Three Dimensional Imaging of Porous Media using Confocal Laser Scanning Microscopy

S.M. Shah[1], J. P. Crawshaw and E.S. Boek

Qatar Carbonates and Carbon Storage Research Centre (QCCSRC)
Department of Chemical Engineering, Imperial College London, London SW7 2AZ, UK

Abstract

In the last decade, imaging techniques capable of reconstructing three dimensional pore-scale model have played a pivotal role in the study of fluid flow through complex porous media. In this study, we present advances in the application of Confocal Laser Scanning Microscopy (CLSM) to image, reconstruct and characterize complex porous geological materials with hydrocarbon reservoir and CO_2 storage potential. CLSM has a unique capability of producing three dimensional (3-D) thin optical sections of a material, with a wide field of view and sub-micron resolution in the lateral and axial planes. However, CLSM is limited in the depth (z-dimension) that can be imaged in porous materials. In this study, we introduce a 'grind and slice' technique to overcome this limitation. We discuss the practical and technical aspects of the confocal imaging technique with application to complex rock samples including Mt. Gambier and Ketton carbonates. We then describe the complete work-flow of image processing to filtering and segmenting the raw 3-D confocal volumetric data into pores and grains. Finally, we use the resulting 3-D pore-scale binarized confocal data obtained to quantitatively determine petrophysical pore-scale properties such as total porosity, macro- and micro-porosity and single-phase permeability using lattice Boltzmann (LB) simulations, validated by experiments.

[1] saurabh.shah10@imperial.ac.uk

1. INTRODUCTION

In the field of engineering, biomedical and geoscience applications, the structure of complex pore geometries strongly influences fluid flow through porous materials and their bulk physical and mechanical properties (Fredrich, 1999). In the last decade, the field of geoscience has developed the need to enhance understanding of the geometry and topology of reservoirs at the pore-scale to better predict transport processes with two main purposes: 1) the trapping of carbon dioxide and 2) production of remaining oil and gas (Andrew, Bijeljic, & Blunt, 2013; Blunt, et al., 2013). Such a pore-scale study is a combination of imaging the 3-D pore space of the rocks and applying accurate numerical methods to model single and multiphase fluid flow directly or indirectly on the 3-D pore space images. Traditional imaging methods such as reflected or transmitted light microscopy and scanning electron microscopy (SEM) using quantitative stereological methods were widely used in the past to estimate parameters such as porosity and specific surface area (Krohn, 1988; Wong, Fredrich, & Gwanmesia, 1989; Fredrich, Greaves, & Martin, 1993). An important shortcoming of these methods is that they produce a two-dimensional (2-D) representation of 3-D objects (Bernabe, 1991), and pores with complex shapes and connectivity are difficult to identify in 2-D images, impeding accurate analysis of rock properties.

This limitation of traditional imaging methods was partially overcome by several innovative imaging techniques. 3-D images of sandstone were generated using 2-D serial sections using SEM, incrementally taking away as little as 1 μm of surface material by polishing and coupled these with image processing techniques (Koplik, Lin, & Vermette, 1984; Lin, Pirie, & Trimmer, 1986). Although effective, this technique is laborious and is limited by the scanned sample volume in z-dimension. In the last decade, X-ray micro-computed tomography (micro-CT) and synchrotron CT have emerged as a powerful alternative to image and reconstruct the 3-D complex geometry of real porous media (Auzerais, et al., 1996). Blunt et al. 2013 have

obtained data for carbonate samples at different voxel resolutions ranging from 2.68μm to 13.7μm. The most important limitation of micro-CT is the compromise between field of view and voxel resolution along with facility access and the availability of instrument time. Confocal Laser Scanning Microscopy (CLSM) has been widely used in the biological community over the past decade (Pawley, 1990; Stevens, Mills, & Trogadis, 1994) and it has been proved effective as a new optical imaging technique in the field of geomaterials. The first application of the CLSM technique to geomaterials started in the early 1990s by Petford and Miller (1990) who used it to study fission tracks. CLSM has been used for examining pores and pore networks in sandstone reservoir rocks (Fredrich, Greaves, & Martin, 1993; Fredrich, 1995; Petford, Davidson, & Miller, 1999; Fredrich, 1999; Menendez, David, & Nistal, 2001), and also for characterising porosity in hardened concrete (Head & Buenfeld, 2006). The CLSM technique has not been widely used in imaging 3D geomaterials due to the limitation of depth information. Petford et al. (1999) obtained a maximum depth of 500 μm compromised by poor axial resolution; Fredrich et al. (1999) stated that the optical sectioning depth in rock samples ranged from 50 to 250μm, depending on the nature of the imaged rock material. In the patent literature, a method to build 3D digital models of porous media has also been reported, using confocal profilometry, transmitted CLSM and multi-point statistics with a depth limit of 500 μm (US Patent No. 20110004448 A1, 2011; US Patent No. 20120281883 A1, 2012)

