

## Contents

### List of Contributors XIII

|                 |                                                                 |          |
|-----------------|-----------------------------------------------------------------|----------|
| <b>Part One</b> | <b>Process Control and Quality Assurance</b>                    | <b>1</b> |
| <b>1</b>        | <b>Industrial Perspectives</b>                                  | <b>3</b> |
|                 | <i>Andreas Kandelbauer, Manfred Rahe, and Rudolf W. Kessler</i> |          |
| 1.1             | Introduction and Definitions                                    | 3        |
| 1.1.1           | Introduction                                                    | 3        |
| 1.1.2           | Historical Aspects                                              | 3        |
| 1.1.3           | Definition of Quality: Product Functionality                    | 4        |
| 1.1.4           | Quality Control                                                 | 8        |
| 1.1.5           | Quality Assurance                                               | 9        |
| 1.2             | Management and Strategy                                         | 9        |
| 1.2.1           | PAT Initiative                                                  | 9        |
| 1.2.2           | PAT Toolbox                                                     | 11       |
| 1.2.3           | The Concept of Quality by Design (QbD)                          | 12       |
| 1.2.4           | ICH                                                             | 13       |
| 1.2.5           | The Concept of a Design Space                                   | 16       |
| 1.2.6           | Implications for Other Branches of the Life Sciences            | 18       |
| 1.2.6.1         | General Remarks                                                 | 18       |
| 1.2.6.2         | Biotechnology                                                   | 19       |
| 1.2.6.3         | Food Industry                                                   | 19       |
| 1.2.6.4         | Summary and Outlook                                             | 20       |
| 1.3             | Toolboxes for Process Control and Understanding                 | 21       |
| 1.3.1           | Introduction: Causality                                         | 21       |
| 1.3.2           | Sampling                                                        | 22       |
| 1.3.3           | Process Validation                                              | 25       |
| 1.3.3.1         | Role of Design of Experiments (DoE)                             | 25       |
| 1.3.3.2         | Role of Failure Mode and Effects Analysis (FMEA)                | 26       |
| 1.3.4           | Measurement Technologies (How to Measure)                       | 27       |
| 1.3.4.1         | Selection of the Appropriate Technique                          | 27       |
| 1.3.4.2         | Working in Aqueous Systems                                      | 28       |
| 1.3.4.3         | Trace Analysis                                                  | 29       |

|         |                                                                            |    |
|---------|----------------------------------------------------------------------------|----|
| 1.3.4.4 | Qualification of a Spectrometer                                            | 29 |
| 1.3.5   | Data Analysis and Calibration (How to Process Data and How to Calibrate)   | 30 |
| 1.3.5.1 | Introduction                                                               | 30 |
| 1.3.5.2 | Spectral Data Pretreatments and Data Cleaning                              | 31 |
| 1.3.5.3 | Chemometrics                                                               | 32 |
| 1.3.5.4 | Regression Analysis                                                        | 33 |
| 1.3.6   | Process Control (How to Control a Process)                                 | 34 |
| 1.4     | Specific Problems Encountered in Industrial Process Analytics              | 37 |
| 1.4.1   | Moisture Measurements (NIR, MW)                                            | 37 |
| 1.4.1.1 | NIR Spectroscopy                                                           | 37 |
| 1.4.1.2 | Microwave Resonance Technique (MWR)                                        | 38 |
| 1.4.2   | Process Analytics of Solids and Surfaces: Specular and Diffuse Reflectance | 39 |
| 1.4.3   | Working in Multiple Scattering Systems: Separating Scatter from Absorbance | 41 |
| 1.4.3.1 | Basics in the Measurement of Opaque Systems                                | 41 |
| 1.4.3.2 | Separation of Scatter from Absorption                                      | 43 |
| 1.4.3.3 | Optical Penetration Depth                                                  | 45 |
| 1.4.4   | Spectral Imaging and Multipoint Spectroscopy                               | 46 |
| 1.5     | Survey Through Industrial Applications                                     | 49 |
| 1.5.1   | Selection of Applications                                                  | 49 |
| 1.5.2   | Pharmaceutical Industry                                                    | 50 |
| 1.5.2.1 | UV/Vis Spectroscopy                                                        | 50 |
| 1.5.2.2 | NIR Spectroscopy                                                           | 50 |
| 1.5.2.3 | Raman Spectroscopy                                                         | 51 |
| 1.5.2.4 | Imaging Techniques                                                         | 51 |
| 1.5.3   | Food and Agriculture                                                       | 52 |
| 1.5.3.1 | UV/Vis Spectroscopy                                                        | 53 |
| 1.5.3.2 | NIR Spectroscopy                                                           | 53 |
| 1.5.3.3 | Raman Spectroscopy                                                         | 53 |
| 1.5.3.4 | Imaging Techniques                                                         | 54 |
| 1.5.4   | Polymers                                                                   | 55 |
| 1.5.4.1 | UV/Vis Spectroscopy                                                        | 55 |
| 1.5.4.2 | NIR Spectroscopy                                                           | 55 |
| 1.5.4.3 | Raman Spectroscopy                                                         | 56 |
| 1.5.4.4 | Imaging Techniques                                                         | 56 |
| 1.6     | Perspectives                                                               | 56 |
| 1.6.1   | Technology Roadmap 2015+                                                   | 56 |
| 1.6.2   | Medical Applications and Tomographic Imaging                               | 58 |
| 1.6.3   | Multi- Point-Information Systems in Manufacturing                          | 59 |
| 1.6.4   | Multimodal Spatially Resolved Spectroscopy                                 | 60 |
| 1.6.5   | Micoreactor Control and Reaction Tomography                                | 61 |
|         | References                                                                 | 63 |

