Quantitative Methods in Scanning Electron Microscopy:

Image Processing

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U.S. Department of Commerce

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Introduction

You've collected all of these wonderful BSE/EDX images, so *Now What*?!

This session will introduce the basic concepts on how to perform **Image Processing** and **Image Analysis** in order to *qualitatively*, and, more importantly, *quantitatively* characterize your data.

Image Processing and Analysis

- We will be demonstrating a simple method for quantitative analysis using two *free* software packages:
  - **ImageJ** http://imagej.nih.gov/ij/
    - ImageJ was developed by NIH. It is open source and operates in Java, which has allowed for extensive usability and accessibility through the creation of thousands of plug-ins.
  - **MultiSpec** https://engineering.purdue.edu/ biehl/MultiSpec/
    - MultiSpec was developed at Purdue University for processing of hyperspectral data. Initially created for analysis of images of the Earth from satellite data, it is widely applicable to any stack of spectral images, such as EDX maps or medical images.

- *In this session, we will show a demonstration of the method. At the start of Practicum 5, we will do a slower demonstration where you can follow along step-by-step on your computer.*

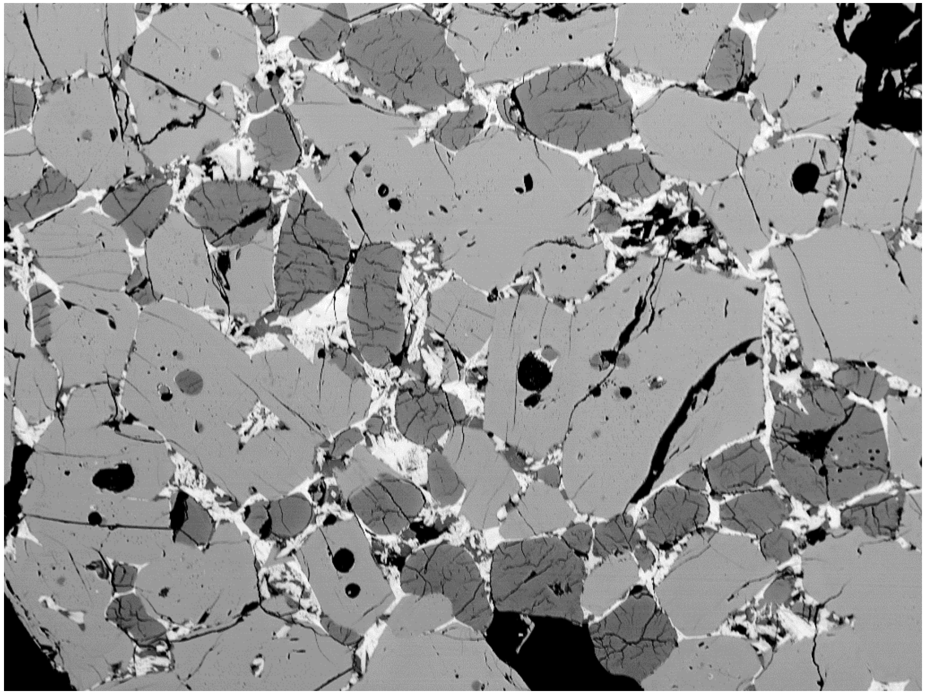
Step-by-Step Analysis

See these references for more detailed instructions:

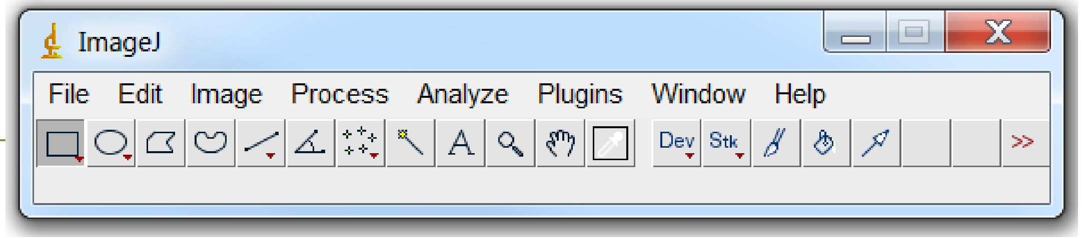
Stutzman, P.E., Feng, P., and Bullard, J.W. (2016). “Phase analysis of portland cement by combined quantitative X-ray powder diffraction and scanning electron microscopy,” *Journal of Research of the National Institute of Standards and Technology*, 121, 47-107. http://dx.doi.org/10.6028/jres.121.004

Stutzman, P.E., Bullard, J.W., and Feng, P. (2015). *Quantitative Imaging of Clinker and Cement Microstructure*, NIST Technical Note 1877, U.S. Department of Commerce, Washington, D.C. http://dx.doi.org/10.6028/NIST.TN.1877