The current paper presents a novel method we call ‘slice, grind and process’ integrated with CLSM to overcome the z-depth limitation. We combine CLSM imaging and reconstruction to obtain a physical field of view 7 x 7 x 2 mm^3 with a lateral and axial resolution of ~ 2.5 μm to obtain true 3D pore-grain geometries to arbitrary depth. The results for a Ketton carbonate compare favourably with other imaging techniques in the compromise between field of view and voxel resolution. The volumetric 3-D confocal rock data is then used quantitatively to

predict macroscopic petrophysical properties including total porosity, macro- and micro-porosity as well as sub-sample single-phase permeability using Lattice Boltzmann simulations.

2. TECHNICAL AND PRACTICAL CONSIDERATION

The concept of confocal microscopy was patented by Minsky (US Patent No. 3013467 A, 1957) at Harvard University in 1957, but advances in optical hardware and software technology allowed the first confocal microscope to be built at the end of 1980s (Mauko, Muck, Mirtic, Mladenovic, & Kreft, 2009). A detailed description of CLSM techniques can be found in Boyde (1990b), Webb (1996), Shah et al. (2013). In 1792, Henry Baker stated "When you employ the microscope, shake off all prejudice, nor harbour any favorite opinions; for, if you do, 'tis not unlikely fancy will betray you into error, and make you see what you wish to see" (North, 2006). This sampling bias can be largely avoided if the volume of material imaged for analysis is large. Therefore, in this study we explain the major technical and practical considerations to obtain optimal 2D and 3D high-resolution confocal images of heterogeneous and porous rocks to quantify macroscopic petrophysical properties accurately.

A pivotal factor in determining image resolution for confocal systems is the correct pinhole diameter and numerical aperture (NA). The NA is defined as $\mathrm{NA} = n_i \sin(\theta_{\max})$, where n_i is the refractive index of the immersing medium (air, water, oil, etc.) adjacent to the objective lens, and θ_{max} is the half-angle of the maximum cone of the light aperture by the lens. The modified pinhole diameter, PD, measured in Airy Units[2], is defined as pinhole diameter/magnification. A geometrical optical confocality analysis is used when PD > 1.0 AU and a wave-optical confocality analysis is used when PD < 0.25 AU (Wilhem, Grobler, Gluch, & Heinz, 2003; Park, Choi, & Kihm, 2004). The quantification of the interaction between the resolution and

[2] Airy unit, AU = $\frac{1.22\,\lambda_{ex}}{NA}$, NA is the numerical aperture and λ_{ex} being the fluorescent excitation wavelength.

noise in the confocal systems is solved by the concept of resolution probability. CLSM tends to work with PD > 0.25 AU; a diameter of 1 AU is a typical setting (Wilhem, Grobler, Gluch, & Heinz, 2003). Table 1 shows the theoretical lateral/axial resolution and optical slice thickness formulae for conventional microscopy, compared to geometrical and wave optical formulae for confocal systems. The lateral resolution in conventional microscopy is primarily based on the Rayleigh criterion given by emitted wavelength (λ_{em}) and NA of the objective lens (Webb, 1996). In this study, we are using PD ~ 1 AU as recommended, dominated by excitation wavelength (λ_{ex}), NA and the refractive index of the immersing medium between rock sample and objective, in our case air (n =1) to determine accurate lateral and axial resolution. The wave optical confocality analysis is dominated by the mean wavelength (λ_m).[3]

[3] The mean wavelength is defined as , $\lambda_m = \sqrt{2}\frac{\lambda_{ex}\lambda_{em}}{\sqrt{\lambda_{ex}{}^2+\lambda_{em}{}^2}}$

To understand the complex flow processes in the porous rock samples, a statistical characterization of the pore geometry in 3-D is necessary to understand the connectivity. According to the literature in the biomedical community, the vertical depth to which the optical slicing can be performed depends on the excitation wavelength (~ absorption peak for fluorescent dye), numerical aperture (NA), and magnification of objective. The concept of "Seeing is believing" led us to research the optimal acquisition parameters to accurately image heterogenous porous materials. We provide here a detailed guide for adjusting the acquisition parameters in the confocal system to obtain optimal 3D rock sample data with experimental validation for future research using confocal systems. The confocal microscope used in this study is a Zeiss LSM 700 (Carl Zeiss Microscopy, USA).