|          |                                                                                             |           |
|----------|---------------------------------------------------------------------------------------------|-----------|
| <b>2</b> | <b>Applications of Spectroscopy and Chemical Imaging in Pharmaceutics</b>                   | <b>71</b> |
|          | <i>Aoife A. Gowen and José M. Amigo</i>                                                     |           |
| 2.1      | The New Paradigm of Process Control                                                         | 71        |
| 2.2      | Overview of Spectroscopic Techniques Commonly Used                                          | 72        |
| 2.3      | The Need for Multivariate Data Analysis                                                     | 75        |
| 2.3.1    | Pre-Processing                                                                              | 75        |
| 2.3.2    | Exploration Techniques                                                                      | 76        |
| 2.3.3    | Regression, Resolution and Classification Techniques                                        | 77        |
| 2.3.4    | Image Processing Techniques                                                                 | 77        |
| 2.4      | Applications in Pharmaceutical Process Monitoring and Quality Control                       | 78        |
| 2.4.1    | Spray Formulations                                                                          | 78        |
| 2.4.2    | Powders                                                                                     | 79        |
| 2.4.3    | Polymorphism                                                                                | 79        |
| 2.4.4    | Solid Dosage Forms                                                                          | 80        |
| 2.4.5    | Root Cause Analysis                                                                         | 80        |
| 2.4.6    | API Distribution                                                                            | 80        |
| 2.4.7    | Coatings                                                                                    | 81        |
| 2.4.8    | Counterfeit Identification                                                                  | 81        |
| 2.4.9    | High Throughput Analysis                                                                    | 81        |
| 2.5      | Issues Facing the Implementation of Spectroscopic Techniques in the Pharmaceutical Industry | 82        |
| 2.5.1    | Sampling                                                                                    | 82        |
| 2.5.2    | Spatial Resolution                                                                          | 83        |
| 2.5.3    | Representativeness of the Measured Surface                                                  | 84        |
| 2.5.4    | Irregularities in the Measured Surface                                                      | 85        |
| 2.6      | Conclusions                                                                                 | 85        |
|          | References                                                                                  | 86        |
| <br>     |                                                                                             |           |
| <b>3</b> | <b>Quality Control in Food Processing</b>                                                   | <b>89</b> |
|          | <i>Colette C. Fagan</i>                                                                     |           |
| 3.1      | Introduction                                                                                | 89        |
| 3.2      | Quality Applications                                                                        | 91        |
| 3.3      | Safety Applications                                                                         | 94        |
| 3.4      | Authenticity Application                                                                    | 96        |
| 3.5      | Process Control                                                                             | 102       |
| 3.6      | Conclusions                                                                                 | 104       |
| 3.7      | Glossary                                                                                    | 104       |
|          | References                                                                                  | 105       |

