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RPPR Final Report
as of 12-Jul-2018

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Agreement Number: W911NF-16-1-0556

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Begin Performance Period: 01-Sep-2016
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RPPR Final Report
as of 12-Jul-2018

STEM Degrees: **STEM Participants:** 16

Major Goals: See uploaded document.

Accomplishments: See uploaded document.

Training Opportunities: See uploaded document.

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Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Robert Brandon Pratt
Person Months Worked: 12.00 **Funding Support:**
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Article Title: Functional lifespans of xylem vessels: Development, hydraulic function, and post-function of vessels in several species of woody plants
Authors: Anna L. Jacobsen, Jessica Valdovinos-Ayala, R. Brandon Pratt
Keywords: active-xylem staining; cavitation; chaparral; dye ascent; hydraulic conductivity; occlusions; tyloses; vessel development; vessel differentiation; wood formation
Abstract: Abstract Premise of the Study Xylem vessels transition through different stages during their functional lifespan, including expansion and development of vessel elements, transition to vessel hydraulic functionality, and eventual transition to post?functionality. We used information on vessel development and function to develop a model of vessel lifespan for woody plants. Methods We examined vessel functional lifespan using repeated anatomical sampling throughout the growing season, combined with active?xylem staining to evaluate vessel hydraulic transport functionality. These data were combined with a literature review. The transitions between vessel functional lifespans for several species are illustrated, including grapevine (Vitis vinifera L., Vitaceae), English oak (Quercus robur L., Fagaceae), American chestnut [Castanea dentata (Marshall) Borkh.; Fagaceae], and several arid and semi?arid shrub species. Key Results In intact woody plants, development and maturation of vessel el
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Article Title: Identifying which conduits are moving water in woody plants: a new HRCT-based method
Authors: R Brandon Pratt, Anna L Jacobsen
Keywords: active xylem staining, cavitation, conductivity, dye ascent, HRCT, microCT, tracheids, vessels, X-rays, xylem
Abstract: In vivo imaging methods are useful for examination of plant vascular tissues, particularly in the identification of fluid vs gas-filled conduits; however, these methods may not allow for the simple identification of conductive conduits. Our aim in the present study was to develop a method that would allow for the in vivo identification of conductive conduits. Intact plants and segments of grapevine (Vitis vinifera L.) and intact American chestnut (Castanea dentata (Marshall) Borkh.) saplings were examined. We found that iohexol, a water soluble iodine-rich molecule, was a useful contrast agent. We also stained the xylem of segments and gas- dried samples to compare between intact scans and excised segments. Iohexol could be readily fed through cut roots or stems into the transpiration stream, was successfully transported through the xylem and marked conductive vessels within high-resolution computed tomography (HRCT) scans. Iohexol results were comparable to those obtained by staining
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14 May 2018

RE: Final report for ARO Proposal No. 68885-EV (Contract: W911NF-16-1-0556)

To whom it may concern:

I am submitting a final report for a grant entitled "Acquisition of a Micro-CT for Multi-Disciplinary Research, Teaching, and STEM Outreach at California State University, Bakersfield". The grant was awarded on 26 Aug 2016 and the original period of the grant was 12 months. A no cost extension was requested and granted and this moved the final end date to 28 February 2018. The total amount granted under this award was $496,665.

The attached report closely follows the guidelines stated in the awarding contract and includes the equipment purchased with the granted funds and a concise summary of research.

This award has greatly advanced the research and teaching capacity of California State University, Bakersfield and we are grateful for the support. If you have any questions regarding this report, please do not hesitate to contact me.

Sincerely,

R. Brandon Pratt (P.I.)
Professor of Biology

Equipment items purchased

There was one piece of equipment requested for this grant: a micro-resolution computer assisted tomographic device (microCT). These instruments use x-rays to illuminate a sample and some of these x-rays are absorbed by the sample while other pass through and hundreds to thousands of high resolution images are collected by a digital camera. These images are then reconstructed into a high resolution 3D model of the scanned object that can be used in research. Since the x-rays have high penetrative capacity, an almost limitless range of specimen types can be examined given a strong enough x-ray source.