The selection of the fluorescent dye to impregnate the pore structures to obtain qualitative and quantitative information from confocal systems is significant (Wilson, 1990; Fredrich, 1999). The absorption peak of the fluorescent dye should be well matched to the imaging wavelength. The two most commonly used fluorescent dyes to visualize pore space in rocks and cements are Rhodamine B (Fredrich, 1999) and Epodye (Hudson Yellow) (Head & Buenfeld, 2006; Fernandes, Broekmans, & Noronha, 2009). We investigated both dyes with different excitation wavelengths to obtain optimal results and most importantly understand the depth of confocal imaging capability. Rhodamine B can be excited with laser line wavelengths 488 nm and 555 nm in our confocal LSM 700 whereas Epodye gives better results when excited at 488 nm. Therefore, we impregnated two different Mt. Gambier carbonate rock samples with resin containing Epodye and Rhodamine B using the sample preparation technique as explained in detail in the section below. The samples had a thickness of several millimetres and in both cases the dyed resin penetrated the full depth of the sample. The samples impregnated with Rhodamine B and Epodye were excited using laser line wavelengths of 555 nm and 488 nm respectively. Two sets of 3-D data were obtained using 10x and 50x magnifications with

numerical apertures (NA) of ~ 0.3 and 0.55 respectively, to investigate the quality of data. This is shown in Figure 1.

The 3D confocal data shown in Figure 1 illustrate the importance of magnification and numerical aperture to the accessible optical depth. Here we note two major limitations of CLSM. Firstly, the attenuation of the signal with increasing depth due to absorption and scattering by the sample lying above the focal plane limits the depth. This phenomenon occurs as the light passes through regions of different absorbance and refractive index within the sample itself (North, 2006). Figure 1 shows that the signal intensity decreases with increasing depth. At 10x magnification with NA ~ 0.3 (fig 1 (a) and (c)) for both the fluorescent dyes, the depth from which any signal can be obtained was limited to approximately 100 μm. At 50x magnification with NA ~ 0.55 (fig 1 (b) and (d)), the maximum scan depth was approximately 35 μm. However, the true depth at which features such as pores show intensity homogeneity was significantly smaller at approximately 20 μm for 10x and 15 μm for 50x magnification. The lateral and axial resolution obtained using 50x magnification and numerical aperture ~ 0.55 gives better resolved features than at a magnification of 10x and numerical aperture ~0.3, at the cost of imaging time and memory space. Of the two dyes, Epodye gives somewhat better depth information. The second major limitation is the optical slice thickness. The numerical aperture of the objective controls the lateral/axial resolution and the optical slice thickness. Figure 2 shows the theoretical lateral/axial resolution for excitation wavelength, λ_{ex} ~ 488 nm, approximately the same results would be obtained for λ_{ex} ~ 555 nm. Figure 3 shows the optical slice thickness for both excitation wavelengths at the same three numerical apertures (NA), using the formula shown in Table 1.

The theoretical calculation of lateral and axial resolution along with the experiments above with different fluorescent dyes and excitation wavelength guide the selection of the best acquisition parameters. The theoretical optical slice thickness for NA = 0.55 and 50x

magnification is approximately 5 μm, with a lateral resolution of 0.5 μm, whereas for NA = 0.3 and 10x magnification the slice thickness is approximately10 μm with lateral resolution of 2.5 μm. The time required to scan a large field of view area of 7 x 7 mm^2 of using 50x magnifications is 10 times larger than for a 10x magnification.

Therefore in this study we subsequently worked with 10x magnification and NA = 0.3 giving lateral resolution ~ 2.5 μm, optical slice thickness ~ 10 μm and good depth information to ~20μm. We used a z-stacking program to build the 3D data sets from the series of optical sections (Wilhelm et al. 2003). We also selected Epodye as the fluorescent dye (using excitation wavelength, λ_{ex} =488nm) over Rhodamine B. A compromise between different technical parameters has to be made to obtain the optimal solution. For example, the laser line excitation wavelength (λ_{ex} = 488nm) is set to the absorption peak of fluorescent Epodye impregnated with a mixture of resin and hardener in the rock sample. The laser power transmission when set to 10% results in observable photobleaching (Fredrich, 1999). However, 2% transmission results in no noticeable bleaching effects. Trying to reduce the optical slice thickness by adjusting the pinhole diameter to the recommended setting of ~ 1AU leads to reduced laser intensity at depth. To compensate for the reduced intensity we increase and balance the gain of the photomultiplier detector when changing the depth of investigation.

