

**APPLICATION OF A NOVEL HIGH RESOLUTION
WIDEFIELD SURFACE PLASMON MICROSCOPE IN CELL
ENGINEERING, WOUND HEALING AND DEVELOPMENT
OF NEW BINDING ASSAYS**

The Widefield Surface Plasmon Resonance Microscope was used for high lateral resolution imaging of: binding events between micropatterned extracellular matrix proteins and antibodies, and the cell/surface interface.

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STATEMENT OF ORIGINALITY

To the best of my Knowledge, the material or the contents presented in this thesis are original except where otherwise noted within the text. None of this research has been submitted in whole or in part for any degree at this or any other university.

Muhammad Mahadi Abdul Jamil



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PTTA
PERPUSTAKAAN TUNKU AMINAH

Application of a Novel High Resolution Widefield Surface Plasmon Microscope in Cell Engineering, Wound Healing and Development of New Binding Assays

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Keywords: Bio-molecular interaction, Antigens/Antibody interactions, Laminin, Fibronectin, Micro-contact printing, Surface Plasmons Microscopy, Transformation Growth Factor β three (TGF β 3), Cell guidance, Tetramethylrhodamine Isothiocyanate (TRITC), High resolution imaging, Cell on a substrate, Live cell imaging.

ABSTRACT: Surface Plasmon (SP) microscope systems are mostly built around the prism based Kretschmann configuration. In these systems the generation of Surface Plasmons (SPs) is achieved by p-polarized light striking a metallised prism surface at a specific angle and then monitoring the intensity of the reflected light. Thus in these systems, an image of the material can be obtained in terms of an intensity map, in which the intensity of the image is dependent on the way the light couples into the SPs. The drawback of these systems is that lateral resolution relies on the ability of plasmons to propagate along the metallised layer. The lateral resolution is thus limited to a few microns. Therefore, a new microscope system was developed, i.e. the Widefield Surface Plasmon Resonance (WSPR) microscope, that is not only capable of analysing molecular interactions at high vertical resolutions, but also enables SP imaging at much higher lateral resolution than prism based systems. The functionality of the novel (WSPR) microscope has been investigated by imaging a sequence of binding events between micropatterned extracellular matrix proteins and their specific antibodies both in air and real-time. Using the WSPR system a change in contrast was observed with each protein binding events. Images produced via the WSPR system were analyzed and compared qualitatively and quantitatively. The preliminary results acquired for these binding studies between antibody/antigens demonstrate that the WSPR system capable of resolving features down to 260nm although the theoretically proven lateral resolution of the WSPR system is \sim 500nm. Cell surface interactions under two different culture conditions, i.e. HaCaTs cultured on SPR substrate with Transforming Growth Factor β 3 (TGF β 3) (50ng/ml) and without TGF β 3 were also investigated. It was found that HaCaTs cultured in the presence of TGF β 3 showed enhanced division and motility along with decreased cell attachment as compared with cells maintained in TGF β 3 free media. It is believed that cellular signalling by TGF β 3 is very important for enhancing tissue development in wound repair. It is confirmed that the WSPR microscope described here can be used to study sequential monomolecular layer of antibody/antigen interactions binding events and examination of cell surface interfacial interactions at lateral scales of less than one micron without the need for traditional immunofluorescent labelling. These results have significant implications in the development of new breed fast binding assays system and in enabling high resolution detailed examination of the cell surface couplings and cell signalling processes involved in cell attachment and migration.

LIST OF ABBREVIATIONS

3D	three dimensional
Au	Gold
Ag	Aluminium
AFM	Atomic force microscopy
ATP	adenosine triphosphate
ATR	attenuated total reflectance
Anti	antibody
BFP	back focal plane
BSA	bovine serum albumin
BSE	bovine spongiform encephalopathies
CCD	charge-coupled device
DIC	differential interference contrast microscope
DNA	deoxyribonucleic acids
Dthiol	1,6-Hexanedithiol
ER	endoplasmic reticulum
EM	electron microscope
ECM	extra-cellular matrix
ESEM	environmental scanning electron microscope
ELISA	enzyme linked immunoabsorbant assay
FCS	foetal calf serum
FGF	fibroblastic growth factor
FIB	fibronectin
GAGs	glycosaminoglycans
HOBs	human osteoblast

