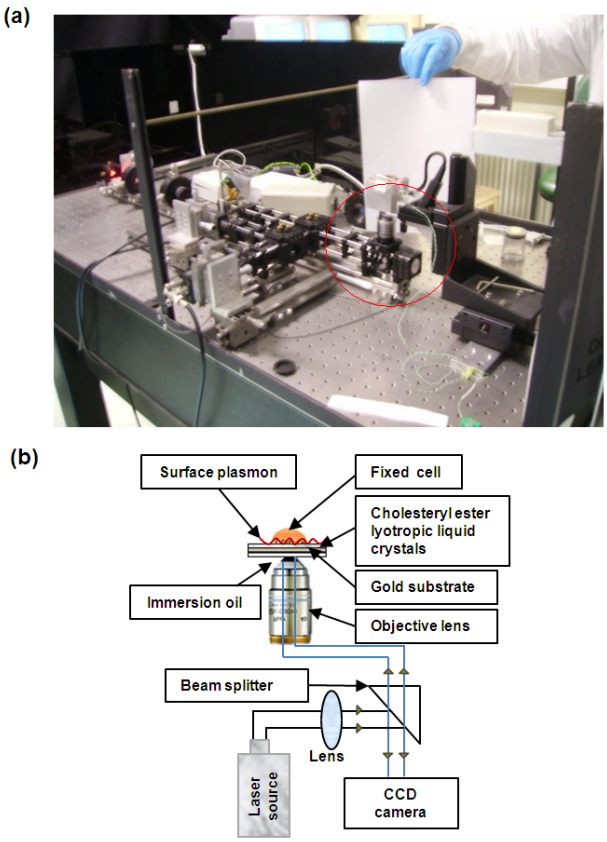


Figure 2. (a) A widefield surface plasmon microscope and (b) the simplied setup for imaging fixed cells. The red circle in image (a) shows the location of the gold substrate holder and the oil immersion lens in the setup.

## III. RESULTS AND DISCUSSION

For the WSPR, p-polarized light was used to generate evanescent wave that interacts with the interfacial structures overlying the metal-dielectric layer which leads to the changes in reflectivity via the objective lens. The incident light must be first filtered by an annular mask which allows the penetration of P-polarized light into the imaging system. Fig. 3 shows an example of the annular mask consisting of a pair of arcs patterns that superimposed on the back focal plane of the WSPR microscope.

A WSPR micrograph of the cholesteryl ester liquid crystal coated on a gold substrate is as shown in Figure 3. Streaks of liquid crystals with monochromatic reflectivity due to the effect of smear coating were found. Cholesteric liquid crystals consist of molecules arranged in twisted helical structures. Colourful birefringence structures are usually displayed by cholesteryl ester liquid crystals in crossed-polarizing microscopy [9]. However, the anisotropic properties of the cholesteric liquid crystals could not be resolved in the monochromatic WSPR microscopy. Due to the anisotropy of the cholesteric liquid crystals, some p-polarized light could be partially diffracted in the liquid crystal samples which has a

mean refractive index of approximately 1.558 [10, 11]. However, a large amount of the p-polarized light was reflected from the oil immersion objective and allowing the imaging of liquid crystals in the WSPR system.

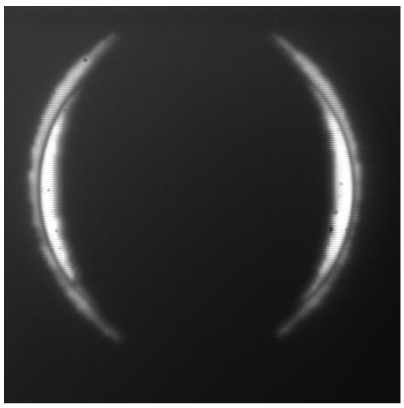

Figure 3. Masking the back focal plane for penetration of p-polarized light

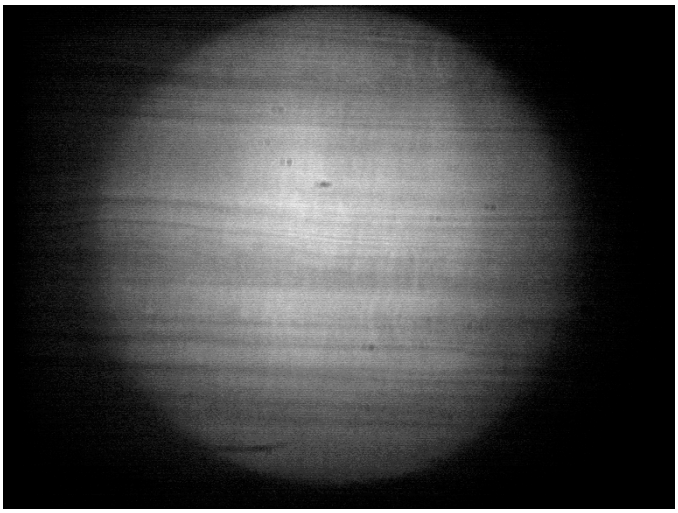

Figure 4. A micrograph of widefield surface plasmon microscopy of liquid crystal coated gold substrate