3. RESULTS

3.1. DEEP 3-D CONFOCAL IMAGING

The main issue regarding 3D confocal imaging remains the depth information. Will the finalised acquisition parameters give us enough depth information to acquire sufficient connectivity in the porous rock sample to predict the petrophysical properties? The answer so far is no, but a new method is proposed here and integrated with the optimized acquisition to help us obtain 3D data from much greater depth at high resolution to quantify pores and throats.

The work flow for the novel method is to image the pore space to the depth which can be accessed by the conventional CLSM approach and then grind away a slightly smaller layer of the rock followed by another imaging step. This process is repeated to acquire a 3D image of unlimited depth as explained in Figure 4. We have successfully applied the workflow and have acquired 100 sets of 3-D confocal data for Ketton carbonate sample and registered all the 100 sets to obtain a larger 3-D volume of depth 2 mm.

3.1.1. Step 1 – CLSM imaging

The first step of scanning was preceded by preparation of the rock sample by impregnation with a low viscosity epoxy doped with fluorescent (Epodye) dye using vacuum impregnation and positive pressure application followed by grinding and polishing to obtain an optically flat surface. (Shah, Crawshaw, & Boek, 2014). The rock sample is then imaged under CLSM using the finalized acquisition parameters (10x magnification and numerical aperture (NA = 0.3)) to obtain ~20 μm depth information. The confocal software uses an efficient z-stack slice algorithm (Wilhem, Grobler, Gluch, & Heinz, 2003). If we use z stack acquisition as shown in Figure 5 (a), where the optical slice thickness is smaller than the slice interval, the structure between the optical slices cannot be detected and ambiguity develops in the 3-D object reconstruction. Therefore we optimise the z-stack parameters as shown in Figure 5 (b), where the second acquired optical z-slice overlaps around half the optical slice thickness of the first one, fulfilling the Nyquist sampling theorem conditions (North, 2006). According to this theorem, the smallest resolvable feature determined by axial resolution (ref fig.2) in the sample can be resolved if and only if the spatial sampling frequency (slice interval ~ 2.5 μm) is more than two times smaller than the smallest resolvable feature in the sample (North, 2006).

We have acquired data at 8-bit resolution (0-255 grey levels) which we found to be sufficient for our analysis. The confocal imaging procedure acquired 10 optical slices using z-stack slice algorithm. The thickness of each optical slice was ~10 µm while the interval between the slices was ~2.5 µm capturing a total depth of ~ 25 µm. As reported in the earlier section, only the data from the first ~20 µm was used in the final image acquired at 10x magnification to avoid problems with intensity homogeneity. Therefore, we have obtained the first set of Ketton carbonate confocal volumetric data of 7359 x 7359 x 20 μm^3 using numerical aperture (NA) ~0.3 and 'tile–scanning' control to obtain a large field of view shown in Figure 6. However, the additional ~5 µm (2 slices) scanned is important additional data for image registration in the later steps.

3.1.2. Step 2 – Grind/Polish

The most significant step to overcome the depth limitation is grinding and polishing to remove the previously imaged slice from the rock sample, exposing new rock surface for re-imaging under CLSM. However, the process of grinding the rock surface with precision requires prior practice and implementing different settings on grinder/polisher equipment for individual rock types. The optimised grinding and polishing was done using a Buehler Auto-Met 300 automatic grinder and polisher. The rock sample scanned in the first step was loaded in the equipment, ground and polished to remove a 20 µm layer from the sample. The removal of the layer is directly dependent on the controls of the equipment such as platen speed, head speed, grinding and polishing disc and time to perform grinding and polishing. We have tested different combinations of the equipment parameters on the trial rock samples to optimise the removal of 20 µm layer. The sample was measured using a digital vernier caliper of high precision to validate each time that 20 µm was ground off the rock surface. Table 2 summarises all settings on the grinder and polisher equipment and Figure 7 shows the work-flow diagram required.

After grinding and polishing the rock sample surface down by just less than the depth already imaged (in this case 25 μm), the newly exposed rock surface is again placed on the sample holder of the CLSM. The major challenge here is to place the rock sample back on the sample holder maintaining the angle and position of the previously obtained first set of 3D confocal data sufficiently well to register accurately with the second data set. This was achieved by drilling small holes in the epoxy at the corners of the sample as shown in Figure 8.

The first data set (Set 1) is scanned along with one of the selected corner drilled holes as shown in Figure 9 (a) for a Ketton carbonate sample. The second data set (Set 2), after grinding and polishing is shown in Figure 9 (b). The selected drilled hole is first located through continuous scanning on the CLSM and then, once it approximately matches the position of the hole in Set 1, the second set of 25 μm confocal depth data is acquired. This process continues alternating between CLSM imaging and grinding/polishing, in this case for 100 times, to obtain depth information over 2 mm.

