

## Index

1. An introduction to digital imaging and processing .....	5
2. IMAGEJ/FIJI Software .....	6
2.1 Introduction.....	6
2.2 Installation and startup.....	7
2.3 Interface .....	7
2.3.1 Commands Menu .....	7
2.3.2 Toolbar .....	15
2.3.3 Status Bar .....	17
2.3.4 Progress Bar .....	17
2.3.5 Information in the opened image .....	17
2.4 Start using Imagej.....	17
2.5 Types of images.....	18
2.5.1 By their origin .....	18
2.5.2 By the interaction of the light source with the material.....	18
2.5.3 By its resolution and depth .....	18
2.5.4 By its dimensionality .....	19
2.5.5 By its compression.....	19
3. Basic Tools .....	20
3.1 Open images.....	20
3.2 Close Images.....	21
3.3 Colors/Channels .....	21
3.3.1 Change colors .....	21
3.3.2 Merge channels.....	21
3.3.3 Split channels .....	22
3.4 Alignments .....	22
3.4.1 Align channels .....	22
3.4.2 Other alignments.....	22
3.5 Scale .....	22
3.5.1 Calibrating an image.....	22
3.5.2 Densitometric calibration.....	23
3.5.3 Rescaling an image .....	24
3.6 LookUp Tables (LUTs) .....	24
3.7 Annotations.....	25
3.7.1 Set a scale bar.....	25

3.7.2 Insert text in an image or stack .....	25
3.7.3 Drawing ROIs, lines or arrows in an image or series of images .....	25
3.8 Region of Interest.....	26
3.8.1 ROI Manager .....	26
3.8.2 Wand Tool .....	27
4. Image Processing.....	28
4.1 Introduction to image processing .....	28
4.2 Adjusting levels .....	28
4.3 Histogram .....	30
4.4 Threshold.....	31
4.5 Stacks/Hyperstacks .....	33
4.5.1 View and record a series of xyz images.....	34
4.5.2 Duplicate a stack .....	34
4.5.3 Delete one or more images from a stack.....	34
4.5.4 Reduce a stack.....	34
4.5.5 Add one or more images to a stack.....	34
4.5.6 Split a series into different segments.....	34
4.5.7 Reverse the order of the sections in a stack .....	34
4.5.8 Convert a stack into a hyperstack (and viceversa).....	35
4.5.9 Display and save an image stack as a movie .....	35
4.5.10 Make a projection .....	35
4.5.11 Make a 3D projection .....	36
4.5.12 Make a coded projection by depth .....	37
4.5.13 Mosaic merging .....	37
4.5.14 Create figures .....	39
4.5.15 Set up a gallery of images z .....	41
4.5.16 Vertical section (xy/z) of a xyz stack.....	42
4.5.17 Surface Render (VolumeJ).....	43
4.5.18 Projection Viewers .....	44
4.6 Transform .....	47
4.7 Filtering .....	47
4.7.1 Point-to-point operations .....	47
4.7.2 Pixel group processing operations .....	48
4.8 Background noise removal.....	49
4.8.1 Subtract Background .....	49
4.8.2 Additional methods to clean up the background of an image.....	50

4.9 Time dimension experiments.....	50
4.9.1 Linking two or more series in z-axis or time dimension.....	50
4.9.2 Stack projections of time-based experiments.....	50
4.9.3 Deleting specific z sections in time experiments .....	50
4.9.4 Keeping specific z sections in time experiments.....	51
4.9.5 Aligning xy-shifted sections in time experiments .....	51
4.9.6 Splitting stacks into substacks.....	51
4.9.7 Sorting the stack slices .....	51
5. Image Analysis.....	52
5.1 Introduction to image analysis.....	52
5.1.1 Preparing images for quantification.....	52
5.1.2 Defining measurement parameters.....	53
5.2 Intensity quantification .....	54
5.2.1 Along a line .....	54
5.2.2 In an area.....	54
5.2.3 In a stack.....	55
5.3 Volume measurement.....	56
5.4 Particle counter.....	56
5.4.1 Point/Multi-point Tool .....	56
5.4.2 Manual counter.....	57
5.4.3 Automatic particle counting.....	58
5.5 Image operations .....	64
5.5.1 Image calculator .....	64
5.5.2 Mathematical processing .....	64
5.6 Colocalization studies.....	64
5.6.1 Colocalization basics.....	64
5.6.2 Quantitative colocalization .....	65
5.7 Particle movement studies.....	67
5.7.1 Basic studies of particle dynamics.....	67
5.7.2 Wound healing experiments .....	67
5.7.3 Particle tracking plugins .....	67
5.8 Gels Densitometry.....	70
6. Plugins .....	71
6.1 Introducing plugins into the software.....	71
7. Macros.....	71
7.1 Introducing macros into the software .....	72

7.2 Generating new macros .....	72
8. Appendices .....	73
8.1 Standard Procedures.....	73
8.1.1 Rescaling an image .....	73
8.1.2 Lookup Tables (LUT) .....	73
8.1.3 Selections .....	74
8.2 Action Bar .....	75
8.3. Common image specifications .....	75
8.4 Biostatistics fundamentals .....	76
8.4.1 Biostatistics concepts .....	76
8.4.2 Correlation coefficients .....	76
9. BIBLIOGRAPHY.....	78
9.1 Books and journals .....	78
9.2 Online resources .....	78