For the WSPR microscopy of cells adhesion to the liquid crystals, HaCaTs were found widely spread on the gold cover slip, a wide band like structure was formed around the nucleus at the centre of the cell as shown in Figure 5. This is supported by more scattered focal adhesions spread on the gold substrates [7]. A large spread area with irregular features showed the need for larger and scattered group of adhesions. Lamellipodia is an extension of the cell body which moves and adheres to a surface around the cell body. In WSPR imaging, the lamellipodia is characterized by a thin membrane which adhered to the surface via small contact points (in bright reflectivity) located at the outer edges of the lamellipodium (Figure 5a). The bright structures shown in high reflectivity represent closer contacts of cells to the gold surface and vice versa. It is the ability of the WSPR to enable the high resolution imaging of cell surface contacts without the need for immunostaining that is particularly valuable.

In high contrast, cells adhered to the liquid crystal coated substrate showed multiple narrow bands of concentric rings under the area covered by the cell (Figure 5b). This gave an indication that the stresses were regularly exerted around edges (narrower band) of the cell membrane when cells were cultured on the liquid crystals. In fact, the concentrated stresses exerted at the outer band was formed by a more uniform arrangement of smaller focal adhesions as evidenced by the vinculin staining reported in our previous work [7]. Cells cultured on the liquid crystal coating region took on a round morphology and attached to a smaller spread area as compared with cells adhered to the plain gold substrate region (Figure 6). This is greatly related to the stiffness of a substrate in which, soft substrate could influence the morphology, cytoskeleton structure and adhesion of a cell [12].

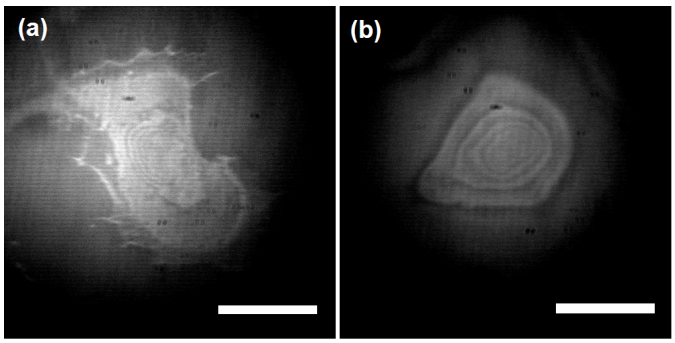


Figure 5. Micrographs of widefield surface plasmon microscopy of human keratinocyte cells cultured on a (a) plain gold substrate and (b) liquid crystal coated gold substrate (scale bar: 25 µm).

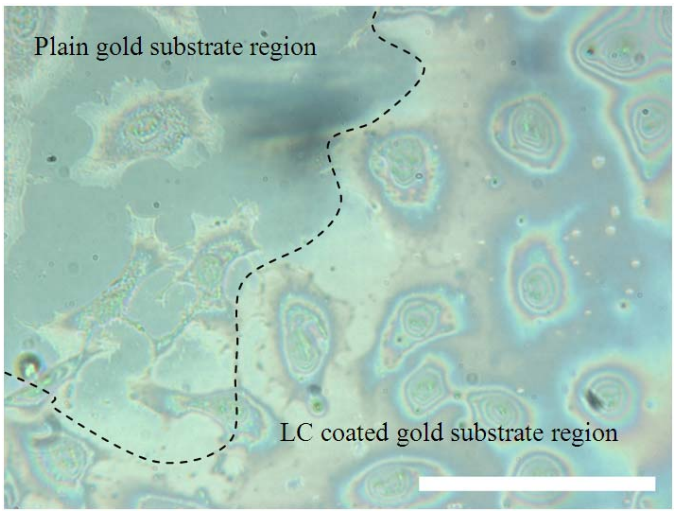


Figure 6. A micrograph of phase conrast imaging of human keratinocyte cell lines cultured on a gold substrate with and without liquid crystal coating (scale bar: 50 µm). The dotted line showing the separation region of plain gold substrate and liquid crystal coated gold susbtrate.

## IV. CONCLUSION

WSPR imaging indicated that liquid crystals had modified the adhesion, morphology and cytoskeleton structure of HaCaT cells. The forces exerted by focal adhesions of HaCaT cells on liquid crystals are more uniform around the periphery of the cells and this was supported by punctuated vinculin expressions that were expressed regularly at the edges of the cells membrane. Furthermore, cells took on a rounded morphology when attached on the liquid crystals compared with those cultured on plain gold substrates. The result presented in this report demonstrated the capability of the WSPR in studying cells-LC interactions.

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