Jessica Douglas is currently working on her M.Sc. at the University of Southern Mississippi, under the direction of Dr. Kenneth Curry. Her research topic is three-dimensional clay nano- and microfabric of marine sediment and laboratory control samples. It should be noted that her proposal received unanimous support from the judges.

Clay fabric (defined as the spatial distribution, orientations, and particle-to-particle relations of the solid particles) has been of scientific and engineering interest since the work of Terzaghi (1925) and Casagrande (1932). Various early models were proposed (Terzaghi-Casagrande Honeycomb Model; Goldschmidt, 1926; Lambe, 1953; and Tan, 1957) to explain the physical and mechanical properties of the soils and sediment but a significant refinement of clay particles and fabric models emerged with the advancement of transmission electron microscopy (TEM) that provided visualization of clay (Rosenqvist, 1959). For a number of years, clay fabric models evolved with concepts that increasingly coupled function with structure. However, these concepts relied upon 2-D TEM images to achieve the high resolution micrographs with great detail. Technological advances in computer capabilities and computer software, first demonstrated by Bennett *et al.* (1977) using traditional 2-D microfabric TEM photomicrographs, are presently more advanced and are helping us elucidate sediment clay fabric in 3-D images. Historically, fabric analysis using 2-D representations addressed physical properties such as porosity, void ratio, and particle size distribution, and particle shape. Important properties such as particle orientation, tortuosity, and the distinction between effective and inaccessible porosity have remained elusive until recently. With the advent of software for nanoscale 3-D reconstruction techniques, 3-D representations of clay now enables us to visualize and study quantitatively those previously elusive properties at the nanometer scale (level) of organization allowing us to refine and improve models of clay fabric from a volumetric perspective. Three-dimensional techniques using tomography and scanning electron microscopy (Keller *et al.*, 2011) are showing the promise of 3-D technology, but TEM takes this technology to the highest level of resolution that can be achieved.

Effects of organic matter (OM) on clay fabric are poorly understood but are significant to in situ depositional properties and dynamic sediment behavior (Bennett *et al.*, 1985, 1999a, 1999b, and 2004). Various investigators (*i.e.*, Mayer, 1994a, 1994b and Ransom, 1997), have proposed that clay can trap and protect OM against enzymatic degradation at the nanometer scale in significant concentrations and, furthermore, this protection extends into geologic time scales (sedimentary rock formations, *etc.*). Evidence of the sequestering process can be found within lithified stratigraphic layers of clay, shale, and mudstone, where ancient OM is still present (*Bennett et al.*, 1985, 1991, 2004).

Mayer (1994a, 1994b) proposed a monolayer-equivalency hypothesis to describe an association between the surface area of clay and organic matter concentration noting that organic matter could coat the surface of clay one molecule thick. The term monolayer-equivalent was used to describe this association because he considered a uniform monolayer unlikely. An irregular coverage of organic matter bridging between clay particles and on clay surfaces also was confirmed by Ransom *et al.* (1998).

The purpose of this project is to develop a technique for 3-D reconstruction of selected marine clay sediment that will enable refinement of nanometer scale models of clay fabric including delineation of relationships of domains and aggregates that form the building blocks of clay sediment. An effort will be to develop techniques for quantifying clay fabric and properties such as particle orientation, tortuosity, effective porosity, and inaccessible porosity. A judicious choice of (1) samples of laboratory consolidated clay for control on the mineralogy and percentages of OM and (2) natural marine sediment samples including polychaete fecal pellets and sediment from the Gulf of Mexico will be examined for comparison of clay fabric, selected physical properties, and micro- and nanofabric differences that can be ascribed to OM concentration and distribution within the different sediment 3-D volumes of study. Furthermore, techniques will be developed to visualize the otherwise electron transparent OM in 3-D representations of fabric.

Materials and Methods

Clay samples for analysis are carefully embedded in resin in a manner that preserves their ultrastructure (Bennett *et al.*, 1977, Baerwald *et al.*, 1991). Ultrathin serial sections (ca. 100 nm) are collected, carbon coated to protect against electron beam damage, and examined with TEM to discover suitable areas for analysis, i.e., long series of contiguous samples free from artifacts. Appropriate areas are photographically mapped, the electron micrographs are rendered in digital form (scanned) and assembled as mosaics in a graphing program (Corel Photo-paint). Each photographic mosaic represents one serial section (ca. 100 nm thick). The serial mosaics are assembled into a stack and converted into a 3-D representation (ImagePro). The 3-D representation can be rendered into an animation that the user can rotate to any vantage point (VRML) and can be made into movie clips (AVI, MP4) for qualitative analysis. Quantitative analysis requires substantial sub-sampling of the 3-D representation in order to perform measurements and calculations on the contiguous clay domains and aggregates. Particle fragments in the sub-samples are measured for volume, surface area, orientation (azimuth and inclination), etc. Representations can be electronically inverted so that voids can be treated as solid volumes and the same quantitative measurements can be effected. Data are exported to a spreadsheet, reorganized, and imported into a plotting program where histograms of parameters of interest are created. Figure 1 is a pictorial representation of our materials and methods. The combination of qualitative data and visualized quantitative data allow for a powerful analysis of clay fabric and various samples can be readily compared.

