Abstract

Image analysis and processing was completed on three different samples, in order to define an imaging procedure which could be used as a step by step guide for analysing greyscale porous images. Titania thin films, Calcite crystals and dentine infiltrated with nanoparticle hydroxyapatite were the three samples, which underwent various processing methods using ImageJ, in order to determine the most ideal method. Through trial and error of image analysis combined with pore data analysis with literature pore data, an accurate step by step procedure for imaging greyscale porous images was achieved, for both a 'well defined image' and an 'impaired image'. The process involved applying Subtract background and Gamma filters in order remove background intensities, and physical impairments if present on the image.

Acknowledgements

Table of Contents

Section 1) Introduction

Porous materials are of increasing interest due to their ability to increase surface area to volume ratio and hence affect many other properties such as strength of the

materials. Porous materials can have a variety of different pore sizes, porosity percentages and geometry of the pores, based mainly on their fabrication methods, for example in titania thin cells for dye sensitised solar cells, the pores are created when the binder, which is used to prevent the film from cracking under sintering, burning off (Reading, 2012). Due to technological advances, porous materials are becoming more important, with different techniques being used to create them, and their applications constantly being updated for various uses (Glover., n.d.).

Porous materials are solids with voids in, which are 'gaps' in the material. These voids can be of varied sizes, and geometries. The different parameters of pore sizes make pores important for scientific and technological advances. The pores have specific and usually varied dimensions, which allow for connectivity and pore distribution to be calculated and related to the properties of the sample for example the strength of the solid, or the efficiency of the material such as in titania thin cell DSSC, which required dye to reach and infiltrate the pores, whether positive or negative.

Porosity is an important parameter of a material and can determine the performance of

the solid or powder. Porosity is being more readily exploited in new technologies, such

as medical and renewable energies. The porosity of the materials allows for bone in-

growth into hip implants for example, and release of energy pockets, respectively.

Porosity of a material can be a strength, for example hydroxyapatite creating the

advantage of bone growth with hip implants, or a weakness, where too much porosity

causes the material to become more likely to fracture as pores can create a path of

weakness, which can cause a crack, causing fractures along that particular line. You

can identify different characteristics from pore size such as connectivity and surface

area, using different measuring techniques e.g. mercury porosimetry and computer

analysis from different imaging methods such as light microscopy, transmission

electron microscopy and scanning electron microscopy.

Imaging of porous material is achieved by microscopy; e.g. light microscope, Scanning

electron microscopy, Transmission Electron Microscopy, X-ray Topography. A group at

the University of Manchester are currently using Light Microscopy in order to image

nuclear graphite reactors, whereas a team at University of Leeds used FIB-SEM in order image dentine tubules infiltrated with nanoparticles hydroxyapatite. This project will focus on imaging SEM images. Analysis of SEM images of porous materials can be achieved using Image analysis software in order to produce pore data such as average pore size, which can be used to compare the pore data to previous data values, or confirm a theory based on porosity.

In this project, image analysis using ImageJ will be used in order to produce pore data from three samples: Titania thin cells used in dye sensitised solar cells, calcite crystals and dentine tubule infiltration. The pore data and visual analysis of the original image to processed image can be used to compare and contrast different imaging processing procedures, in order to define the best method to analyse a porous SEM image.

The aim of this project is to image SEM images, in order to produce quantifiable data that can be analysed with the purpose of defining an image analysis technique for SEM images of porous materials. The porosity and pore data are important parameters

to know and understand about materials, and hence an accurate image analysis

procedure is essential.

Section 2) Literature review

Examples of different porous materials

Below are examples of different porous materials. In this project, analysis on a three

materials will be completed.

2.1.1. Nuclear graphite is currently being researched by the Nuclear Graphite

Research Group by University of Manchester and University of Leeds. The research

has been taking place since 2010. The nuclear graphite is porous, in order to reduce

the internal stress from irradiation. Radiolytic oxidation reduced the mechanical

properties of nuclear graphite, and hence affects the porosity (Li et al.). The aim of the

research group is to understand the graphite materials properties as there are fast

neutron damage and radiolytic oxidation, to understand the graphite component stress

analysis, the whole behavior of the nuclear graphite, and how to dispose of the nuclear

graphite waste (Nuclear Graphite Expert Group, 2012).

2.1.2. Calcite crystals have important biomaterial properties the control of the size of the crystals formed by adding different additives and porosity control can be manipulated, i.e. adding different amounts of Aspartic acid affects porosity. The porosity of the material can alter the efficiency of the calcite crystals as a material because controlled porosity is essential for applications like catalysis and filtration.

2.1.3. Shale rock contains natural gas in the pores which is a new and interesting energy source. The reservoirs of gas found in these rocks are hard to analyze due to the nano-sized pores, and so varied imaging techniques are required.

2.1.4. Dye-sensitized Titania solar cells are most effective when highly porous. The dye absorbs the photon, and creates an electron hole pair, the electron is then transferred into the conduction band of the titania creating energy, hence large interfacial pore area is very important. The TiO2 layer, adsorbs a dye, hence if there are more pores, more dye is adsorbed, the dye is subsequently oxidized and electrons are transported to the working electrode where the electrochemical circuit is completed, and hence as an energy source can be deemed as potentially very effective.

2.1.5. Porous hydroxyapatite is a key material for artificial bone implants. The porosity of the hydroxyapatite determines the degree of bone ingrowth, and hence the mechanical and biocompatibility of the new foreign implant. Nanoparticles of hydroxyapatite can also be used in order to infiltrate open dentine tubules which cause discomfort to a person due to liquid flow through the voids.

Defining pores and how to determine porosity

Pores can be classified by shape, size and whether they are open and closed. Pores are classed via a standard rating system now, the International Union of Pure and Applied Chemistry (IUPAC). The void (pore) is classified by IUPAC into three size categories: pores of less than 2nm are micropores, mesopores are between 2 and 50 nm and pores larger than 50 nm are macropores (Kaneko et al., 1994).

2.2.1. Pores in a material can be defined as open or closed. Pores communicating with external surfaces are open pores, and closed pores are hidden within the material and are internal pores, usually from the material not being heated enough and hence collapsing (Kaneko et al., 1994).

2.2.2. The shape (geometry) of pores can be determined by electron microscopy

imaging, and visual analysis. IUPAC have classified pores into one dimensional, two

dimensional and three dimensional pores, and the following shapes, seen in figure 1.

 Figure 1, Pore shapes (Kaneko et al., 1994)

2.3) Determining porosity from different methods

2D pore analysis

2.3.i. Stereology

Using images from various methods, porosity can be defined using software in which

size and shape can be determined. This is achieved by preparing a sample, and taking

an image. The different imaging methods that are used are mainly light microscope,

scanning electron microscopy and transmission electron microscopy. The data from

this can then be used qualitatively to determine characteristics such as pore size,

surface area and fractal geometry and inputted into image analysis software such as

ImageJ. Usually the 2D image has to be stacked with a selection of other slices from

the same sample. This allows a 3D image to be produced. Some calculations can be achieved from 2D images and then verified with 3D analysis; however for accurate analysis of a sample a 3D analysis is preferred. Therefore 2D images can be used to extract 3D data.

2.3. iii. Petrographic method

This method involves injecting a high contrast medium into the sample in order for higher definition between pores and solid. The 2D porosity is measured using image analysis methods (Glover., n.d.). The 2D pore data, perimeter and pore area can be used to determine surface area of the pores. This will be discussed further in the following section.

2.3.iv. Image analysis software

The two most common pieces of analysis software are ImageJ and Fiji. The porosity, pore area and average pore area can be mathematically calculated via pixel variations and alterations of the original digital SEM, AFM, TEM or light microscopy image. The

process involves thresholding and binarising an image (these are explained in 'Method'), in order to define the pores (objects of interest) from the background.

3D pore analysis

2.3. ii. Mercury porosimetry Injection

The sample is placed in mercury, and the pressure is raised stepwise. This method can measure porosity because in normal laboratory pressures, mercury will not enter the pores of the sample, and hence by altering the pressure, the mercury will begin to enter the pores, until and ideal maximum pressure has been identified where all pores are invaded. Hence this displacement can be used to calculate the volume of the sample and the amount of mercury that has entered the pores, which can then be used to calculate porosity. The disadvantage with this method is that the smallest pores will inevitably not be invaded as the mercury will not be able to enter all pores; however the porosity will only be marginally lower than if calculated with other methods (Glover., n.d.).

2.3. ii. Gas adsorption method

A known volume of gas e.g. helium, is injected into a sample, which then adsorbs to the surface of the porous material. A measure of the amount of gas that has adsorbed can determine many characteristics of porosity such as pore size distribution. This method is done under constant temperature whilst measuring volume and pressure (Dullien, 1979). The BET method can then be used to determine algorithmic data for calculating surface area.

2.3.iv. NMR Cryoporometry

This method involves injecting a liquid into the sample which fills the pores. However the liquid can only fill open pores, and hence full porosity data cannot be achieved. The liquid is frozen and then heated in order to determine the melting temperature, which can give information of the pore size distribution. The amount of mobile liquid is then measured using NMR, where the melting point is inversely proportional to the pore size distribution (Mitchell et al., 2008).

Similarly, differential scanning calorimetry can be used to determine porosity. The transient heat flows through phase changes and can determine the melting point.

However pore volume and pore size are difficult to obtain as the method cannot be suspended to achieve individual pore data (Brunl et al., 1997).

