

Contents

1	Thesis Overview	6
1.1	General Introduction	6
1.2	Contributions	6
1.3	Thesis Overview	6
2	Introduction	8
2.1	Introduction	8
2.2	Fluorescence Microscopy	8
2.3	Forster Resonance Energy Transfer	8
2.4	Single Molecule Fluorescence Microscopy	9
2.4.1	Overview	9
2.4.2	Confocal Microscopy	9
2.4.3	Total Internal Fluorescence Microscopy	10
2.4.4	Epifluorescent Microscopy	10
2.4.5	Super-Resolution Microscopy	10
2.5	Probabilistic Inference and Bayesian Statistics	10
2.5.1	Bayesian Statistics	10
2.5.2	Sampling Techniques	10
3	Analysis Tools for Single Molecule Confocal Microscopy	11
3.1	Overview	11
3.2	Introduction	12
3.2.1	The Single Molecule Fluorescence Experiment	12
3.3	Data Analysis in Confocal smFRET Experiments	13
3.3.1	Continuous Excitation	14
3.3.2	Alternating Laser Excitation	15

3.4	Development of Scientific Software	16
3.5	pyFRET: Design and Implementation	17
3.5.1	Code Layout and Design	17
3.5.2	Simple Event Selection and Denoising	19
3.5.3	Burst Search Algorithms	22
3.6	RASP: Recurrence Analysis of Single Particles	22
3.6.1	Compatibilities	23
3.7	Experimental Methods	24
3.7.1	Benchmarking the Gaussian Fitting Using Simulated Datasets	24
3.7.2	Data to Evaluate the Simple Event Selection Algorithms	24
3.7.3	Data to Evaluate Event Selection Using the Burst Search Algorithms	25
3.7.4	Performance Analysis Using Mixtures of DNA Duplexes	26
3.7.5	Testing the RASP Algorithm	26
3.8	Performance Analysis of smFRET Analysis Algorithms	27
3.8.1	Evaluating Performance with DNA Duplexes	27
3.8.2	Evaluating the Burst Search Algorithms	28
3.8.3	Evaluating Performance of the Gaussian Mixture Model	32
3.8.4	Benchmarking the RASP Algorithm	35
3.9	Conclusions	36
3.10	Availability and Future Directions	38
4	Bayesian Inference of Intramolecular Distances Using Single Molecule FRET	39
4.1	Overview	39
4.2	Introduction	40
4.2.1	A smFRET Experiment	40
4.2.2	Approaches to Analysis of smFRET Data	42
4.2.3	Model Based Inference	44
4.3	Theory	49
4.3.1	A Physical Model of a smFRET Experiment	49
4.3.2	Inference of Model Parameters	57
4.3.3	The Metropolis-Hastings Algorithm	61
4.4	Experimental Methods	63
4.5	Results	67
4.5.1	Justification of the Gamma-Poisson Mixture Model	77
4.6	Conclusions and Future Work	79

5	Bayesian Inference of Oligomer Sizes Using Single Molecule FRET	81
5.1	Overview	81
5.2	Introduction	82
5.2.1	Diseases of Protein Aggregation	82
5.2.2	Studying Protein Aggregation	83
5.2.3	The Relationship Between Size and Photon Emission is Complex . . .	84
5.2.4	The Effect of Confocal Excitation Heterogeneity on Photon Emission	85
5.2.5	Controlling Confocal Excitation Heterogeneity	86
5.2.6	The DNA Holliday Junction as a Model Oligomer	87
5.3	Theory	87
5.3.1	A Simple Poisson Model of Oligomer Photon Emission	90
5.3.2	A Gamma-Poisson Mixture Model of Oligomer Photon Emission . . .	91
5.4	Experimental Methods	93
5.4.1	Labelling of Protein Monomers	93
5.4.2	Protein Aggregation Experiments	93
5.4.3	Preparation of DNA Holliday Junctions	93
5.4.4	Simple FRET Measurements of DNA Holliday Junctions	94
5.4.5	Flattening the Confocal Volume Using Acousto-Optic Deflection: A Modified Single Molecule Fluorescence Microscope	94
5.4.6	Preparation of Microfluidic Channels	95
5.4.7	smFRET Measurements to Determine the Effect of Unequal Excitation on Photon Emission	95
5.4.8	Counting Photobleaching Steps Using TIRF Imaging	96
5.5	Results	96
5.5.1	The need for a Generative Model	96
5.5.2	Understanding the Relationship Between Size and Photon Emission .	99
5.5.3	Inferring Event Brightness Using the Gamma-Poisson Model	102
5.5.4	How Bright Are Holliday Junction Events	108
5.5.5	Photobleaching Steps Analysis Reveals Additional Source of Overdis- persal	112
5.6	Conclusions	113
5.6.1	Complex Relationship between Size and Photon Emission	113
5.6.2	Implications for Future Work on Molecular Sizing	114

6 Probabilistic Inference for Error Detection in De Novo Genome Assem-

blies	116
6.1 Overview	116
6.2 Introduction	116
6.3 Theory	119
6.4 Experimental Methods	124
6.5 Results	128
6.6 Conclusions	130
7 Conclusions and Future Work	134
7.1 General Conclusions	134
7.2 Applications	134
7.3 Future Work	134