Work Accomplished and Work to be Done (Research)

We have developed and refined techniques for creating serial sections, photographic mosaics, and 3-D representations using our control samples with 1 and 10% OM (chitin). Creating 3-D representations for qualitative analysis has been mastered, and we have developed a procedure for sub-sampling and quantitative analysis. These techniques can be readily applied to other clay sediment samples; *e.g.*, Gulf of Mexico, and polychaete fecal pellets for comparative analyses of fabric characteristics and types.

We have developed a technique using silver proteinate for visualizing the location of polysaccharides, a class of organic molecules, in relation to nanofabric signatures observed in 2-D electron micrographs; now we must refine and extend the technique for 3-D application. We also have experience with visualizing specific types of organic molecules with colloidal gold and intend to explore this powerful technique for visualization of OM in 3-D clay fabric representations. The interaction with crude oil and marine clay is a topic of current interest for the Gulf of Mexico, and we are developing a technique for visualizing that broad class of OM.

A number of samples are available to us that would make meaningful geological comparisons of clay fabric and sediment properties. We have refined our techniques using the laboratory control consolidated smectite-illite rich samples with 1 and 10% chitin added as an organic substrate that can be visualized with silver proteinate (Curry *et al.*, 2009) and colloidal gold. This includes sub-samples that have been enzymatically digested with chitinase to analyze the protective properties of clay fabric and physico-chemistry for sequestered OM. We have samples of marine polychaete fecal pellets that represent natural, bioturbated clay, sub-samples of which have been enzyme digested to analyze the protective mechanisms of bioturbated clay sediment microstructure. We also have acquired GOM samples near the recent Deepwater Horizon oil spill that provides an additional natural sediment with the novelty of impregnated crude oil-rich mud.

Anticipated Results and Benefits

The proposed research effort will address important issues regarding the 3-D clay fabric characteristics of fine-grained clay mineral and OM-rich sediment deposits that largely control and thus determine the fundamental convection, diffusion, OM sequestering, and sediment physical and early diagenetic properties of marine muds. Important comparisons of observed 3-D nano- and microfabric will be made with earlier published 2-D fabric models to develop an understanding of potential differences in the interpretation of volumetric versus two dimensional quantitative and numerical analysis of the fundamental fabric and sediment properties as delimited and measured in the TEM 2-D and 3-D fabric models. Hypotheses will be tested by direct observation with transmission electron microscopy using appropriate staining techniques for OM and hydrocarbons (HC). Appropriate samples from the GOM will be prepared for examination. The patterns of HC distribution is best assessed using three-dimensional reconstructions of sediment clay fabric. Our recent on-going NSF funding is providing the opportunity to develop and become proficient in creating three-dimensional reconstructions of clay fabric using the control laboratory samples, and with computer software we are visualizing and characterizing both qualitatively and quantitatively the nature of the fabric, pores, and the distribution of OM; thus an excellent opportunity to address natural sedimentary deposits including the Gulf of Mexico HC-sediment issue.

We address the important issue of the long term fate of the OM and HC by natural containment through processes and mechanisms of OM and HC interaction with clay sediment.

In summary the research proposed is to:

Develop a quantitative three-dimensional visual model of OM protection in natural clay sediment pores and adsorbed on clay surfaces based on direct TEM observations that reveal the distribution of OM with respect to pores and potential energy fields developed by the clay microstructure. Marine fine-grained clay sediment represents the globally most important OM depocenters. We plan to reconstruct:

1. pore throat size and geometry and associated pores that may demonstrate a potential relationship with OM protection,

2. micro- and nanofabric pores of varying tortuosity developed by the fabric that also may demonstrate a relationship with OM protection, and

3. micro-and nanofabric in order to demonstrate the distribution of OM on edges of clay platelets (domains) which define pore openings and pathways into pores and that influence the depositional porosity, permeability and compressibility of marine fine-grained sediments.

