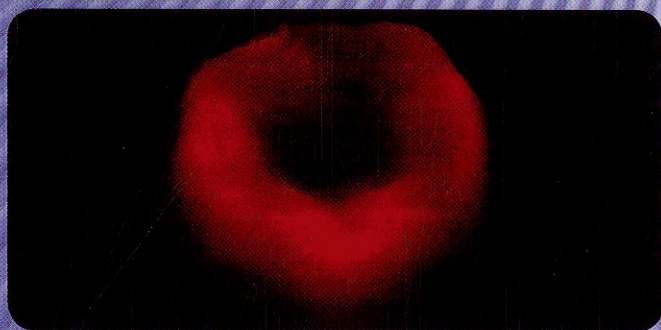


McGRAW-HILL | BIOPHOTONICS

# Quantitative Phase Imaging of Cells and Tissues



Gabriel Popescu



---

# Contents

|                                                                                              |           |
|----------------------------------------------------------------------------------------------|-----------|
| Foreword .....                                                                               | xv        |
| Preface .....                                                                                | xvii      |
| Aknowledgments .....                                                                         | xix       |
| <b>1 Introduction .....</b>                                                                  | <b>1</b>  |
| 1.1 Light Microscopy .....                                                                   | 1         |
| 1.2 Quantitative Phase Imaging (QPI) .....                                                   | 2         |
| 1.3 QPI and Multimodal Investigation .....                                                   | 3         |
| 1.4 Nanoscale and Three-Dimensional Imaging ...                                              | 4         |
| References .....                                                                             | 6         |
| <b>2 Groundwork .....</b>                                                                    | <b>9</b>  |
| 2.1 Light Propagation in Free Space .....                                                    | 9         |
| 2.1.1 1D Propagation: Plane Waves .....                                                      | 9         |
| 2.1.2 3D Propagation: Spherical Waves ....                                                   | 11        |
| 2.2 Fresnel Approximation of Wave Propagation ...                                            | 14        |
| 2.3 Fourier Transform Properties of Free Space ...                                           | 16        |
| 2.4 Fourier Transformation Properties of<br>Lenses .....                                     | 18        |
| 2.5 (The First-Order) Born Approximation of Light<br>Scattering in Inhomogeneous Media ..... | 22        |
| 2.6 Scattering by Single Particles .....                                                     | 28        |
| 2.7 Particles Under the Born Approximation ...                                               | 30        |
| 2.7.1 Spherical Particles .....                                                              | 30        |
| 2.7.2 Cubical Particles .....                                                                | 33        |
| 2.7.3 Cylindrical Particles .....                                                            | 34        |
| 2.8 Scattering from Ensembles of Particles<br>within the Born Approximation .....            | 35        |
| 2.9 Mie Scattering .....                                                                     | 36        |
| Further Reading .....                                                                        | 37        |
| <b>3 Spatiotemporal Field Correlations .....</b>                                             | <b>39</b> |
| 3.1 Spatiotemporal Correlation Function:<br>Coherence Volume .....                           | 39        |
| 3.2 Spatial Correlations of Monochromatic<br>Light .....                                     | 42        |
| 3.2.1 Cross-Spectral Density .....                                                           | 42        |
| 3.2.2 Spatial Power Spectrum .....                                                           | 44        |
| 3.2.3 Spatial Filtering .....                                                                | 48        |

|          |                                                       |           |
|----------|-------------------------------------------------------|-----------|
| 3.3      | Temporal Correlations of Plane Waves                  | 48        |
| 3.3.1    | Temporal Autocorrelation Function                     | 48        |
| 3.3.2    | Optical Power Spectrum                                | 50        |
| 3.3.3    | Spectral Filtering                                    | 51        |
|          | References                                            | 52        |
| <b>4</b> | <b>Image Characteristics</b>                          | <b>55</b> |
| 4.1      | Imaging as Linear Operation                           | 55        |
| 4.2      | Resolution                                            | 56        |
| 4.3      | Signal-to-Noise Ratio (SNR)                           | 57        |
| 4.4      | Contrast and Contrast-to-Noise Ratio                  | 59        |
| 4.5      | Image Filtering                                       | 60        |
| 4.5.1    | Low-Pass Filtering                                    | 61        |
| 4.5.2    | Band-Pass Filter                                      | 62        |
| 4.5.3    | High-Pass Filter                                      | 62        |
|          | References                                            | 64        |
| <b>5</b> | <b>Light Microscopy</b>                               | <b>65</b> |
| 5.1      | Abbe's Theory of Imaging                              | 65        |
| 5.2      | Imaging of Phase Objects                              | 72        |
| 5.3      | Zernike's Phase Contrast Microscopy                   | 74        |
|          | References                                            | 78        |
|          | Further Reading                                       | 78        |
| <b>6</b> | <b>Holography</b>                                     | <b>79</b> |
| 6.1      | Gabor's (In-Line) Holography                          | 79        |
| 6.2      | Leith and Upatnieks's (Off-Axis) Holography           | 81        |
| 6.3      | Nonlinear (Real Time) Holography or Phase Conjugation | 84        |
| 6.4      | Digital Holography                                    | 85        |
| 6.4.1    | Digital Hologram Writing                              | 85        |
| 6.4.2    | Digital Hologram Reading                              | 88        |
|          | References                                            | 89        |
| <b>7</b> | <b>Point-Scanning QPI Methods</b>                     | <b>91</b> |
| 7.1      | Low-Coherence Interferometry (LCI)                    | 91        |
| 7.2      | Dispersion Effects                                    | 95        |
| 7.3      | Time-Domain Optical Coherence Tomography              | 98        |
| 7.3.1    | Depth-Resolution in OCT                               | 99        |
| 7.3.2    | Contrast in OCT                                       | 101       |
| 7.4      | Fourier Domain and Swept Source OCT                   | 102       |
| 7.5      | Qualitative Phase-Sensitive Methods                   | 106       |
| 7.5.1    | Differential Phase-Contrast OCT                       | 106       |

