Image Analysis – Overview of Analysis Code

3. Crust Analysis

X a, Crust

1. Image Prep

X a, Hole

2. Global Analysis

X a, Global

4. Pore Analysis

X a, Particle

X a, Add to all

Start

1. Image Prep
- Saves the image in a new folder for results
- Transform image to 8 bit
- Local-Threshold [minimum] and analyse, to select the slice outline
- Use outline to crop out the background
- Threshold [Bernsen] the cropped image, plus use MorphotLibJ plug-in to fill holes and kill boarders (fill bubbles thresholder into rings and half in crust), to prepare for analysis

Is the product a ring? Yes / No

- Use MorphotLibJ plug-in to identify the largest area (the hole) and measure.
- Use MorphotLibJ plug-un to remove the largest area

Uses Original image

2. Global Analysis
- Measure properties of the thresholded image, within slice outline
- Produce heat map by applying a mean filter to the thresholded image
- Produce Contour map by thresholding filtered image at set values, analysing, using BAR plug-in to produce contours of intensity.

Uses Thresholded image

Lighter = more bubbles = higher porosity = hotter

3. Crust Analysis
- Split the Blue intensity image from the original
- Local Threshold [contrast] to highlight the crust
- Analyse partials with a high minimum (20,000 sq.pixels)

Dark brown [crust] has more blue than light brown [crumb]

This will only measure the large crust area

4. Pore Analysis
- Run particle analysis
- Save results to the large, all results, document using the Read_And_Write_Excel plug-in
- Save colour maps and distribution plots with the BAR plug-in
- Run Granulometry to 20 pixel radius
- Close all open windows

End / Move on to next image

Saves all results to one document, side by side, on the desktop

Repeating code running ROI-color-coder and distribution plotter for area, circularity, ferret length and ferret angle