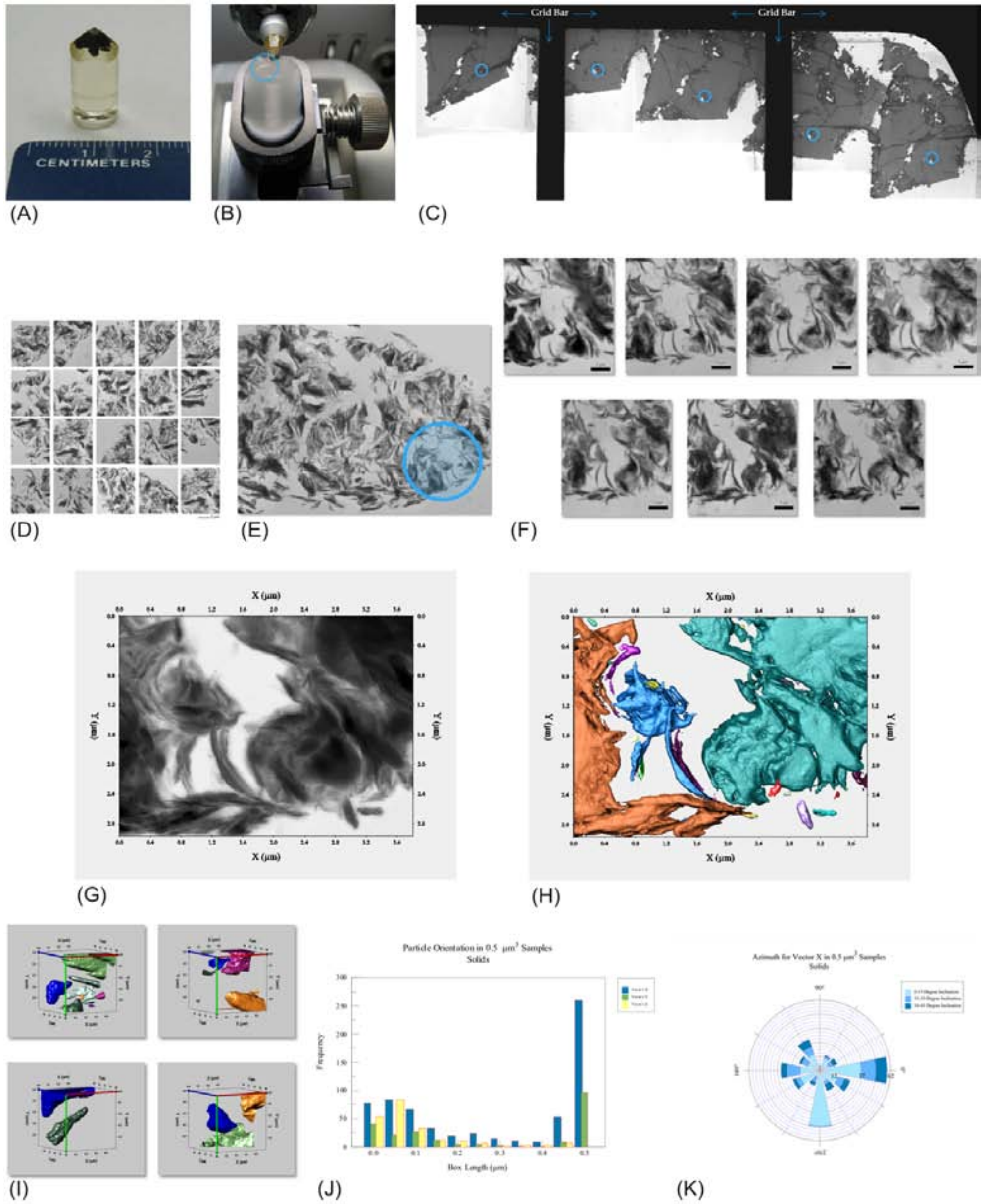

Figure 1. Pictorial description of method. (A) Resin embedded sediment, (B) Serial sections (blue circle) from an ultra microtome, (C) Electron micrographs of serial sections at low magnification with areas of interest circled, (D) Photographic map of one serial section (12,000X), (E) Mosaic of D with area of interest circled, (F) Serial set of mosaics, (G) 3-D representation, (H) 3-D representation with isosurfaces necessary for quantitative analysis, (I) Representative sub-samples of H, (J) Histograms of orientation data. See accompanying MP4 files for animations of G and H.

Bibliography

Baerwald, R.J., P.J. Burkett, and R.H. Bennett, 1991, Techniques for the preparation of submarine sediments for electron microscopy, pp. 309–320, i*n* R.H. Bennett, W.R. Bryant, and M.H. Hulbert (eds.), Microstructure of Fine-Grained Sediments: From Mud to Shale. Springer-Verlag, New York, 582 pp.

