Multiscale Hessian fracture filtering – User guide

Maarten Voorn, Februari 2013

The following manual is intended for explaining how to use implementations of the multiscale Hessian fracture filter and other data processing steps, in ImageJ/FIJI and MATLAB®. The main details of the technique are explained in the paper:

Voorn, M., Exner, U. and Rath, A., 2013. Multiscale Hessian fracture filtering for the enhancement and segmentation of narrow fractures in 3D image data. Computers and Geosciences.

Some basic prior knowledge on the usage of ImageJ/FIJI and MATLAB® can be an advantage, but is not required. All ImageJ/FIJI macros can run with a GUI, the MATLAB® script does not.

Sections 1 to 5 cover the main part of the multiscale Hessian fracture filtering and the required preparations. Later sections cover optional additional steps (calibration, connectivity filtering and binarisation).

The consecutive numbering in the file names will – if all goes well – always correspond to the input files. For example, an input file named (e.g.) "Data_0100.tif" corresponds to output containing "Data_0100" in its filename as well.

1. Included data

1.1 Files for running the code, the preparations and optional additional steps

- MSHFF_Prep.ijm   ImageJ macro for ROI and control lines preparation
- MSHFF.ijm   Main multiscale Hessian fracture filtering ImageJ macro
- MSHFF_Cal.ijm   ImageJ macro for calibration using a cosine curve
- MSHFF_Conn.m   MATLAB® script for connectivity filtering
- MSHFF_Block.xls   Spreadsheet file for determining the most suitable block size

1.2 Other data

- Testdata   Folder containing 100 test slices of data
- Testdata_results   Folder containing results of an analysis on above test data

2. Preparation checklist before running any code

2.1 Image files
- 3D dataset should be divided into a series of 2D slices, with consecutive numbering.
- Filenames should start with letters or numbers.
- 8 bit and 16 bit greyscale are supported. Other formats may work, but conversion is advised.
- "Pseudo 8 bit greyscale", 24 bit RGB images should be converted to true 8 bit greyscale first!
- The TIFF file format is recommended, although other formats may work.
- The features of interest (e.g. fractures) should be dark (have a lower greyscale value) than the surrounding material. If not, one can try to invert the image data first.
- The input data should be spatially isotropic, i.e. the resolution is equal in all 3 directions (x, y, z). This means all voxels are cubic. Anisotropy is NOT supported. During the filtering process every voxel is regarded to have a dimension of 1x1x1. The true resolution is thus not of importance for the filtering.

2.2 Downsampling
- Downsampling data speeds up the filtering process severely. In the example of a 2-fold downsampling in all three directions, there is 8 times less data to process in the filtering routine. We have reached very reasonable results with filtered downsampled data compared to its filtered, full scale counterpart. From a data perspective, to prevent additional artefacts or loss of information, it is however not generally advised to use downsampling. It is therefore best to use it only for memory reasons or testing purposes. Downsampling is not included in this code, so should be carried out manually if desired.

2.3 Disk space
- Make sure enough HDD space is available. The minimum amount of required disk space for the Hessian filtering process can be calculated by:

$$Diskspace(MB) = \frac{32}{8 \cdot 1024 \cdot 1024} \cdot pixelsize\ x \cdot pixelsize\ y \cdot number\ of\ slices \cdot (number\ of\ scales + 3)$$

  The *number of scales* is the number of scales used for the Hessian filtering, i.e. the number of different apertures considered ((s_{min}-s_{max})/s_{step}, as determined in a later stage). Note the amount of required HDD space may run into the 100s of GBs, for large image datasets.

2.4 Folder structure
- Create an empty folder for the processing steps. Hereafter this is termed *rootfolder*. Short paths are advised.
- Create a subfolder termed *Input*, and copy the stack of images into this folder. Do not put any other files than the image files into this folder.
- The macro codes and scripts can be copied into the *rootfolder*.

2.5 Software
- Download (fiji.sc) and install FIJI.
- Open the programme and go to *Edit → Options → Memory & Threads*.
- Set the maximum memory high, i.e. as close as possible to your computer's RAM memory. The higher the memory setting, the faster the multiscale Hessian fracture filter will work.
- Close FIJI. (This is done after every step in this analysis to prevent conflicts and memory issues.)
- If connectivity filtering is required, make sure MATLAB® with the Image Processing Toolbox is installed.

3. Determining analysis parameters

3.1 List of chosen parameter values (to be filled in when going through the following sections):

Parameter	Value	Value to use when unspecified	Section
s_{min}		-	3.2, 3.2.1
s_{max}		-	3.2, 3.2.1
s_{step}		-	3.2, 3.2.1
$uses_{min}$		$=s_{min}$	3.2.1
$uses_{max}$		$=s_{max}$	3.2.1
$uses_{step}$		$=s_{step}$	3.2.1
blocksize		-	3.2, 3.2.2
avgmat		Maximum greyscale (8 bit: 255, 16 bit: 65535)	3.3, 3.3.1
consthresh		0	3.3, 3.3.2
maxmat		Maximum greyscale (8 bit: 255, 16 bit: 65535)	3.3, 3.3.3
padding		0 (recommended)	3.3, 3.3.4

NOTE: The values need to be ordered $s_{min} \leq s_{max}$, $uses_{min} \leq uses_{max}$, $s_{min} \leq uses_{min}$, $uses_{max} \leq s_{max}$, $s_{step} \leq uses_{step}$ and *constresh < avgmat ≤ maxmat*. Furthermore, *blocksize* ≤ total amount of slices. Else, the values have not been determined correctly.

3.2 Required parameters
- The following parameters are required in every analysis:

| Name/symbol	Parameter	Description	Section
s_{min}	minimum Gaussian smoothing scale	relates to the minimum aperture to detect	3.2.1
s_{max}	maximum Gaussian smoothing scale	relates to the maximum aperture to detect	3.2.1
s_{step}	stepsize between Gaussian smoothing scales	controls the intervals between the apertures to detect	3.2.1
blocksize	blocksize in amount of slices	amount of data to be analysed per consecutive step	3.2.2