Image Analysis (Fiji)
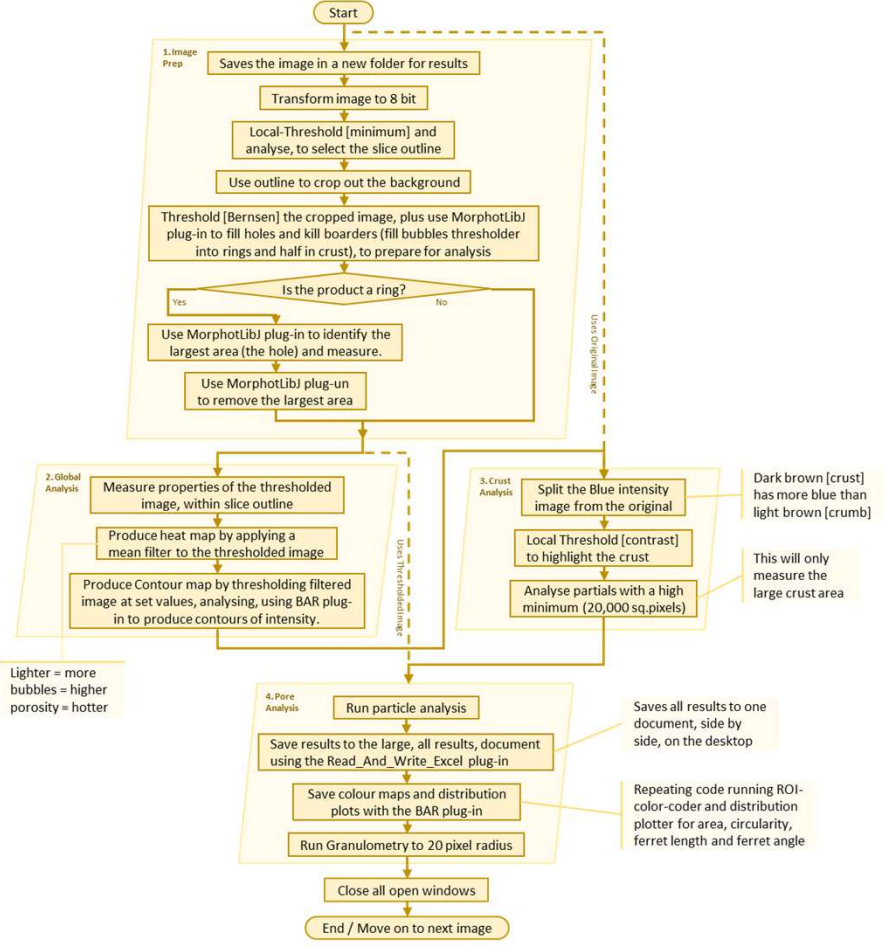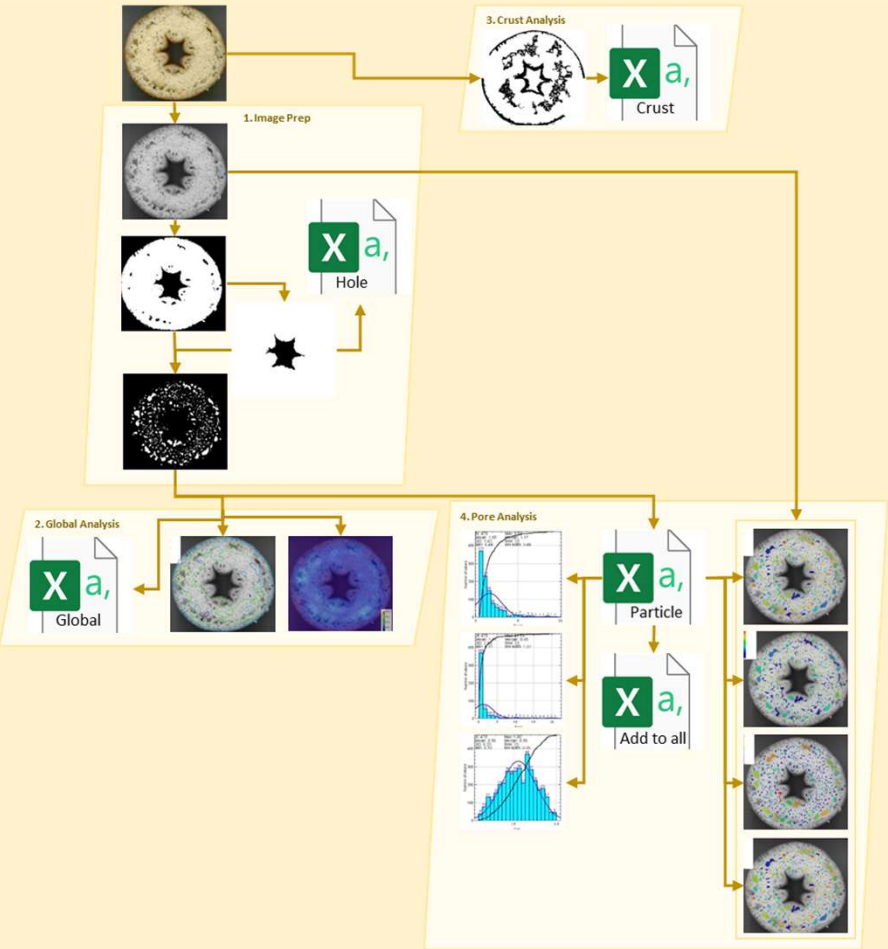
Code available at: https://lunet-my.sharepoint.com/:f:/r/personal/mmct_lunet_lboro_ac_uk/Documents/2021%20Internship%20-%20B%20Sargeant/Completed%20Macro_Bread_Analysis?csf=1&web=1&e=Dbk3m5
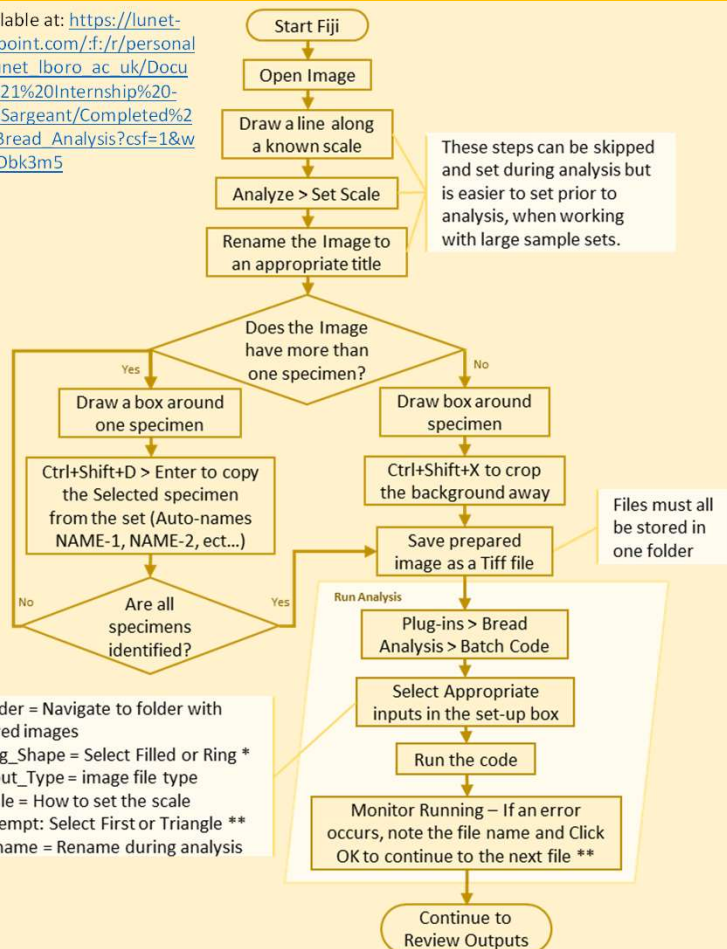
Required Add-ons - As well as downloading Fiji, additional plug-ins are required:
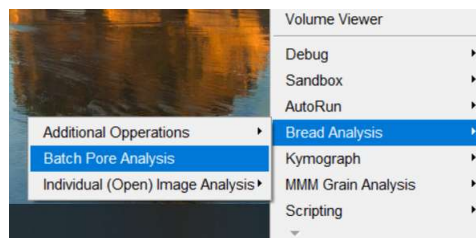- **Install Bread Analysis Macros:** Copy the whole Complete Bread Analysis Marco folder into the local Fiji program files (ie. C:\Program Files\Fiji.app\plugins) > Restart Fiji
- Activate the **Bar**, **MorphotLibJ** and **Results_to_Excel optional plug-ins**. Open Fiji > Help > Updates > Manage update Sites > Check BAR, IJPB-plugins and ResultsToExcel > Close > Apply Changes > Close > Restart Fiji