2.3.v. X-Ray Tomography

Even though x-ray tomography is used primarily for medical uses in order to image a specific area of the body using 2D x-ray images around a single axis to produce a three dimensional image of the inside of an object (Herman., 2009), their potential use in imaging other materials and the 3D nature of a porous material are evident.

Focusing on imaging and analysis

The project will focus on determining porosity from image analysis. Using different image samples from SEM, a procedure to analyse porous materials can be developed.

From literature examples of how these porous materials have been prepared and imaged are given below. The way in which they are prepared and imaged can remove the risk of producing inaccurate data. For example, if the sample required pretreatment before imaging, this could affect the overall image analysis.

2.4.1. Nuclear graphite

Thermally and radiolytically oxidised Gilsocarbon graphite has been imaged with a Olympus light microscope inside a glove box, to reduce the risk of radiation.

Three different types of microscopy are used to image the graphite: bright field imaging, polarised light microscopy and using a fluorescent dye to image the porosity.

The sample were prepared by thermal oxidation at 900°C under a flowing gas stream of a CO2-5%CO mixture in a tube furnace at to simulate the radiolytic oxidation in an AGR. Images have been produced and analysed using ImageJ (Nuclear Graphite Expert Group, 2012).

Figure 2, An example of a light microscopy image is highlighted below, with the ImageJ altered version on the right. The alterations involved using ImageJ, to brightness/contrast, threshold and binarise the original image, so that the pores and background were well defined for pore data analysis (this method is explained for a 'well defined image' in 'Method'.

Figure 2, The ImageJ image has numbered all the pores that were distinguished by thresholding the original image. The numbered pores are used in analysing the particles and the size distribution.

2.4.2. Shale rock

Shale rock sample was imaged as received from a production well in North America. It was imaged using focused ion beam scanning electron microscopy (FIB-SEM). Once imaged using FIB-SEM, the image was binarised and underwent threshold in order to determine the ideal image for characterization. Top hat segmentation was used as thresholding gave varied pore size distribution at different threshold ranges (Zhang, 2011).

Segmentation involved separating the organic matter from the mineral matrix. Initially a small threshold is applied (120-255) (Zhang, 2011) in order to remove high intensity pores in the mineral matrix, and then small pores are neglected as these do not affect porosity. There is an assumption that there are no nanoscale pores smaller than 5nm, due to the acquired resolution of 5nm. Porosity results are given for different threshold

values between 0-100 and 45-65 thresholds appears to give the best results, which

gave porosity between 4-13% (Zhang, 2011).

NB. The following materials are the three samples that I will be analyzing in order to

produce the best image analysis procedure.

2.4.3. Dye-sensitised Titania solar cells

The sample of Titania film to be images was prepared by screen printing of titania

paste onto a conducting glass substrate, and undergoes heating to maintain

mechanically robustness. The sample was made porous by sintering the substrate and

paste together at 550 °C for 30 minutes. The sample was then soaked in a dye (Ru

(II)L2(NCS)2Cl2 (L=2,2´-bipyridine-4,4´dicarboxylic acid) (Tesfamichael et al., 2003) to

sensitise the film, for different time periods. Finally the sample was washed with

deionized water and dried with air (Tesfamichael et al., 2003).

The samples were characterized using a transmission electron microscope at 200KeV.

The image was analysed and characteristics were produced. Size pores between 10-

25nm were found with geometries of tetrahedral and rhombohedra shape

(Tesfamichael et al., 2003).

Another method used to produce titania thin films for DSSC and imaging, involves

mixing a binder (used for good connectivity in sintering of the titania powder) and

powder together (different sized titania nanoparticles). Three different powders are

used, which contain different sizes of particles. Once the binder, usually ethyl cellulose,

has been burnt away (to produce pores), the paste that is produced is then screen

printed onto a titanium substrate, and a thin film is produced. The film is then heated at

520 °C, for efficient connectivity of the titania aggregates. Opacifiers can be added to

the paste, which can increase efficiency by scatter the photons in the film and hence

produce better use of all pores. The titania thin cell is imaged using FIB-SEM, and a

peak pore area at 50 nm2 for one type of titania powder, with aggregate size of 25nm

(Reading, 2012).

The three titania samples (with varying powders) I will analyze are shown in table 1:

Classification in report Name of sample Description of sample A H110 70% PCX20 and 30% 250nm anatase opacifier B PCX20 Powder with particle size around 20nm and average aggregate size around 40nm C 18NRT Colloid with particles around 10nm length and 20nm width, and similar average aggregate sizing.   Table 1

2.4.4 Dentine tubules infiltration with hydroxyapatite

Nanoparticle hydroxyapatite was produced via hydrothermal route. The hydroxyapatite was used to infiltrate the dentine tubules in order to block them, and hence reduce fluid motion and eventually aid the reduction of sensitivity in teeth caused by pores in dentine. The dentine without hydroxyapatite infiltration and with infiltration were imaged using FIB-SEM. The infiltrated dentine was analysed and 90% of the tubules had been partially or completely occluded (Earl et al., 2006).

2.4.5. Calcite crystals

I am going to analyze calcite crystals imaged by SEM. The calcite crystals were prepared in the lab. Glass slides are placed into piranha solution (piranha solution is 70% sulphuric acid, 30% hydrogen peroxide and is an extremely oxidising solution) to

clean the slides and also etch them. This is left overnight. The slides are removed and

rinsed thoroughly with deionised water. They are then placed into petri dishes. A

solution containing $CaCl_2$ (usually 10mM) and aspartic acid (ranges from 5 to 50mM)

is poured into the petri dish so that it covers the crystals. These petri dishes are placed

into a desiccator along with solid ammonium carbonate and left overnight. The slides

are removed, rinsed with deionised water and then ethanol. On the top surface of the

slides (the side exposed to the solution) are the crystals. I will use ImageJ to analyse

these images (Lee, 2013). In this project three samples of Calcite crystal will be used,

all fabricated the same, except Samples A, B and C have increasing amounts of

aspartic acid, 5mM, 20mM and 50mM respectively (Lee, 2013).

Figure 3 shows the FIB-SEM image of the calcite crystal (left), and the altered image.

The image was altered using techniques discussed in 'Method' and 'Results and

discussion'.

The pores highlighted in the right image can be dark or white depending on preference.

Advantages and Disadvantages of imaging techniques

Different imaging techniques have varied pros and cons. There are many microscopy techniques such as SEM, TEM, FIB-SEM, Atomic Force Microscopy, Infrared Microscopy, Light Microscopy, backscattered scanning electron microscopy and scanning probe microscopy. The main techniques I will focus on are SEM, TEM and light microscope, however if during my research I find images from a different imaging technique I will use analyse them.

The images that are obtained from the different techniques will be of varied quality. This fact can determine how accurate the image analysis data will be.

Advantages of light microscope are that the process is relatively simple and quick, no pre-treatment is required, and however the resolution is only a couple of hundred nanometres (250 nm) and so is very poor (UOU lecture slide, u.d.).

SEM can be imaged in all directions, so a 3D image can be produced and the surface of an sample can be imaged very well, i.e. average pore area can be calculated relatively easily in ImageJ. However the resolution is relatively poor at a ten's of

nanometres and stain coating is required if biological species are being imaged, which

could affect the accuracy of the results (UOU lecture slide, u.d.).

TEM has a resolution as low as 0.2 nm which can define very minute objects; however

the sample has to be very thin in order for the electrons to pass through the sample

(UOU lecture slide, u.d.).

These can affect the analysis when using ImageJ and hence this needs to be taken

into account during the initial preparation of the image on ImageJ.

When imaging porous materials for image analysis, any type of image technique can

be used to achieve the images i.e. light microscopy. However the advantages and

disadvantages described above are indications as to what limitations will be

discovered when analysing an image. Resolution and magnification of an image is very

important in order for accurate pore data to be achieved, as distinguishing objects in

the background of a sample image is required.

 Image analysis

Image analysis is a way in which digital images from microscopy techniques can be altered via software techniques in order to produce quantitative data. In order for this to be achieved the image has to be binarised, segmented, filtered and smoothed, which are all described in 'Method'. The software can then produce information on pore size and porosity once this has been determined manually (Ross J., ImageJ user guide) (Horovisitz., 2011).

The analysis software being used is ImageJ, which is software developed by Wayne Rasband at the Institute of Health in order to quantify data by processing and analysing digital images. The predecessor was NIH Images, however ImageJ has varied filtering and alteration techniques for different images, and the ability to download plugins for more in depth and specific image analysis of an image, for example Weka Segmentation is used in order to define objects from the background when there is a small pixel intensity difference and furthermore more than two colours can be segmented and thresholded from one another (Ross J., ImageJ user guide). FIJI is an additional imaging software for image analysis. It is based on ImageJ

(F.I.J.I.- 'Fiji Is Just ImageJ'), however the software has plug-ins built in. Other image analysis software exists, such as 'CoLocalizer Pro' which analyzes the colocalization in images from fluorescence imaging, however the best imaging method for imaging porous materials is ImageJ or FIJI (Ross J., ImageJ user guide).

Once the sample has been imaged, initial pore size can be determined using the scale and resolution. The image can then be input into an image analysis software know as digital image analysis (DIA) such as ImageJ, which can determine pore size distribution, porosity and surface area. The data can then be used to determine other and more accurate calculations by stacking layers of the same image taken at different cross sections, to produce 3D images which can be further analysed values for the above parameters. This technique requires cross section images of the same sample in order to produce a 3D image of the sample (Reading, 2012).