Example: SRM2688



Step-by-Step Analysis



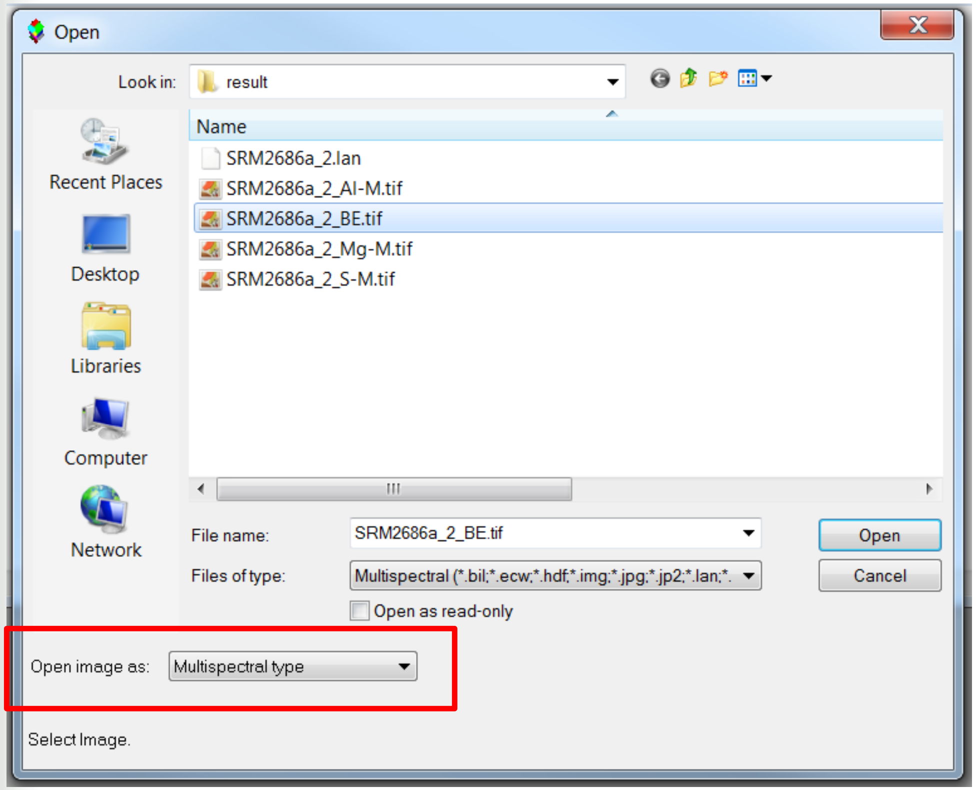
Step 1. ImageJ

1. Open BSE and EDX images in ImageJ and Tile (*Window -> Tile*).
   - Typically just look at BSE image and EDX maps of Al, Mg, K, Na, and S
   - You can open images by dragging files from the folder to ImageJ.
2. Adjust each image's contrast and brightness (*Image -> Adjust -> Brightness Contrast*) to see the background noise more clearly
   - Just move the "Maximum" slider (2^{nd} bar from top) in B&C box
3. Subtract background noise (*Process -> Math -> Subtract*). Use the preview check box in the Subtract window to interactively adjust the noise level selection. Focus on removing the majority of the noise.
   - Filter out any additional noise (*Process -> Noise -> Despeckle*)
     - Typically, if your data are high quality, you do NOT need to use any filters
4. Save (*File -> Save As -> TIFF*) the modified images with an "M" or other identifier so that the original (unmodified) image is not over-written. (Note: You may need to convert images to 8 bit)

Step-by-Step Analysis

Step 2. MultiSpec

1. Read original (or modified) BSE image into MultiSpec (*File -> Open Image*). Check the “Multispectral Type” box.



2. Link the **modified** EDX images to the BSE image. Use (*File -> Open Image*) and select “Link to active image window” and individually select each modified EDX image. Write down the order in which you link each element (*e.g.*, 1=BSE, 2=Al, 3=Mg, 4=S…) Click *Cancel* to complete linking.
3. Save this linked image set (*Processor -> Reformat -> Rectify Image*). Be sure to retain the .LAN extension.

Step-by-Step Analysis

Step 2. MultiSpec

4. Close all images. Open (*File -> Open Image*) the .LAN image set to confirm everything is correct. A window called “Multispectral Display Specifications” will appear; this is where you can assign color channels (RGB format) to each image. Use (*Processor -> Display image*) to switch between channels.

- For Clinkers, assign RGB channels to correspond to:
  - R, G, B = BSE, Mg, Al -> this is most useful to differentiate alite, belite, aluminate, ferrite, periclase, lime, and voids
  - R, G, B = BSE, S, Al -> this is useful to see sulfates
  - [Also: R, G, B = BSE, S, (Na,K) -> if you want to differentiate sodium sulfate (aphthitalite) from potassium sulfate (arcanite)]

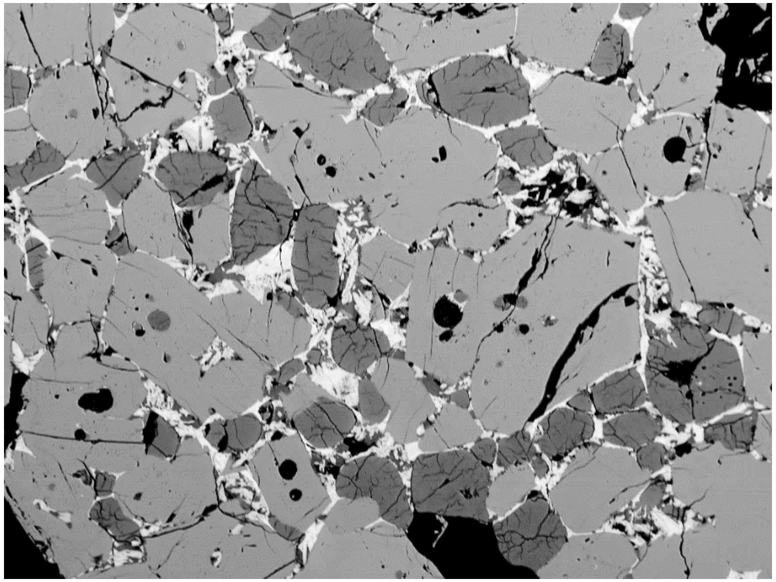
Step-by-Step Analysis

Step 2. MultiSpec

Alite (light red) Ferrite (pink)

Belite (dark red) Aluminate (blue)

Voids (black) Periclase (green)