Flowchart:
- Start Fiji
- Open Image
- Draw a line along a known scale
- Analyze > Set Scale
- Rename the Image to an appropriate title

These steps can be skipped and set during analysis but is easier to set prior to analysis, when working with large sample sets.

- Does the Image have more than one specimen?
  - Yes: Draw a box around one specimen → Ctrl+Shift+D > Enter to copy the Selected specimen from the set (Auto-names NAME-1, NAME-2, ect...)
    - Are all specimens identified?
      - No → (loop back)
      - Yes →
  - No: Draw box around specimen → Ctrl+Shift+X to crop the background away
- Save prepared image as a Tiff file

Files must all be stored in one folder

Run Analysis
- Plug-ins > Bread Analysis > Batch Code
- Select Appropriate inputs in the set-up box
- Run the code
- Monitor Running – If an error occurs, note the file name and Click OK to continue to the next file **
- Continue to Review Outputs

Folder = Navigate to folder with saved images
Ring_Shape = Select Filled or Ring *
Input_Type = image file type
Scale = How to set the scale
Attempt: Select First or Triangle **
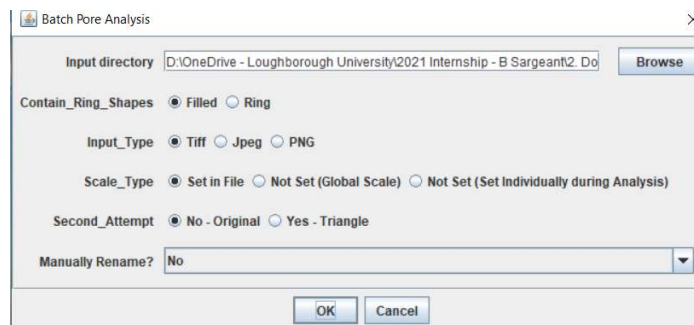Rename = Rename during analysis

Test 5 Power 0.5 Location 1+2.jpg
(1000x758 pixels)

T5_Pw0-5_Loc_1+2.tif
(80x96 mm)

It is optional to run this code as a batch process (folder), individually (open in Fiji and run *_All macro), or run analysis step-by-step (open in Fiji and run *0, *1, *2, *3, *4).
* All variations of the code can be ran – Normal or Ring, and Original or Triangle.

Menu:
- Volume Viewer
- Debug
- Sandbox
- AutoRun
- Additional Opperations
- Batch Pore Analysis
- Individual (Open) Image Analysis
- Bread Analysis
- Kymograph
- MMM Grain Analysis
- Scripting

Batch Pore Analysis
- Input directory: D:\OneDrive - Loughborough University\2021 Internship - B Sargeant\2. Do | Browse
- Contain_Ring_Shapes: ● Filled ○ Ring
- Input_Type: ● Tiff ○ Jpeg ○ PNG
- Scale_Type: ● Set in File ○ Not Set (Global Scale) ○ Not Set (Set Individually during Analysis)
- Second_Attempt: ● No - Original ○ Yes - Triangle
- Manually Rename? No
- OK | Cancel

