

## SAXS4COLL - User Guide (for Windows)

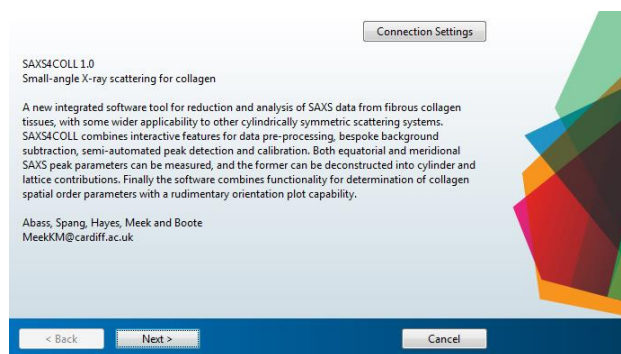
### 1. System and requirements

SAXS4COLL is a GUI-based software developed in MATLAB. It is supplied as a stand-alone executable which does not require MATLAB or its compiler to be installed on the computer. However, before processing, files should be converted to a standard high resolution image file format compatible with the imread.m function in MATLAB (e.g. TIFF, JPEG or PNG). The program requires Java - for smooth operation ensure Java is updated as necessary.

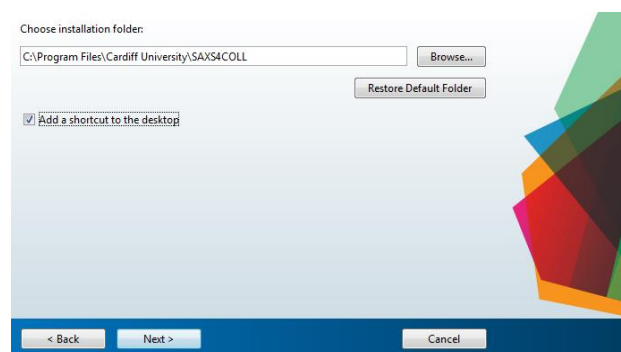
### 2. Installing/Uninstalling the program

To install SAXS4COLL:

- Double-click on executable file "SAXS4COLL.exe"
- Click "Next"

