Basic processing in Fiji

Bio-formats import option (appears automatically for some image formats)
View stack with: Hyperstack → OK
Select and image → OK

ZOOM
Magnifying glass ison or +/- → zoom in (mouse click) / zoom our (mouse click + Ctrl) / restore orig. size (mouse click + shift))

Open PROPERTIES
Image → Properties or Ctrl+Shift+P

Display METADATA
Image → Show Info or Ctrl+I

Open CHANNELS TOOL window (allows to simply change views – composite/color/greys)
Image → Color → Channels Tool or Ctrl+Shift+Z
Color more: LUT (upper bar) choose color: switch to the glow mode

Make Z-PROJECTION
Image → Stack → Z-project ….
Type – max. intensity: nice contrast usually
– average

Change LUTs to "glow"
to see over/under exposure → good: do it close to the max. (blue)
Image → Lookup tables or LUT icon
To see all LUTs – Image → Color → Display LUTs

Open BRIGHTNESS/CONTRAST
Image → Adjust → Brightness/Contrast or Ctrl+Shift+C
use pref. min/max

⇨ ADJUST each channel of the image
(adjust min and mac values, do not change gamma)
⇨ Press APPLY for each channel

Change LUTs back to desired color
Image → Lookup tables or LUT icon
→ Blue, green red/cyan, magenta → do not use green and red together

Switch to COMPOSITE using CHANNELS TOOL
Image → Color → Channels Tool or Ctrl+Shift+Z

SAVE the image as TIFF (stack of 3 grayscale images)

Add SCALE BAR
Analyze → Tools → Scale Bar ….

MAKE RGB (1 RGB image)
Image → Color → Stack to RGB
Image → Type → RGB color/RGB stack

Save the image as TIFF
File → Save as → Tiff

Processing all images in one file

OPEN
- Bio-format Import Option
- Hyperstack
- (open all series shen compatible)
- Color mode: Colorized/(Composite → split channel)
- (Autoscale)

MACRO
→ record
→ create

Back to original size of image
(yellow square at left upper corner
Shift + magnifying glass (bookmark bar)

Saving all images – opened as cocantenate
Adjust images as usual ….
a) Image → Type → RGB
File → Save as → Image as sequence
b) Image → Color → Split Channels
Image → Adjust → Brightness/Contrast
→ ! must be´ Apply´, otherwise it is not apply does not work with export !
Plugins → Bio-format → Bio-format Exporter TIFF Write each time point to a separate file → Uncompressed or LWZ compression
c) Image → Adjust …..
Image → Color → Merge channels → File → Save as → Image as sequence
↔ for each channel do not: "Image Type RGB "

Other useful tools in Fiji

Look on OTHTOGONAL VIEWS
Image → Stack → Orthonogonal views or CTRH+SHIFT+H

SPLIT CHANNELS
Image → Color → Split Channels

MERGE CHANNELS
Image → Color → Split Channels
Recommendation: check "keep source"

PLOT PROFILE
Draw line → Analyze → Plot profile or Ctrl+K

MAKE SUBSTRACK (define a list of slices or a range of slices with an increment)
Image Stack Tools Make Substrack …

MAKE DIMENSIONALITY, try various options
Image → Hyperstack → Reduce Dimensionality
- isolate one time-frame, keep slices, keep channels
- isolate one slice, keep frames, keep channels
- isolate one channel …

CROP the image (the original image down)
Draw a square → Image Crop or CTRL+Shift+X

DUPLICATE a cut-out of the image (the original image stays open)

Adjust IMAGE SIZE (re-define image height, width and/or depth)
Image Adjust Size ….

Save as MOVIE
(File → Save as → AVI …

SYNCHRONIZE windows
(Analyze → Tools → Synchronize windows → Synchronize All)

Final handling in Photoshop/Illustrator

Only for pictures for publication, not analysis

Open the TIFF image

CROP a region and PASTE it to a new window
Rectangular marquee tool → CTRL+C Ctrl+N Ctrl+V

Draw SCALE BAR
Rectangular tool set fixed pixels
or line with different stroke
→ must be done before resolution on 300 dpi (resampling)
merge layers afterwards

Set IMAGE SIZE and RESOLUTION on 300 dpi (check Resample image)
Image Image size or Ctrl+Alt+I
Resample image (tich) than write resolution + contrast proportion

Open a NEW FILE in Illustrator (set desired size and change the color mode to RGB)
File → New

COPY composite and each channel individually to Illustrator
Ctrl+C and Ctrl+V

Align images
Window → Align

Scale bar issues ….

Resampling is better in ….

Scale bar:
e.g. 10 = 10 000 → 68. 5 pix

ImageJ → Image → Show info down
Width: in microns (pixels)
Height: in microns (pixels)

Scale bar
LAS X: physical length = local pixels * voxel size = pixel size

Size of image in Illustrator: physical length * required scale bar = "real size of scale bar in illustrator"

e.g. 1398 * (0) * 203 232 = 147.32 : 203 232 *15 = 10.87

!!! FiJi - ImageJ: !!!
Analyze Tools Scale Bars Save as jpeg or png; if TIFF no scale bar

Gels and membrane analysis - quantification

Open images in ImageJ

Optional:
Adjust Brightness/contrast
We aware of control – everything has to be the same !!!!

Processing
- draw rectangle around firs band
  should be big enough to fit band and also for the rest
- Analyses → Gels → select first line (ctrl+1)
- Drag rectangle form the first line (with arrow, rectangle will duplicate) and label second, etc. ….. the last line (! all rectangles have to have the same size !)
- Analyses → Gels → Plot lanes
- Graph: integration of peaks: use line tool (+Shift) or draw line with pencil
  - For WB (white background and black bends): area under {maybe above] just check peak
  - For ELFO gel (black background and white bends): area above peak, or invert colors and process as Wb membranes
- Magic wand tool → to select area under graph → Measure …. To see numbers copy to excel
- Results can be saved as Excel file

Optional
- Draw one rectangle for all bands integrate in one graph …. !!! Do not mix up the samples!!!
- Note: in some Fiji versions that doesn't work properly

Note
- There is many videos online on YouTube