Brightness and Contrast adjusted BSE image

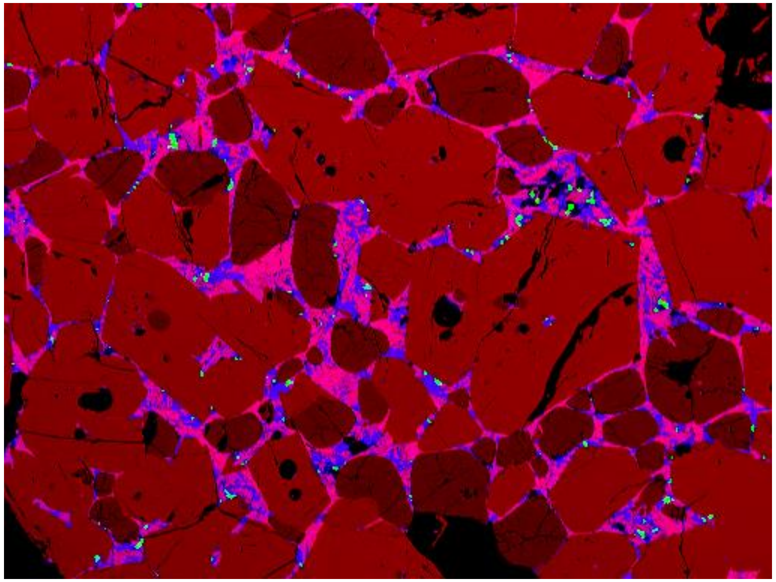


Image in MultiSpec after Item 4: RGB channels corresponding to BSE, Mg, Al

Step-by-Step Analysis

Step 2. MultiSpec

5. Set up the training classes (*Processor -> Statistics*). Accept the default options in “Set Project Options” window.

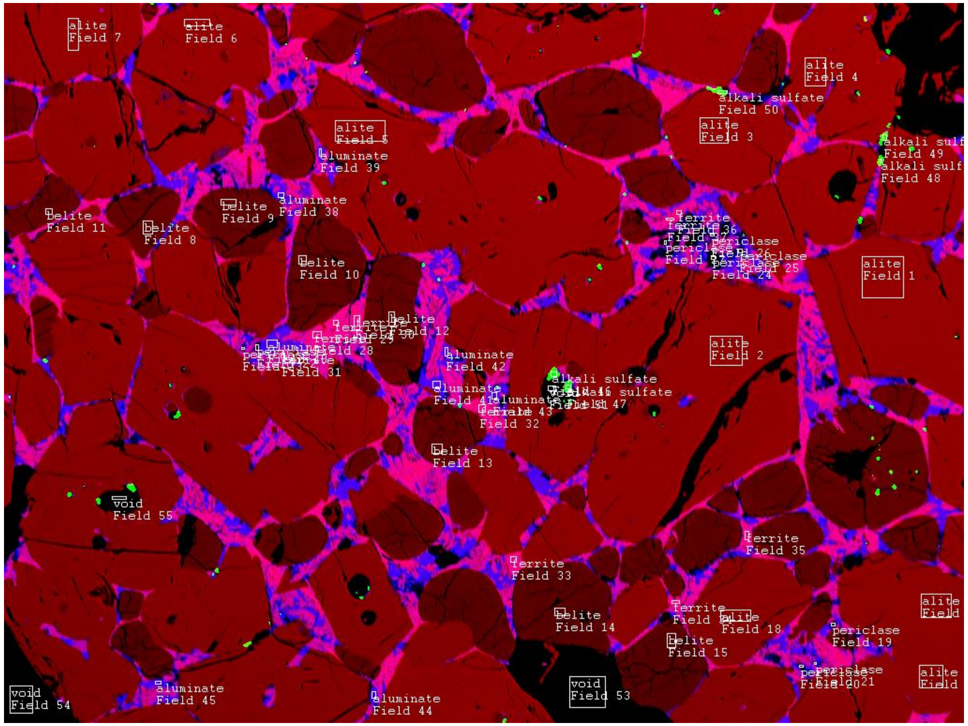
- In the “Class Designation” window, make sure “Class: ‘New’ ” is selected. Select a region of the image corresponding to a certain phase, then click “Add to List” and name the phase accordingly. Repeat for all other phases:
  - For clinker, assign: alite, belite, aluminate, ferrite, periclase, alkali sulfates, voids
  - For cement, assign: same as clinker, may also have gypsum and limestone
- Add training classes by selecting the class from the list and then specify “Add to List.” Adding more training pixels will help train MultiSpec to identify phases.
- Following this order will help with implementation in other programs later, such as VCCTL and THAMES:

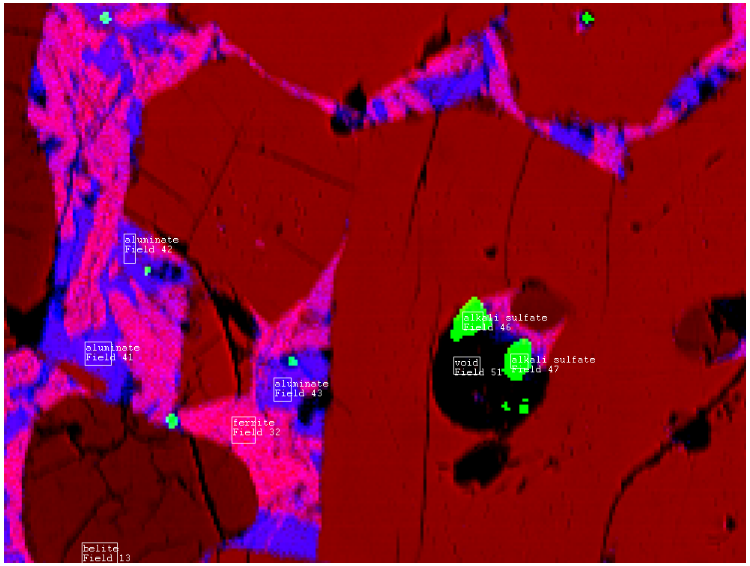
1 = alite	2 = belite	3 = aluminate	4 = ferrite
5 = periclase	6 = arcanite	7 = aphthitalite	8 = void

Step-by-Step Analysis

Step 2. MultiSpec

Item 5: Assigning training fields



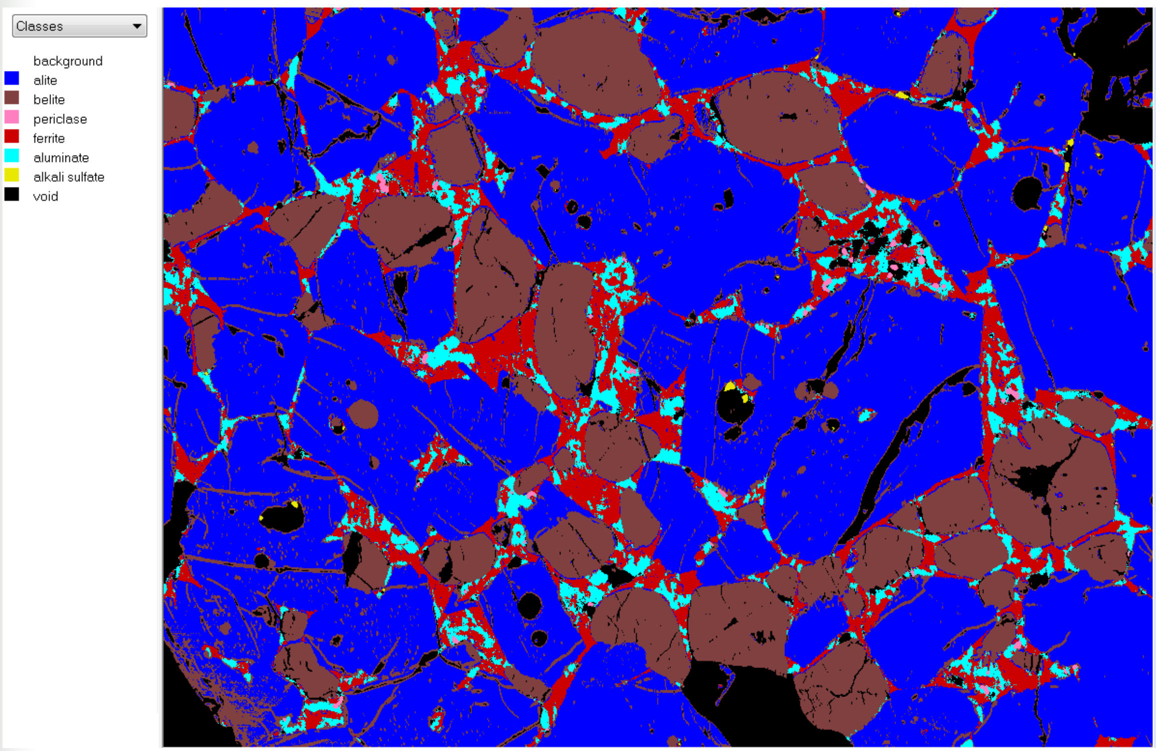


Step-by-Step Analysis

Step 2. MultiSpec

6. Classify the data (*Processor -> Classify*).

- Use the “Minimum Euclidean Distance” algorithm
- Check the box “Write Classification Results” to disk and be sure to check that the file path is correct and that the file has .GIS extension



CLASS DISTRIBUTION FOR SELECTED AREA

	Class	Number Samples	Percent
1	alite	440,833	56.055
2	belite	184,152	23.416
3	periclase	1,780	0.226
4	ferrite	71,837	9.135
5	aluminate	34,820	4.428
6	alkali sulfate	414	0.053
7	void	52,596	6.688
	Total	786,432	100.000