3.2.1 Smoothing scales

- Open FIJI
- Go to *File → Import → Image Sequence*
- Browse to the *Input* folder
- Press *Open*
- In the dialog, leave all settings as they are but check *Use virtual stack*
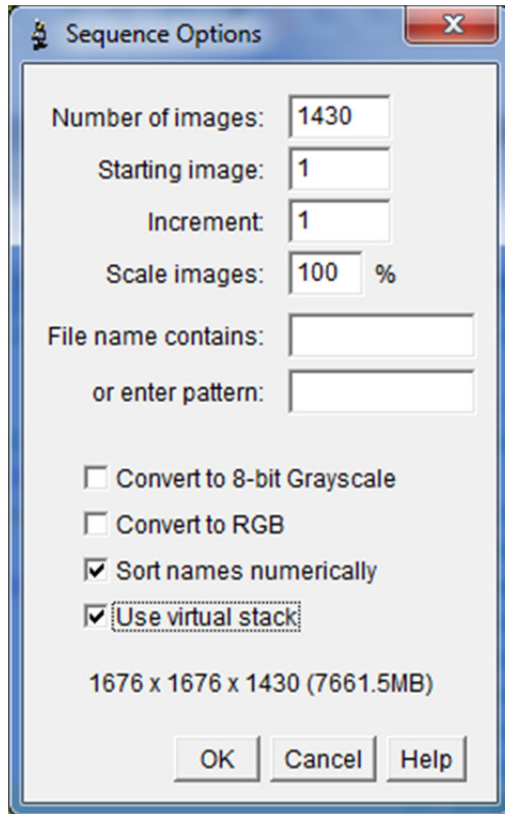

- Press OK. The image stack is opened. The slider at the bottom can be used to go through all the images.

- Make sure the image properties are set to isotropic voxels. This is required for determining the correct Gaussian smoothing scales. To do this, go to *Image* → *Properties*. The following dialog box appears:
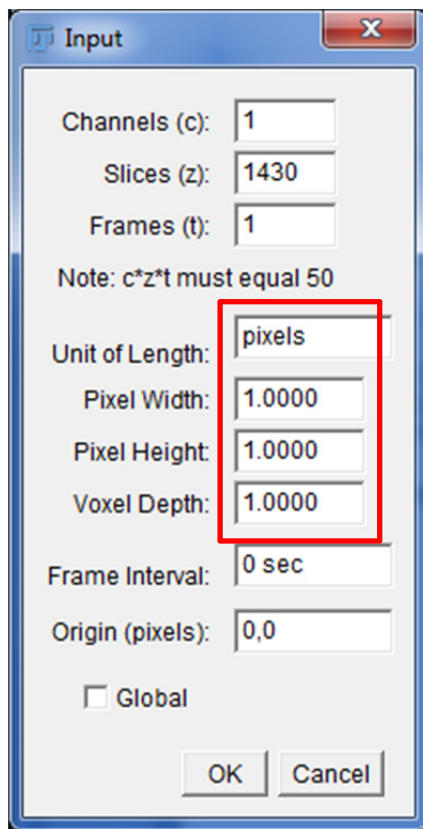
- In the indicated area, the *Unit of Length* should be set to *pixels* (this can be typed in), and the *Pixel Width*, *Pixel Height* and *Voxel Depth* should all be set to 1. Press OK.

- Use the straight line tool to draw a line over one fracture or other planar feature of interest.

- Press CTRL + K (or *Analyze* → *Plot Profile*). A line plot appears:
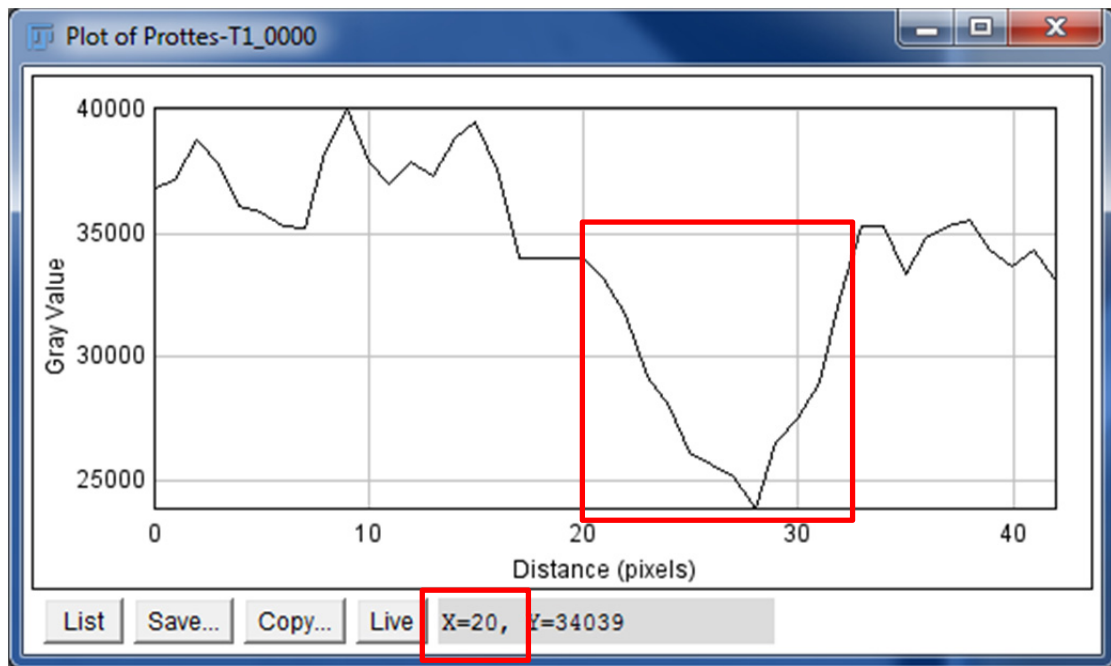
- Drag the cursor over the plot. On the lower end, the relative X position can be read.
- Determine the width of the feature of interest, i.e. the width of the "valley" in the plot. In the example, this width is ca. 12 pixels.
- The best-fitting Gaussian smoothing scale for a feature is its width <u>DIVIDED BY 2</u>. In the example, a suitable smoothing scale would thus be 6.
- Close the plot.
- Repeat the procedure for several (representative) features and write down the results.
- Determine a suitable range of Gaussian scales (minimum scale to maximum scale, and the stepsize in between; e.g., 2 to 6 with a stepsize of 1: scales 2, 3, 4, 5 and 6 are calculated).

NOTE: Although fractions are supported, it is best to keep the smoothing scales integer. This also has a theoretical basis as voxels are discrete too.

NOTE: Of course, this approach is NOT exact and the chosen values can differ per user. This will affect the final output of the technique, but variations can be minute. Of main importance is NOT to choose too low smoothing scales (these will introduce noise) or too high smoothing scales (these will result in unrealistically broad features). The user is of course also free to use a different or more sophisticated technique to find a suitable range of smoothing scales.

NOTE: One has the option in the code to calculate more Gaussian scales than are actually used eventually. This can be beneficial for comparing for example the inclusion of an additional scale in the analysis. Every additional added scale takes longer to calculate, but calculating an additional scale separately later will take even longer! The combination of several scales can be run separate from the main code, in a later stage.

s_{min}, s_{max} and s_{step} define the scales that are calculated.
$uses_{min}$, $uses_{max}$ and $uses_{step}$ define the scales that are actually included in the final, combined result.
If you do not wish these to differ, use the same values as for the calculated scales.