The image is put through a set of step procedures. The software which I plan to use for image analysis is ImageJ. The software enables you to threshold, binarise, smooth and filter images in order to get quantitative data.

Plug-ins can be added in order to solve many imaging problems, for example Maureen

Haverty at the University of Manchester has discovered a plug-in which can define

open and closed pores and with various different filters: Sobel filter which detects

edges of pores, Hessian filter which determines blob-like, line-like and plane-like

features and Difference of Gaussians which 'subtracts one blurred version of an

original grayscale image from another, less blurred version of the original.' (Haverty M.,

2013)

 Image processing

Previous processing methods were applied in order to have a basic system, (Labino,

n.d.). Once an image has been produced by a particular imaging technique, the image

is opened in ImageJ and has to be undergo digital image analysis. This involves using

brightness/contrast, threshold and binarisation techniques in order to define the pores

from the background of the image. This is discussed further in 'Method'.

A process used to determine the best threshold value (pixel intensity maximum and

minimum values for the image) for optimum pore data, involves determine porosity

from different threshold values, seen in figure 4. There has been success with this

(Zhang, 2011) in shale rock analysis.

Figure 4, (Zhang, 2011).

Initially, matter is determined from pore voids by brightness/contrast altering and then

the image is binarised (black/white image) (Ross J., ImageJ user guide) (Horovisitz.,

2011). Thresholding can define a binarised image more accurately, as usually an

image cannot be binarised easily due to different topographies and differences in initial

brightness (Ross J., ImageJ user guide) (Horovisitz., 2011). The threshold sets a

maximum and minimum pixel brightness for the image and consequently each

individual pixel is determined as a pore or matter (above the brightness threshold is

matter and vice versa) (Ross J., ImageJ user guide) (Horovisitz., 2011). Smoothing

involves removing any noise in the image which could be inferred as a void.

 Image Analysis

Once the image has been processed by ImageJ, classification of the pores can be

obtained. Calculations can be used for parameters such as pore distribution. The

calculations for these parameters are usually done automatically via ImageJ, however

other more complex calculations can be achieved by inputting data from ImageJ into

Excel (Ross J., ImageJ user guide).

Connectivity

Connectivity refers to how pores connect to themselves and also whether they are

open or closed to the atmosphere. Open and closed pores have been defined in

previous text. Connectivity is also found to be inversely proportional of density of the

sample, and also pore connectivity was sensitive to anisotropy (Hing et al., 1999).

Connectivity can be defined as the number of whole pores identified within

measurement from the area (Hing et al., 1999).

Surface area

Surface area defined by IUPAC is the total area of the material including open and

closed pores. This can be calculated using gas adsorption techniques, and then using

the data from the amount of gas adsorbed in the BET method.

Surface area can also be calculated using Petrographic image analysis which involves using the following equation:

S_{pv}= Specific surface area, m2/per unit volume

L_p = Perimeter of pore

A_p= Pore area

2D image analysis pore data can be used to determine 3D pore surface area information.

Geometry

Geometry determines the shape of the pore which can alter the surface area of the material. See figure 1 for the types of pore shapes.

Pore geometry can be defined using 3D analysis of the sample.

Individual pore size (diameter) / Average pore area

Pore size can be very varied and there are three classifications for pore size stated in my previous text. Pore size is inversely proportional to density of the sample, and the total numbers of pores at various sizes are usually compared on a graph against frequency and hence this determines the pore size distribution.

Pore size obviously relates to geometry, however these classifications by IUPAC, are only for spherical pores hence the data can be inaccurate.

A new way of defining pores is to measure the diameter, this is stated in Hing's literature Hing et al., 1999).:

P.D= ?((Pore are)/?)?^(1/2) × 2

This equation will be referred to as 'Area and pore diameter equation/Equation calculation' throughout this project.The feature area is the whole image sample and the equation is a variation of the 'Area of a circle' equation.

There are ways to define pores that are not cylindrical also, using Dubinin's method

which defines ellipsoidal shapes by calculating the distance between two sides, and

other non-pores based on their smallest dimension (Dubinin et al., 1960).

However using Image analysis software, pore area and size are automatically

calculated, and output in a 'Results table'

Fractal geometry

Fractal geometry can be defined as the specific outline of a pore under high resolution

and magnitude; this can slightly alter the pore size if there is a large difference in

fractal geometry at different resolutions and magnifications. In effect the fractal

geometry can be defined as the exact perimeter of a pore and how this alters with

varying magnifications and resolutions of imaging techniques e.g. SEM.

Porosity

This is the measure of how many pores there are in a sample compared to the whole

sample, e.g. percentage area of pores compared to the whole sample. Pore

distribution can be calculated, which is the percentage distribution of pores of a various

sizes. When measuring porosity of a material through image analysis, usually only

open, outer pores are taken into account as these are more visually present.

Intraparticle and interparticle pores

When using ImageJ, the pores are defined by a measure of dark/light pixels due to

segmentation and thresholding. If pores are clumped together, known as interparticle

pores, this is measured as one pore and hence can give inaccurate data on pore size

and porosity (Kaneko et al., 1994).

ImageJ pore classification of data

Imaging analysis and pore analysis in ImageJ can be used to determine pore area,

porosity, average circularity ratio of pores, average perimeter and average Feret's

Diameter. These are all described in 'Method'.

Average Feret's diameter is the average of the maximum Feret's diameter of a pore

and the minimum Feret's diameter of a pore shown in the figure 5 below.

Figure 5, (Sympatec.com, n.d.)

Stereology: 3D analysis and processing with ImageJ

From literature and guides on 3D image analysis and processing, using ImageJ, you stack the 2D images to produce a whole 3D image. This is done using an ImageJ plugin such as 'Stacks' and then you can visualize the 3D image using 'ImageJ 3D viewer'. There are many problems with this method in terms of achieving a perfectly contrasted 3D image from different 2D images, however there are settings which can be applied which can set the brightness and contrast of all the 'slices' in order to achieve a uniform image (Boudier., 2008).

Segmentation and thresholding of the 3D images is completed as well in order to produce a uniform image, and the various intensities of the different slices can be normalized (Boudier., 2008). A review on segmentation and binarisation is commented on by Salzer (Salzer., 2012).

Success with 3D visualization has been achieved in a shale rock experiment (Zhang, 2011) shown in figure 6.

Figure 6 (Zhang, 2011).

ImageJ plugins use the z-section of the 2D image to display 3D images, and in order to

visualize the entire 3D image, the non-important pixel intensities need to be removed,

because not all pixel intensities will be visible and if kept, the data will be inaccurate

(Boudier., 2008)

Analysis of the image and pore calculations are obtained using the ImageJ software

and plug-ins; many different parameters can be achieved such as shape and size of

the pores, as well as the 3D sphericity of the pores, which can be compared with the

circularity found from the 2D images. The main plugin used is the 3D manager which

measures various different parameters shown in figure 7 below:

Figure 7- shows 3D manager in use (Boudier., 2008).

 Objectives and aims of project

Tasks and aims of this project in chronological order:

Through literature reading and 'trial and error' determine a imaging technique for SEM images which produces a processed images with similar pore sizes as the original image

Visit Maureen Haverty at the University of Manchester in order to understand different and more advanced imaging techniques

Image a 'well defined' image from Titania thin film samples, and determine the best imaging process.

Image an 'impaired image from titania thin film samples, and determine the best imaging process.

From this the best 'imaging procedure' will be defined. Use this to determine the imaging process for different magnifications of the same image and then image analyse the two other samples: Dentine tubules and Calcite crystals. Are different methods required? Determine what data is required from each sample in order to determine whether the process was accurate

Produce surface area data for the Titania samples.

Compare my data to (Reading, 2012), and understand and explain why his porosity

data was very different.

Use data from image analysis, i.e. pore size, percentage of pores, and used literature

reviews to compare values obtained, and further confirm the image analysis procedure

Produce a 'Flow chart' for the procedure to process and analyse SEM greyscale

porous images

The method, section 3, will chronologically go through the 'trial and error' process used

in order to determine pore data and the best analysis procedure and results and

discussion in section 4 will analyse the processed image visually with the original

image and also analyse pore data in comparison to literature review. Section 5 will

contain a summary of the results I obtained, and section 6 is the final procedure in a

'Step-by-Step' manner. The Project is concluded in section 7.

Section 3) Method

3.1. Titania thin cells: Method Introduction

Based on previously reported methods for porosity (Labino, n.d.), the basic method for image analysis involves using brightness/contrast[1], threshold[2] and binarise[3] in order then be able to determine particle parameters from the 'analyse particles' application. The image analysis system used was ImageJ, which has its own user guide manual, which can be useful in identifying features and tools (Ross J., ImageJ user guide).

NB Definition of 'well defined'/'good images' is an image which visually has a strong contrast between pores and background, with no physical impairments such as 'cracks' in the sample. An 'Impaired'/ 'bad image' is defined as an image which has visual physical impairments, which impair the contrast between pore and background. Also in each case, using 'threshold' in ImageJ, as a primary starting point will determine whether intensity variations on the image exist.

An image was opened for analysis by using 'File--> open'. Initially the 'well defined image' was analysed by setting the scale, cropping, brightness/contract, subtract background, despeckle[4], threshold and analyse particles were used in order to

achieve pore data, these processes are described in the next section. This involved

visual analysis and trial and error of the stated applications. Analysis and the process

to achieve data for a 'good image' is more concise and easier that for a bad image.

However I will discuss these applications in more detail for the 'impaired image'.