|                   |                                                                                                                                 |            |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>4</b>          | <b>Application of Optical Methods for Quality and Process Control of Topically Applied Actives in Cosmetics and Dermatology</b> | <b>111</b> |
|                   | <i>Juergen Lademann, Martina C. Meinke, Maxim E. Darwin, and Joachim W. Fluhr</i>                                               |            |
| 4.1               | Introduction                                                                                                                    | 111        |
| 4.2               | Laser Scanning Microscopy                                                                                                       | 112        |
| 4.2.1             | Fluorescence Measurements                                                                                                       | 112        |
| 4.2.2             | Remission Measurements                                                                                                          | 119        |
| 4.2.3             | Multiphoton Measurements                                                                                                        | 119        |
| 4.3               | Raman Spectroscopic Measurements                                                                                                | 121        |
| 4.4               | Resonance Raman Spectroscopy                                                                                                    | 123        |
| 4.5               | Conclusions                                                                                                                     | 123        |
|                   | References                                                                                                                      | 124        |
| <b>Part Two</b>   | <b>On-Site Analysis</b>                                                                                                         | <b>127</b> |
| <b>5</b>          | <b>Agricultural Applications: Animal Epidemics and Plant Pathogen Detection</b>                                                 | <b>129</b> |
|                   | <i>Robert Möller</i>                                                                                                            |            |
| 5.1               | Introduction                                                                                                                    | 129        |
| 5.2               | Diagnosis Under Field Conditions                                                                                                | 130        |
| 5.3               | Immunological Based-Techniques                                                                                                  | 131        |
| 5.3.1             | Flow Through Format                                                                                                             | 132        |
| 5.3.2             | Lateral Flow Assays                                                                                                             | 133        |
| 5.4               | Nucleic Acid-Based Testing                                                                                                      | 134        |
| 5.5               | Emerging Technologies                                                                                                           | 135        |
| 5.6               | Conclusion                                                                                                                      | 137        |
|                   | References                                                                                                                      | 138        |
| <b>6</b>          | <b>On-Site Analysis</b>                                                                                                         | <b>141</b> |
|                   | <i>Günther Proll and Günter Gauglitz</i>                                                                                        |            |
| 6.1               | Introduction                                                                                                                    | 141        |
| 6.2               | Substances to be Monitored                                                                                                      | 143        |
| 6.3               | Optical Methods for Monitoring                                                                                                  | 144        |
| 6.4               | Assays                                                                                                                          | 154        |
| 6.5               | Applications                                                                                                                    | 158        |
| 6.5.1             | Chemical Contaminants, EDCs, Pharmaceuticals and Toxins                                                                         | 158        |
| 6.5.2             | Pathogens                                                                                                                       | 161        |
| 6.6               | Perspectives and Visions                                                                                                        | 162        |
|                   | References                                                                                                                      | 163        |
| <b>Part Three</b> | <b>Security Applications</b>                                                                                                    | <b>173</b> |
| <b>7</b>          | <b>Body Scanner</b>                                                                                                             | <b>175</b> |
|                   | <i>Torsten May and Hans-Georg Meyer</i>                                                                                         |            |
| 7.1               | Introduction                                                                                                                    | 175        |
| 7.2               | X-Ray Techniques                                                                                                                | 176        |

|                  |                                                                                 |     |
|------------------|---------------------------------------------------------------------------------|-----|
| 7.2.1            | Overview                                                                        | 176 |
| 7.2.2            | Physical and Technical Background                                               | 176 |
| 7.2.3            | Backscatter Imaging                                                             | 177 |
| 7.2.4            | Transmission Imaging                                                            | 179 |
| 7.3              | Millimeter Wave Electronic Techniques                                           | 180 |
| 7.3.1            | Overview                                                                        | 180 |
| 7.3.2            | Physical and Technical Background                                               | 180 |
| 7.3.3            | Active Imaging                                                                  | 182 |
| 7.3.4            | Passive Imaging                                                                 | 184 |
| 7.4              | Sub-Millimeter Wave (Terahertz) Photonic Techniques                             | 186 |
| 7.4.1            | Overview                                                                        | 186 |
| 7.4.2            | Physical and Technical Background                                               | 187 |
| 7.4.3            | Active Imaging                                                                  | 188 |
| 7.4.4            | Passive Imaging                                                                 | 189 |
| 7.5              | Conclusion and Outlook                                                          | 191 |
|                  | References                                                                      | 191 |
| <b>8</b>         | <b>Detection of Explosives</b>                                                  | 195 |
|                  | <i>Wolfgang Schade, Rozalia Orghici, Mario Mordmüller, and Ulrike Willer</i>    |     |
| 8.1              | Introduction                                                                    | 195 |
| 8.2              | Optical Methods for the Detection of Explosives – Overview                      | 196 |
| 8.2.1            | State of the Art Spectroscopic Methods                                          | 199 |
| 8.2.1.1          | Absorption Spectroscopy                                                         | 199 |
| 8.2.1.2          | Detection of Decomposition Products and Fragments                               | 200 |
| 8.2.1.3          | Laser Induced Breakdown Spectroscopy (LIBS)                                     | 201 |
| 8.2.1.4          | Raman Spectroscopy                                                              | 202 |
| 8.2.1.5          | Photoacoustic Spectroscopy of Explosives                                        | 204 |
| 8.2.1.6          | Cavity Ring Down Spectroscopy                                                   | 206 |
| 8.2.2            | Novel Approaches                                                                | 207 |
| 8.2.2.1          | Femtosecond Coherent Control                                                    | 207 |
| 8.2.2.2          | THz-Spectroscopy                                                                | 208 |
| 8.2.2.3          | Photonic Ring Resonator Sensors                                                 | 209 |
| 8.3              | Summary                                                                         | 213 |
|                  | References                                                                      | 213 |
| <b>Part Four</b> | <b>High Throughput and Content Screening</b>                                    | 219 |
| <b>9</b>         | <b>High-Throughput and -Content Screening/Screening for New Pharmaceuticals</b> | 221 |
|                  | <i>Astrid Tannert and Michael Schaefer</i>                                      |     |
| 9.1              | Introduction                                                                    | 222 |
| 9.2              | Targets                                                                         | 223 |
| 9.3              | Substance Libraries                                                             | 224 |
| 9.4              | Biomarkers and Labels                                                           | 226 |