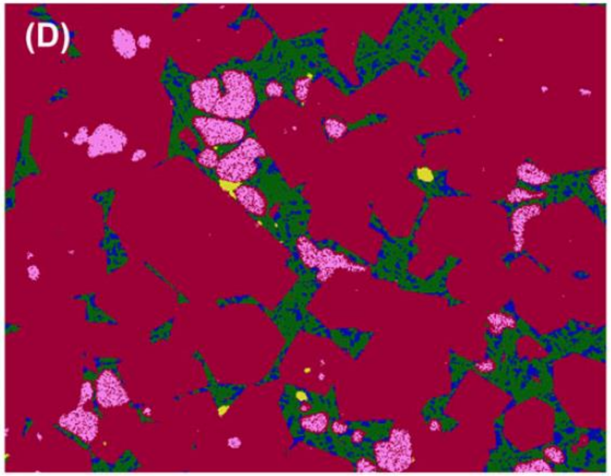
Step-by-Step Analysis

Step 2. MultiSpec

7. Save the work as a Project. This way you can go back and change the training fields, try different classification algorithms, etc.
8. Save the .GIS file as a .TIFF file. This will retain the color assignments. Do NOT use .JPG as this will not retain the color assignments correctly.
9. Open the .TIFF file in ImageJ and save as ASCII file (*File -> Save As -> Text Image*)

- This is so you can have an ASCII file with the indexed phases for input to other programs (such as MicroChar)

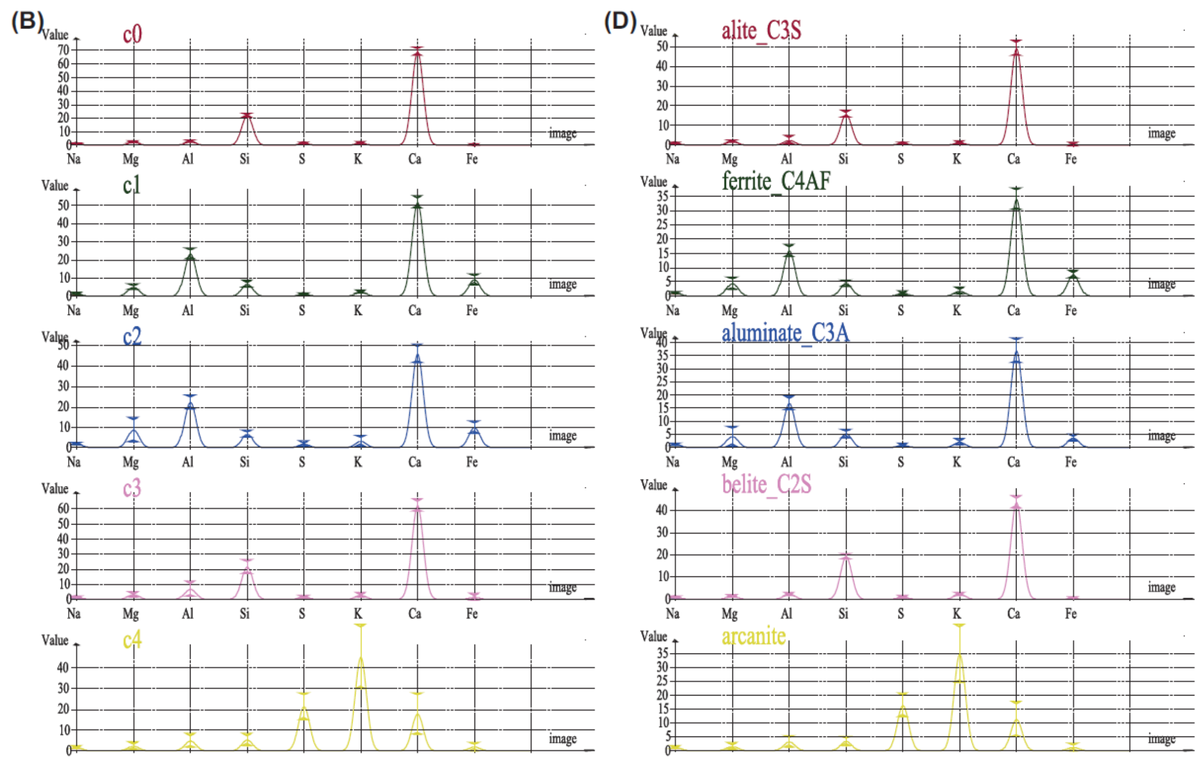
Another ImageJ Technique



Münch, B., Martin, L., and Leemann, A. (2015). "Segmentation of elemental EDS maps by means of multiple clustering combined with phase identification," *Journal of Microscopy*, 260 (3), 411-426. doi:10.1111/jmi.12309

Get the ImageJ plugin at: http://imagej.net/Xlib

- Input the EDX maps
- The program will look at the chemistry at each pixel and use clustering algorithms to group those pixels into an unknown phase
- Unknown phases are then compared to the chemistry of known phases to predict what that group of pixels most likely corresponds to



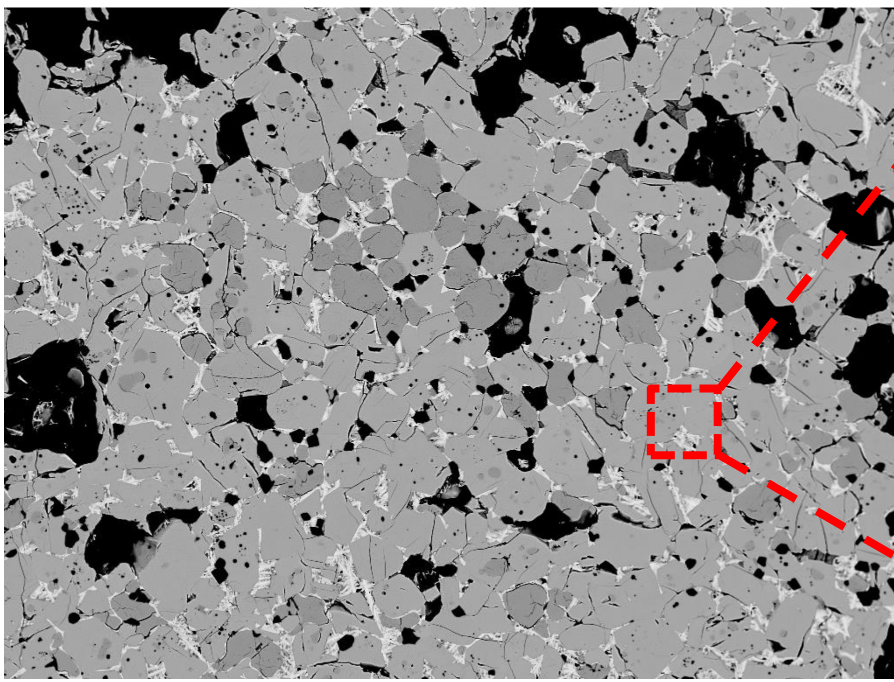
What if I have too many images?