HaCaT	Human Keratinocytes
HBSS	Hank's balanced salt solution
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
IgG	immunoglobulin
LAM	laminin
LCD	liquid crystal display
MCP	micro contact printing
MRI	magnetic resonance imaging
MIMIC	micromolding in capillaries
mRNA	messenger ribonucleic acids
NA	numerical aperture
OWLS	optical waveguide lightmode spectroscopy
PCL	polycaprolactone
PEG	poly (ethylene glycol)
PEO	polyethylene oxide
PHA	polyhydroxylkanoate
PHB	polyhydroxybutyrate
PLA	poly(lactic acid)
PLG	poly(glycolic acid)
PMNs	polymorphonuclear leukocytes
PPF	poly(propylene fumarate)
PPO	polypropylene oxide
PDGF	platelet derived growth factor
PDMS	polydimethylsiloxane
PLGA	poly(DL- lactic -co- glycolic acid)
PLLA	Poly (L-lactic acid)
PDLLA	Poly (D,L-lactic acid)

PHSRN	Pro-His-Ser-Arg-Asn
PLLACL	Poly (L-lactic acid-co-ε-caprolactone)
PDLLACL	Poly (D, L-lactic acid-co-ε-caprolactone)
QCM-D	Quartz crystal microbalance with dissipation
RER	rough endoplasmic reticulum
RGD	Arg-Gly-Asp
RNA	ribonucleic acids
RPMI	Rosewell Park Memorial Institute
RRETAWA	(Arg-Arg-Glu-Thr-Ala-Trp-Ala)
SP	surface plasmon
SPs	surface plasmons
SAM	self assembled monolayer
SER	smooth endoplasmic reticulum
SEM	scanning electron microscope
SFM	scanning force microscope
SPR	surface plasmon resonance
STM	scanning tunnelling microscope
SCOM	scanning confocal optical microscope
TEM	transmission electron microscope
TGF-β	transformation growth factor beta
Thiol	2-Aminoethanethiol
TRITC	tetramethylrhodamine isothiocyanate
UV	ultra violet
WSPR	Widefield Surface Plasmon Resonance
IWSPR	Interferometer Widefield Surface Plasmon Resonance

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LIST OF ASSOCIATED PUBLICATIONS

1. A. J. M. Mahadi, M. Youseffi, S. T. Britland, S. Liu, C. W. See, M. G. Somekh, and M. C. T. Denyer, "High resolution imaging of TGF β 3 treated human keratinocyte via a newly developed widefield surface plasmon resonance microscope," IFMBE proceedings: vol. (15) pp. 286-290, 3rd Kuala Lumpur International Conference on Biomedical Engineering, Kuala Lumpur, Malaysia, 11-14 December 2006.
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3. A. J. M. Mahadi, M. Youseffi, S. T. Britland, J. Zhang, S. Liu, C. W. See, M. G. Somekh, and M. C. T. Denyer, "Advancement in protein/DNA microarray chip development: High resolution real-time monitoring of biomolecular interfacial interactions via a novel widefield surface plasmon microscope," IRCE: Pharmacy and Pharmacological, Institute of Pharmaceutical Innovation (iPi), University of Bradford, 13th September 2006. Available online: http://www.irce.org/va_html_ai=82 **(Received the best poster prize award from School of Life Sciences)**
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10. M. Mahadi Abdul Jamil, M.C.T. Denyer, M. Youseffi, S.T. Britland, S. Liu, C.W. See, M.G. Somekh, and J. Zhang. "The lab on a chip: Imaging of interfacial bio-molecular interactions by high lateral resolution Widefield Surface Plasmon Resonance microscopy". (In preparation and will be submitted to the *Journal of Microscopy*, 2007).
11. M. Mahadi Abdul Jamil, M. Youseffi, S.T. Britland, S. Liu, C.W. See, M.G. Somekh, J. Zhang and M.C.T. Denyer, "High resolution imaging of the cell surface interface using the new widefield surface plasmon microscope". (In preparation and will be submitted to the *Journal of Structural Biology*, 2007).



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