|          |                                      |     |
|----------|--------------------------------------|-----|
| 9.4.1    | Labels for Cell-Free Assays          | 227 |
| 9.4.2    | Labeling of Cells                    | 227 |
| 9.4.2.1  | Synthetic Fluorophores               | 227 |
| 9.4.2.2  | Genetically-Encoded Marker Proteins  | 228 |
| 9.5      | Instrumentation                      | 229 |
| 9.6      | Assays                               | 230 |
| 9.6.1    | Fluorescence Polarization            | 231 |
| 9.6.2    | Time-Resolved Fluorescence           | 234 |
| 9.6.3    | Proximity Assay                      | 235 |
| 9.6.4    | Protein Complementation Assays       | 236 |
| 9.6.5    | Resonance Energy Transfer            | 236 |
| 9.6.6    | Fluorescence Fluctuation Approaches  | 237 |
| 9.6.7    | Reporter Gene Expression             | 238 |
| 9.6.8    | Measurement of Intracellular Calcium | 238 |
| 9.6.9    | Indicators for Ion Channel Activity  | 241 |
| 9.6.10   | Flow Cytometry                       | 242 |
| 9.6.11   | Automated Microscopy                 | 243 |
| 9.6.11.1 | Image Analysis                       | 245 |
| 9.7      | Data Mining and Quality Control      | 246 |
| 9.7.1    | Quality Control                      | 246 |
| 9.7.1.1  | Random and Systematic Errors         | 247 |
| 9.7.1.2  | Compound Interferences               | 248 |
| 9.7.2    | Identification of Hits               | 249 |
| 9.8      | Conclusions                          | 249 |
|          | References                           | 250 |

## **10 Optical Measurements for the Rational Screening of Protein Crystallization Conditions** 257

*Christoph Janzen and Kurt Hoffmann*

|        |                                                                                       |     |
|--------|---------------------------------------------------------------------------------------|-----|
| 10.1   | Introduction                                                                          | 257 |
| 10.2   | State of the Art of Protein Crystallization Techniques                                | 258 |
| 10.3   | A New Crystallization Method that Enables the Use of Optical Measurement Technologies | 262 |
| 10.4   | Optical Measurements for a Rational Crystallization Process                           | 263 |
| 10.4.1 | Static Light Scattering for the Analysis of the Pre-Nucleation Phase                  | 264 |
| 10.4.2 | Dynamic Light Scattering for the Analysis of the Nucleation Phase                     | 266 |
| 10.4.3 | Quantitative Polarization Microscopy for the Analysis of the Post-Nucleation Phase    | 268 |
| 10.5   | Development of a New Optical Instrument                                               | 271 |
| 10.5.1 | Measurement of Static and Dynamic Light Scattering in Small Volumes                   | 271 |
| 10.5.2 | Quantitative Polarization Microscopy                                                  | 274 |
| 10.5.3 | System Integration, Automation and Data Processing                                    | 274 |
| 10.6   | Optical Measurements                                                                  | 275 |

|          |                                                                           |            |
|----------|---------------------------------------------------------------------------|------------|
| 10.6.1   | Static Light Scattering                                                   | 276        |
| 10.6.1.1 | Instrument Performance                                                    | 276        |
| 10.6.1.2 | Measuring the Virial Coefficient of Proteins                              | 277        |
| 10.6.1.3 | Development of the Virial Coefficient with Different Solvent Parameters   | 280        |
| 10.6.2   | Dynamic Light Scattering                                                  | 281        |
| 10.6.3   | Quantitative Polarization Microscopy                                      | 284        |
| 10.7     | Outlook – An Iterative Optimization Process Based on Optical Measurements | 285        |
|          | References                                                                | 288        |
|          | <b>Index</b>                                                              | <b>291</b> |