- ImageJ and MultiSpec work very well, but they can be tedious if you have a lot of images
  - Consider Zhao and Darwin (1992) argued that you may need upwards of 150 BSE images to be 95% confident in your quantification of uhydrated cement content at 1000x magnification
- A lot of the tedious button-clicking in ImageJ and MultiSpec can be automated (or at least semi-automated) in platforms like MATLAB
  - Use MATLAB to:
    - Successively or iteratively read in multiple (or all) files
    - Automatic/Semi-automatic greyscale (or other) thresholding
    - Automatic collation and statistics of data from all images
    - Automatic image properties (*e.g.*, shapes, geometries, distances)
    - More than likely whatever else you can think of!!

Zhao H. and Darwin D. (1992) Quantitative backscattered electron analysis of cement paste. *Cem. Concr. Res.* **22**, 695–706.

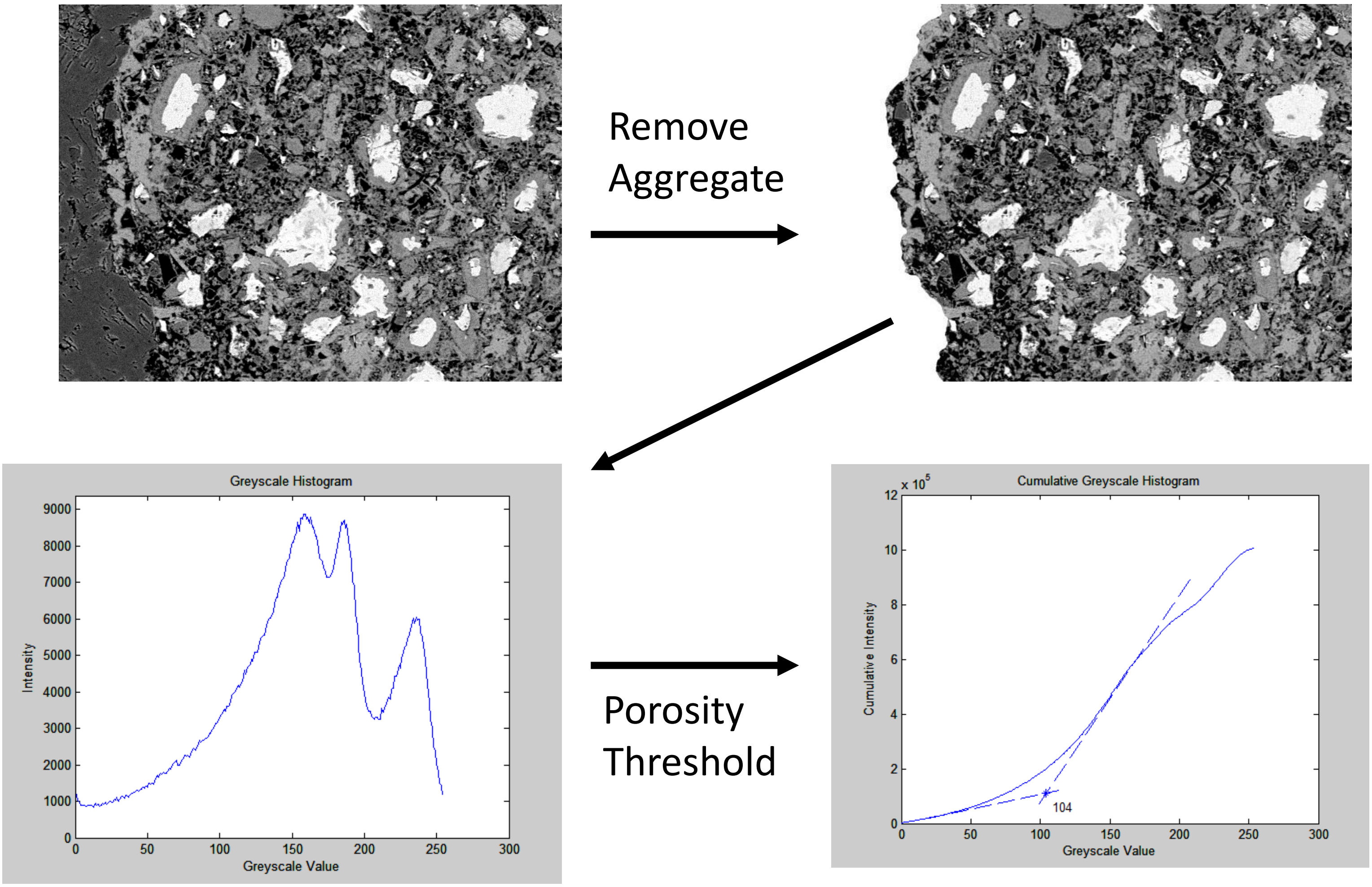
Why MATLAB?

- A programmable computing environment optimized for matrix manipulation and computing
  - MATLAB = **Mat**rix **Lab**oratory
- Images are just matrices!



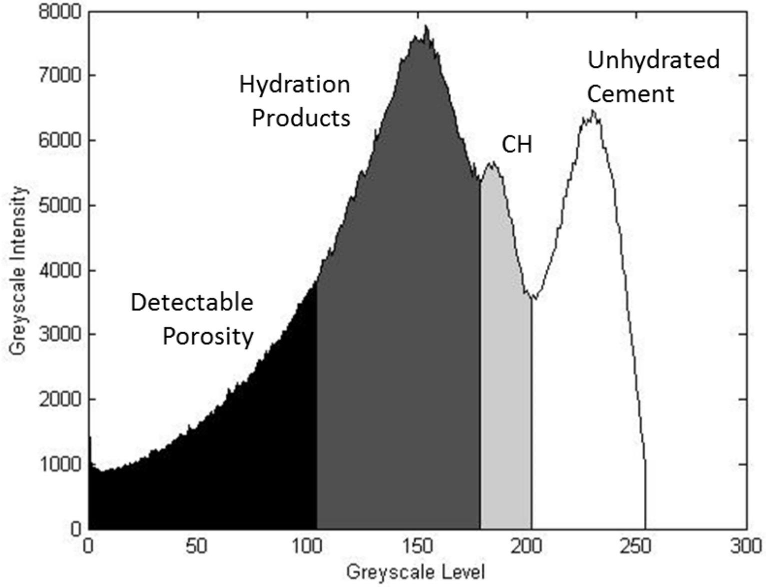
169	168	172	120	158	169	168	165
169	171	143	148	169	165	164	165
170	167	145	163	168	169	167	166
168	138	150	152	163	168	168	168
165	134	164	184	168	167	167	168
163	140	184	187	166	166	165	168
141	150	170	151	159	167	162	166
148	151	126	122	157	167	166	168
153	144	121	118	149	167	168	169
136	133	120	120	137	162	167	168
172	176	178	185	175	165	168	168
144	158	171	194	197	169	166	166
154	159	162	162	179	175	165	165
164	160	167	166	163	179	166	164
169	155	165	167	164	168	162	165