Included in the original grant submission was a quote for a microCT instrument (Skyscan model 1272) from the Bruker Corporation. While this instrument was well suited for some of our research needs, this instrument was not the one we ended up purchasing. Instead, we purchased an instrument with far greater flexibility in the context of the range of research projects that could be undertaken. The instrument that we did purchase (Skyscan model 2211; Fig. 1) offers numerous advantages relative to the 1272 model. For starters, it has a much larger internal compartment that allows us to scan a far wider range of sample sizes. Another key to the flexibility of this instrument is that it has two cameras, a large panel-type lower resolution camera and a small high resolution camera. This has two important benefits. First, this instrument can produce very high-resolution images (as high as 100 nanometer resolution) using the small high resolution camera. The drawback is that the scans have to be very long (4-8 hours) to get the highest levels of resolution. The larger lower resolution camera allows for very fast scan times (4-20 minutes at 3 micrometer resolution), which is crucial for some studies such as scanning live organisms (we proposed to examine plants, insects, rats, marine microorganisms, and microbial pathogens). Another advantage of the 2211 is that is has an x-ray source that produces x-rays with a wider range of energy and intensity outputs. This has the benefit of being able to study a wider range of materials, including those that are very low x-ray absorbing (like thin biological samples) and those that strongly absorb x-rays, such as thick rock samples.

Due to its obvious benefits of model 2211 over model 1272, we would have proposed to purchase the 2211 instrument in the original proposal, but the cost of the 2211 was beyond the maximum amount possible for this particular award. The cost, without tax, of the model 2211 was $700,000. Two things allowed us to ultimately purchase the 2211 with this award. The first is that the Bruker Corporation gave us a 25% academic discount bringing the pre-tax purchase price down to $525,000. The second factor was that two other PIs on campus had active National Science Foundation grants that stood to greatly benefit from the added capabilities of the model 2211 instrument relative to those of the model 1272. These PIs were able to adjust their budgets to come up with some funds to support the purchase of the 2211. A final factor was that CSUB was able to provide some internal funds to make up the difference. To summarize, the vast majority of the purchase of the 2211 instrument came from this award, with 100% of the award going to purchase the instrument ($496,665). The difference between the awarded amount and the final cost of the model 2211 instrument ($525,000 + tax) came from the aforementioned sources. By leveraging the funds from this award and other awards on campus, we were able to get a far more capable instrument that allows us to examine a far wider range of research topics and greatly enhances the impact of this equipment on our research and educational activities.

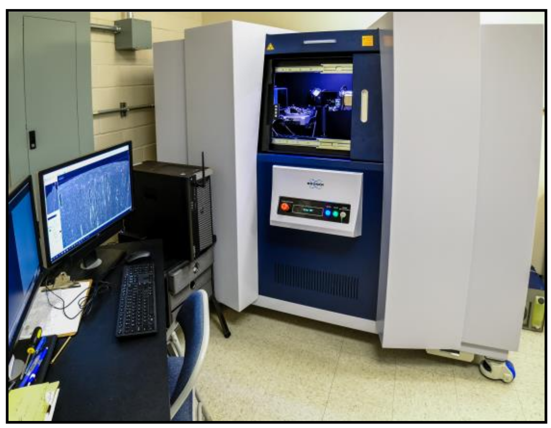

Figure 1. Image of the Bruker Skyscan 2211 installed at CSUB.

Concise summary of the research

We proposed to examine a range of research topics using microCT. Two of these projects are already completed and published (Pratt and Jacobsen 2018, Jacobsen et al. 2018), others are getting started, and others are still in the planning stage. As other researchers have learned about this instrument being on campus, there has been great interest in incorporating it into research, with multiple studies that have been undertaken that were not described in the original proposal. Moreover, because of the power of this instrument and method in general, there has been broad interest among outside groups coming to CSUB to collaborate on projects using microCT. One exciting aspect of this is that a number of students have been involved in research using microCT so the presence of the instrument is having a strong educational impact. What follows is a brief summary of work completed and ongoing using this instrument using the original headings from the proposal. I also discuss some work that was not originally proposed.

Field/Title: Biology- *Understanding the limits to plant water transport during drought* and
Field/Title: Biology- *Determining the three-dimensional structure of plant hydraulic and biomechanical networks*

One of the transformative features of this instrument is the ability to examine the internal workings of live organisms. My work in plant biology examines vascular function of plants. I have been able to scan live plants and have developed a new method of feeding medical iodine into plants and examining the water transport pathways in real time (Pratt and Jacobsen 2018; Fig. 2). This new method is now being applied to understanding how plants respond to drought. This work is ongoing. In addition, the data collected provide information on the 3D nature of the vascular system (woody tissues) of plants. These data are being used to test ideas about the role of vascular structure and biomechanical strength.