* Ring setting will measure the middle hole and them remove it from the analysis.
** Triangle Analysis will use the Triangle thresholding method, instead of the original Minimum thresholding method. This is a common method to successfully run analysis on images that produce and error during the first run
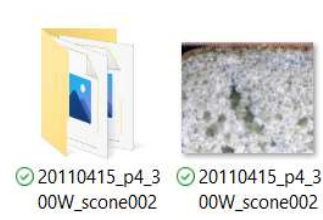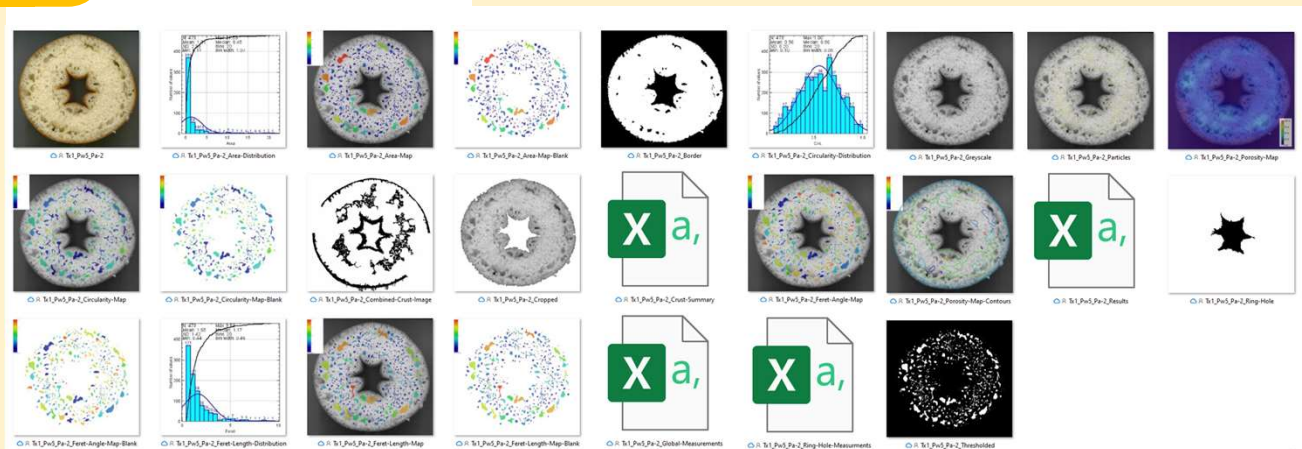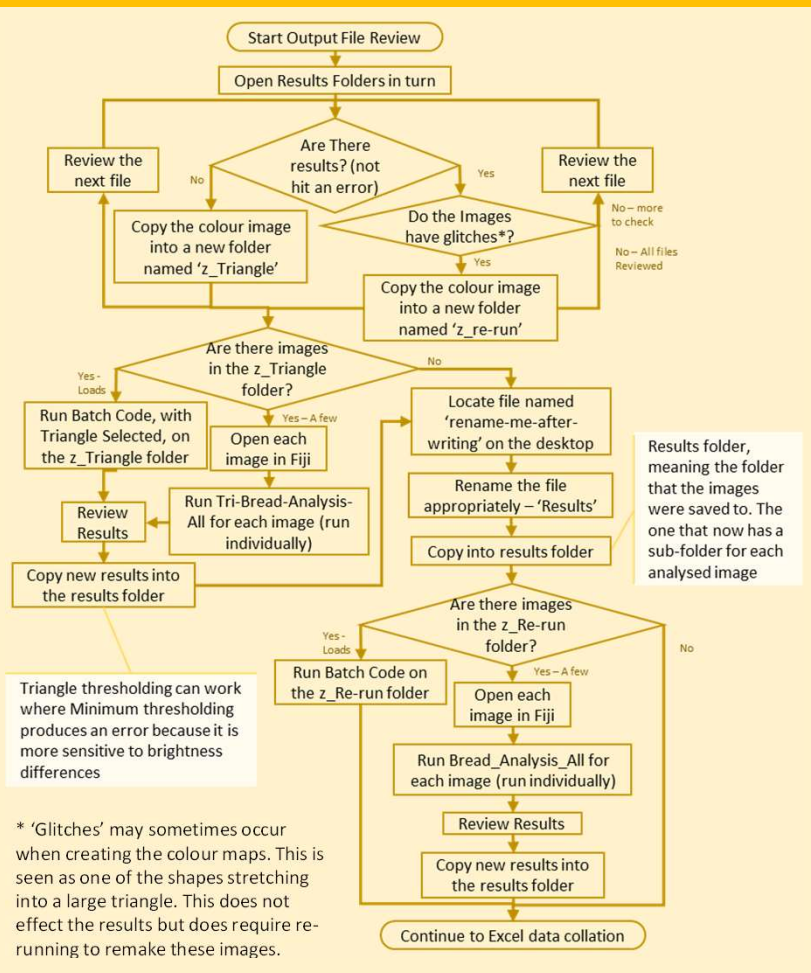
Image Analysis - Output

Once Analysis has been completed, the folder with the test images will be filled with sub-folders. Each subfolder is contains the results for one image and is named after that image.
All results are also saved to one document, in the Desktop. This must be coped to this folder before data analysis

20110415_p4_300W_scone002   20110415_p4_300W_scone002

Flowchart

Start Output File Review

Open Results Folders in turn

Are There results? (not hit an error)
- No → Review the next file
- Yes → Review the next file

No – more to check / No – All files Reviewed

Do the Images have glitches*?
- Yes → Copy the colour image into a new folder named 'z_re-run'
- No → Copy the colour image into a new folder named 'z_Triangle'

Are there images in the z_Triangle folder?
- Yes - Loads → Run Batch Code, with Triangle Selected, on the z_Triangle folder
- Yes – A few → Open each image in Fiji → Run Tri-Bread-Analysis-All for each image (run individually)
- No

Review Results → Copy new results into the results folder

Triangle thresholding can work where Minimum thresholding produces an error because it is more sensitive to brightness differences

Locate file named 'rename-me-after-writing' on the desktop → Rename the file appropriately – 'Results' → Copy into results folder

Results folder, meaning the folder that the images were saved to. The one that now has a sub-folder for each analysed image

Are there images in the z_Re-run folder?
- Yes - Loads → Run Batch Code on the z_Re-run folder
- Yes – A few → Open each image in Fiji → Run Bread_Analysis_All for each image (run individually)
- No

Review Results → Copy new results into the results folder → Continue to Excel data collation

* 'Glitches' may sometimes occur when creating the colour maps. This is seen as one of the shapes stretching into a large triangle. This does not effect the results but does require re-running to remake these images.