After determining the basic method in order to obtain 'well defined image' data, this

was used on the 'impaired image' data; using the basic applications of

brightness/contrast, threshold and binarise. The particular image being used is not

very good because it has physical streak marks in it from when it was cut. You cannot

get rid of these streaks because they are physical features than are underneath the

pores in the background. Filters can be applied which reduce the streak imposition. In

this case, the data will slightly be off. However there are very good filters which can

remove the streak presence as much as possible, and produce the best possible data.

3.2) Titania thin films: 'Impaired' image analysis

Set the scale on the scale bar at the bottom as seen in the figure 8 below, using

'Analyze --> Set scale', Select global if using a large range of images, in order for the

scale to be set for all. However check that magnifications are the same, and the image

sample is the same and obviously scale is the same.

Figure 8- Setting the scale

Any areas which are not of interest, and that can visibly seen as not a part of the

image, and an obvious error were disregarded and cropped out of the image in order

to maintain , accurate and precise data. To crop the image, the selection shape was

selected on the tool bar and 'image--> crop' applied. Using the 'Image-->type' tab, the

image was set as greyscale, '8-bit'.

Brightness and contrasting the image was based on your personal judgement,

however the minimum and maximum values were altered to either side of the

histogram graph on the selection tab. Also auto was selected, which is a mathematical

selection of the optimum brightness and contrast using the histogram values, by

saturating a small percentage of the pixels, to obtain a mathematical suggestion. This

is selected using 'image-->adjust-->brightness/contrast'.

The 'well defined image' of titania DSSC was defined as bad due to the thresholded image and the obvious physical streaks. Thresholding the image provided evidence to suggest that areas that are not pores were highlighted as such, which can be seen in figure 9 below as red sections.

Figure 9 – Threshold intensity variations

Once threshold had been applied, 'Wand tracing tool'5 was used, which is highlighted in the tool bar, it was possible to manually select the background and then click on the bucket at the end of the tool bar, and click on the pores that were to be altered into the background. The wand was used to highlight connected pores, which shouldn't have been, and the erode binarisation filter6 was applied by 'Process --> Binary --> Erode'. An application called 'Watershed' can be used which automatically erodes pores in the image based on maxima and minima values of pixels, however when this was applied to the image, the erosion achieved was too much, and gaps were created between whole pores.

In this image, the areas which are highlighted as 'pores' which shouldn't be were

located near the bottom of the image. This is due to intensity differences on the image

from when it was initially imaged in the Scanning Electron Microscope. Using the

wand tool and erode with comparison of the ImageJ altered image and the original,

pore anomalies in the altered image were corrected.

An assumption was made that all the dark objects that were set in a linear

configuration, especially the ones that had no break and were one long jagged lines

were from the physical streak marks, rather than pores.

This was a long method, and would not be appropriate for use on multiple images.

However for one or two images, which was ideal for this project, this method seemed

the most desirable.

From this image below, the grey highlighted areas on the bottom of the image are

those which had been altered, shown in figure 10.

Figure 10

Once this has been completed, the image was binarised by 'Process--> Make binary, convert to mask'.

This method is not ideal, as when the streak lines are highlighted which are identified as pores, the actua pores are more than likely to be removed as well, however it is hard to differentiate between the two unless full visual analysis of the altered image to the original image is completed. The process of using the 'wand tool' is very manual and time consuming, due to having to select 'non pores' using the ImageJ tools. With the mask and wand tool, the large pores near the top appeared as separate pores which skew the results slightly, hence this can deem that using just the basic steps for a 'bad image' is not sufficient and filters are required.

3.2.1. Filters: 'Process --> Filters -->'

In light of this, user guide depiction determined that filters were required for 'bad images' in order to reduce imperfections as much as possible.

Gaussian Blur7:

Figure 11 shows: Process involved Brightness/contrast, Gaussian blur,

brightness/contrast, threshold, in stated order. This method appeared to detect pores,

however data analysis was needed in order to verify this.

Convolve8:

Figure 12 shows: Convolve created an image that was difficult to interpret. However

data analysis was also required to determine this.

Median9:

The figure 12 below, shows the results once thresholded. The intensity variations are

too vast, and hence data for pore size is impossible. A filter to smooth the background

intensities was required.

Figure 12

The same visual image results are shown using 'mean filter'10.

3.2.2. Smoothing: 'Process --> Smooth'

Smoothing11 had little effect but still was used as it reduced background streaks slightly.

Completion of all filters, and the data analysis confirmed that none are acceptable either due to incomparable pore size data, or vast image intensity background variations.

3.2.3 Improving background intensities

Subtract background12:

Figure 13

Using subtract background at a specified rolling ball radius, allowed for the intensity of the background to appear more uniform. Light background, sliding paraboloid and disable smoothing13 were all selected in the menu, shown in figure 13.

Although the subtract background removed the unevenness and produced better visual results for the streaks, the pores remained and contributed towards the pore data shown in figure 14.

Figure 14

Using the subtract background, the large pores at the top remained separate, a

process was applied to combine separated pores and make visually obvious small

pores larger, this was achieved by using dilate14, when pores which were smaller than

on the original image or broken up are dilated. However using the wand tool and

'Process-->Binary --> erode', 'clumps of pores' which were present at the bottom of the

image, due to the physical streaks , removed present pores. This posed a problem.

Using subtract background was visually be seen as positive, however there were some

streak marks still which contributed to pore data, as well as a variation in intensities

across the background. The Math filter 'gamma' was applied15 using 'Process -->

Math --> Gamma', in order to determine if using gamma proved positive. Using

gamma as a way to reduce the background noise in order for thresholding to be

carried out quickly and as efficiently as possible, visually appeared very well, and data

analysis of pore size confirmed this.

Using gamma and subtract background together produced visual results which were most acceptable. Gamma was applied first followed by subtract background.

Brightness and contrast was applied again, in order to further distinguish pores from background.

Figure 15

Gamma math filter and a subtract background value of a low size was applied, shown in figure 15, after which the image was thresholded, shown in figure 16.

Figure 16

Pore analysis was achieved once the image was binarised, 'Process --> binary --> Make binary'.

3.2.4. Pore data analysis of images

When analysing the particles the measurements were set, 'Analyse-->Set measurements', which were calculated in regards to particular preference and what you are trying to find out from your image, shown in figure 17.

Figure 17

The area, average area, circularity, minimum Feret's diameter and area fraction of

pores16 were selected in order to achieve data on pore sizes and distribution.

The best 'analyse particle' outline for binarised pores was the 'mask' from 'Analyse -->

Analyse Particles drop down menu', as the pores are dark opaque regions, and hence

comparison to the original image was easier. With the mask – the definition is '8-bit

binary image containing filled outlines of the measured particles', which was ideal for

the pores.

Once the images were analysed and data saved, 'Summary' with averages and

'Results' of each individual pore), pore distribution based on percentage of a certain

diameter of pore and average pore size and area were compared with previous

experimental work. This was achieved in 'Excel' using the formula:

=SUMPRODUCT((Excel range initial:Excel range final>=X)*(Excel range initial: Excel

range final <Y)),

in order to determine the number of pores within the range X-Z.

The same method was applied to the PCX20 and 18NRT images. However, manual filling of the 18NRT pores was necessary as outer pores were being analysed, in order for thresholding of the image to work effectively, using the 'Flood Fill' and 'Paintbrush' tool 17 in imageJ. When determining whether different magnifications had any effect on the image analysis method, the 'good image' method and 'bad image' method were used simultaneously to identify which method, if any was ideal for different magnifications.

3.3) Calcite crystals

The calcite crystal sample images (A,B and C) underwent a trial and error of the 'good image' method and the 'bad image' method, after being determined through thresholding and intensity variations. The images were cropped as physical impairments meant that neither method worked effectively. The impairments were not physically underlaying the sample, but instead were presented as cracks or other material on the sample surface. All samples but C, conformed to the 'bad image'

method, with sample C undergoing the 'good image' method. The image data was

compared to Lee's data, whose samples they were.

3.4) Dentine/Hydroxyapatite infiltration samples

The aim of analysis for this sample involved using the 'paintbrush' tool on three

separate images of the same image. Each image involved filling all pores, filling the

partially filled pores, and filling the completely filled pores in ImageJ in order to define

porosity in terms of fully occluded, partially occluded and not occluded compared to

before infiltration. After the filling of the pores, the 'good image' method was applied,

and porosity percentage values compared, for each of the pore filled images.

Figure 18 shows partially filled pores that have been manually filled using the ImageJ

tool.

Figure 18, (Earl et al., 2006)

Section 4) Results and discussion

The experimental section of this project consisted of image analysis of three different types of porous material. Initially, the visual comparison and secondly the quantitative results for each of the three materials, will be discussed. The results for the titania samples will be compared and contrasted with the results for the three separate types of titania sample. Secondly discussion of the results from calcite crystal image analysis and thirdly the results from dentine/hydroxyapatite image analysis.

Visual comparison of altered/analysed image to original images of porous materials

Titania samples

Classification in report Name of sample Description of sample A H110 70% PCX20 and 30% 250nm anatase opacifier B PCX20 Powder with particle size around 20nm and average aggregate size around 40nm C 18NRT Colloid with particles around 10nm length and 20nm width, and similar average aggregate sizing.

Table 1

4.1.1

Analysis of 'well defined image' of H110 titania sample

When analysing a good image, the expected procedure is simple with very few steps.