Another aspect of plant vascular function that has been examined is the water storage capacity of vascular tissue. The amount of water stored in a tissue can buffer it from dehydration and is an important component of drought tolerance. This study was undertaken by MS Biology student Alex Baer. The data we have generated have been an important part of his MS work and have allowed him to confirm another more destructive method used to estimate water content of his tissue. The micro-CT was also used to examine how living cells in the xylem may respond to drying and the potential impacts of these changes on the functional lifespan of water transport conduits within plants (Jacobsen et al. 2018).

We have plans to test how plant tissue, specifically wood, deforms when dehydrated, which will help us understand the fundamental mechanical properties of this important material. Understanding plant water transport has biomimetic applications for and improving pumps that operate under negative pressures and microfluidic systems. This area of research is also one of basic biophysics, which is a Department of Defense (DoD) research priority.

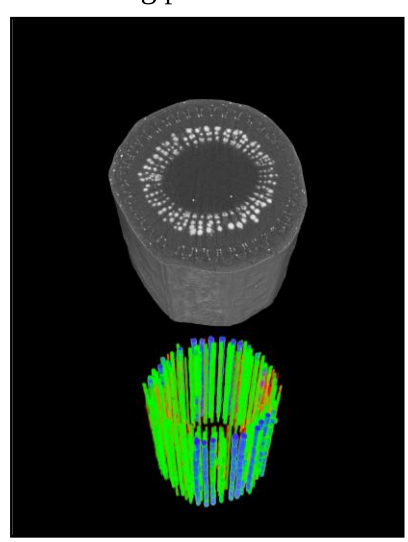

Figure 2. 3D reconstruction of the stem of a grapevine from Pratt and Jacobsen (2018). The white spots in the upper image is iodine that we fed into the root system using a new method. The colored image below are the conduits that are specialized to transport water separated from the surrounding tissues. The different colors correspond to different diameters of the conduits.

Field/Title: Biology- *Changes in muscle cross sectional area and morphology following repeated bouts of eccentric contractions.*

Dr. Todd McBride, a muscle physiologist, has been developing methods to examine muscle characteristics using microCT. One of the challenges is that muscle tissue is not inherently x-ray dense, so he has been studying and developing methods to use contrast agents that will allow the tissues to be readily visualized. The most promising protocol thus far involves submerging muscle tissues in Lugol's iodine solution for 24 hours (Metscher 2009). Another faculty member has recently joined our department, Charlotte Stinson, and she is also working on examining muscle tissues in vertebrates so this method is being developed in two separate labs.

This research on the basic understanding of muscle physiology has connections to DoD research priorities. In particular, in the context of war fighter performance (BAA for Navy and Marines, code 34), under the sub-focus of basic biomedical science (a) and stress physiology (o).

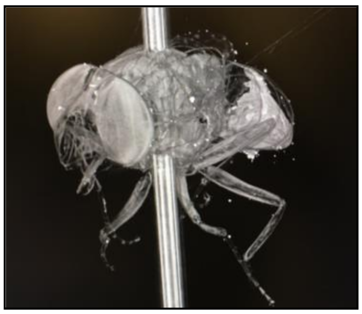

Figure 3. 3D reconstruction of pinned fly at 10 micrometer resolution.

Field/Title: Biology- *Biological investigations of the petroleum fly,* Helaeomyia petrolei, *using 3D CT scan technology.*

Dr. Paul Smith is developing methods to examine insects using HRCT with an aim to study petroleum flies. These flies live in crude oil where it bubbles up to the soil surface, which can be found in a number of areas in southern California such as the La Brea Tar Seeps. Dr. Smith has already successfully scanned some non-petroleum flies to work out methods. He is the process of collecting petroleum flies that he can use in his imaging study and he will also assess population-genetic characteristics of these interesting insects.

Field/Title: Biology- *Investigation of the interactions of marine intertidal sediment structure and microphytobenthic species composition*

Dr. Jeroen Gillard proposed to study marine intertidal sediment structure and microphytobenthic species composition. As a step toward this goal, Dr. Gillard has scanned algae from his lab. The initial results were not satisfactory because the algae are mobile and they moved during the scan. Future attempts are going to use a more concentrated agar medium during the scan, which should slow the algae and keep them still long enough to scan.

One practical benefit from these studies is that it will allow for the determination of the structural properties that are associated with high species diversity and primary productivity. This information is essential in order to artificially recreate benthic growth condition for biofuel applications.

Field/Title: Biology- *Characterization of soil microhabitats that support the growth of* Coccidioides *spp. the causative agent of valley fever in California*

This work is in its preliminary stages and is headed by microbiologist Dr. Antje Lauer. One thing that we have done to facilitate this study as it progresses is the development of methods to scan and quantify different soil types. We have had success in scanning and characterizing soils, so we are optimistic that this study will yield exciting new data. One challenge we will face is to impart sufficient contrast from fungus in the soil compared to the surrounding organic and inorganic soil matrix. We will try a range of common contrast agents highlighted in Metscher (2009) and are cautiously optimistic that iodine stain (Lugols solution) will prove fruitful.

