

# Contents

<b>I</b>	<b>Introduction: Medical context and goal of the thesis</b>	<b>1</b>
1	CONTEXT OF THE THESIS	3
1.1	Importance of tissue-based biomarkers in histopathology . . . . .	3
1.2	Whole slide scanners . . . . .	6
1.3	From digital pathology to computational pathology . . . . .	7
1.4	Tissue microarrays and multiplex immunohistochemistry: contribution to computational pathology . . . . .	9
2	GOALS OF THE PH.D. THESIS	15
2.1	Goal 1: Automating TMA core identification . . . . .	16
2.2	Goal 2: Providing digital solutions to the inter-batch variability of immunohistochemical staining . . . . .	16
2.3	Goal 3: Compartmentalizing IHC quantification . . . . .	17

2.4	Goal 4: Analyzing multiplex IHC assays and staining colocalization . . . . .	18
<b>II Original developments</b>		<b>21</b>
3	OVERVIEW OF THE ORIGINAL DEVELOPMENTS	23
4	CORE DETECTION AND IDENTIFICATION FOR AUTOMATING TMA ANALYSIS	29
4.1	ROIs in virtual TMA slides . . . . .	30
4.2	Method of automatic grid fitting on tissue micro-array images . . . . .	31
4.3	Method validation and uses . . . . .	35
5	INTER-BATCH STAINING NORMALIZATION	37
5.1	Causes of IHC staining variability . . . . .	38
5.2	State of the art . . . . .	41
5.3	Methods . . . . .	44
5.4	Experimental design for quantitative evaluation . . . . .	52
5.5	Results . . . . .	56
5.6	Discussion . . . . .	70
6	GLAND SEGMENTATION TO COMPARTMENTALIZE IHC QUANTIFICATION	75
6.1	Previous work and novel contributions . . . . .	76
6.2	Methods . . . . .	78

6.3	Evaluation methodology and results . . . . .	87
6.4	Discussion and conclusion . . . . .	102
7	<b>IMAGE REGISTRATION AND COLOCALIZATION</b>	<b>107</b>
7.1	Registration of serial TMA slides . . . . .	108
7.2	Colocalization measurements: from fluorescence to brightfield IHC . . . . .	112
7.3	Validation of colocalization measurements for IHC biomarkers . . . . .	114
7.4	discussion and conclusion . . . . .	121
<b>III</b>	<b>Future works and conclusions</b>	<b>123</b>
8	<b>FUTURE WORK</b>	<b>125</b>
8.1	Using IHC biomarkers to generate supervised training sets . . . . .	126
8.2	Compartmentalized multimarker analysis . . . . .	128
8.3	Improving the deep neural network architecture developed during this thesis . . .	128
9	<b>DISCUSSION AND CONCLUSIONS</b>	<b>131</b>
9.1	Normalization and data augmentation . . . . .	132
9.2	On the current limitations of deep learning . . . . .	133
9.3	Deep learning at the service of pathology. . . . .	136
	<b>REFERENCES</b>	<b>139</b>