A well defined image can be determined visually by clear and defined pores, with a

large contrast to the background intensity of the image.

A minimum pore size of 2nm was allocated in data analysis, as mesopore range (2-

50nm) is the expected pore range for titania DSSC's. These are the specified values in

order to characterise the void as a pore. Also the size of an atom is 0.1nm, and in

order for porosity to have an effect of parameters, liquids, solutions are required to

penetrate these pores in order for their desired effect. Pores are also used to increase

surface area of a sample as well, however increase in surface area is usually required

in order for a reaction to occur quicker or more efficiently, hence liquids, and gas

compounds are required to penetrate the samples. Hence a compromised minimum

pore size of 2nm is stated as ideal for these properties.

Once the image of the H110 sample had been cropped, scaled and the type of the image (8-bit) confirmed, the image analysis results can be produced. The method used requires brightness/contrast, thresholding and binarisation.

As mentioned in the method, subtract background appears to be a common application that is required in order to remove intensity variations on an image, which are frequently discovered. These are due to the preparation methods, the electrons backscatter at different angles and so brightness intensities vary when there is an uneven surface. This could be due to the surface not being smoothed and polished enough, or the general topography of the sample.

The sample required the background to be subtracted due to an uneven brightness intensity, which was discovered once the image was thresholded.

The first trial of imaging produced, figure 19, which possessed uneven connectivity and larger pores than what can be seen on the original image. This connectivity is mainly at the bottom of the image, where the high noise and intensity appears when the image undergoes thresholding.

Figure 19

Subtraction of the background during the procedure removed variations in intensity.

The resulting analysed image is A, and the original image B, shown in figure 20:

A   B

Figure 20, (Reading,2012)

A visual comparison of the images shows that the main large pores are visible. Issues

arose with the image as can be seen as the arrow points on image A, figure 20, shows

the connection of pores which are actually separate.

Eroding using the binarise tool, was used in order to break up connected pores, seen

in figure 21. This was achieved by comparing the original image with the previously

altered image for alterations. An application called 'Watershed' can be used which

automatically erodes pores in the image based on maxima and minima values of pixels,

however when this was applied to the image, the erosion achieved was too much, and

gaps were created between whole pores.

Figure 21,

(Reading,2012)

Final image compared with original image, figure 22

Figure 22, (Reading,2012)

Image analysis of a 'impaired image' of Titania H110

The results from the visual analysis of H110 sample at 100,000 times magnification

showed that subtract background is required in order to achieve visible pore results

comparable with the original image. The image that is being defined as a 'bad image'

is of the same sample but at 20,000 magnification, hence more defects can be seen,

i.e. the physical streak marks.

The final imaging method which was defined consisted of using gamma and subtract

background, subsequently with erode and dilate tools in order to manually define pores,

or undefine pores which have been selected. Also the wand tool proved useful in

manually selecting areas which were not within the correct threshold. However other

filters had been applied previously, to determine whether they would be effective. The

visual results were seen in a previous section, and the quantitative data in the following

section.

Using gamma and subtract background applications together allowed for the difference

in background intensity on the image, to be subdued in order for thresholding to be

achieved efficiently and accurately.

The final image is shown in figure 23.

Figure 23

Using all the applications necessary to achieve this binarised image are stated in the

method. Visually, in comparison to the original image, the final imageJ product

contains the key pores that can be seen.

4.3.1 ImageJ final image in comparison with original image, figure 24

Figure 24, (Reading,2012)

4.4) Results from visual image analysis of samples Titania PCX20, and NRT18

PCX20 final image from the specified H110 method shows that only a few of the pores that can be seen on the original image are 'mesopores', and hence suitable for Dyesol use as solar cells. This is because the dye needs to enter the titania nanostructure through the pores.

4.4.1. Comparison of Titania PCX20 with the original image, figure 25

Figure 25, (Reading,2012)

The PCX20 image analysis shows that visually the pores seem similar sizes and image processing and analysis has defined the pores efficiently, however the image had to be cropped slightly due to intensity variations on the edges of the image which could not be removed effectively.

4.4.2. Image comparison of Titania NRT18 with original image

The NRT18 image is based on the outer pore and inner pores inside those. Focus on analysing the outer pores was important as this was the first contact with the dye. Once the image had been altered in 'Paint' in order to define the outer pores by

darkening them for thresholding and binarisation, the image was analysed, seen in figure 26.

Figure 26, (Reading,2012)

This image had to undergo another initial step that other samples did not need to. Due to the three dimensional nature of the image, there appeared to be pores within pores. Hence due to this the, thresholding of the image was difficult as the pixel range was not within the limits in order to distinguish between the outer pores and the outer pores. Using 'Paintbrush tool', definition of outer pores was achieved. This allowed for thresholding to occur successfully for the outer pores. This type of analysis was used a very basic method once 'paint' had been used though, due to the strong intensity pixel differences so thresholding was easy.

The images for the three samples of titania DSSC's have all been analysed visually. The visual analysis involved comparing the original image with the altered image. The altered image data can then be analysed for porosity and pore distribution in order to determine whether the pore size of the particular sample are in correlation with other

reports. This will verify the ImageJ analysis method used and determine whether it has

been successful.

Do different magnifications of images require different applications for analysis?

Using the H110 titania sample, three images of the same sample at different

magnifications were analysed. Determination of applications required in order to

produce accurate data for different magnifications were investigated. The different

magnifications are 20,000, 50,000 and 100,000 times, shown in figure 27. The analysis

procedure stated in 'method' shows that initially, the basic method was used, and then

subtract background and gamma applications used, due to positive results from

analysing previous results.

20,000   50,000

100,000

Figure 27, (Reading,2012)

The magnification of images affects the clarity of the image, hence the ability to threshold the image without the use of other applications. This is usually due to no other physical effects or intensity variations which alter the threshold values. Gamma was used in the two largest magnifications, but subtract background and erode filters were used in all three.

4.6.) Calcite crystals

Three different calcite crystal images (based on different aspartic acid amounts added) were imaged using the method identified for Dyesol. The images produced were visually 'good images' hence it was predicted that the method of image analysis would involve only a few steps. The first image analysed was 'Calcite crystal A'.

 Sample A

Figure 28, (Lee, 2013)

The image was cropped, shown in figure 28, as deformities in the sample imaging, such as the top left corner produced results which were anomalous. This meant that

the same method applied to the titania samples had to be applied on all the calcite

crystal images i.e. subtract background and gamma, except sample C, which was

cropped and two 'good images' from part of the sample were analysed. This was due

to the background light intensity differences, which proved thresholding to be difficult.

 Sample B

Figure 29 (Lee, 2013)

Owing to the physical height differences and physical impairments on the image

especially the darker background which hinders the ability for ImageJ to distinguish

between pixel intensities, the image had to be cropped in order to accurately measure

the average pore size, pore size distribution and pore percentage coverage, shown in

figure 29.

 Sample C

Similarly to the other two Calcite crystal samples, the image needed to be cropped. In the original image there was a noticeable crack which could be calculated as a pore, and also ridges which presented the same issue, shown in figure 30.

Figure 30 (Lee, 2013)

In all of the images except sample C, the same procedure was used as the titania DSSC samples. In sample C, the basic 'good image' method was used, however noise in terms of speckles in the background of the image existed, which were removed using the 'noise' application.

Dentine/hydroxyapatite porous materials

An etched dentine sample shows similar pores that would occur in teeth due to dental conditions. The pores represent open dentine tubules, shown in figure 31.

Figure 31, (Earl et al., 2006)

Hydroxyapatite rod-like nanoparticles were used to infiltrate the pores and block them, with an expected 90% blockage or partial blockage of all pores (Earl et al., 2006).

Using the image analysis, percentage pore distribution can be calculated, which can be compared to the original etched dentine sample.

The pores had to become more defined by using the 'tool' application in ImageJ, i.e. visually completely occluded pores were filled with the tool 'fill' application in order to determine porosity in comparison with original porosity.

The SEM image produced below, in figure 32-35, shows the infiltration of the hydroxyapatite rod-like nanoparticles. Analysis of the open pores after infiltration, expected to be 10% of total area of the sample was undertaken. Similar analysis for all occluded pores, and all partially occluded particles was completed. The area percentage results of the pore distribution was compared and correlated with the 40% and 50% distributions relative to what was discussed in previous publications (Earl et al., 2006).

Non occluded pore visual analysis, figure 32

Partially occluded pore analysis, figure 33

Fully occluded pore analysis, figure 34

Owing to the nature of this image analysis i.e. manual definition of the specific pores was required, extra vigilance was taken in visual comparison to the original image.

Pore analysis from data of ImageJ samples

Titania DSSC samples

4.8.1. Well defined image sample

Initially a 'well defined image' of titania DSSC H110 sample was analysed. This occurred so that a general basic method could be determined as a primary point. The image analysis of the sample in order to extract pore data was based mainly of visual comparison to the original image, figure 35. However quantitative data was used in order to develop the procedure, by relying on comparison to previous pore data reviews of similar samples.

The well defined image (titania H110) sample underwent two procedures; one including the erosion application and one without.

The average pore volume for the method one, which involved no erosion application

gave an average pore area of 819nm2, whereas with the erode applications, the

average pore area was 622nm2.