|           |                                                                                                |            |
|-----------|------------------------------------------------------------------------------------------------|------------|
| 7.5.2     | Interferometric Phase-Dispersion<br>Microscopy .....                                           | 107        |
| 7.6       | Quantitative Methods .....                                                                     | 110        |
| 7.6.1     | Phase-Referenced Interferometry ....                                                           | 110        |
| 7.6.2     | Spectral-Domain QPI .....                                                                      | 112        |
| 7.7       | Further Developments .....                                                                     | 115        |
|           | References .....                                                                               | 116        |
| <b>8</b>  | <b>Principles of Full-Field QPI .....</b>                                                      | <b>121</b> |
| 8.1       | Interferometric Imaging .....                                                                  | 121        |
| 8.2       | Temporal Phase Modulation: Phase-Shifting<br>Interferometry .....                              | 122        |
| 8.3       | Spatial Phase Modulation: Off-Axis<br>Interferometry .....                                     | 124        |
| 8.4       | Phase Unwrapping .....                                                                         | 126        |
| 8.5       | Figures of Merit in QPI .....                                                                  | 127        |
| 8.5.1     | Temporal Sampling:<br>Acquisition Rate .....                                                   | 128        |
| 8.5.2     | Spatial Sampling: Transverse<br>Resolution .....                                               | 129        |
| 8.5.3     | Temporal Stability: Temporal-Phase<br>Sensitivity .....                                        | 131        |
| 8.5.4     | Spatial Uniformity: Spatial Phase<br>Sensitivity .....                                         | 135        |
| 8.6       | Summary of QPI Approaches and Figures<br>of Merit .....                                        | 137        |
|           | References .....                                                                               | 138        |
| <b>9</b>  | <b>Off-Axis Methods .....</b>                                                                  | <b>141</b> |
| 9.1       | Digital Holographic Microscopy (DHM) ....                                                      | 141        |
| 9.1.1     | Principle .....                                                                                | 141        |
| 9.1.2     | Further Developments .....                                                                     | 146        |
| 9.1.3     | Biological Applications .....                                                                  | 147        |
| 9.2       | Hilbert Phase Microscopy (HPM) .....                                                           | 151        |
| 9.2.1     | Principle .....                                                                                | 151        |
| 9.2.2     | Further Developments .....                                                                     | 153        |
| 9.2.3     | Biological Applications .....                                                                  | 155        |
|           | References .....                                                                               | 163        |
| <b>10</b> | <b>Phase-Shifting Methods .....</b>                                                            | <b>169</b> |
| 10.1      | Digitally Recorded Interference Microscopy<br>with Automatic Phase Shifting<br>(DRIMAPS) ..... | 169        |
| 10.1.1    | Principle .....                                                                                | 169        |
| 10.1.2    | Further Developments .....                                                                     | 170        |
| 10.1.3    | Applications .....                                                                             | 171        |