- Write down the chosen ranges in the table at section 3.1.

3.2.2 Blocksize

- The *blocksize* can be chosen but more optimum values can be calculated.
- Open the spreadsheet MSHFF_Block.xls
- Enter the required values (RAM memory as set in FIJI (better: slightly lower), number of files (=number of slices), pixelsize x and pixelsize y, plus the maximum Gaussian smoothing scale chosen above (s_{max})).
- The maximum possible, most efficient *blocksize* will appear in green. If a value higher than the number of files is given, one should of course use this number of files (*blocksize* ≤ number of files)

NOTE: If the multiscale Hessian fracture filter still crashes on memory issues, the blocksize should be lowered. Such crashes are possible if the "optimum" blocksize results in a RAM usage very close to the maximum in FIJI, since memory overhead is difficult to assess. Therefore, to prevent issues, one can choose to set the memory in the spreadsheet slightly lower.

- Write down the chosen *blocksize* in the table at section 3.1.

BACKGROUND: Visual explanation of the blockwise analysis

The filtering process is characterised by a blockwise serial approach for a full 3D analysis of a large dataset, basically compensating for lack of RAM memory with disk space. Generally speaking the Hessian part of the analysis is characterised by:
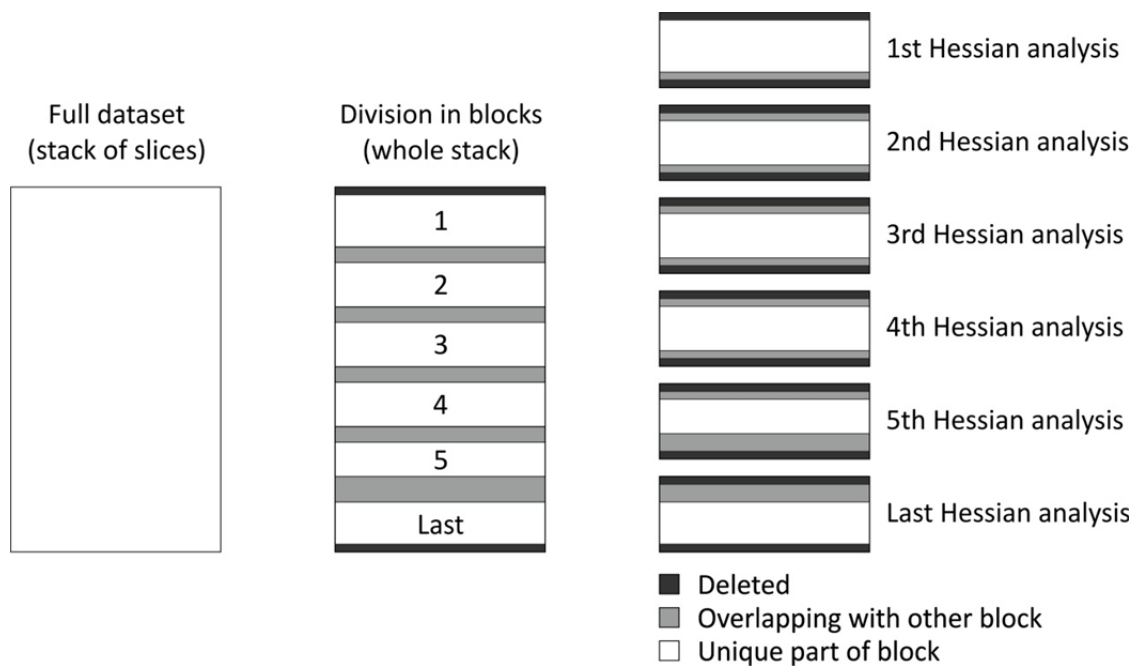
The number of deleted slices is set at 2x the maximum Gaussian scale calculated (s_{max}), corresponding to the significant range of influence of the Gaussian curve on the end result in this analysis. This ensures the combined output stack is fully 3D processed.

3.3 Optional parameters

- The following parameters are optional, but can improve the analysis:

Name/symbol	Parameter	Description	Section
avgmat	Average material greyscale	Greyscale value that characterises the material outside the fractures. Used for control lines.	3.3.1
consthresh	Conservative threshold of fractures	Greyscale value that estimates the greyscales present in the fractures. Used for control lines.	3.3.2
maxmat	Maximum material greyscale	Greyscale value that can be used to remove unusually bright spots in the dataset to prevent artefacts in the final output.	3.3.3
padding	Percentage of padding to be performed at start and end of input stack	First and last slices can be copied several times and included in the analysis. Extends the range of output slices but introduces "pseudo-3D" information.	3.3.4

3.3.1 Average material greyscale (avgmat)

- If not opened anymore, open the image stack in FIJI as in Section 3.2.1
- Use one of the area selection tools to select a region of average material greyscale, hence NOT including fractures or porosity.
- Press CTRL + M (or *Analyze → Measure*). A *Results* window opens.
- In the *Results* window, there is a column termed *Mean*.

NOTE: If the mean is not shown, one can turn this measurement on under *Analyze → Set Measurements* from the main window of FIJI. Check *Mean gray value*, and press OK.

- Repeat the procedure for different areas, at different positions in the image stack. New measurements are added to the list.
- After taking several (at least 10-20) measurements, determine an overall mean. This can be used as the average material greyscale (*avgmat*).
- Write down *avgmat* in the table at section 3.1.

NOTE: The main use of choosing an average material greyscale is for the control lines. The results of normalisation performed during the multiscale Hessian fracture filtering are therefore more realistic relative to the input data, and easier to visualise. However, with calibration steps performed afterwards, the actual influence of the average material greyscale on the result is limited. An exact value is therefore not required.

NOTE: For the Hessian analysis, only contrast is of interest. One can therefore also choose to set the *avgmat* to the greyscale difference of the material relative to the fractures, and keep the *consthresh* value at 0. This may also be useful when a dataset contains a lot of shading, where a true *avgmat* is difficult to define

NOTE: When no *avgmat* is specified, use the values 255 (8 bit data) or 65535 (16 bit data).