Example: Semi-Automated Analysis of ITZ Characteristics

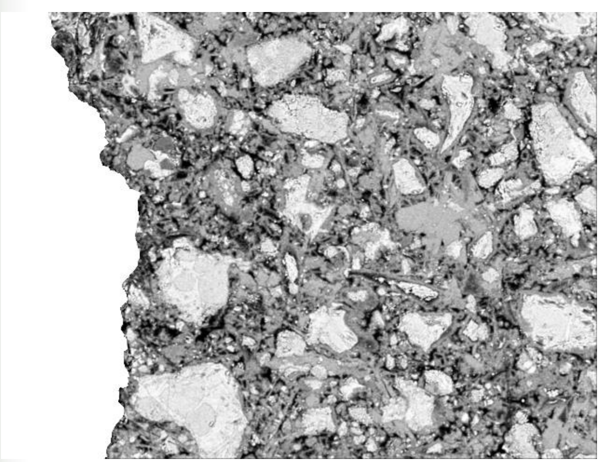


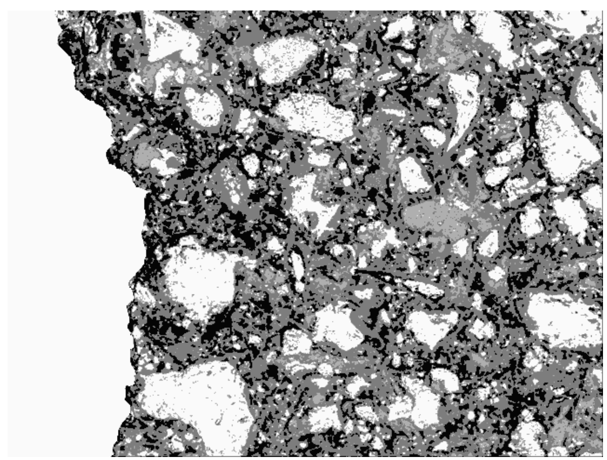
Brand A. S. (2015) *Interfacial transition zone composition and bonding in cementitious systems with asphalt-coated particles*. PhD Dissertation, University of Illinois, Urbana, IL.

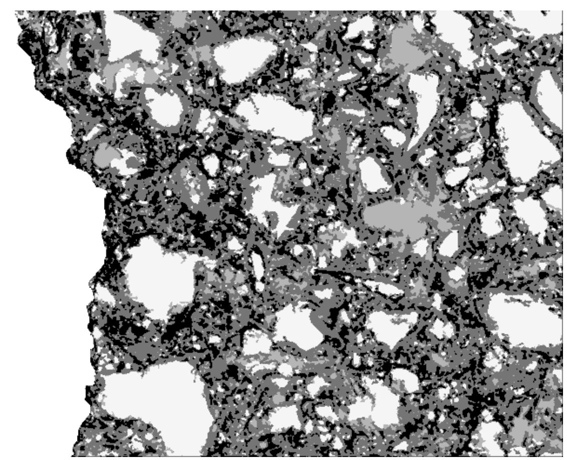
Example: Semi-Automated Analysis of ITZ Characteristics