Bennett, R.H., W.R. Bryant, and G.H. Keller, 1977, Clay Fabric and Geotechnical Properties of Selected Submarine Sediment Cores from the Mississippi Delta: U.S. Dept. of Commerce, NOAA Prof. Paper 9, 86p.

Bennett, R.H., L. Lehman, M.H. Hulbert, G.R. Harvey, S.A. Bush, E.B. Forde, P. Crews, and W.B. Sawyer, 1985, Interrelationships of Organic Carbon and Submarine Sediment Geotechnical Properties: Marine Geotechnology, v. 6, no. 1, p.61-98.

Bennett, R.H., N.R. O'Brien, and M.H. Hulbert, 1991, Determinants of Clay and Shale Microfabric Signatures: Processes and Mechanisms, p.3-32, *in:* Microstructure of Fine-Grained Sediments: From Mud to Shale, Springer-Verlag, 582p.

Bennett, R.H., H.W. Olsen, M.H. Hulbert, R.J. Baerwald, W.B. Sawyer, and B. Ransom, 1999a, Organic Matter and Geotechnical Properties Interrelationships: Marine Sediments: Proceedings of the 13th ASCE Engineering Mechanics Division Specialty Conference, Johns Hopkins University, Baltimore, June 13-16, (CD-ROM).

Bennett, R.H., B. Ransom, M. Kastner, R.J. Baerwald, M.H. Hulbert, W.B. Sawyer, H. Olsen, and M.W. Lambert, 1999b, Early Diagenesis: Impact of Organic matter on Mass Physical Properties and Processes, California Continental Margin: Journal of Marine Geology, v. 159, no. 1-4, p.7-34.

Bennett, R.H., M.W. Lambert, M.H. Hulbert, C.W. Curry, H.W. Olsen, and A. Lowrie, 2004, Invited paper, Microfabric and Organic Matter Impact on Burial Diagenesis From Mud to Shale: SEPM-AAPG Special Symposium Short Course 9: Siltstones, Mudstones and Shales: Depositional Processes and Reservoir Characteristics, April 17, 2004, Dallas, Texas, available on CD through SEPM.

Casagrande, A., 1932, The structure of clay and its importance in foundation engineering: Journal of the Boston Society of Civil Engineers, p. 168.

Curry, K.J., R.H. Bennett, P.J. Smithka, and M.H. Hulbert, 2009, Hierarchical modeling of biogeochemical processes and mechanisms that drive clay nano- and microfabric development *in* A. Malik and R.J. Rawat eds., New Nanotechniques: Nova Science Publishers, Inc., Hauppauge, NY, p. 287-317.

Goldschmidt, V. M., 1926, Undersokelser over lersedimenter: Nordisk Jordbrugs forskuing , v. 4-7, p. 435 - 445.

Keller, L.M., Holzer, L., Wepf, R., and P. Gasser, 2011, 3-D geometer and topology of pore pathways in Opalinus clay: Implications for mass transport: Applied Clay Science. v. 52. p. 85-95.

Lambe, T.W., 1953, The structure of inorganic soil: Proceedings of the American Society of Civil Engineers, v. 79, separate 315, p. 1-49.

Mayer, L.M., 1994a, Surface area control of organic carbon accumulation in continental shelf sediments: Geochim. Cosmochim. Acta v. 58, p. 1271–1284.

Mayer, L.M., 1994b. Relationships between mineral surfaces and organic carbon concentrations in soils and sediments: Chem. Geol., v. 114, p. 347–363.

Ransom, B., R.H. Bennett, R. Baerwald, and K. Shea, 1997, TEM study of in situ organic matter on continental margins: occurrence and the “monolayer” hypothesis: Mar. Geol. v. 138, p. 1–9.

Ransom, B., D. Kim, M. Kastner, and S. Wainwright, 1998, Organic matter preservation on continental slopes: importance of mineralogy and surface area: Geochim. Cosmochim. Acta, v. 62, p. 1329–1345.

Rosenqvist, I.T., 1959, Physico-chemical properties of soils: Soil-water systems: Journal of the Soil Mechanics and Foundation Division, Proceedings of the American Society of Civil Engineers, v. 85, p. 31-53.

Tan. T. K., 1957, Soil properties and their measurement: 4th International Conference on Soil Mechanics and Foundation Engineering, London: International Soil Mechanics and Foundation Engineering, v. 3, p. 87-89.

Terzaghi, K., 1925, Erdbaumechanik auf bodenphysikalischer grundlage: Leipzig und Wien, Franze Dutike. 399 p.