3.1.3. Step 3 3-D Registration and Image Processing

The problem of registering the 20 data sets of 3-D confocal Ketton carbonate data is minimized by the introduction of the drilled holes but still some manual error is introduced. There may be a small shift in lateral x and y direction and a small change in angle on placing the sample back on confocal stage after the grinding and polishing step that can be visualized by careful analysis of Figures 9 (a) and 9 (b). Therefore, the next step is the implementation of an accurate algorithm integrated with an image processing technique to register each set of true depth (20 μm) 3-D data acquired with the next set, avoiding the shifting error in x and y direction and maintaining the angle of reference data set 1 for all the incrementing sets of data down to the depth required. Each 3-D acquired data sets consists of overlapped ~ 5 μm (2 slices) section for accurate registering in z-plane.

Image processing acts as a channel to connect and register each set of acquired 3D confocal data accurately. Moreover, it provides a tool to compensate for intensity inhomogeneity and filter the noise in the images, enhancing the edges without removing any crucial information and assisting in simple segmentation of pore and grain space required as an input to our simulators to predict the petrophysical properties. A major challenge before 3D registration is the intensity inhomogeneity in the each set of acquired 3-D confocal data which comprises of 2-D stacks of images. The intensity inhomogeneity is observed for the first few and last few 2-D confocal images compared with the intensity obtained for the central 2-D images which builds each set of 3-D confocal data, shown in Figure 10 (a) and (b).

To remove this artefact, we use the 'Match Contrast' module from Avizo Fire Edition 8.0 (FEI, http://www.fei.com/software/avizo3d/) on the first and last few 2-D images with reference to the central 2-D image information. The module corrects the intensity of the first and last 2-D image with respect to the mean and variance intensity information of the reference central 2-D image of the 3-D confocal data, shown in Figure 10 (c).

After correcting each set of 3-D data for intensity inhomogeneity, the next step is to register all the single sets to construct a 3-D model. The registration of two different 3-D data sets is a common problem in 3-D imaging. The best solution to our problem of registering is an algorithm implementing rigid body transformations, normally used for registering images in the medical field. The rigid transformations consist of translation and rotation of geometrical data represented as point sets (Nikolaidis & Pitas, 2000). In this study we are using an open source ImageJ plugin *TurboReg* developed by Thevenaz et al. (1998). The plugin can automatically register 3-D data sets, but unfortunately does not give the required output. Therefore, we first manually use the plugin to register the last two slice of set 1 as reference for the first two slice of data set 2 (overlapped section). Now the registered first two slice of

set 2 is considered as a reference and the remaining slices in set 2 are registered using the plugin. The overlapped 3-D section (~5 µm, 2 slices) scanned for each data-sets proved to be effective in registering the z-plane. This process continues for all the remaining 3-D data sets.

The real question is whether the rigid body transformation algorithm registers the 2-D slices in the third (z-) dimension accurately with respect to the x-y dimensions. Figures 11 and 12 show the registered data at two positions in x-z and y-z sections. The registration is satisfactory as features are continuous in the z-direction.

The visual appearance of the registered x-z and y-z sections is satisfactory showing the pore space features, seen as bright in Figures 11 and 12. But comparing the x-z dimension and y-z dimension of micro-CT image, the edges are not smooth but instead show streaking effects when one stack of slices is registered with another. In this study, we use the rigid transformation algorithm to stack 3D confocal rock data but further research is recommended.

A 3D median filter was used to smooth the 3D registered data and subsequently each voxel was segmented into pore phase (0) and grain phase (1). A major advantage of optical imaging is that the two phases of porous material are quite distinct and simple to segment compared to micro-CT imaging. CLSM imaging has the ability to quantify sub-resolution porosity directly using the fluorescent intensity which is proportional to the voxel porosity whereas the equivalent CT number in a microporous region in a micro-CT scan depends on both both sub-resolution porosity and mineralogy. Micro-CT images are much more difficult to segment even after appropriate filtering due to the absorption of X-rays across the sample limiting the contrast of different phases in the porous material (Shah, Crawshaw, & Boek, 2015). In our study, the filtered 3D data preserves and enhances the edges to segment pore and grain space accurately using the segmentation module, ‘Interactive Thresholding’ in Avizo Fire Edition

8.0. This interactive thresholding tool is simple and allows the user to select the thresholds interactively matching the pore and grain space in 2D slices in all dimensions. The pore and grain space are represented in binarized form assigning the pore voxels in 3D data with 0's and grain voxels with 1's. The 3-D binarized data is prerequisite for any modelling method predicting the petrophysical properties. The 3-D binarized data is volumetrically rendered in Avizo Fire 8.0. The pore phase (blue colour) with isolated small pores is shown in Figure 13 (a) and the grain phase (red colour) is shown in Figure 13 (b)

3.2. Estimation of Petrophysical Properties and Validation

Once the 3-D image of the pore space has been reconstructed by the novel approach described above, it can be used to calculate petro-physical properties of the rock by already established digital rock approaches. Here we show an estimation of orthogonal flow properties as a further demonstration of the validity of the new technique.