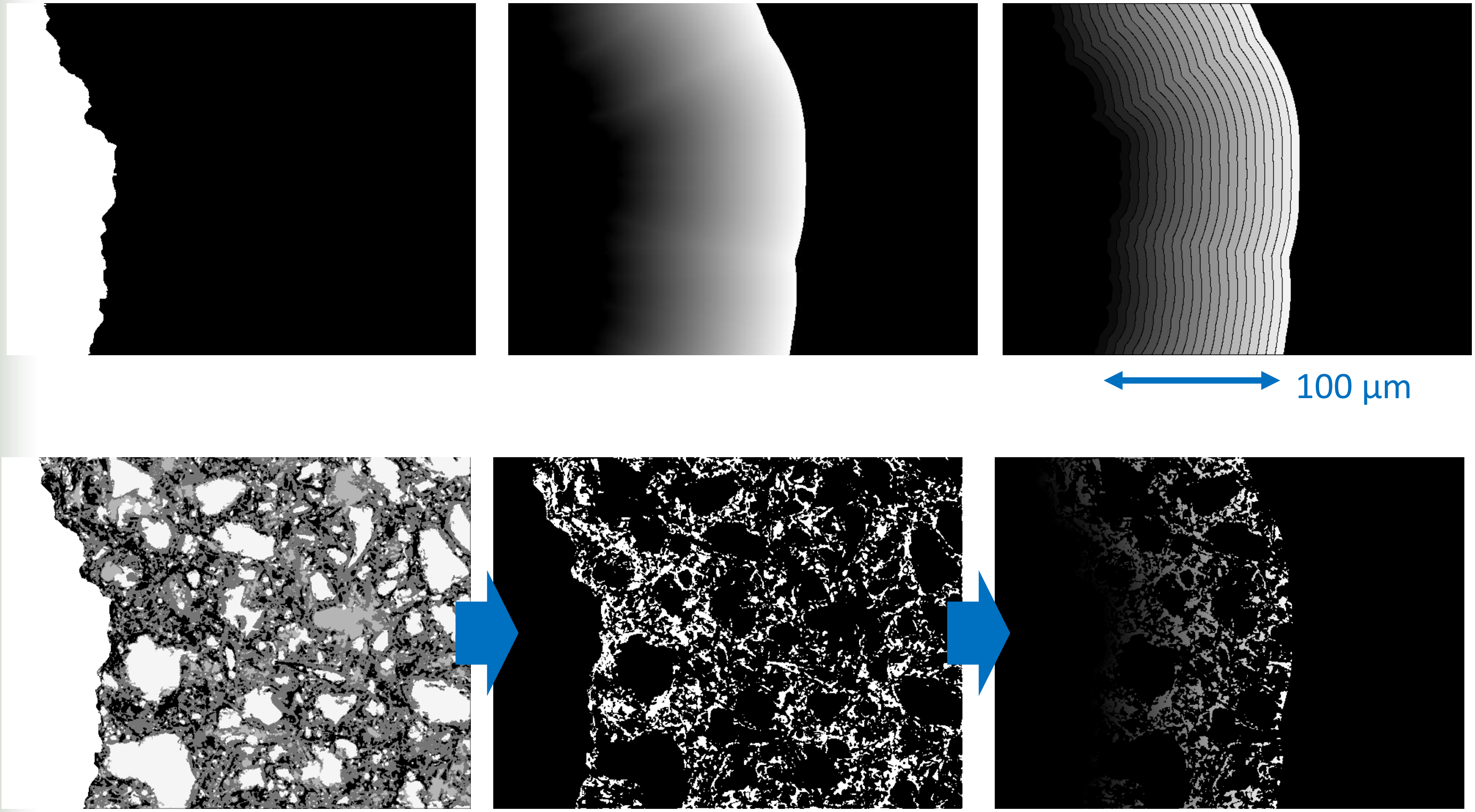
Automated morphological filtering



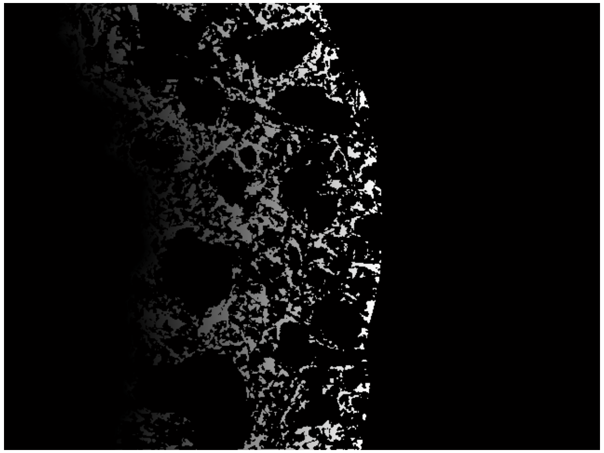


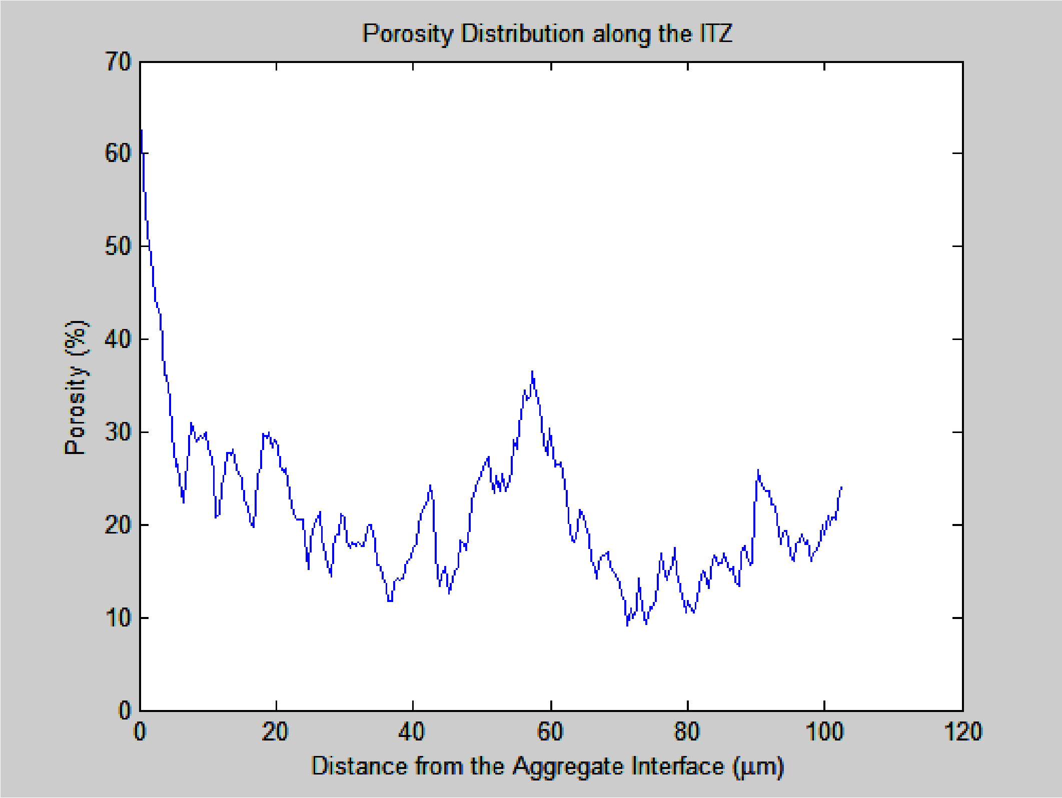


Example: Semi-Automated Analysis of ITZ Characteristics

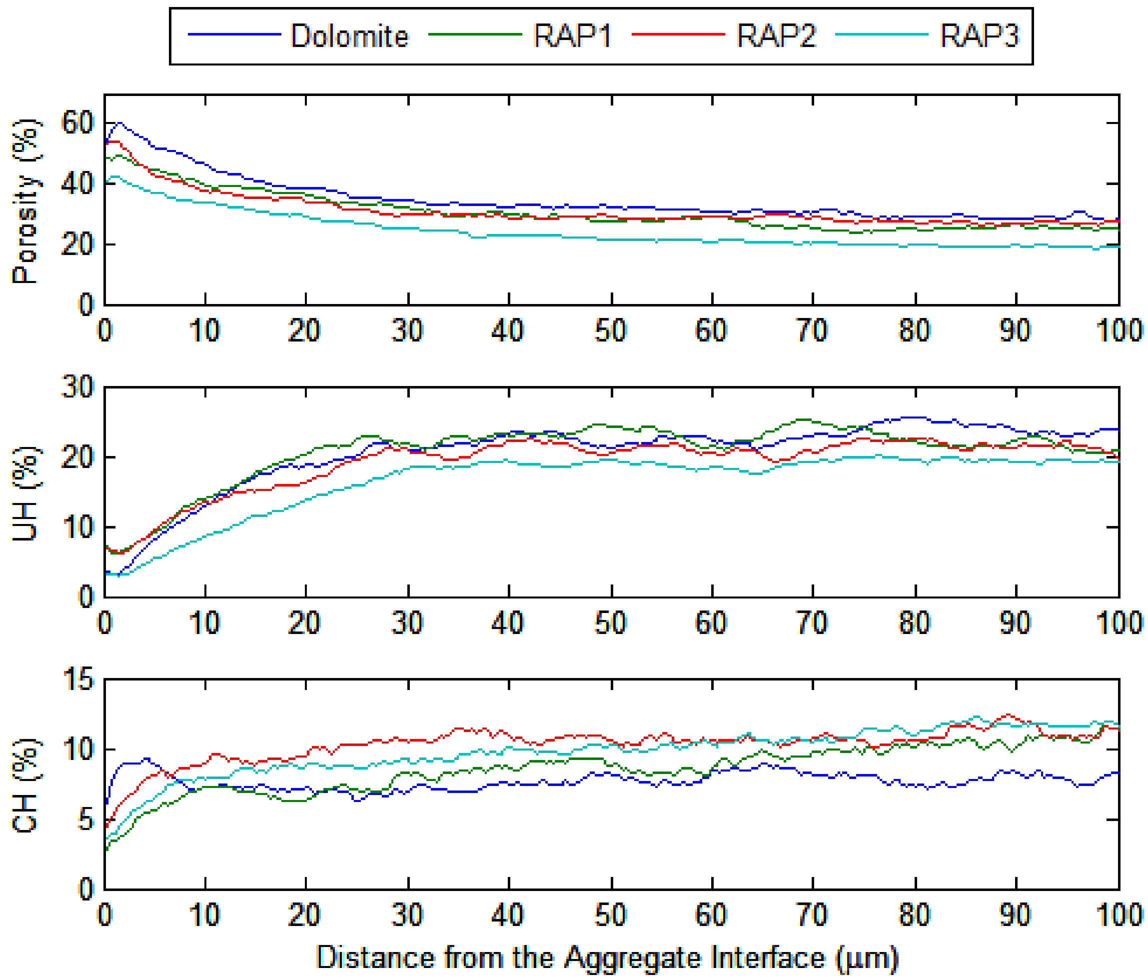


Example: Semi-Automated Analysis of ITZ Characteristics



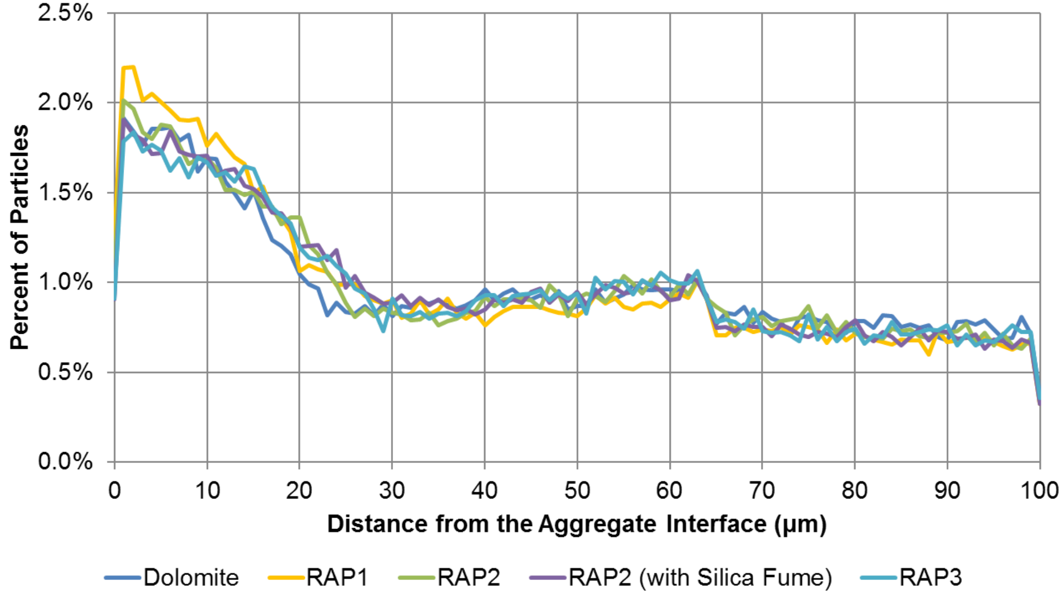


Example: Semi-Automated Analysis of ITZ Characteristics

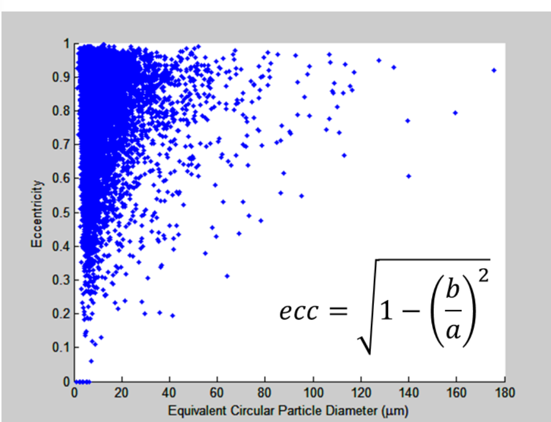


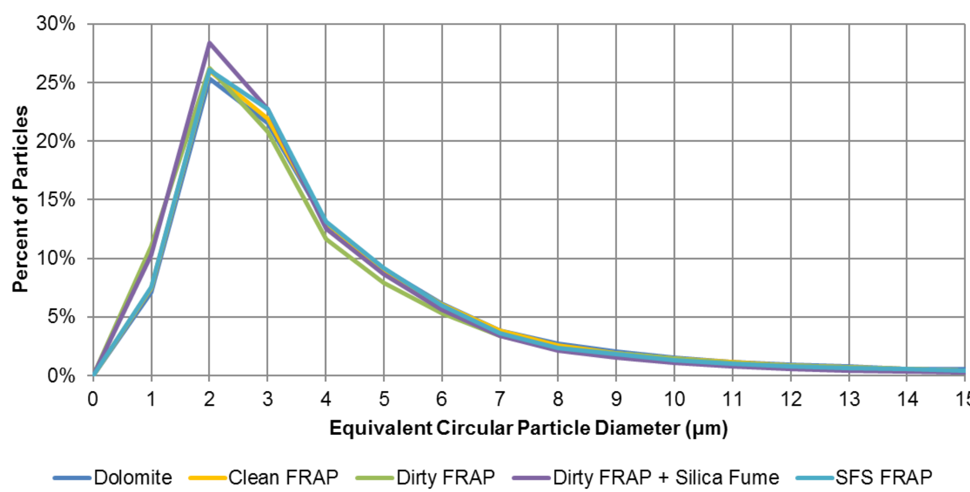
Collate all of the data to get ITZ distributions averaged over 50-60 images

Example: Semi-Automated Analysis of ITZ Characteristics



Automated calcium hydroxide morphological analysis





$$d_{eq} = s\sqrt{\frac{4}{\pi}A}$$

Recommended References

Overview Documents with Useful Step-by-Step Instructions for ImageJ and MultiSpec

Stutzman, P.E., Feng, P., and Bullard, J.W. (2016). "Phase analysis of portland cement by combined quantitative X-ray powder diffraction and scanning electron microscopy," *Journal of Research of the National Institute of Standards and Technology*, 121, 47-107. http://dx.doi.org/10.6028/jres.121.004

Stutzman, P.E., Bullard, J.W., and Feng, P. (2015). *Quantitative Imaging of Clinker and Cement Microstructure*, NIST Technical Note 1877, U.S. Department of Commerce, Washington, D.C. http://dx.doi.org/10.6028/NIST.TN.1877

Stutzman, P.E. (2012). "Microscopy of clinker and hydraulic cements," in *Reviews in Mineralogy and Geochemistry*, 74, 101–146. doi:10.2138/rmg.2012.74.3.