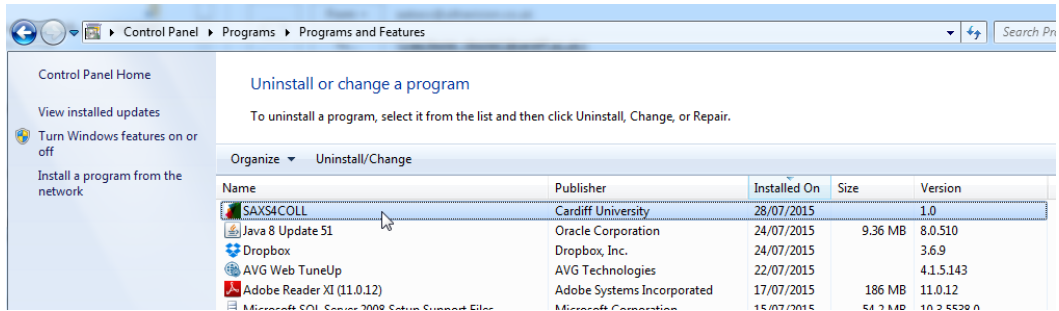


- Choose installation folder, and add desktop shortcut if required

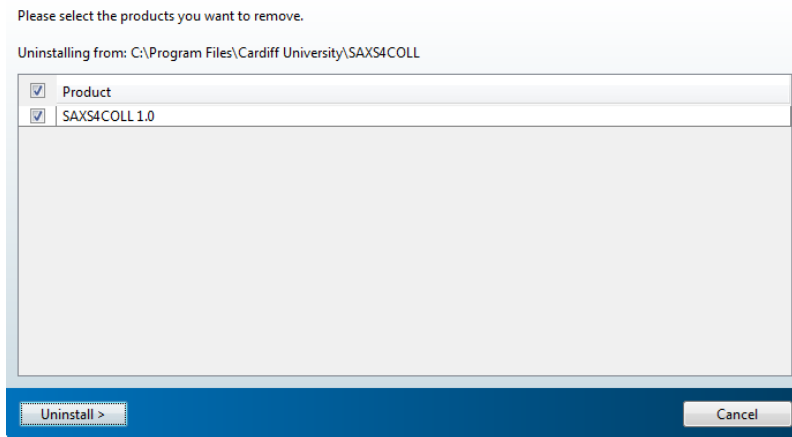


To uninstall SAXS4COLL:

- Open Control Panel
- Choose "Uninstall program"
- Select SAXS4COLL
- Click "Uninstall/Change"



- select products to remove and click "Uninstall"

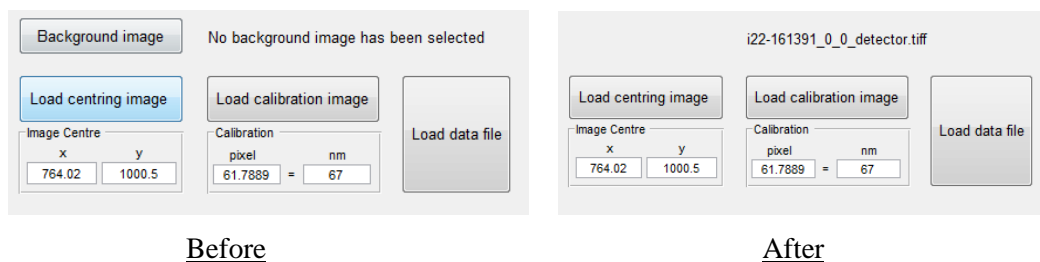


### 3. File input order

**Note that files should be inputted in the following order: Background image, Centring image, Calibration image, Data image.** This will ensure that all calibration and pre-processing is applied to the data image before return of the output collagen structure parameters.

### 4. Background image subtraction

A background image (e.g. empty specimen cell) can (optionally) be subtracted by clicking on the "Background image" button and navigating to the chosen background file. Once loaded, the background image will stay in the memory and be automatically subtracted from all data files subsequently inputted. The "Background image" button will also disappear and be replaced by the loaded image filename:



Before

After

## 5. Image Centring

Click on "Load centring image" button to load an appropriate image for finding the image centre for a particular experimental run (e.g. a powder SAXS pattern). The "Centring" (image) and "Figure navigation" windows will open:

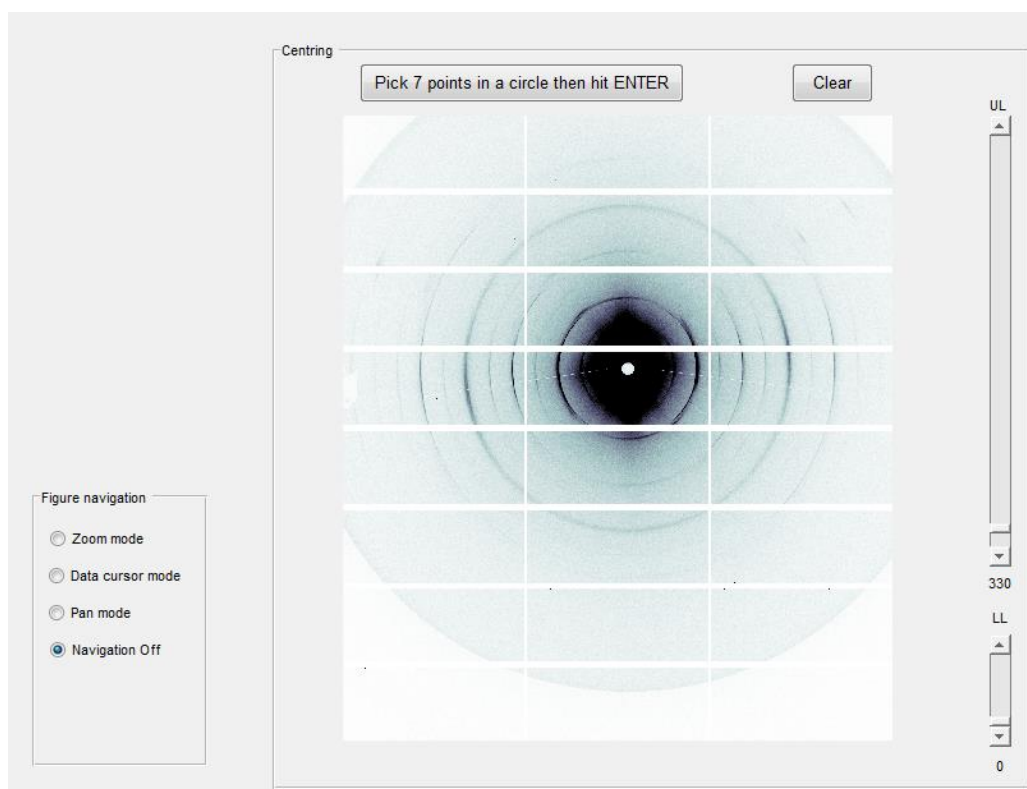


Figure navigation has 4 selectable modes:

1. Navigation Off.
2. Pan mode - left mouse click and hold to grab and move image in window.
3. Data cursor mode - left mouse click to see x,y coordinates and intensity ("Index") of selected points.
4. Zoom mode - left/right mouse click to zoom in/out or left click and hold to drag out a zoom window.

Image intensity thresholds can be adjusted with the upper limit ("UL") and lower limit ("LL") vertical sliders on the right hand side of the image window. The max/min values of all sliders can be adjusted by clicking on the "Adjust sliders" button and manually changing the values in the "Slider Adjustment" window that pops up:

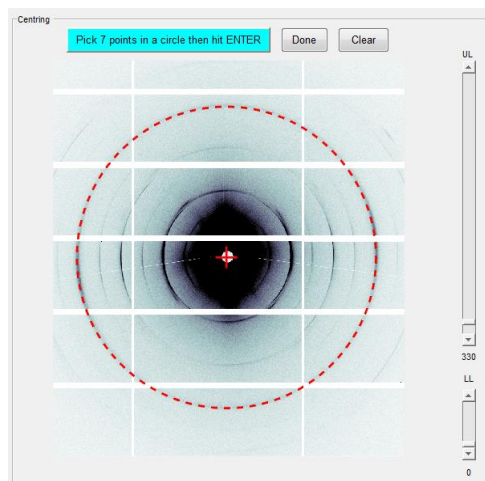
Slider Adjustment

	Slider	Slider
Ri	0	100
Rio	700	700
UL	100	1000000
LL	0	100
X	100	300
Y	0	1
UL2	1	1000

Update Sliders

Reset Sliders

Once navigation and thresholding are complete, follow instructions to choose (circle) points for centring. Click "done" to return to the main window. The image centre will be stored for all subsequent processing:





Load centring image

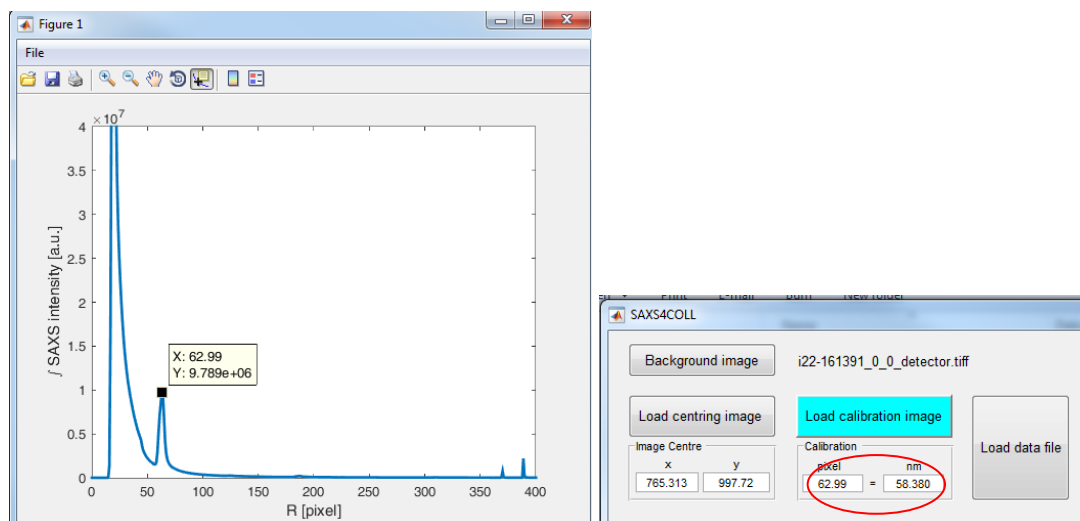
Image Centre

x	y
762.407	994.256

## 6. Calibration

Click on "Load calibration image" and select a calibration standard file (e.g. rat-tail tendon, silver behenate etc). A MATLAB image window will pop-up with the azimuth-integrated radial SAXS profile of the calibrant image. Use the zoom  and data cursor  icons of the MATLAB image window toolbar to select and measure an appropriate calibration peak.

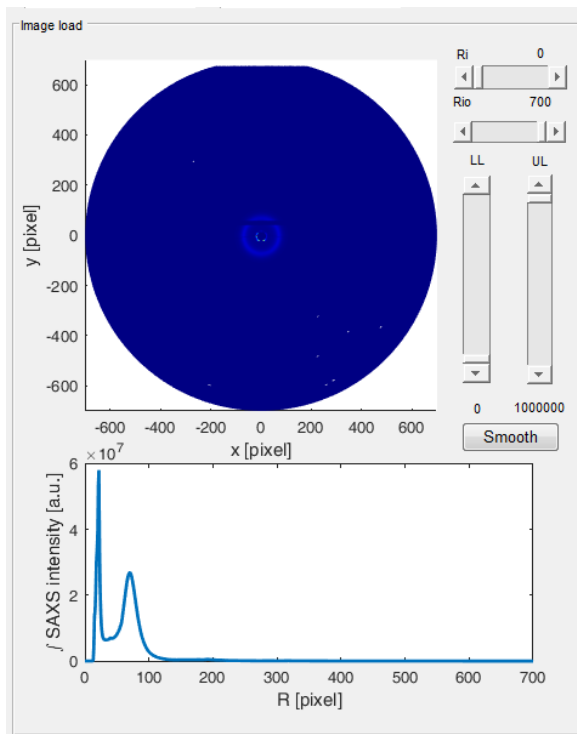
The corresponding pixel and spacing (in nm) can then be manually entered in the "Calibration" boxes in SAXS4COLL. For example, for the first order peak of silver behenate ( $d_{001} = 58.380\text{\AA}$ ) at pixel position 62.99:



Loading an image for calibration will not show the calibration image itself - the calibration image can be views by loading it as a data file.