3.3.2 Conservative threshold for clear fractures (consthresh)
- If not opened anymore, open the image stack in FIJI as in Section 3.2.1.
- Press CTRL + SHIFT + A (or *Edit → Selection → Select none*) to ensure no selection is active.
- Go to *Image → Adjust → Threshold*. The following dialog appears:
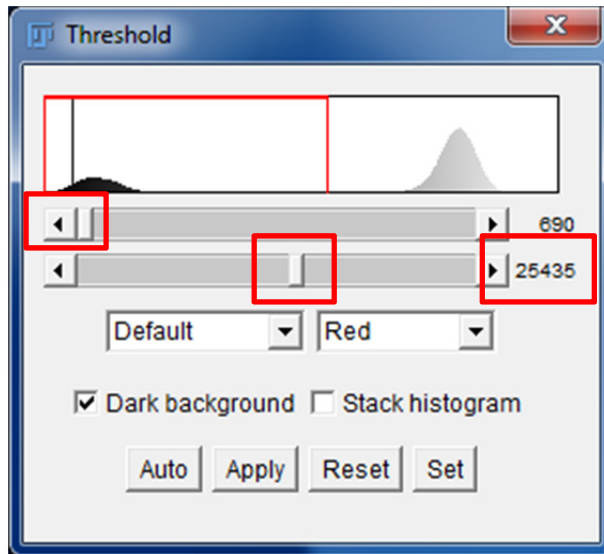
- In this dialog, one can find threshold values.
- Set the slider displayed at the top to its minimum.
- Shift the slider displayed at the bottom until a point is found where only the clearest fractures and open porosity are selected. Hence, this threshold should be very conservative.
- Test the chosen value throughout multiple locations in the image stack.
- When a proper setting is found, write down the value displayed next to the lower slider at *consthresh*, in the table at section 3.1.

NOTE: When not specifying a conservative threshold here, use the value 0 in further processing steps. Also use the value 0 when using a relative value for *avgmat* (see note in section 3.3.1).

NOTE: As for *avgmat*, the value used here is of main importance for the control lines and the normalising, and does therefore not have to be exact. However, this value can also be used in the calibration step, if one chooses to include a conservative threshold there. In that case, extra care for selecting a proper value (including no noise at all) should be taken here.

3.3.3 Maximum material greyscale, for removing bright spots (maxmat)

- One can find in a similar way as in section 3.3.2 a maximum material greyscale that can be set to remove bright spots in the data, which can otherwise introduce artefacts in the final outcome.
- Open the threshold dialog:
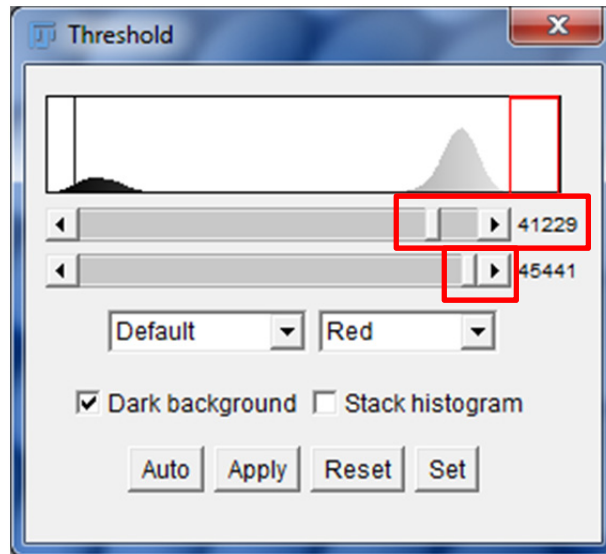
- Set the displayed at the top to its maximum.
- Shift the displayed at the bottom until only anomalously bright areas are selected.
- Test the chosen value throughout multiple locations in the image stack.
- When a proper setting is found, write down the value displayed next to the upper slider at *maxmat* in the table at section 3.1.
- During the multiscale Hessian fracture filtering, the greyvalues of the here selected areas are replaced by the *maxmat* value itself.
- Close FIJI.

NOTE: When no *maxmat* is specified, use the values 255 (8 bit data) or 65535 (16 bit data).

NOTE: Although artefacts due to bright spots are reduced by this approach, they can still appear. For data with a lot of bright areas, the multiscale Hessian fracture filter may not be suitable.

3.3.4 Padding

- *Padding* can be used to extend the range of output data. This reduces the amount of deleted slices at the start and end of the stack in the output data.
- When *padding* is turned on, the first and last slices of the full stack are copied several times.
- The amount of *padding* is set as a percentage of the overlap (=the amount of deleted slices at each end, so 2x s_{max}). 0% *padding* thus means no copied slices, 100% *padding* means the first and last slices are both copied (2x s_{max}) times.
- With an increasing *padding* setting, one increases the amount of "pseudo-3D" data output at the start and end of the stack. Therefore, to ensure 3D output, it is advised to set the *padding* to 0.

NOTE: It is best to leave the *padding* at 0% to ensure 3D output. On small stacks of data, one might however choose to turn *padding* on, to not delete a relatively large amount of the stack.