Porosity analysis carried out directly on the filtered 3D image provides quantitative information on the total porosity; macro- and micro-porosity were validated with mercury intrusion capillary pressure (MICP) experiments on a Ketton carbonate sample from the same block. The porosity estimation from confocal images is less ambiguous than that from micro-CT imaging for the following reasons. The confocal image of any rock sample is composed of two constituents, grains (black) and pores (fluorescent green). In confocal imaging, therefore, the grey value of each pixel is directly proportional to porosity and the pure resin surrounding the sample provides a reliable calibration value for 100% porosity (Shah, Crawshaw, & Boek, 2014). The quantitative total porosity, macro-porosity and micro-porosity shown in Table 3

were calculated directly from the filtered confocal Ketton carbonate data for comparison with MICP porosity data. The macro- and micro-porosity from the MICP data is defined by the cut-off value chosen to be the voxel resolution, ~2.5 μm in this case. The small discrepancy in the split between micro- and macro-porosity results from the difficulty of choosing the cut-off value.

To calculate the single-phase permeability directly on the 3D binarized confocal data, we use a single-phase D3Q19 lattice Boltzmann (LB) model with a Multiple Relaxation Time operator (Yang & Boek, 2013). The original binarized Ketton confocal data has a size 2800 x 2800 x 827 with voxel size 2.5 μm. The current capability of our single phase LB code is limited to a maximum size of 1200^3. As earlier work (Shah, Crawshaw and Boek 2015) showed that, for Ketton, single-phase permeability estimates were unaffected by voxel size up to 10 μm, in this study we have resampled the binarized Ketton data to a larger voxel size, 5.8 μm. This produced a geometry of size 1200 x 1200 x 343 voxels for the LB estimation of permeability in the x, y and z directions. Note that other techniques such as network modelling (Blunt et al. 2013) could produce permeability estimates on larger images if the rock structure demanded both small voxel and large image sizes. This was validated approximately with single-phase experiments done on a 5 mm cylindrical Ketton carbonate core of length 20 mm from the same block, shown in Table 4. We observe that the experimental permeability is in reasonable agreement with the mean permeability (2800mD) obtained from the simulations. This lends credibility to the novel imaging method developed.

4. CONCLUSION

The rapid development in three-dimensional imaging offers an exceptional prospect to characterize the complex geometry of porous media at high resolution to understand the physics of complex fluid flow processes. In this study, we have presented a novel method combining CLSM with sequential grinding and polishing to overcome the limitation of other imaging techniques. A wide field of view can be obtained at high resolution in combination with arbitrary depth. Fewer grinding steps are required compared with conventional serial sectioning using 2D microscopy and the image quality does not degrade with sample size as in micro-CT.

Acknowledgements

This work was carried out as part of the activities of the Qatar Carbonates & Carbon Storage Research Centre (QCCSRC) at Imperial College London. We gratefully acknowledge the funding of QCCSRC provided jointly by Qatar Petroleum, Shell, and the Qatar Science and Technology Park, and their permission to publish this research. S.Shah is grateful to BG Group for funding his PhD project through Imperial College Centre for CCS. Farrel Gray (Department of Chemical Engineering, QCCSRC) and Alex Toth (Department of Earth Sciences and Engineering, Imperial College London) are gratefully acknowledged for their kind help.

References

Andrew, M., Bijeljic, B., & Blunt, M. J. (2013). Pore-scale imaging of geological carbon dioxide storage under in situ conditions. *Geophysical Research Letters, 40*(15), 3519-3918.

Auzerais, F. M., Dunsmuir, J., Ferreol, B. B., Martys, N., Olson, J., Ramakrishnan, T. S., . . . Schwartz, L. M. (1996). Transport in sandstone: A study based on three dimensional microtomography. *Geophysical Research Letters, 23*(7), 705-708.

Bernabe, Y. (1991). Pore geometry and pressure dependence of the transport properties in sandstones. *Geophysics, 56*(4), 436-446.

