

# Measuring Surface Roughness Using Confocal Microscopy and ImageJ (or Fiji variant)

## Collecting Data Set

Depending upon the sample's composition, the confocal microscope is used in either fluorescence or reflection mode. The former could be used for samples which were inherently fluorescent (auto-fluorescence) or have been coated with a fluorescent marker, the latter is used on surfaces which reflect light, such as metals.

Once a z-series is collected it is in a format suitable for roughness analysis.

## Analysing Data

The weapon of choice for determining the Ra value of the surface if a variant of ImageJ called 'Fiji'. Its appearance and operation is the same as ImageJ, but it comes with many pre-installed plugins and can automatically look for, and install, updates. The two plugins used for this procedure are 'Extended Depth of Field' and 'SurfaceChJ 1q'. The first generates a height profile image, the second calculates the Ra value (along with a number of other surface parameters) based upon the height map.

### Height Maps

Usually, the pixels in an image represent intensity, a higher pixel value produces a brighter pixel. In 8 bit images a pixel value of 0 appears as black, a pixel value of 255 produces white. Values in between produce the intermediate grey intensities.

In a height map all the pixels which occur in the same vertical plane have the same value; a value which represents the height at that plane. If the confocal z-series has for example 10 slices, the first image's pixels will all have the value of '1'. The second image '2' and so on up until '10'. It's these values which determine the Ra.

## Plugin #1: Extended Depth of Field

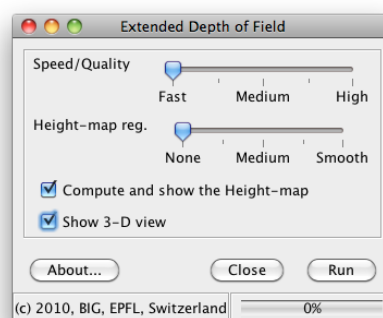
Download from <http://bigwww.epfl.ch/demo/edf/index.html> and install in 'Plugins' folder of ImageJ. Restart ImageJ and locate plugin at: Plugins / Extended Depth of Field / Easy Mode ...

When activated, the following dialogue box appears.

There is a trade-off between speed and quality, so decide what is appropriate for your sample. Repeating the analysis on the same sample with different settings may show if significant differences occur.

Make sure you have ticked the 'Compute and show height map' option.

The 'show 3-D view' can be useful to visualise the surface properties.



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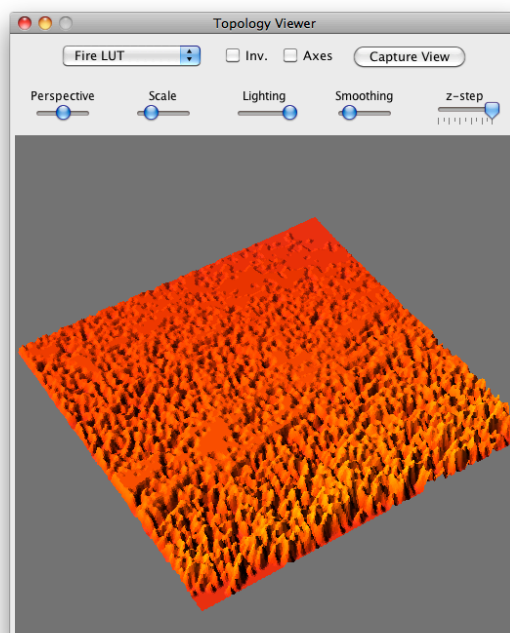
## Output Files

The plugin will generate a number of files, depending upon which options are chosen.

Output: An extended focus image where all the sharpest regions from each focus plane are compiled into a composite image. In effect, everything from the top to the bottom of the slide is in focus.

Height Map: The pixels represent height, not intensity, as explained above. This is the image the second part of the analysis is performed upon to determine the Ra value.

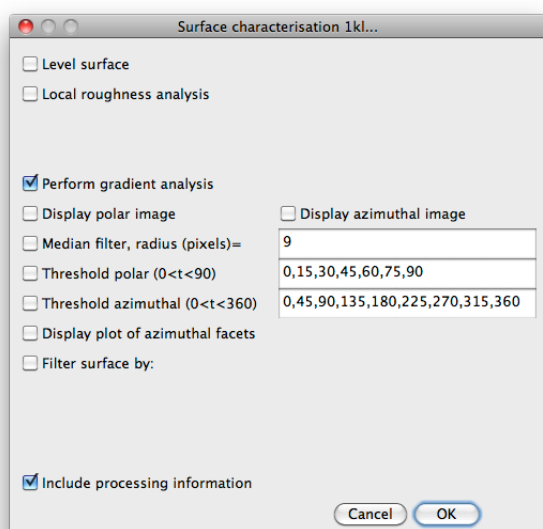
Topology Viewer: This allows a degree of interaction to adjust the appearance of the surface, including the exaggeration of the z axis (z step option). No numbers are generated, but it can be useful to obtain an idea of what the surface's general properties are. If used with caution, the smoothing option can improve the surfaces appearance and may better represent what it really is. (See notes later in text.)



If nothing else, save the Height Map file for analysis.

## Plugin #2: SurfCharJ

Download plugin from <http://www.gcscs.net/IJ/SurfCharJ.html> and install as described above

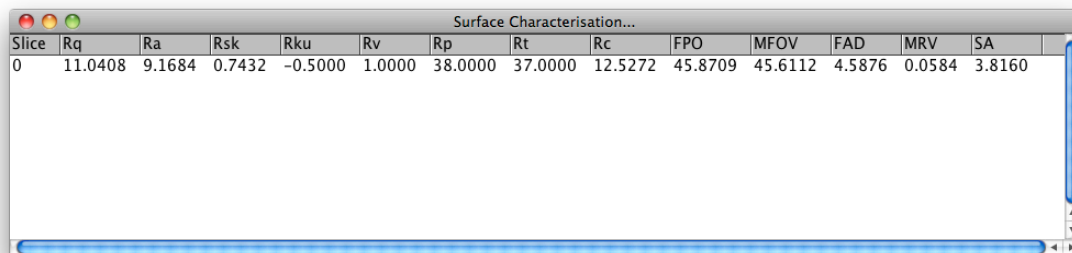


With the Height Map file as the active window, run the SurfCharJ plugin, the window shown here appears. For detailed descriptions of each of the options, see the plugin website.

Once the appropriate options have been selected, press 'Ok' to perform the surface analysis.

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The results window, shown below, contains the Ra, Rv, Rp values, etc. which relate to the measured surface.



Slice	Rq	Ra	Rsk	Rku	Rv	Rp	Rt	Rc	FPO	MFOV	FAD	MRV	SA
0	11.0408	9.1684	0.7432	-0.5000	1.0000	38.0000	37.0000	12.5272	45.8709	45.6112	4.5876	0.0584	3.8160

### Notes

Often the raw data contains a significant amount of noise as a consequence of random light scattering under the confocal microscope. A judgment call can be made on the contribution of such noise, as it increases the measured roughness of the surface.

In some cases it is probably best to remove the noise in ImageJ (or Fiji) prior to beginning analysis. Suitable filters may be:

Process / Noise / De-speckle

Process / Noise / Remove Outliers

Process / Filters / Gaussian Blur ...

Obviously, care is required if this step is used, as results could easily be skewed. Also, it may be appropriate to apply the same correction across all similar samples - or not, it really is a case of understanding your sample.

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