Legend

NAME: Original File Input
*_Area-Distribution: Plot of Frequency against Area
*_Area-Map: Superposed colour map denoting the area of each pore
*_Area-Map-Blank: Image of pores, with colour representing the Area
*_Border: Image with Minimum Thresholding applied, used to identify the outer perimeter
*_Circularity-Distribution: Plot of Frequency against Circularity
*_Circularity-Map: Superposed colour map denoting Circularity
*_Circularity-Map-Blank: Image pores, with colour representing Area
*_Cropped: 8bit image with the outer perimeter (*_Border) used to crop the background
*_Crust: Image after isolating the blue intensity and contrast local threshold. This should show the crust of the product
*_Crust-Summary: Table of *_Crust average measurements – minor axis best represents crust thickness
*_Feret-Angle-Map: Superposed colour map denoting the Feret's Angle
*_Feret-Angle-Map-Blank: Image of pores, with colour representing Feret's Angle

*_Feret-Length-Distribution: Plot of Frequency against Feret's Length
*_Feret-Length-Map: Superposed colour map denoting Feret's Length
*_Feret-Length-Map-Blank: Image pores, with colour representing Feret's Length
*_Global-Measurements: Table of measurements recoded from the whole of the slice (withing perimeter identified by *_Border)
*_Greyscale: 8bit image
*_Particles: Superposed outlines of identified pores
*_Porosity-Map: Heat map of the global porosity
*_Porosity-Map-Contours: Map showing contours of global porosity
*_Results: Table of measurements taken from each pore. These results were also copied into one document, saved to the desktop, collating all results in one file.
*_Thresholded: Image after Bernsen Local Thresholding, 'kill borders' and 'fill holes' from MorphotLibJ Plug-in
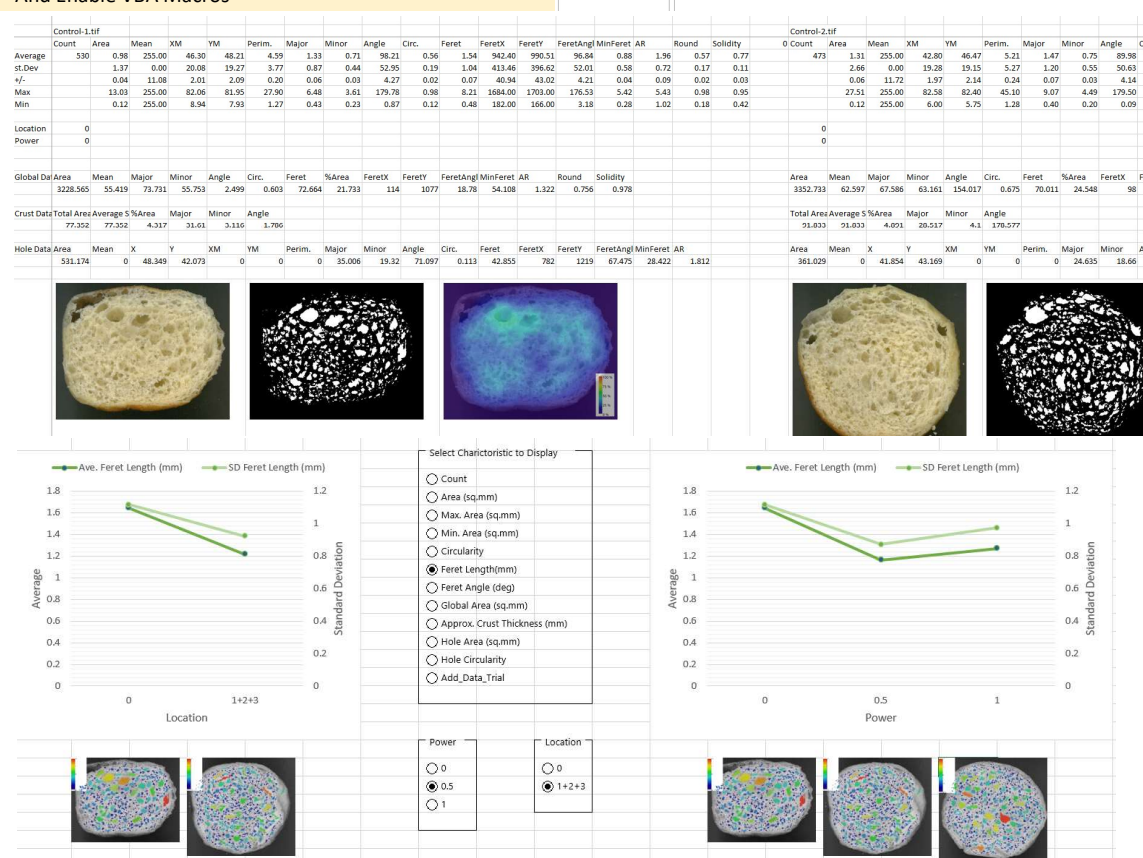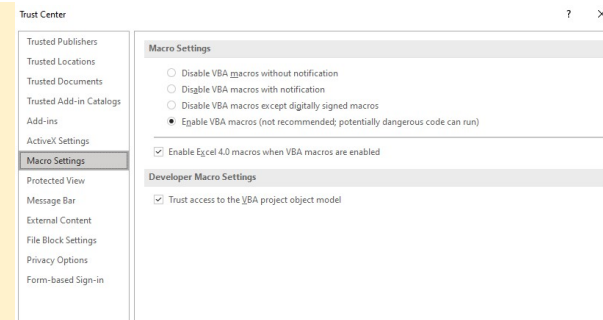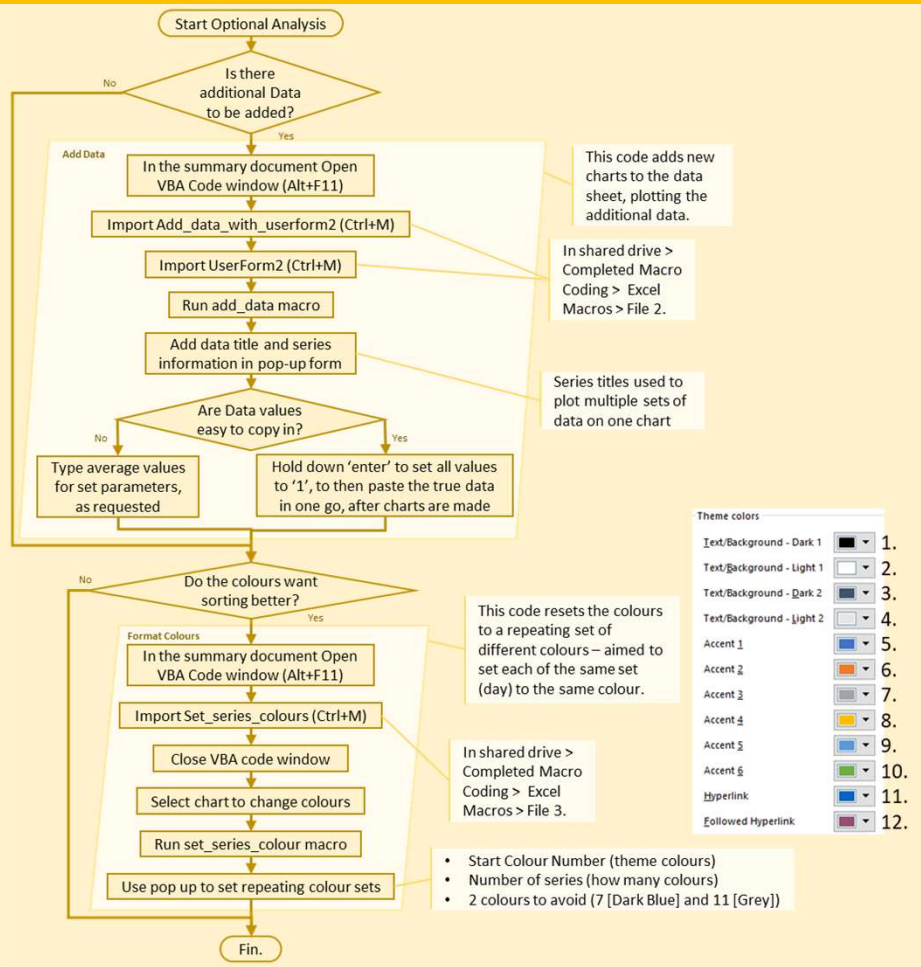
Data Analysis (Excel)
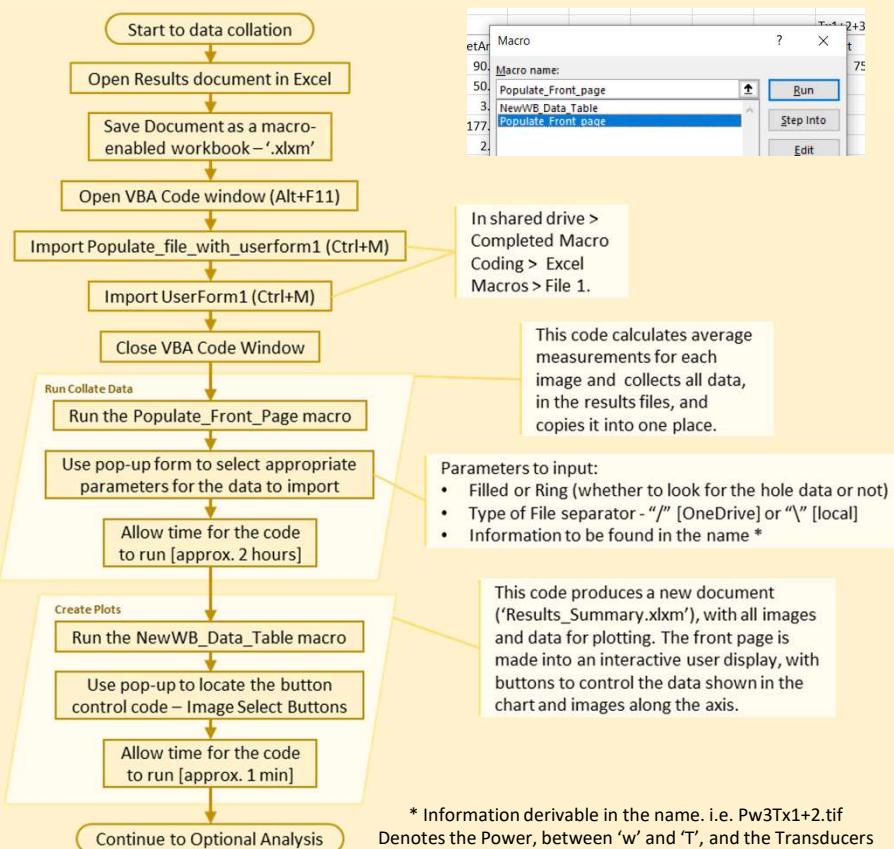
Requirements
- Results Document must be saved as a macro-enabled ("xlsm") file.
- Save file in the results folder, with individual results in the subfolders
- Add the Developer tab to the menu, or access VB code window using Alt + F11.
- Set Trust Centre to allow macros and VBA objects:
  - Open Excel > File > Options > Trust Centre > Trust Center Settings… > Macro Settings
  - Ensure the Check box "Trust access to the VBA project object model" is checked
  - And Enable VBA Macros