Blunt, M. J., Bijeljic, B., Dong, H., Gharbi, O., Iglauer, S., Mostaghimi, P., . . . Petland, C. (2013). Pore-scale imaging and modelling. *Advances in Water Resources, 51*, 197-216.

Boyde, A. (1990b). Confocal optical microscopy. In P. J. Duke, & A. G. Michette (Eds.), *Modern Microscopies: Techniques and Applications* (pp. 185-204). New York: Plenum Press.

Fernandes, I., Broekmans, M. A., & Noronha, F. (2009). Petrography and Geochemical Analysis for the Forensic Assessment of Concrete Damage. In K. Ritz, L. Dawson, & D. Miller, *Criminal and Environmental Soil Forensics* (pp. 163-180). Netherlands: Springer.

Fredrich, J. T. (1995). Imaging the Pore Structure of Geomaterials. *Science, 268*(5208), 276-279.

Fredrich, J. T. (1999). 3D Imaging of porous media using laser scanning confocal microscopy with application to microscale transport processes. *Physics and Chemistry of the Earth, Part A: Solid Earth and Geodesy, 24*(7), 551-561.

Fredrich, J. T., Greaves, K. H., & Martin, J. W. (1993). Pore geometry and transport properties of Fountainebleau Sandstone. *Internation Journal of Rock Mechanics and Mining Sciences & Geomechanics Abstracts, 30*(7), 691-697.

Head, M. K., & Buenfeld, N. R. (2006). Confocal imaging of porosity in hardened concrete. *Cement and Concrete Research, 36*(5), 896-911.

Hurley, N. F., Zhang, T., Xu, G., Xu, L., & Slim, M. (2011). *US Patent No. 20110004448 A1*.

Hurley, N. F., Zhang, T., Zhao, W., & Al Ibrahim, M. (2012). *US Patent No. 20120281883 A1*.

Koplik, J., Lin, C., & Vermette, M. (1984). Conductivity and permeability from microgeometry. *Journal of Applied Physics, 56*, 3127-3131.

Krohn, C. E. (1988). Sandstone fractal and Euclidean pore volume distributions. *Journal of Geophysical Research, 93*(B4), 3286-3296.

Lin, C., Pirie, G., & Trimmer, D. A. (1986). Low permeability rocks: Laboratory measurements and three-dimensional microstructural analysis. *Journal of Geophysical research, B2*, 2173-2181.

Mauko, A., Muck, T., Mirtic, B., Mladenovic, A., & Kreft, M. (2009). Use of confocal laser scanning microscopy (CLSM) for the characterization of porosity in marble. *Materials Characterization, 60*(7), 603-609.

Menendez, B., David, C., & Nistal, A. M. (2001). Confocal scanning laser microscopy applied to the study of pore and crack networks in rocks. *Computers & Geosciences, 27*(9), 1101-1109.

Minsky, M. (1957). *US Patent No. 3013467 A.*

Nikolaidis, N., & Pitas, I. (2000). *3-D Image Processing Algorithms3-D Image Processing Algorithms.* New York, NY, USA: John Wiley & Sons, Inc.

North, A. J. (2006). Seeing is believing? A beginners' guide to practical pitfalls in image acquisition. *The Journal of Cell Biology, 172*(1), 9-18.

Park, J. S., Choi, C. K., & Kihm, K. D. (2004). Optically sliced micro-PIV using confocal laser scanning microscopy (CLSM). *Experiments in Fluids, 37*(1), 105-119.

Pawley, J. B. (1990). *Handbook of Biological Confocal Microscopy.* New York: Plenum Press.

Petford, N., & Miller, J. A. (1990). SLM confocal microscopy: an improved way of viewing fission tracks. *Journal of the Geological Society, 147*, 217-218.

Petford, N., Davidson, G., & Miller, J. A. (1999). Pore structure determination using Confocal Scanning Laser Microscopy. *Physics and Chemistry of the Earth, Part A: Solid Earth and Geodesy, 24*(7), 569-567.

Shah, S. M., Crawshaw, J. P., & Boek, E. S. (2014). Preparation of microporous rock samples for confocal laser scanning microscopy. *Petroleum Geoscience, 20*(4), 369-374.

Shah, S. M., Crawshaw, J. P., & Boek, E. S. (2015). Micro-Computed Tomography Pore-scale Study of Flow in Porous Media:Effect of Voxel Resolution. *Advances in Water Resources*, Available online 21 July 2015.

Shah, S. M., Yang, J., Crawshaw, J. P., Gharbi, O., & Boek, E. S. (2013). Predicting Porosity and Permeability of Carbonate Rocks From Core-Scale to Pore-Scale Using Medical CT, Confocal Laser Scanning Microscopy and Micro CT. *SPE 166252.* New Orleans, Louisiana, USA.