Helpful How-To and Troubleshooting Guide

Winter, N.B. (2012). *Scanning Electron Microscopy for Cement and Concrete,* WHD Microanalysis Consultants: Woodbridge.

Overview Document on Theory and Literature

Scrivener, K.L. (2004). "Backscattered electron imaging of cementitious microstructures: Understanding and quantification," *Cement and Concrete Composites*, 26 (8), 935–945. doi:10.1016/j.cemconcomp.2004.02.029

Diamond S. (2001). "Considerations in image analysis as applied to investigations of the ITZ in concrete," *Cement and Concrete Composites*, 23 (2-3), 171–178. doi:10.1016/S0958-9465(00)00085-8

Other Useful Image Processing Techniques and Tools

Wong, H.S., Head, M.K., and Buenfeld, N.R. (2006). "Pore segmentation of cement-based materials from backscattered electron images," *Cement and Concrete Research*, 36, 1083–1090. doi:10.1016/j.cemconres.2005.10.006.

Wong, H.S., and Buenfeld, N.R. (2006). "Euclidean distance mapping for computing microstructural gradients at interfaces in composite materials," *Cement and Concrete Research*, 36, 1091–1097. doi:10.1016/j.cemconres.2005.10.003.

Münch, B., Martin, L., and Leemann, A. (2015). "Segmentation of elemental EDS maps by means of multiple clustering combined with phase identification," *Journal of Microscopy*, 260 (3), 411-426. doi:10.1111/jmi.12309 (and also see http://imagej.net/Xlib for accompanying software and ImageJ plug-in)