4. Selecting ROI and control lines settings

- Open FIJI.
- Drag and drop the macro MSHFF_Prep.ijm on the main FIJI screen (or open it manually in FIJI)
- Press *Run*. The following dialog box appears:
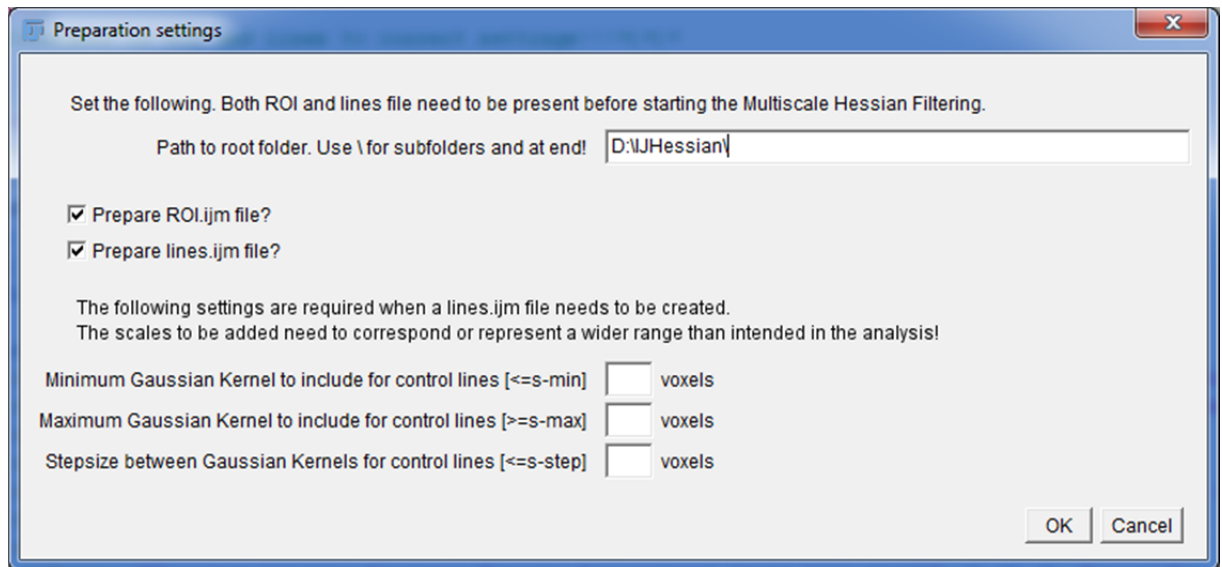
NOTE: When restarting the macro, previously chosen settings are NOT remembered.
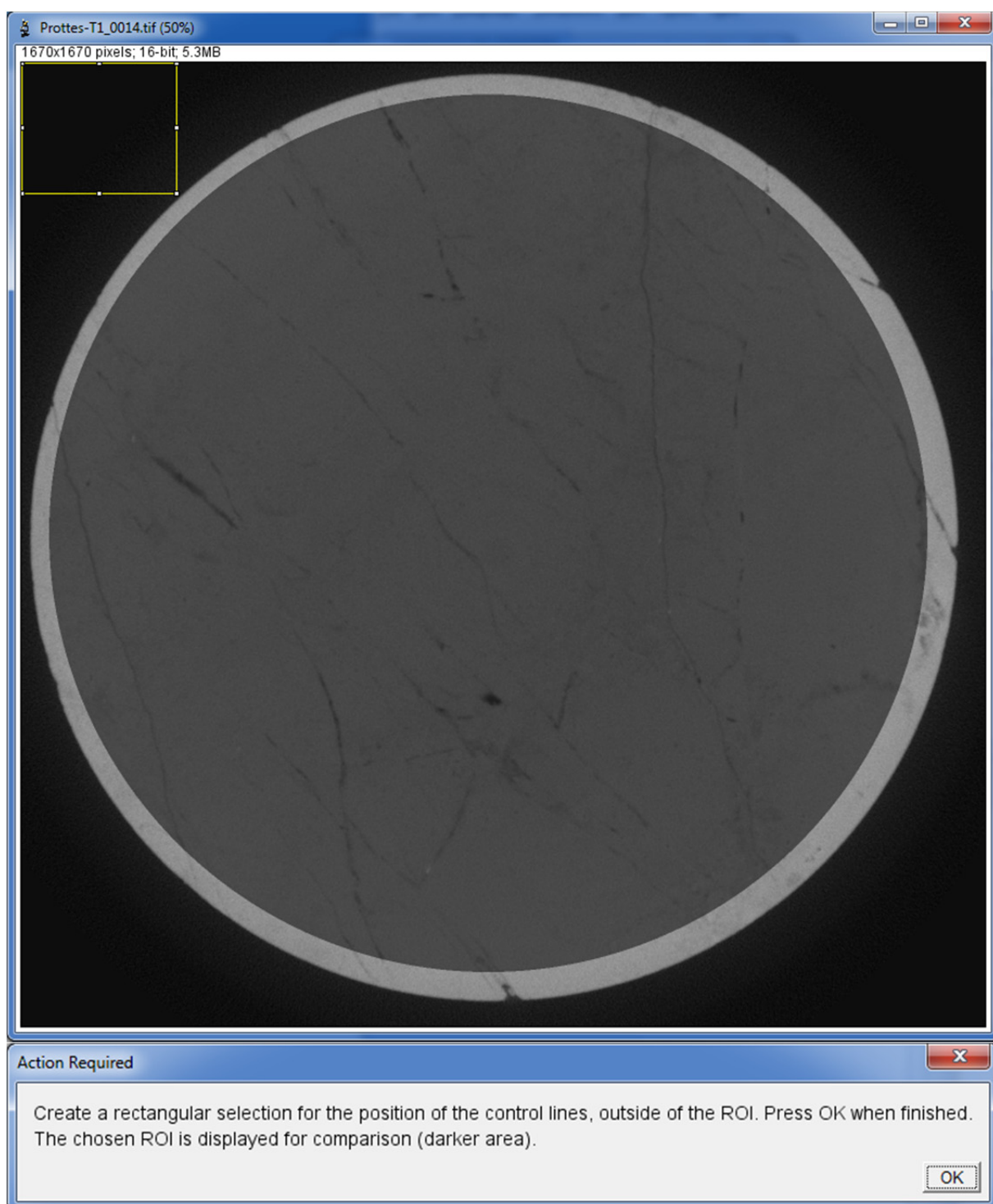
- Enter the *rootfolder* (hence, WITHOUT the *Input* folder). Be sure to put a " \ " at the end of the folder name!
- Leave the two options checked. Both files are required for the multiscale Hessian fracture filtering.
- Enter a minimum and maximum Gaussian smoothing scale, and the stepsize in between.

NOTE: The minimum needs to be equal or smaller than s_{min}, the maximum needs to be equal or larger than s_{max}, and the stepsize needs to be equal or an integer division of s_{step}. It is advised to keep the stepsize equal to s_{step}, and add two or more additional lines outside the range s_{min}-s_{max}.

- Press OK.
- The stack will open and a dialog box will appear.
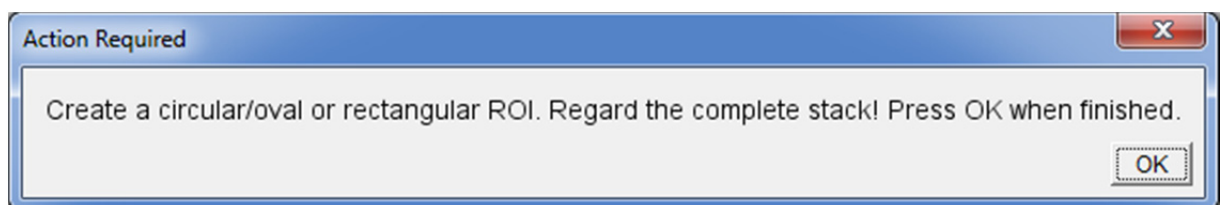
- Draw a suitable ROI (oval or rectangular using ; keep the SHIFT key pressed for a perfect circle or square). Check if the ROI is suitable throughout the whole stack. If one wants to include the most possible data, one should create the largest possible fitting ROI. Press OK.
- The selected ROI is indicated (dark) in an image and a new dialog box appears:

Prottes-T1_0014.tif (50%)

1670x1670 pixels; 16-bit; 5.3MB

Action Required

Create a rectangular selection for the position of the control lines, outside of the ROI. Press OK when finished. The chosen ROI is displayed for comparison (darker area).

