



Characterization of the cell-substrate interface using surface plasmon resonance microscopy

Eva Maria Kreysing

Schlüsseltechnologien / Key Technologies

Band / Volume 189

ISBN 978-3-95806-369-3

Forschungszentrum Jülich GmbH
Institute of Complex Systems
Bioelectronics (ICS-8)

Characterization of the cell-substrate interface using surface plasmon resonance microscopy

Eva Maria Kreysing

Schriften des Forschungszentrums Jülich
Reihe Schlüsseltechnologien / Key Technologies

Band / Volume 189

ISSN 1866-1807

ISBN 978-3-95806-369-3

Table of Contents

Acknowledgment	i
Zusammenfassung	iv
Abstract	vii
Table of Contents	ix
1 Introduction	1
2 Biological background	7
2.1 Cell types	8
2.1.1 Cellular structure of neurons	8
2.1.2 Cardiomyocytes	10
2.2 Cell membrane	12
2.2.1 Membrane structure	12
2.2.2 Membrane potential	14
2.3 Cell adhesion	22
2.4 Cell dynamics at the interface	26
2.4.1 Cell migration	27
2.4.2 Excitation-contraction coupling in cardiomyocytes	29
3 Imaging techniques for the cell-substrate interface	33
3.1 Fluorescence	34
3.1.1 Super-resolution microscopy	36
3.2 RICM	37
3.3 FLIC	40
3.4 MIET	43
3.5 FIB	46
3.6 Surface plasmon resonance imaging	48

Table of Contents

4 Surface plasmon resonance theory	51
4.1 Surface plasmon excitation	51
4.2 Transfer matrix method	53
4.3 SPRM types	55
4.3.1 Prism based SPRM	56
4.3.2 Objective-based SPRM	56
5 SPRM Setup	63
5.1 Introduction of the SPRM at the ICS-8	64
5.1.1 Prior work on the SPRM at the ICS-8	64
5.1.2 Overview on the modifications of the setup	67
5.2 General description	68
5.3 Live imaging	70
5.3.1 Laser live-imaging	70
5.3.2 Projector live-imaging	74
5.3.3 Comparison of laser live-imaging and projector live-imaging SPRM	77
5.4 Scanning	82
5.4.1 Physical realization of the scanning modes	82
5.4.2 Scanning acquisition modes	84
5.4.3 Processing of the scanning data	85
5.5 Further setup elements	96
6 Validation	99
6.1 Angle validation	100
6.2 Refractive index validation	107
6.2.1 Critical angle dependence on last layer	107
6.2.2 Refractive index validation measurements	109
6.3 Distance validation	112
6.3.1 Measurement of microlenses	113
6.4 Discussion	120

7 Cardiomyocyte experiments	123
7.1 Experimental	124
7.2 Live-imaging of cardiomyocytes	127
7.2.1 Correlation of live- and confocal imaging after labeling	129
7.3 Single-spot measurements	131
7.4 Time dependent area scan	135
7.4.1 Discussion	140
7.4.2 Calculations of changes in the RI	145
8 Neuron experiments	147
8.1 Correlation of SPRM with FIB-SEM	147
8.1.1 Experimental	148
8.1.2 Analysis	150
8.1.3 SPRM measurements	153
8.1.4 Discussion	162
8.2 Other experiments with neurons	167
8.2.1 Measurement of cell organelles	167
8.2.2 Measurement of growth cones	171
8.2.3 Failed measurement series	177
8.2.4 Time series of neurons	178
9 Discussion	181
9.1 Development of the technique	181
9.1.1 What has been done?	181
9.1.2 What remains to be done?	183
9.1.3 Problems and issues	184
9.1.4 Comparison with other techniques	187
9.2 Biological measurements	190
9.2.1 What has been measured?	190
9.2.2 What could be measured in the future?	191
10 Abbreviations	193

Table of Contents

11 Protocols	195
11.1 Sample production	195
11.1.1 Chip design	195
11.1.2 Layer deposition	196
11.1.3 Sample assembly	197
11.2 Coatings	198
11.2.1 Fibronectin coating	198
11.2.2 PLL coating	198
11.3 Cell culture of cardiomyocytes	199
11.3.1 Thawing, plating, maintenance	199
11.3.2 Calcium imaging of cardiomyocytes	200
11.4 Cell culture of neurons	200
11.5 Preparation of samples for FIB	202
11.5.1 Fixation with glutaraldehyde	202
11.5.2 Heavy metal staining and resin embedding	202
11.6 Fixation and staining for immunofluorescence	204
11.6.1 Fixation and staining	204
11.6.2 Antibodies and additional stainings	205
11.6.3 CB Buffer	205
11.7 Scripts	206
11.7.1 Grid markers on chips	206
11.7.2 Background correction	207
11.7.3 Animation of contracting cardiomyocyte	208
11.7.4 SPRM profile cuts along the entire interface	215
11.7.5 Align and compare distance profiles	218
12 Appendix	237
12.1 Angle validation	237
12.2 Additional measurements and specifications	238
12.2.1 Scanning parameters	238
12.2.2 Detached lamella	238
12.2.3 Surface defects	238

12.3 Analysis software optimization	240
Bibliography	249

Schlüsseltechnologien / Key Technologies
Band / Volume 189
ISBN 978-3-95806-369-3