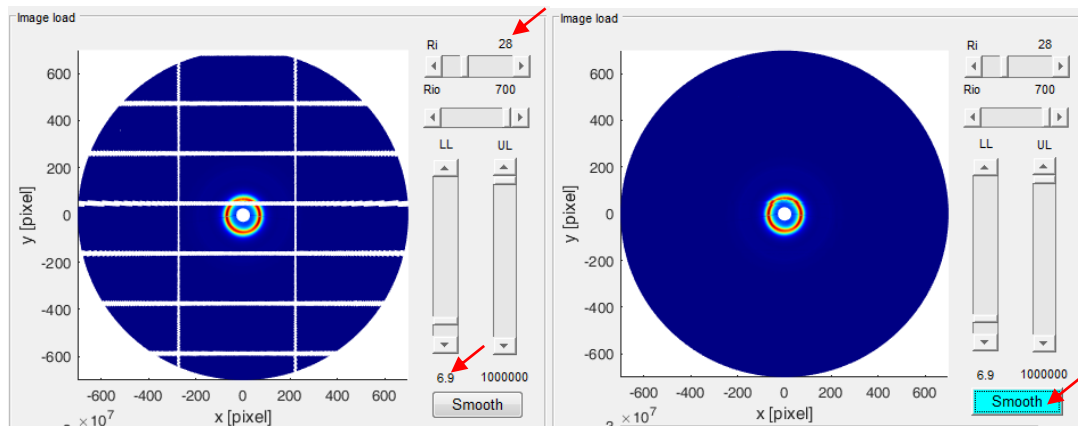
## 7. Loading data files

Data files are loaded by click the "Load data file" button and navigating to the chosen file. The "Image load", "Power fitting" and "Figure navigation" windows will open and the data load button will then display "Next image". The Image load window will display the data image after background image removal (if chosen), with the (azimuth integrated) radial SAXS profile underneath:



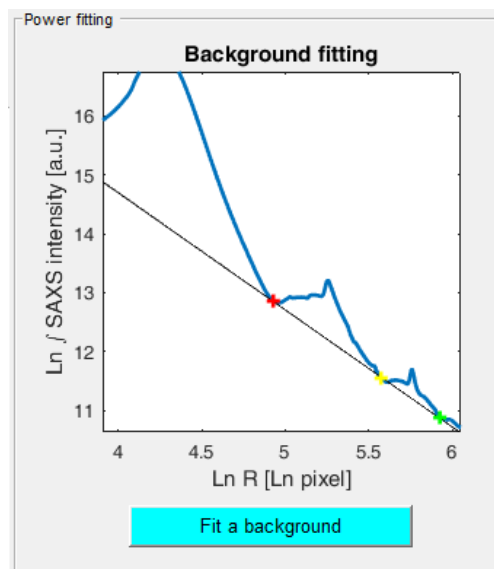
## 8. Selection of data, outlier removal and recovery of missing data.

The inner and outer limits of the pattern can be set with the Ri and Rio sliders. For example Ri can be set to mask out the beamstop area, as shown in the left panel of the figure below (Ri has been increased to 28). Outlier data points, such as beam-stop flare and "dead pixels" from detector module gaps, can be excluded from the processing by adjusting the lower limit (LL) and upper limit (UL) intensity sliders. For example in the left panel of the figure below the LL slider has been increased to 6.9 to re-set the detector gap regions to zero (white colour). To optionally recover missing data (e.g. detector module gaps) click the "Smooth" button to invoke a combination of cubic surface interpolation/pattern symmetry enforcement. The result of image smoothing is shown in the right panel of the figure below:



## 9. Background fitting

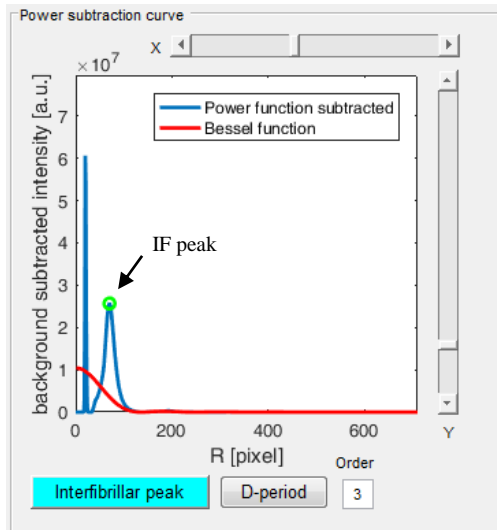
The "Power fitting" window shows the (azimuth integrated) radial SAXS profile displayed as a double-logarithmic plot. To fit a bespoke power-law back-ground, click on the "Fit a background button" and then select 3 background points for the linear fit using the cursor arrow. The zoom feature of the "Figure navigation" window may be used to aid the selection and the program will automatically detect the nearest data point to the selected position. Once 3 points are selected the program will show the fitted background line and open the "Power subtraction curve" window. The process can be repeated/re-started by re-clicking the "Fit a background" button at any time.