In other pore area data from previous experiments, the average mesoporous diameter

range for ordered porous titania thin cells is between 10-50nm (Karthikeyana et al.,

2005). However the average diameter is reported with variations from 15 nm

(Stathatos, 2011), to 17-25nm (Fang et al., 2011) to 25-30 nm (Rawolle M., 2012) and

to 70 nm (Ali et al, 2011). In light of this, the combined range of pores expected is

between 15-70nm, however because titania thin film pores are mesoporous, i.e. 2-

50nm, it is implied that the range will be altered to 15-50nm. Average pore area data is

difficult to compare to previous recorded data, as many scientists record pore diameter

as this is in conjunction with IUPAC mesoporous range (2-50nm) based on the

diameter of pores. However using the pore area that has been output from ImageJ,

diameter can be found using the equation from Hing's literature:

P.D= ?((Feature area)/?)?^(1/2) × 2

Equation (1)

The only implication is that the pores are not completely circular, and therefore the diameter value will not be ideal, however as a comparative, using the diameter found from this equation using known pore area, Feret's median diameter and pore size (diameter) values from literature, a determination on whether the image process and analysis is acceptable can be achieved.

The circularity of the pores for with and without the erode application is 0.25 and 0.211 respectively. It can therefore be presumed that the pore shape is irregular, as a complete circle has a circularity value of 1, becoming more elongated as the circularity value decreases.

The data achieved states that the diameter of the pores, based on the pore area/diameter equation, applying the erode filter and not applying the filter were, 28 nm and32 nm, respectively. These values represent the use of the erode filter. The pore diameter using erode filter was 28 nm, which is within the range, however when

evaluating the Feret's diameter for pore diameter, the value was around 36nm, which again is within range, however the circularity ratio of 0.25 implies a rather oblong circle allowing for the equation calculating pore area and diameter to not be deemed completely accurate, nonetheless, this value is bound to be slightly inaccurate, due to the pores not being completely circular, and instead oblong and irregular, however the two sets of diameter values obtained are similar and so using the cited pore diameters as a guide, the calculated average pore diameter is effective in determining the fact the erode filter is a useful filter to be used in the image analysis procedure, due to all pore diameter values calculated being within the 15-50nm pore diameter range.

As pore area is used to calculate pore diameter values, and pore area literature for titania thin films is not widely available, the pore size distribution graphs will be based on Feret's average diameter of pores, hence pore diameter distribution as a function of number of pores of that pore size. This is most useful in comparison to literature values as pore sizes (diameter) are readily available and the mesoporous range (which is the range for titania thin cell pores) is based on diameter, in nanometres.

Figure 35, (Reading, 2012)

Pore size (nm)	2.0-10	10.0-50	50-100	100-200	200-300	300-400
Number of pores	303	625	155	64	23	2
Pore size distribution (%)	25.8	53.3	13.2	5.5	2	0.2

Table 2

Graph 1

The graph 1 and table 2 shows the pore size distribution of H110 titania thin films. Visually analysing the original image to the processed image, the average pore size appears to correlate with the data shown in the graph. The peak at 10-50 nm shows that the pore diameter range of 15-50nm is accurate, however there are pores present at 2-~350nm, which suggests that the aggregate size of H110 Titania film (around 40nm and 250nm opacifier present) determines the ranges of sizes and infers the presence of very large pores.

Therefore it can be inferred that the erode application is required in the procedure for porosity and pore size data, mainly confirmed by visual analysis and comparison, but reinforced with quantitative data analysis.

The procedure for analysing porosity in a 'well defined' image can be defined as using the most basic method, and is detailed in a following section. The pore distribution validates that the pore diameter range 15-50 nm is still acceptable and hence the image analysis method has been sound.

4.8.2. Impaired image samples

Convolve and Gaussian blur filters

Owing to the nature of this project, different samples had to be analysed in order to define an accurate procedure to define porosity from SEM images. Analysis of three different samples of titania DSSC, and then subsequent analysis of a sample at different magnifications allowed for unique and common formats to be addressed.

From visual comparison of the first titania DSSC sample (H110) to be analysed, it was clear that this was defined as a 'bad image' due to the physical streaks which the SEM imaging technique picked up. Due to this additional filters and applications were used. Through reviewing different applications that could be used on the image the ones that prevailed included filters such as Gaussian Blur, as well as gamma math filter and subtract background, which are all discussed in a previous section.

The method developed for the 'well defined image' was applied to the H110 titania impaired image, and further filters were applied separately in order to determine porosity and whether the application was viable. Filters used were Gaussian blue and convolve as all the others visually were unacceptable and hence would give obscure quantitative data.

The results for the convolve and Gaussian blur filter suggests that the average pore area is around 425nm2 for each, this is smaller than the pore area for 'well defined' image which was of the same type of titania sample. As the well defined image was clear and easily processed and analysed, comparisons to the pore area are validated.

The ideal diameter range is 15-50 nm, and the average Feret's diameter was 20 nm

and 24 nm respectively. This is just within the specified range, however the values are

on the range limit and so accurate depiction on whether the image processing method

was accurate is difficult to validate. Comparison of the original image with the

processed image, highlights the inaccuracies further, as area in the processed image

are highlighted as pores which are not as such in the original image and vice versa.

The reason for the pore data being on the smaller end of the pore diameter range was

probably due to very small pores being highlighted, which were actually grooves from

the physical streaks on the image. The pore diameter value using the equation gives a

pore diameter of around 23 nm, which is within the middle of the Feret's diameter size;

however this is due to the fact that the circularity ratio is 0.886. This implies a nearly

perfect circular shape, and hence suggests a reason why the two diameter values

(Feret's minimum diameter and pore diameter from the pore area equation) are similar.

The pore diameter sizes is a little smaller than expected but and this is probably due to

the filters not detecting pore edges correctly due to the background interference of the

SEM image, i.e. the physical streaks. The physical streaks are interpreted as pores by the ImageJ software, and hence contribute to the pore size diameter and consequently pore size distribution. Hence an issue has occurred in that the physical streaks in the background of the H110 SEM image are required to be 'dulled' or removed as much as possible. As they are physically part of the image, it is unlikely they can be removed completely, and instead their intensity, compared to the rest of the image, lowered.

Pore size distribution graphs were not used for Convolve and Gaussian Blur filter data, because it was clear from visual analysis of the original image and processed, altered image that the procedure of image analysis used was not suitable, this was further highlighted with summary data for Feret's diameter and pore area, which inferred lower vales than expected compared to the 'well defined' image data.

In light of this, subtract background and gamma math filters were used independently in order to reduce the intensity of the background, in order to highlight the pores more effectively.

4.8.3. Subtract background and gamma math filters: applied separately

These two applications were used separately and results for average pore area were 838 nm 2 and 829 nm2 for subtract background and gamma math filter respectively.

These values are a lot larger than the 'well defined' image pore area average of ~600 nm2, this is probably due to grooves from the physical streaks in the background of the image contributing to pore area and also visually conjoining to other pores, hence pores will seem larger than they are in reality. Average pore diameter from the pore area and diameter equation calculated the pore diameter as 33nm and 32nm, where as pore diameter based on Feret's average diameter output values for 'Subtract Background' filter as 47 nm and 44nm using the 'Gamma filter'. These values are in correlation with the pore size range of 15-50nm, however graph 2 and table 3 show that the maximum pore size is very large, and out of the mesoporous range. This is possibly due to similar reasons as to why the average pore area was larger.

Gamma filter	Pore size (nm)	10.0-50	50-100	100-200	200-300	300-400	400-500	500-600
	Number of Pores	4736	1310	374	49	12	1	3
	Pore size distribution (%)	73	20.2	5.8	0.75	0.2	0.015	0.046

Subtract background filter

Pore size (nm)	2-10.0	10.0-50	50-100	100-200	200-300	300-400	400-500	500-600	600-700	700-800
Number of pores	1232	3104	1032	245	90	43	45	22	12	3
Pore size distribution (%)	21.1	53	18	4.2	1.54	0.74	0.77	0.4	0.21	0.05

Table 3

Graph 2

In this case there is not a large difference between the two diameter values (from Feret's diameter and calculated equation), which again is probably due to the fact that the average circularity ratio is on the high end of the scale, 0.88, and therefore infers near circular pores. Also the pore diameter values are similar and within range for the ideal pore diameter 15-50nm suggesting that using the Gamma and Subtract background filters are required for image processing and analysis. However the pore area is large compared to the 'well defined' image data, which suggests that an ideal image processing procedure has not been identified, nonetheless, the pore diameter averages are well within the ideal average pore range 15-50nm which suggests that using Subtract background and gamma filters are required for image processing.

The total percentage of pores between mesoporous range for each filter is above 70%, which further suggests that using the filters in the imaging procedure is essential in order to analyse mesopores in titania thin films. However the average percentage of pores that are within ideal pore diameter range determined by literature is around 53% for Subtract background and 73% for Gamma filter.

Although both of these values are large and suggest a good analysis process, processing and analysis of gamma and subtract background filters applied together was tested.

4.8.4. Subtract background and gamma math filters: applied together

Using both applications simultaneously on the 'bad image', the background, i.e. physical streaks, which were effecting the data analysis of the image were removed as much as possible in order to achieve average pore size data which was within the allocated range, shown in the table 4 and graph 3 below.

Pore size (nm)	17-50	50-100	100-200	200-300	300-400	400-500	500-600
Number of pores	4837	1119	294	24	6	1	1
Pore size distribution (%)	77	17.8	4.7	0.4	0.1	0.00016	0.00016

Table 4

Graph 3

The average pore area was calculated at 690 nm2 which is very similar to the 'well defined' image of 622nm2. This average area is used in the area and diameter calculation in order to define pore diameter, which was 28nm. In conjunction, the Feret's average diameter was 39 nm, however the circularity ratio was not 1 (0.89), hence there was some degree of irregularity which suggests why the equation calculated pore diameter is slightly lower than Feret's average pore diameter.