Flowchart:

- Start to data collation
- Open Results document in Excel
- Save Document as a macro-enabled workbook – '.xlsm'
- Open VBA Code window (Alt+F11)
- Import Populate_file_with_userform1 (Ctrl+M)
- Import UserForm1 (Ctrl+M)
- Close VBA Code Window

In shared drive > Completed Macro Coding > Excel Macros > File 1.

Run Collate Data
- Run the Populate_Front_Page macro
- Use pop-up form to select appropriate parameters for the data to import
- Allow time for the code to run [approx. 2 hours]

This code calculates average measurements for each image and collects all data, in the results files, and copies it into one place.

Parameters to input:
- Filled or Ring (whether to look for the hole data or not)
- Type of File separator - "/" [OneDrive] or "\" [local]
- Information to be found in the name *

Create Plots
- Run the NewWB_Data_Table macro
- Use pop-up to locate the button control code – Image Select Buttons
- Allow time for the code to run [approx. 1 min]

This code produces a new document ('Results_Summary.xlsm'), with all images and data for plotting. The front page is made into an interactive user display, with buttons to control the data shown in the chart and images along the axis.

- Continue to Optional Analysis

* Information derivable in the name. i.e. Pw3Tx1+2.tif Denotes the Power, between 'w' and 'T', and the Transducers used, between 'x' and '.' - control gives 0's.
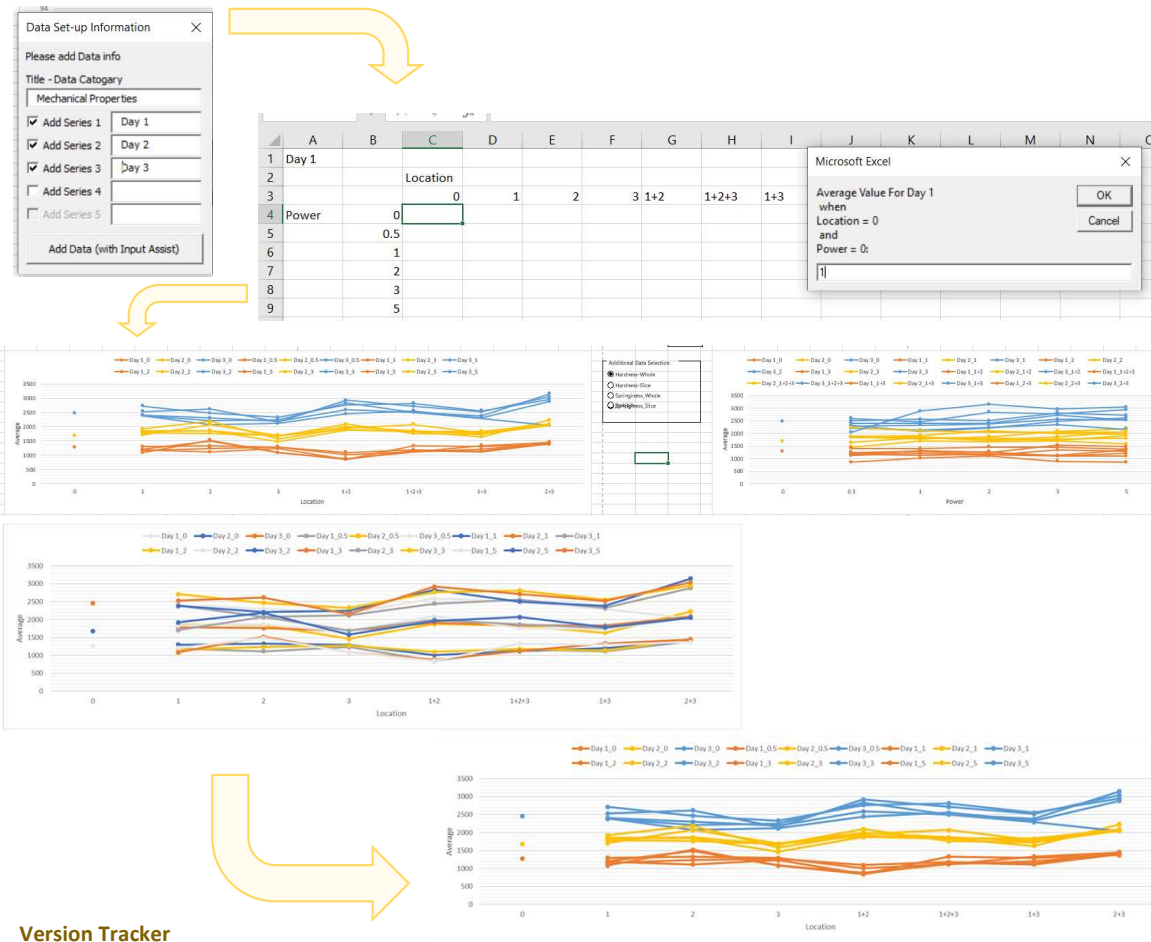
Optional Excel Analysis

Code available at: https://lunet-my.sharepoint.com/:f:/r/personal/mmct_lunet_lboro_ac_uk/Documents/2021%20Internship%20-%20B%20Sargeant/Completed%20Macro_Bread_Analysis?csf=1&web=1&e=Dbk3m5

Data Set-up Information

Please add Data info

Title - Data Catagory

Mechanical Properties

☑ Add Series 1   Day 1
☑ Add Series 2   Day 2
☑ Add Series 3   Day 3
☐ Add Series 4
☐ Add Series 5

Add Data (with Input Assist)

Microsoft Excel

Average Value For Day 1 when Location = 0 and Power = 0:

1

[OK] [Cancel]

Flowchart

Start Optional Analysis

Is there additional Data to be added? — No / Yes

Add Data

In the summary document Open VBA Code window (Alt+F11)
→ *This code adds new charts to the data sheet, plotting the additional data.*

Import Add_data_with_userform2 (Ctrl+M)

Import UserForm2 (Ctrl+M)
→ *In shared drive > Completed Macro Coding > Excel Macros > File 2.*

Run add_data macro

Add data title and series information in pop-up form
→ *Series titles used to plot multiple sets of data on one chart*

Are Data values easy to copy in? — No / Yes

No → Type average values for set parameters, as requested

Yes → Hold down 'enter' to set all values to '1', to then paste the true data in one go, after charts are made

Do the colours want sorting better? — No / Yes

Format Colours

In the summary document Open VBA Code window (Alt+F11)
→ *This code resets the colours to a repeating set of different colours – aimed to set each of the same set (day) to the same colour.*

Import Set_series_colours (Ctrl+M)
→ *In shared drive > Completed Macro Coding > Excel Macros > File 3.*

Close VBA code window

Select chart to change colours

Run set_series_colour macro

Use pop up to set repeating colour sets
→ • Start Colour Number (theme colours)
• Number of series (how many colours)
• 2 colours to avoid (7 [Dark Blue] and 11 [Grey])

Fin.

Theme colors

Color	Number
Text/Background - Dark 1	1.
Text/Background - Light 1	2.
Text/Background - Dark 2	3.
Text/Background - Light 2	4.
Accent 1	5.
Accent 2	6.
Accent 3	7.
Accent 4	8.
Accent 5	9.
Accent 6	10.
Hyperlink	11.
Followed Hyperlink	12.

Version Tracker

Version	Updated	Changes Made
Beta	06/2021	First completion of the Image analysis
Version 1	07/2021	Image analysis adjusted to repair bugs, found during use. Also includes completed Excel data analysis
Version 2	08/2021	User interface made more user friendly and code more universal, to mend bugs found during use.
Version 3	09/2021	Code reduced to aid licencing, Name prep changed to remove naming restrictions (auto replace), additional input types, scaling options, min circularity set to 0.1 [no pores are below 0.1] and re-written steps to make easy use