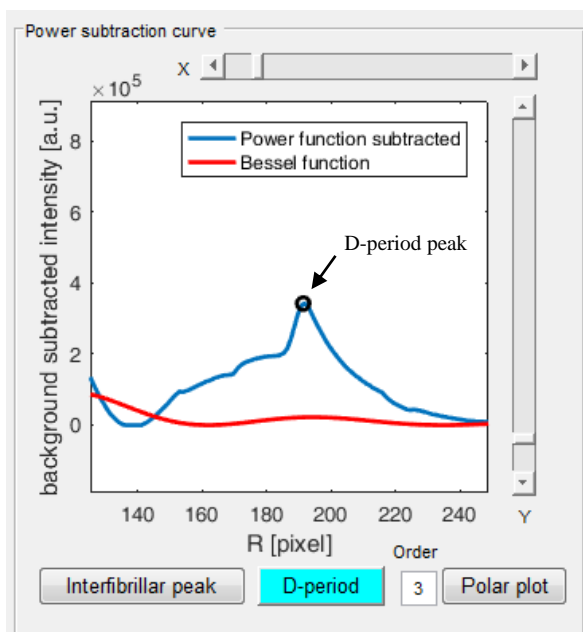
## 10. Peak detection (uncompensated)

The "Power subtraction curve" window shows the radial SAXS data after background subtraction alongside the fitted fibril transform ("Bessel function"). The interference function

peak before compensated by Bessel division, is selected by clicking the "Interfibrillar peak" button. Use the cursor arrow to select the interference function peak - the program will automatically detect the data point closest to the selected point. Use Figure navigation to zoom in if necessary. The selected IF peak position will be indicated by a green circle:



Click the "D-period" button to select the meridional D-period peak in a similar fashion, inputting the order number (1, 2, 3 etc....) in the adjacent box. The detected D-period peak will be indicated by the black circle:



Upon selecting the D-period, the "Interference function window" will be opened - showing the isolated interference function (i.e. the SAXS profile after division by the fibril transform) - from which the Bessel-compensated IF peak can be selected after fitting of the fibril

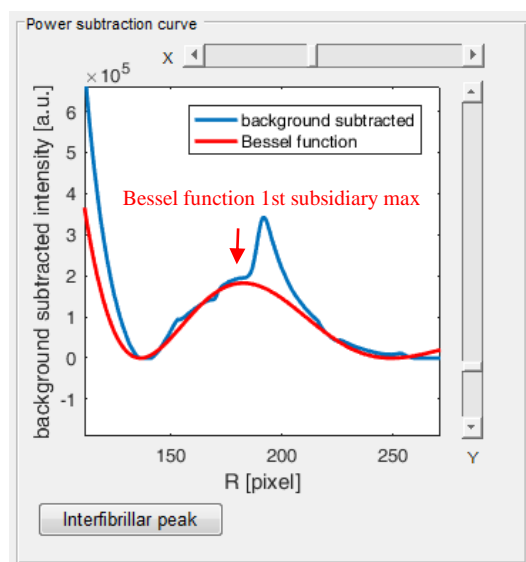


transform. Also, the calibrated values for the (uncompensated) IF peak position (average fibril spacing), IF peak Height/HHW (fibrillar spatial order), axial D-period and average fibril diameter will be shown in the "Uncompensated" parameter window:

Uncompensated	
Interfibrillar Peak (nm)	59.1
Height/HHW (a.u./nm*10 <sup>6</sup> )	4.3
D Period (nm)	64.9
Fibril Diameter (nm)	36.867

## 11. Fibril transform fitting

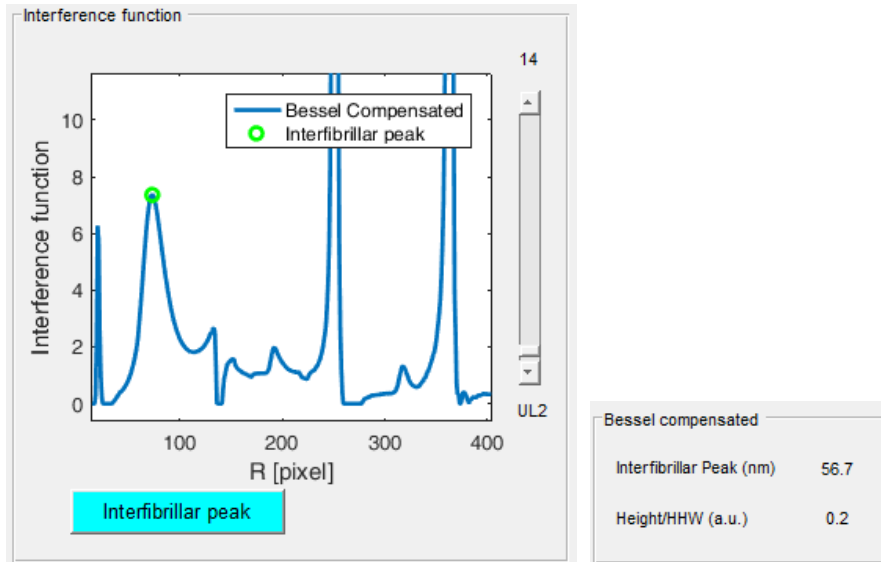
Use the X and Y sliders to set the position and amplitude of the Bessel curve for the best fit to the (background subtracted) equatorial SAXS data - if necessary ignoring any meridional diffraction peaks. Use the Figure navigation to zoom in and ideally choose a region where the equatorial SAXS profile has only the contribution from the fibril transform. In the example below (from corneal collagen) the first subsidiary maximum peak of the Bessel function is fitted, as the interference function contribution is negligible at this point, while the (sharper) 3rd order meridional peak is ignored:



The "Interference function" window will be updated automatically.

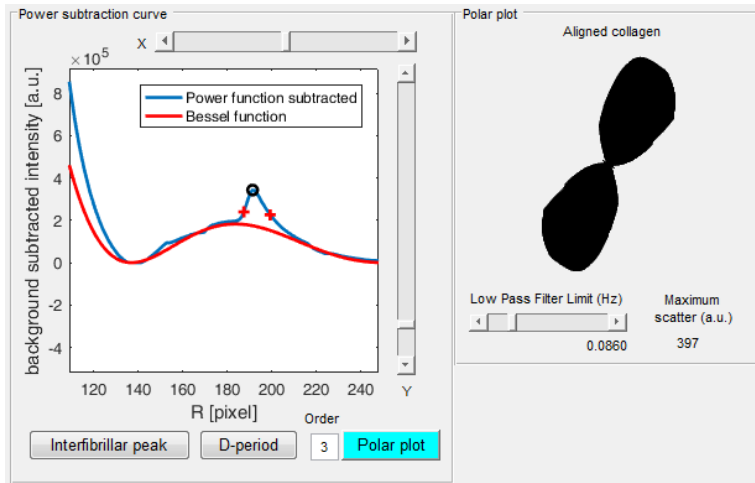
## 12. IF peak detection (Bessel-compensated)

In the "Interference function" window, use the UL2 slider and Figure navigation to clearly visualise the IF peak and then click on the "Interfibrillar peak" button. Use the cursor arrow to select the IF peak. The program will again detect the nearest data-point to the selected point. The Bessel-compensated IF peak will be indicated by a green circle and the updated interfibrillar distance and H/HHW values displayed in the "Bessel compensates" parameter window:



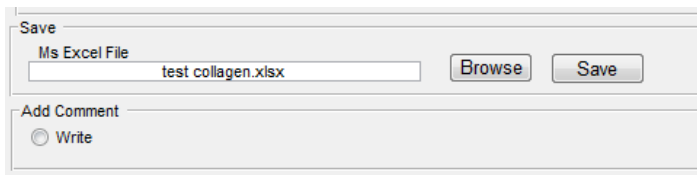
### 13. Collagen orientation (polar) plot

Click on the "Polar plot" button of the "Power subtraction curve" window to produce a polar vector plot of preferential collagen fibril orientation. Use the cursor arrow to select the radial limits of an appropriate peak for integration (in the example below the 3rd order meridional peak is used) - the chosen points will be shown by red crosses. The azimuth profile of the selected (radially integrated) peak will be displayed in polar coordinates in the "Polar plot" window of the GUI and also shown in a pop-out MATLAB figure window. The latter will automatically be saved as a TIFF file and can also be manually saved in any other MATLAB-compatible format. Use the "Low Pass Filter Limit" to select the desired degree of smoothing of the plot (the plot will be automatically updated). The polar plot data will be automatically saved to an Excel spreadsheet named polar\_{Excel save name}.



#### 14. Exporting results to Excel

In the "Save" window click "Browse" to navigate to the save location and set the filename for the Excel export file. An optional comment can be added by checking the "Write" button of the "Add comment" window:



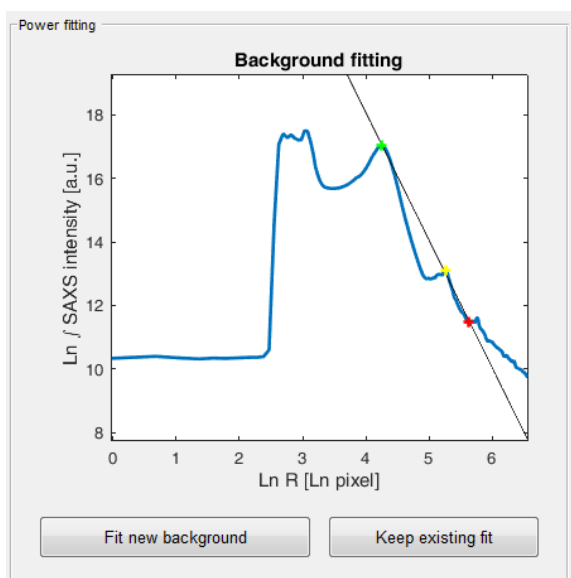
Click "Save" to save the image ID, collagen structure parameters and optional comment to the .xlsx file:

1	2	3	4	5	6	7	8	9
Image ID	Interfibrillar Peak (nm)	Fibril Diameter (nm)	D Period (nm)	Ratio of hBC Interfibrillar	BC Ratio of	Comment		
i22-16139	59.1	36.867	64.9	4.3	56.7	0.2	No comment	

In addition, on completion of processing a jpeg screen capture of the GUI display for each image will automatically be saved in the working directory as a visual record all processing steps and outputted structure parameters.

#### 15. Batch processing mode.

Rapid processing of similar image files in a common directory can be achieved using the semi-automated batch processing feature, which is the default status of the program (cancel the current batch with the “Clear” button). Clicking the “Next image” button will load the next batch image in a directory with the same background image subtraction and interactive background fitting points selected. The background fit can be kept or modified as desired:



Following background fitting/subtraction the remaining processing will proceed as normal with the default starting parameters of the Bessel function kept from the previous image. Unless the Excel save name is changed by the user, polar plot and structure parameter data from images in a given batch will be written into common spreadsheets. A new batch is started once the “Clear” button is clicked.