OK

- Draw a rectangular selection outside the ROI. It should be sufficiently large for the control lines (example: in image above). The best position is at the left upper corner, and completely in this corner. Press OK.
- An example is shown, as well as a dialog box:
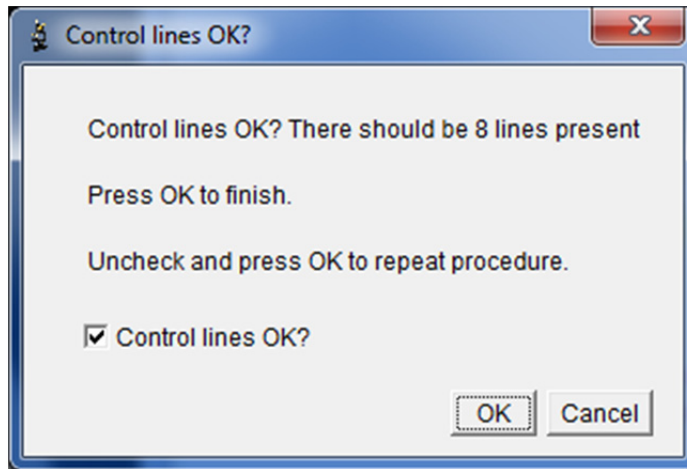
- The amount of control lines that should be present is indicated. Check if these are indeed present in the image. If all is well, leave the *Control lines OK?*-check on and press OK.
- If the choices are not correct, uncheck *Control lines OK?* and press OK. The control lines procedure will restart.
- Close FIJI. All preparation files are now created.

NOTE: The preparation of the input files itself is not yet done. This will be performed in the main multiscale Hessian fracture filtering step (see next section). The outside of the ROI will be set to NaN (not a number), to prevent any non-3D information to influence the end result. Note that due to this, the final ROI is somewhat smaller than the input one.

NOTE: If the control lines cannot be drawn outside the ROI, it is best to shrink the ROI, or to add an additional empty area to the input files first to make space for the control lines box.

NOTE: If the ROI is very small compared to the full images, it is advised to crop the input data first manually to a more reasonable size. This is for memory reasons. Since FeatureJ does not support calculations in a limited area only, applying the ROI does not mean the calculations are only performed inside this area. Every voxel, including all NaNs outside the ROI, require being loaded into the RAM memory in the main multiscale Hessian fracture filtering code. If cropping is applied, remember to also recalculate the *blocksize* and to redo this Section 4.

5. Running the multiscale Hessian fracture filter

- Open FIJI.
- Drag and drop the macro MSHFF.ijm on the main FIJI screen (or open it manually in FIJI)
- Press *Run*. The following dialog box appears:

NOTE: When restarting the macro, previously chosen settings are NOT remembered.

NOTE: Although the three separate parts of the analysis (Input files preparation, Hessian calculations, Combination of outputted scales) can be run separately, it is strongly advised to run all analyses at once. Although some checks are built in the code, there remains a possibility of erroneous output when options are unchecked. In some cases, it may be necessary to run part of the code separately (for example, if the combination of several scales needs to be altered afterwards). In that case, make sure the same settings as used in previous analysis steps are taken!

- Set the following parameters (Assumes a full analysis. Settings as entered in Section 3.1, but note that the order is different):
  - *rootfolder* (hence, WITHOUT the *Input* folder). Be sure to put a " \ " at the end of the folder name!
  - *avgmat*
  - *consthresh*
  - *maxmat*
  - *padding percentage*
  - *blocksize*
  - s_{min}
  - s_{max}
  - s_{step}
  - $uses_{min}$
  - $uses_{max}$
  - $uses_{step}$
- Press OK. The analysis will start.
- Several dialog boxes will appear to check some of the settings. This also includes warnings about possible non-empty folders. On severe errors in settings or input files, the macro will terminate and (usually) display the reason.
- A detailed log-file is created and shown on screen. This log-file is automatically saved in the *rootfolder*, including the chosen settings and the progress of the code.

NOTE: This main part of the multiscale Hessian fracture filtering can take very long and require a lot of HDD space, depending on the amount and size of the input files, the chosen settings, and of course the specifications of the computer. Several examples of runtimes are given in the paper, but with a very different range of settings possible, it is difficult to calculate the runtime in advance. If the analysis really seems to take too long, consider including less high Gaussian smoothing scales, or resampling the input data.

- When finished, close FIJI to clear the RAM memory.

FOLDERS: The *rootfolder* now contains the following subfolders (assuming the full process has been performed):
- *Input*:   Original input files.
- *InputHess*:   Altered input files with ROI applied and control lines added.
- *Calc*:   Normalised Hessian filtering results, per Gaussian smoothing scale.
                 This folder *could* be deleted for space reasons, if the results in the *Output* folder are satisfying.
- *Output*:   Combined Hessian scales, according to the chosen settings.

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6. Calibration

6.1 Background and required parameters

NOTE: Calibration is not required, but in many cases it is desirable. In this implementation, a cosine curve is applied on the data, and values between 0 and 100 are saved in 8 bit greyscale output files. This reflects the link to porosity for our research, and is hence tailored to this (see paper for more details).

- A curve similar to the following will be used for the calibration:


Cosine Calibration Curve (example)
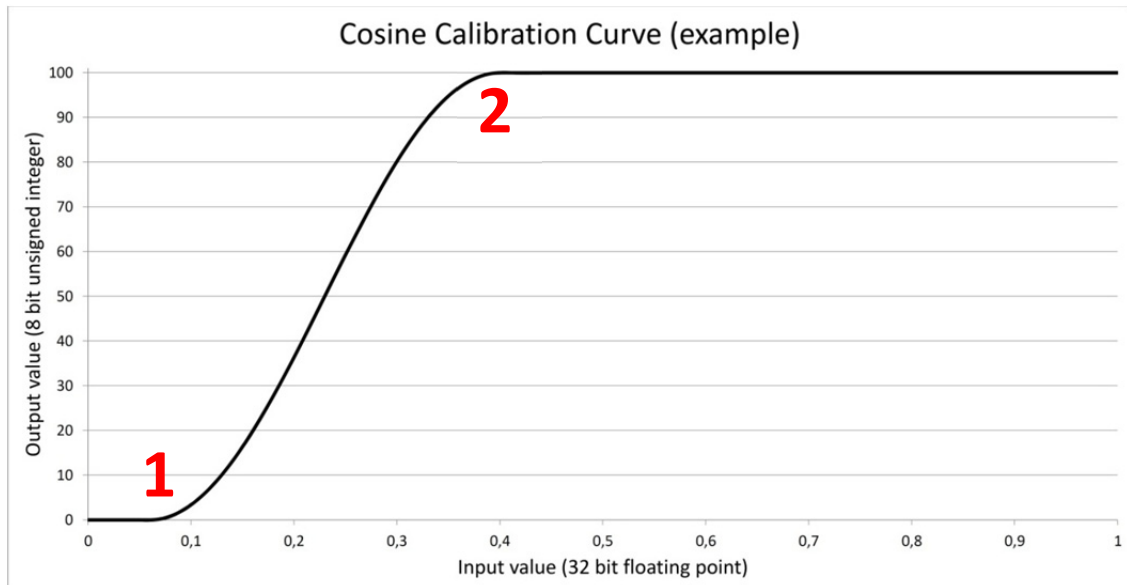
- As visible in the curve, the values from 0 to 1 in the 32 bit floating point data are replaced by output values between 0 and 100. The S-shaped curve (cosine from π to 2π) ensures softening at its start and end points.
- Two values are required:
  - 1) A lower cut-off for noise (*mincal*)
  - 2) An upper cut-off for "maximum porosity" (*maxcal*)
- The values are obtained when following the instructions below. Write them down in this table:

Parameter	Value (between 0 and 1)
1) *mincal*	
2) *maxcal*	

NOTE: The values require user input and introduce some ambiguity. There is no single answer on how to choose these values, and it is depending on the desired results. The following is an empirical approach to determine these values, which works rather well on the data we have.

- Open FIJI.
- Open the data from the *Output*-folder (see Section 3.2.1). Do not use a virtual stack. If there is not enough memory to open all data, open a smaller, but representative part of the stack.
- Go to *Process → Math → Multiply*.
- Type in 1000 and press OK. This makes the selection of the values easier.
- For 1:
  - Use the Threshold sliders as in Sections 3.3.2 and 3.3.3 (*Image → Adjust → Threshold…*) to find a cut-off value for the noise. This means, finding a value where noise levels are just yet tolerable (select the fractures plus a tolerable amount of noise). For example:
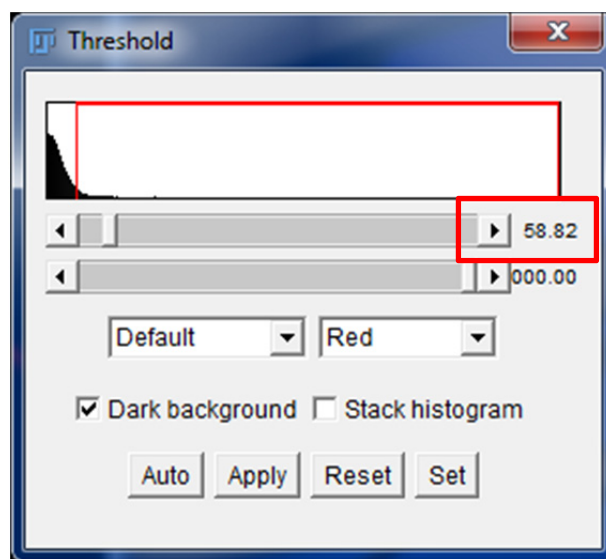

  - Note that due to the shape of the calibration curve, the effect is softened near this value, so even values slightly above the cut-off value will be outputted as very low (near 0 on a scale of 0-100).
  - Divide the found value again by 1000, and write this value down in the table at *mincal*.

- For 2:
  - Empirically, the "last threshold value before the lowest included Gaussian smoothing scale (uses$_{min}$) gets too wide" is a reasonable point for the upper cut-off value. Intensities above this value will all be set to fully porous in the calibrated output.

 - Use the zoom tool to zoom in at the control lines. For example:

  - In this example, 8 control lines from 1-8 were added, with a stepsize of 1. In the example output however, only scales 2-5 (uses$_{min}$-uses$_{max}$) were included. Hence, the lowest included Gaussian smoothing scale was 2, so this corresponding control line should be checked. This is indicated with the arrow.