Stevens, J. K., Mills, L. R., & Trogadis, J. E. (1994). *Three-Dimensional Confocal Microscopy: Volume Investigation of Biological Specimens.* London: Academic Press.

Thevenaz, P., Ruttimann, U. E., & Michael, U. (1998). A Pyramid Approach to Subpixel Registration Based on Intensity. *IEEE TRANSCATIONS ON IMAGE PROCESSING, 7*(1), 27-41.

Webb, R. H. (1996). Confocal optical microscopy. *Reports on Progress in Physics, 59*, 427-471.

Wilhem, S., Grobler, B., Gluch, M., & Heinz, H. (2003). *Confocal laser scanning microscopy: Principles.* Retrieved from http://zeiss-campus.magnet.fsu.edu/referencelibrary/laserconfocal.html

Wilson, T. (1990). The role of the pinhole in confocal imaging systems. In J. B. Pawley, *Handbook of Biological Confocal Microscopy* (pp. 167-182). New York: Plenum Press.

Wong, T.-F., Fredrich, J. T., & Gwanmesia, G. D. (1989). Crack aperture statistics and pore space fractal geometry of westerly granite and rutland quartzite: Implications for an elastic contact model of rock compressibility. *Journal of Gephysical Research, 94*(B8), 10267-10278.

Yang, J., & Boek, E. S. (2013). A comparison study of multi-component Lattice Boltzmann models for flow in porous media applications. *Computers & Mathematics with Applications, 65*(6), 882-890.

Figure Captions

Figure 1 Confocal 3-D data sets obtained for a Mt. Gambier carbonate sample, impregnated with (a) Epodye fluorescent dye and excited using 488nm wavelength, scanned using 10x magnification, NA ~0.3.. (b) Epodye fluorescent dye and excited using 488nm wavelength, scanned using 50x magnification, NA ~0.55 (c) Rhodamine B fluorescent dye, excited using 555nm wavelength, scanned using 10x magnification, NA ~0.3. (d) (c) Rhodamine B fluorescent dye, excited using 555nm wavelength, scanned using 50x magnification, NA ~0.55

Figure 2 Theoretical calculated lateral/axial resolution for λ_{ex} =488nm as a function of three available numerical apertures (NA) in our LSM confocal system for n=1.0 (air).

Figure 3 Theoretical calculated optical slice thickness as a function of three available numerical apertures (NA) in our LSM confocal system for n=1.0 (air) for both excitation wavelengths λ_{ex} =488nm and λ_{ex} =555nm

Figure 4 Work-flow explaining the 'grind and slice' technique to obtain higher 3-D depth information of rock samples imaged using the confocal laser scanning microscopy (CLSM) technique.

Figure 5 3-D slice confocal acquisition settings, (a) based on slice interval method and (b) recommended by CLSM software packages called optimal z-stack slice program

Figure 6. (a) Two dimensional x-y cross-sections of 3-D confocal Ketton carbonate sample (b) Volume rendered first set of 3-D confocal Ketton carbonate sample with imaged physical volume 7359 x 7359 x 20 μm^3 obtained at voxel resolution 2.5 μm.

Figure 7 Work-flow to grind and polish accurately 20μm of rock layer using Buehler Auto-Met 300 automatic grinder with appropriate grinding and polishing disc

Figure 8 Diagrammatic representation of the additional sample preparation by drilling holes at the corners of the rock sample for precise registration after each acquisition of 3-D confocal data

Figure 9 2-D confocal cross sections of Ketton carbonate sample (a) Set 1- representing the first reference confocal data and (b) Set 2- representing the slice after grinding and polishing 20 μm rock surface explored using continuous scanning controls assisting in 3-D registration.

Figure 10 Implementation of match contrast module on each set of 3-D confocal set to compensate for intensity inhomegenity for first and last few 2D confocal images.

Figure 11 Two dimensional x-z cross-sections of 3-D confocal Ketton carbonate sample showing 3-D rigid registration for 2 mm depth resolving the pore space (bright) and grain space (dark) in x-z dimension.

Figure 12 Two dimensional y-z cross-sections of 3-D confocal Ketton carbonate sample showing 3-D rigid registration for 2 mm depth resolving the pore space (bright) and grain space (dark) in y-z dimension.

Figure 13 Volume rendered 3-D binarized confocal Ketton carbonate sample with 2800 x 2800 x 827 voxel size with scanned physical volume of 7 mm x 7 mm x 2 mm and voxel resolution 2.5 μm (a) pore phase represented in blue and (b) grain phase represented in red along with pore phase in blue