|           |                                                                         |            |
|-----------|-------------------------------------------------------------------------|------------|
| 10.2      | Optical Quadrature Microscopy (OQM) . . . .                             | 174        |
| 10.2.1    | Principle . . . . .                                                     | 174        |
| 10.2.2    | Further Developments . . . . .                                          | 175        |
| 10.2.3    | Applications . . . . .                                                  | 176        |
|           | References . . . . .                                                    | 178        |
| <b>11</b> | <b>Common-Path Methods . . . . .</b>                                    | <b>183</b> |
| 11.1      | Fourier Phase Microscopy (FPM) . . . . .                                | 183        |
| 11.1.1    | Principle . . . . .                                                     | 184        |
| 11.1.2    | Further Developments . . . . .                                          | 186        |
| 11.1.3    | Biological Applications . . . . .                                       | 190        |
| 11.2      | Diffraction-Phase Microscopy (DPM) . . . . .                            | 196        |
| 11.2.1    | Principle . . . . .                                                     | 196        |
| 11.2.2    | Further Developments . . . . .                                          | 199        |
| 11.2.3    | Biological Applications . . . . .                                       | 202        |
|           | References . . . . .                                                    | 216        |
| <b>12</b> | <b>White Light Methods . . . . .</b>                                    | <b>221</b> |
| 12.1      | QPI Using the Transport of Intensity<br>Equation . . . . .              | 221        |
| 12.1.1    | Principle . . . . .                                                     | 222        |
| 12.1.2    | Further Developments . . . . .                                          | 223        |
| 12.1.3    | Biological Applications . . . . .                                       | 224        |
| 12.2      | Spatial Light Interference Microscopy<br>(SLIM) . . . . .               | 226        |
| 12.2.1    | Principle . . . . .                                                     | 226        |
| 12.2.2    | Further Developments . . . . .                                          | 234        |
| 12.2.3    | Biological Applications . . . . .                                       | 236        |
|           | References . . . . .                                                    | 241        |
| <b>13</b> | <b>Fourier Transform Light Scattering . . . . .</b>                     | <b>245</b> |
| 13.1      | Principle . . . . .                                                     | 245        |
| 13.1.1    | Relevance of Light-Scattering<br>Methods . . . . .                      | 245        |
| 13.1.2    | Fourier Transform Light<br>Scattering (FTLS) . . . . .                  | 247        |
| 13.2      | Further Developments . . . . .                                          | 252        |
| 13.3      | Biological Applications . . . . .                                       | 254        |
| 13.3.1    | Elastic Light Scattering of<br>Tissues . . . . .                        | 254        |
| 13.3.2    | Elastic Light Scattering of Cells . . . .                               | 258        |
| 13.3.3    | Dynamic Light Scattering of<br>Cell Membrane Fluctuations . . . . .     | 260        |
| 13.3.4    | Dynamic Light Scattering of Cell<br>Cytoskeleton Fluctuations . . . . . | 262        |
|           | References . . . . .                                                    | 265        |

|           |                                                                             |            |
|-----------|-----------------------------------------------------------------------------|------------|
| <b>14</b> | <b>Current Trends in Methods</b>                                            | <b>271</b> |
| 14.1      | Tomography via QPI                                                          | 271        |
| 14.1.1    | Computed Tomography (CT)<br>Using Digital Holographic<br>Microscopy         | 272        |
| 14.1.2    | Diffraction Tomography (DT) via<br>Spatial Light Interference<br>Microscopy | 275        |
| 14.2      | Spectroscopic QPI                                                           | 277        |
| 14.2.1    | Spectroscopic Diffraction-Phase<br>Microscopy                               | 279        |
| 14.2.2    | Instantaneous Spatial Light<br>Interference Microscopy (iSLIM)              | 281        |
|           | References                                                                  | 285        |
| <b>15</b> | <b>Current Trends in Applications</b>                                       | <b>287</b> |
| 15.1      | Cell Dynamics                                                               | 287        |
| 15.1.1    | Background and Motivation                                                   | 287        |
| 15.1.2    | Active Membrane Fluctuations                                                | 288        |
| 15.1.3    | Intracellular Mass Transport                                                | 292        |
| 15.2      | Cell Growth                                                                 | 298        |
| 15.2.1    | Background and Motivation                                                   | 298        |
| 15.2.2    | Cell Cycle-Resolved Cell Growth                                             | 299        |
| 15.3      | Tissue Optics                                                               | 301        |
| 15.3.1    | Background and Motivation                                                   | 301        |
| 15.3.2    | Scattering-Phase Theorem                                                    | 302        |
| 15.3.3    | Tissue-Scattering Properties from<br>Organelle to Organ Scale               | 306        |
| 15.4      | Clinical Applications                                                       | 308        |
| 15.4.1    | Background and Motivation                                                   | 308        |
| 15.4.2    | Blood Screening                                                             | 310        |
| 15.4.3    | Label-Free Tissue Diagnosis                                                 | 313        |
|           | References                                                                  | 317        |
| <b>A</b>  | <b>Complex Analytic Signals</b>                                             | <b>323</b> |
|           | Further Reading                                                             | 326        |
| <b>B</b>  | <b>The Two-Dimensional and Three-Dimensional<br/>Fourier Transform</b>      | <b>327</b> |
| B.1       | The 2D Fourier Transform                                                    | 327        |
| B.2       | Two-Dimensional Convolution                                                 | 329        |
| B.3       | Theorems Specific to Two-Dimensional<br>Functions                           | 329        |
| B.4       | Generalization of 1D Theorems                                               | 330        |
| B.5       | The Hankel Transform                                                        | 332        |
| B.6       | The 3D Fourier Transform                                                    | 335        |

|          |                               |            |
|----------|-------------------------------|------------|
| B.7      | Cylindrical Coordinates ..... | 335        |
| B.8      | Spherical Coordinates .....   | 336        |
|          | References .....              | 338        |
| <b>C</b> | <b>QPI Artwork.....</b>       | <b>339</b> |
|          | Index .....                   | 351        |