- o Use the threshold slider as in Section 3.3.3 to fill up the control lines with red.
- o Continue until the control line of interest is just as wide as it should be. In the case of control line 2, which was originally 4 pixels wide, it should as well be 4 pixels wide.
- o For example, the following is <u>too wide</u> (note the little red pixel on the left bottom):
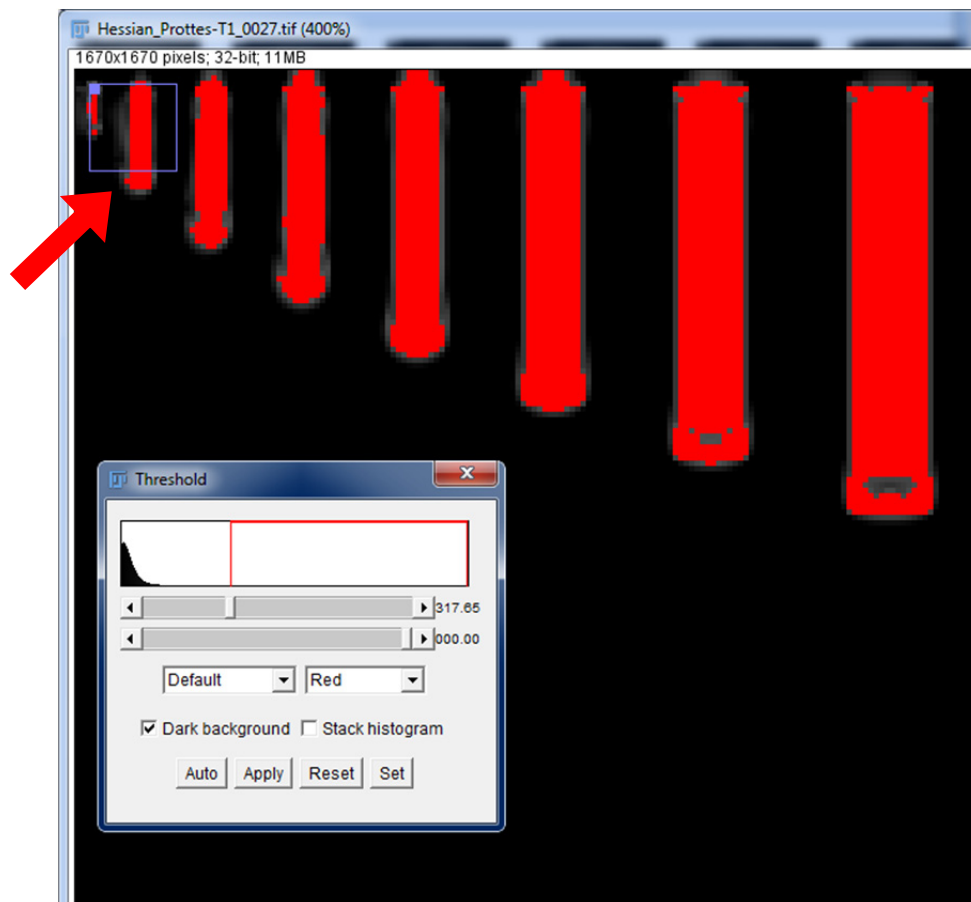
o Whereas the following (one threshold step further) is just correct:
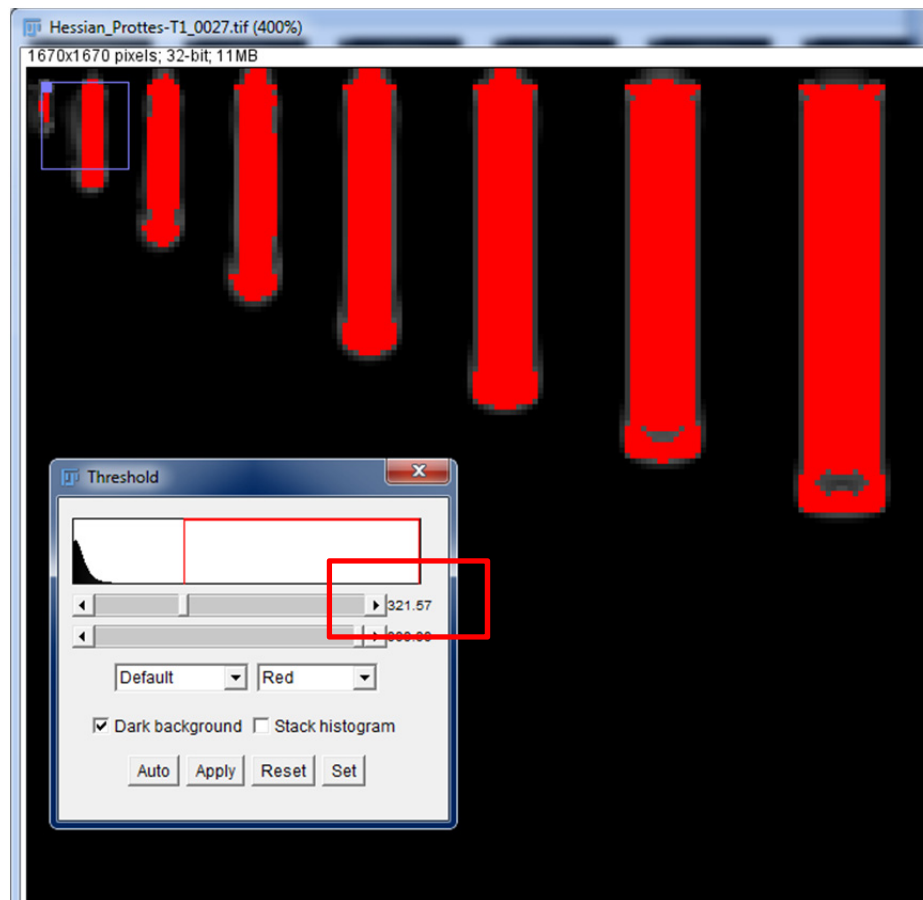
o In this case, one would thus find an upper cut-off of 321.
o Divide the found value again by 1000, and write this value down in the table as *maxcal*.
o Close FIJI.

NOTE: Especially for larger control lines, there may be some distortion at the end points. In that case, look mainly at the centre of the control line for determining the upper cut-off value.

6.2 Applying the calibration
- Open FIJI.
- Drag and drop the macro MSHFF_Cal.ijm on the main FIJI screen (or open it manually in FIJI)
- Press *Run*. The following dialog box appears:

NOTE: When restarting the macro, previously chosen settings are NOT remembered.

- Enter the *rootfolder*. Be sure to put a " \ " at the end of the folder name!
- Enter the found *mincal* and *maxcal* values.
- Enter the used maximum Gaussian smoothing scale ($uses_{max}$). This is required to shrink the ROI accordingly (to ensure 3D output).
- A conservative threshold can be added. If desired, leave the checkmark on and enter a suitable value. In most cases, one can use *consthresh*.
- The calibration can be tested. If this is desired, enter an amount of test slices, and where to start in the stack. The test is performed and displayed on screen. If not testing (i.e. processing of full dataset), set the number of test slices to 0.
- Press OK. The calibration is performed. The ROI (shrunk because of non-3D edge effects) is applied, setting the material outside the ROI to 0 as well. The control lines are removed too.
- A detailed log-file is created and shown on screen. This log-file is automatically saved in the *rootfolder*, including the chosen settings.
- Close FIJI after the calibration is finished.
- The calibrated output is saved in the folder *CosineCal*.

7. Connectivity filtering (in MATLAB®)

7.1 Background and required parameters

NOTE: The included MATLAB® script is provided "as-is". No additional information on the usage of MATLAB® will be given here. The script assumes all previous processing steps have been taken, including a calibration (i.e. input data must be 8 bit TIFF). Current implementation DOES require a lot of RAM memory and may hence not work on every dataset.

- A threshold is set to binarise the data for the use in MATLAB.
- The MATLAB® script looks for connected clusters in the z (consecutive slices) direction of the input data, using *bwconncomp*. All clusters are labelled.
- Only the clusters present in both the first and last slice of data are kept. These are connected throughout the stack.
- After only the fully connected clusters remain, the intensities from the calibration are taken again (instead of the binary intermediate step). Output files are saved.

- To find a suitable threshold, use the threshold slider in FIJI on the calibrated data. If any noise is left in the data, this should be excluded by setting a threshold higher than this noise.
- The lowest possible – and default – threshold value is here 0.9. This assumes, all calibrated output values of 1 to 100 are considered porous for the connectivity testing.

NOTE: In MATLAB®, the threshold value itself is NOT included in the selected material, whereas in ImageJ and FIJI, the threshold value IS included. This means for example on 8 bit data, a threshold of 0.9 in MATLAB® yields the same result as a threshold of 1 in ImageJ and FIJI (in both cases, all values above 1, including 1 itself, are selected).

7.2 Applying the connectivity filter
- Open the script MSHFF_Conn.m in MATLAB®.
- Enter the *rootfolder* (with forward slashes /, including one at the end) and the desired threshold (Section 7.1). For example:

```
rootfolder='D:/IJHessian/';
threshold = 1;
```

- Run the script (▶).
- The progress of the script is indicated.
- When the script is finished, MATLAB® can be closed.
- The connectivity filtered output files are saved in the subfolder *Connected*.

8. Binarisation
No comments are made here for the best way to binarise the data eventually, if this is desired. Many different approaches exist, and different ways can be suitable for different datasets. To apply a threshold, one can open a stack in FIJI, choose a threshold value (as done in previous sections), and press *Apply*. Go to *File → Save As → Image Sequence* to save the output manually. One can enter a name or use the names of the input files.