Figure 36, (Reading, 2012)

The minimum pore size is 17nm and the largest 560nm, however the average pore diameter area is within the ideal range, and larger pores are expected on the titania thin film due to fabrication methods, and aggregate size and opacifier used (size of 250nm), which means that a variety of pore sizes will be present.

The graph 3 shows that after a pore diameter of 50nm, the pore distribution decreases rapidly, further highlighting the fact that the mesoporous range and the ideal pore range from literature of 15-50nm is well suited and accurate. The percentage of pores between 15-50nm is 77% of the total number of pores, shown in table 4, which again implies that the ranges are precise and accurate for the sample used.

These values are well within the pore diameter range for titania thin cells, 15-50 nm, and from this it can be implied that applying Subtract background and Gamma filter together, along with the 'well defined' image 'basic procedure' is the most ideal processing method, shown in figure 36.

4.9) PCX20 and NRT18 titania DSSC samples

4.9.1. NRT18 sample

Owing to the determination that using gamma and subtract background filters were the best applications to use, they were applied to the two other titania samples, in order to determine whether the ImageJ analysis procedure was valid for other image samples.

The image analysis for the 18NRT sample produced data which was completely

different to the H110 titania film image samples. The pore area was 4090 nm2 and the

average pore Feret's diameter was 94nm, with an area and diameter equation pore

diameter of 72nm, however the circularity ratio was 0.74 which suggests that the value

is inferred. This difference in data is obvious when visually analysing the 18NRT image,

shown in figure 37, to the H110 titania image. A suggestion for this is probably due to

the powder which is used to make the titania films. The powder has irregular particle

sizes which could reflect in how the binder flows in between them, hence creating large,

irregular pores. Also the pores that were images appeared to be 'outer pores' and

within these pores 'inner' smaller pores could be seen which could suggest the large

pore area and pore diameter differences.

Figure 37, (Reading, 2012)

The Feret's diameter is above the allocated range (15-50nm), shown in graph 4 and

table 5, however the diameter value is not extremely different, and suggestions for this

are the same as for pore area differences. The minimum pore diameter was around

6nm. This implies that there are pores within the specified ideal range present, however due to fabrication methods and 'outer' pores, these have not become paramount.

Visual comparison of the original NRT18 titania image to the processed image, does not suggest that any object have been highlighted which shouldn't have been and vice versa, therefore the image analysis method is still accurate. The pore distribution data shows that 50% of the samples pores were between 6-50nm diameter, however the larger pore diameter determined as 94nm, is due to very large pores of 900nm diameter influencing the average. These large pores are the 'outer pores' which can be seen on the original sample SEM image.

Pore size (nm)	6.0-50	50-100	100-200	200-300	300-400	400-500	500-600	600-700	700-800	800-900	900-1000
Number of pores	84	50	15	7	7	0	3	1	0	1	1
Pore distribution (%)	49.7	29.5	9	4.1	4.1	0	1.8	0.6	0	0.6	0.6

Table 5

Graph 4

4.9.2. PCX20 titania sample

The PCX20 titania sample had a slightly lower average pore area of 392 nm2; however

this could be due to a larger percentage of smaller pores which reduced the average.

The Feret's diameter value was 29.5nm, which is within the ideal range. The smaller

pore area cannot be compared directly to the 18NRT and H110 titania film samples

because they are each made up of different powders which form various sizes of pores

due to the aggregate size. The PCX20 aggregate size is 40nm, which is higher than

other samples, however 18NRT has various and irregular shaped particles, whereas

H110 contains 70% PCX20 titania but also combines an opacifier (a size of 250nm),

which suggests why the pore area and pore diameter is larger. The range of pore

diameters is between 6 – 170nm, which suggests pores of similar size, which can be

seen on the original SEM image shown in figure 38.

Figure 38, (Reading 2012)

The PCX20 sample is comparable with the ideal pore diameter range of 15-50nm, as 86.2% of the pores in the sample were between this ideal average range, shown in table 6 and graph 5.

Pore size (nm)	6.0-50	50-100	100-200
Number of pores	468	66	9
Pore size distribution (%)	86.2	12.1	1.7

Table 6

Graph 5

Pore percentage per sample (porosity)

The porosity values for the samples are shown in the table 7.

Sample	H110 'Good image	H110 'Bad image' (Subtract background and gamma math filters)	H110 (Gaussian blur)	H110 (Convolve)	H110 (Subtract background individual)	H110 (Gamma math filter individual)	PCX20 18NRT
Porosity (% area)	7	3.859	12.1	9.1	13.3	4.76	4.39 12.08

Table 7

Porosity of a titania DSSC sample in order for maximum efficiency is required to be as high as possible . However, the data does not appear to show any correlation, other than the 'ideal' procedure of subtract background and gamma filter having a relatively low porosity value. In correlation to other porosity data, (Reading, 2012) the values that were achieved in these experiments were much lower, possibly due to the fact that the previous individual who completed the ImageJ porosity task, was focussing more on gas absorption data, and hence did not 'find' or highlighted areas on the SEM sample image which were not actually pores. The porosity of the H110 well defined image and impaired image are different, and this could be due to a higher magnification of the 'good image' meaning that pores could be highlighted more accurately, and smaller pores not be missed. Although with the 'impaired image', all obvious visual pores were highlighted, the image was at a lower magnification, and so obvious smaller pores, would be harder to threshold. Also, the good image is taken of a different section of the H110 sample, and so this could explain the different porosity.

4.10.1. Do different magnifications require different ImageJ procedures?

The ImageJ visual analysis of the three different magnification images of H110 in

figure 27, demonstrated that at higher magnifications less applications and filters are

needed because fewer defects are obvious on the image. On all the images, subtract

background had to be used, which is probably due to light intensities and general

fabrication errors, which occur on many SEM images that I have encountered. Data

analysis of the images showed that the three different magnifications had an average

pore size of around 4nm, which is within range for these titania DSSC. Also, circularity

values for each are below 0.5 so this explains why the average pore size is on the

lower end of the scale.

4.10.2. Surface area of the 3 samples of titania

Equation (2)

Spv= Specific surface area, m2/per unit volume

Lp = perimeter of pore perimeter

Ap= Pore area

Literature information on this technique was vague in regards to what units were required, and also whether the surface area was a ratio, hence any units could be used, or whether any unit of data had to be converted to metres squared. However as the scale on the image was in microns, I am assuming that the units remain in microns, and hence the final unit will be m2/per unit volume3 (inferred as microns).

Using the above equation known as Petrographic image analysis (REFERENCE), surface area (m2/per unit volume) of the titania samples could be calculated. The images are all 2D SEM images, so this equation allows 3D information to be achieved from 2D data. The titania DSSC thin films have been reported as having a surface area range of 50-360 m2/per unit volume (Reading, 2012), with a previous report stating an average surface area of around 130 m2/per unit volume (Tao et al., 2007).

Titania Sample	H110 good image	H110 Bad image	18NRT	PCX20
Surface area (m^2/per unit volume)	201.14	160.5	71.9	223.8

Table 8

The surface area values that were calculated for each of the samples are well within range for titania film cells, shown in table 8. All, but the 18NRT sample, correlate with each other. However the sample that was used to calculate that value was from a completely different study, and so relying on the surface area data cannot be deemed acceptable on its own, albeit it is a good comparative study. The 18NRT data is relatively low compared to the other samples, and as previously stated this is probably due to the fact I analysed the outer pores, which were bigger and fewer in number.

Comparing this surface area data in TABLE X, to Reading's data, there is a signification difference. Reading's surface area data is between 23.7-27.7 m2/g, which is not in range for titania thin films, and my only suggestion as to why is due to misinterpreted data. Surface area of the H110 images and PCX20, all appear to correlate, due to their similar titania powder properties, hence porosity properties.

However, the values of surface area pose a problem in that the units required are unknown, therefore determination and validation of the results is not accurate.

4.10.3. Comparison of the three different titania samples

The samples were each made up of different sized particles, which aggregated at different sizes. The PCX20 and the H110 samples are each made up of similar particles (H110 is made up of 70% PCX20), and therefore the data represents this. The average pore size for each of the samples is 3 and 5nm respectively. This difference in pore size is probably due to the fact that H110 is also made up of 30% 250nm anatase opacifier which would create larger gaps between the particles, hence more space for pores to form.

The 18NRT sample is made up of irregular sized pores which are not circular (10nm by 20nm), this could suggest why there is a larger average pore size, due to the fact the packing of the particles could be irregular.

4.11) Calcite crystals

The Calcite crystals were made by Phillip Lee, and so correlation of this data with his data will allow me to identify whether the procedure used for titania thin cells is also acceptable for other samples.

The three samples of Calcite crystal (A, B and C), were all made up of increasing

amounts of aspartic acid, respectively. As the amount of aspartic acid was increased,

Lee stated that the average pore size and the porosity percentage increase.

In table 9 is Lee's data for each of the samples (Lee, 2012):

SAMPLE A B C Average pore size (nm2) 489.131 691.499 888.386 Porosity (%) 2.698

3.838 9.049 Table 9

In table 10 are the results from my image analysis of the samples. As seen in the

previous visual analysis section, samples B and C were cropped and two sections from

sample C were imaged. Due to this an average of the two sections has been

calculated to achieve the data sample

C.

SAMPLE A B C Average pore size (nm2) 410.70 417.47 435.82 Porosity (%) 2.609

4.157 8.975 Table 10

As can be seen in tables 9 and 10, the average pore sizes that are calculated from my image analysis are a lot lower than from Lee. However the porosity percentage values are all very similar. The difference in porosity could be due to the fact that I imaged one SEM sample, whereas Lee took an average of many different samples of each type. Nonetheless, the pore area for sample A is very similar, and therefore this can be deemed to correlate with Lee's data.

The graphs below show the pore size distribution for sample B, shown in graph 6, and sample C, shown in graph 7. The two lines on sample C graph 6 represent the two different sections that were analysed.

Graph 6

Graph 7

The graph for sample B, graph 6, shows a range of 20-2000 nm2. This range can be used to help understand why the data I analysed is lower than Lee's. The graph 6 shows that larger pores are present and therefore in other samples that were analysed, there may be more of the larger pores, hence giving a larger average pore size.

The graph 7 for sample C shows an additional peak around 1000-2000nm2 which can correlate with Lee's average pore size of 888.386nm2. A similar suggestion as to why my average pore size is lower is the same as for sample B.

Hence the one sample that I imaged of each could have posed the lowest pore sizes. The fact that the porosity is extremely similar, and that, although the data is lower than Lee's, my data analysis shows that the average pore size is increasing with increased amounts of aspartic acid added, verifies that the ImageJ analysis method was sound. Also, there are two peaks on the sample C graph 7, which indicates that two types of pores exist, hence the reduction and lower average pore size.

Figure 39, (Lee, 2013)

A suggestion as to why my average pore area is lower could be because use of a more specific imaging process means that smaller pores are highlighted and thresholded for particle analysis, shown in figure 39.

Dentine/hydroxyapatite nanoparticles samples

The nature of this image analysis was to determine whether using the tools in ImageJ to manually define pores was efficient in order to define percentage porosity compared to the a similar sample. In this case the sample involved defining whether the hydroxyapatite nanoparticles had infiltrated the dentine tubules. The hydroxyapatite nanoparticles had either: completely infiltrated, partially infiltrated, or not infiltrated the dentine tubules at all. The data in table 11 shows the porosity percentage of the three different data values: Completely open pores (dentine tubules), completely closed, and partially closed.

Table 11

Dentine Sample	Before infiltration	Completely closed pore	Partially closed pores
Porosity (%)	34.067	16.258	11.634

The team which reported this data (Earl et al., 2006), stated that the total number of pores that had been infiltrated (completely and partially) was 90%, with 40% partially occluded and 50% completed occluded. My analysis in table 11, shows that 47.7% of pores had been completely infiltrated and 34% of pores had been partially occluded.

Owing to this positive correlation between my data and the reported data, using tools

to define pores in order for porosity comparison, is a simplistic and efficient way to

achieve comparable data. Also, a suggestion as to why the partially occluded data was

slightly lower than the expect 40%, is probably due to the fact the partly occluded

pores had to be at least 50% occluded, and as this was based on purely looking at the

SEM sample, some inaccuracy could have been presented.

Section 5) Summary

From the results, discussion and analysis, it is inferred that;

In terms of the titania thin cells:

 The type of powder used effects the pore diameter volume

 Pore diameter is an important parameter for porosity information, and can be achieved

using two methods (Area and pore diameter equation (equation (1)).

 The best and most accurate pore diameter values were from Feret's diameter

Different imaging procedures and applications produce different pore data results, the most ideal shown in section 6.

The average pore diameter for H110 titania thin cell samples is around 40 nm, for NRT18 94nm an PCX20 powder 29.5nm.

Different magnifications of the same imaged sample did not require different imaging techniques, instead slightly less varied pixel ranges.

Porosity for the samples was on average around 10%.

Specific surface area of the three titania samples was calculated and results within the specified range were calculated.

In terms of calcite crystals:

The porosity and average pore size of the calcite crystals increased with increasing amounts of Aspartic acid added (From sample A to C).

The values I achieved were in the 410-435 nm2 range, which were much lower that Lee's values.

The porosity values 2-8% were comparable to Lee's data.

The imaging method used, applying the 'impaired' image analysis method was deemed accurate due to porosity comparability.

In terms of dentine tubule infiltration with nanoparticles hydroxyapatite:

The definition of pores was improved by manually using the paintbrush tool to increase this.

The porosity values and percentages were comparable with published data, both for partially infiltrated and completely infiltrated pores.

Section 6) Step-by-step procedure

This flow chart is a guide for image analysis of greyscale porous materials from SEM imaging. Step 6 in blue should be followed if the image is 'impaired'. Step 7, in red, should be followed for a well defined image.

Step 1:

Step 2:

Step 3:

Step 4:

Step 5:

Step 6:

Step 7:

Step 8:

Section 7) Conclusion

The main aim of this project was to produce a step-by-step- method which could be

used by anyone in order to produce accurate pore data from greyscale SEM porous

images.

I feel as though the main aim of my project has been reached, and that an easy

procedure has been produced (Section 6), which can be replicated for many different

types of images.

The SEM images for the three samples, each produced data which was comparable to

literature data, and the processed image was visually comparable to the original image.

In cases where there were slight variations from the identified ideal range (15-50nm),

there was a sound explanation which could be derived, usually based on fabrication

and powder composition.

The three types of samples, however all required slight variations of the ''impaired'

image analysis', either through cropping or increasing the definition of pores through

using the manual fill tool.

Each of my aims stated at the end of section 2, have been completed, however some

in not as much detail as I wanted, for example the surface area of titania (m2/g) was

difficult to obtain.

Although, my project and results seemed to be a success, for future work I would like

to:

 Use plug-ins in my method, i.e. Weka Segmentation, which would allow me to

compare manual vs. plug-ins pore data. In my project, plug-ins were not required, due

to the fact that my data was achieved without them, and I wanted to keep my final

procedure as simple as possible, with as few steps necessary.

Compare and use my final method (Section 6) on a variety of different images, to see

if it worked on an even larger scale.

Used gas sorption techniques on the three samples in order to verify if my data was

accurate, and depict any anomalies or variations.

Compare a larger catalogue of 'Calcite crytsal' images in order to define whether the

image I was analysing contained smaller pores, and hence that was why my pore data

was smaller than Lee's.

Compare the surface area values I calculated to gas adsorption data, and try and

speak to someone who has used to Petrographic Image Analysis equation themselves,

in order for verification on units.

Obtain 3D data of each of the samples from gas absorption methods

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1 Brightness/contrast involves altering the lookup table, rather than pixel values. Using the line graph as a guide in the opened box, minimum and maximum values can be readjusted, with pixels displayed as black below the minimum and pixels above the maximum as white (Ross J., ImageJ user guide).

2 Thresholding involves segmenting greyscale images into background and object of interest. 'Red' selection displays the thresholded objects in red. Using the sliding tools, the threshold limits can be altered between 0-255pixels, with 255 as completely white i.e. background (Ross J., ImageJ user guide).

3 Binary converts a thresholded image to black and white pixels. The thresholded objects are black and the background is white (Ross J., ImageJ user guide).

4 Despeckle replaces each pixel with its neighbouring 3x3 pixel values (Ross J., ImageJ user guide).

5 Wand tool works by detecting an pixels of uniform threshold. The wand takes the pixel value from where you initially click as the first pixel (Ross J., ImageJ user guide).

6 Removes one pixel from the edge of the selection (Ross J., ImageJ user guide

7 Out-of-image pixels have a value equal to the nearest edge pixel, and when smoothing, an average of the corner and outer pixels is used (Ross J., ImageJ user guide).

8 Spatial convolution using a kernel entered into a text area. The kernel contains a centre corresponding to the centre pixel, and all outer elements correspond to outer pixels. Multiplying the source with the corresponding kernel coefficient and adding the results, forms the final pixel (Ross J., ImageJ user guide).

9 Each pixel is replaced with the median of the neighbouring pixel, in order to reduce noise, with an optimum pixel for filtering selected (Ross J., ImageJ user guide).

10 Replaces each pixel with the neighbouring mean, in order to smooth (Ross J., ImageJ user guide).

11 The image is blurred, by each pixel being replaced with its surrounding neighbour pixels (Ross J., ImageJ user guide).

12 Smooths a continuous imposing background. If an image has a noticeable background, i.e. height which disrupts the image analysis, a rolling ball radius can be applied which should be the radius of the largest object not part of the background. The ball is theoretically rolled over the 'height' surface (Ross J., ImageJ user guide)

13 Light background selected if there are dark objects on a lighter background. Sliding paraboloid involves replacing the 'rolling ball' with a paraboloid that has the same curvature at the apex as a ball of that radius. Any value of radius is allowed (Ross J., ImageJ user guide).

14 Pixel is added to the edge of the selection (Ross J., ImageJ user guide)

15 Produces an average of the pixels in order to remove large intensity variations. 'Applies the function f(p)?=?(p?255)??×?255 to each pixel (p) in the image or selection, where 0.1???????5 .0' (Ross J., ImageJ user guide)

16 Area of selection in square calibrated units, average circularity, where 1.0 is a complete circle and 0.0 is not, is calculated from $4\pi \times [Area]/[Perimeter]^2$. Minimum Feret's Diameter is the minimum (i.e. width) distance between two points of an individually thresholded area (i.e. a pore) (Ross J., ImageJ user guide)

17 A freehand brush tool for manual filling (Ross J., ImageJ user guide)