The Department of Defense has recently granted support for a pilot study to CSUB and the Desert Research Institute (DRI) to investigate soils from *Coccidioides* endemic areas at Naval Air Station Lemoore in

Kings County and Fresno County (San Joaquin Valley) and Twentynine Palms Marine Corps Air Ground Combat Center in San Bernardino County (Mojave Desert) in California.

Field/Title: Biology- *Study of vasculature changes and other structural plant deformations caused by plant pathogens on important agricultural crops*

Dr. Isolde Francis' lab is attempting to characterize how plant pathogens of crops are modifying and negatively affecting crop structure. This project is underway and plants are growing that will be examined later this year.

Field/Title: Earth Sciences- *An investigation of how changes in pore structure correlate with strain, effective viscosity, and the development of obsidian in welded tuffs as an analog for volcanic conduit processes*

Dr. Graham Andrews has left CSUB and is now working in West Virginia, thus he is no longer pursuing this research at CSUB.

Field/Title: Earth Sciences - *Textural, Mineralogical, and Geochemical Characterization of Geologic Formations Proposed for Carbon Sequestration*

Work on this project by, Dr. Dirk Baron and two MS Geology students, is ongoing. The work is nearly finished and at this stage microCT is not being planned. The next step of this work will use microCT to expand on this initial study.

Field/Title: Earth Sciences- *Determination of physical characteristics and shell distributions within seafloor sediment cores*

This work is underway and plans have been made to obtain seafloor cores by Dr. Anthony Rathburn. In related work, deep sea dwelling foraminifera have been acquired from over 10,000 feet below the ocean surface. We scanned these unique creatures with an eye to how they are able to attach to their substrate and the structures they use to feed. We got some very nice scans of these diverse creatures and this work is being written up for a publication.

Broadly this work will help elucidate anthropogenic chemical transformations, transport, and fates in in aqueous media, and the properties of natural Earth surfaces including understanding their history, both of which are DoD priorities (Army BAA Earth Sciences section 2.1 and 2.2)

Field/Title: Engineering- *Research Applications of microCT (HRCT) in materials science*

Dr. Luis Cabrales, a materials scientist, studies the structure of soft matter, such as polymer foams, and the relationship with its physical properties. This work is underway and Dr. Cabrales and his students plan to scan some materials that have been subjected to stresses that have led to plastic strains. Examining these samples using microCT will help elucidate precisely how the materials fail and will inform subsequent material formulations to enhance strength.

This project described falls within the interests of the Army Research Offices Interest areas 9.2: Mechanical Behavior of Materials, 9.3: Synthesis and processing of materials, and 9.4: Physical properties of materials. Furthermore, these projects fall within the areas of interest of the Office of Naval Research Code 33: The Sea Warfare and Weapons Department in the focus area Naval Materials Science and Technology. These projects also support the research interest of the Air Force Office of Scientific Research, Complex Materials and Devices, focus on Organic Materials Chemistry.

Field/Title: Engineering-Petroleum- *HRCT Scan Based Study of Petrophysical Properties and Multi-Phase Flow Behavior in Porous Media*

Dr. Dayanand Saini, a petroleum engineer, is interested in studying petrophysical and geomechanical properties of high-porosity and -permeability sandstone and carbonate and low-porosity and -permeability shales. Experiments are being planned to investigate distribution and saturation of different fluids these rock types. The collected CT scan data can be used for developing, evaluating, or proving governing physics of pore scale wettability behaviors, thus devising means for improved methods for producing difficult to recover oil. The availability of HRCT scanner will assist us in developing both 2-D and 3-D digital models of porous media and in comparing the results of physical flow experiments obtained either from 2-D pore network models or actual (3-D) rock samples. His work is in the development phase and ongoing.

Projects that have been undertaken that were not part of the original proposal

At CSUB, a number of colleagues and students have learned about the microCT instrument and have undertaken projects that were not part of the original proposal. One colleague in the Department of Anthropology, Dr. Robert Yohe, is interested in using the microCT in dating Native American artifacts. This can be done by examining the water content of certain artifacts made of obsidian such as arrowheads. This is an exciting possibility because it could allow for non-destructive dating of samples, as the current method of dating requires removing a significant portion of the artifact and that permanently damages it. The initial results are promising and work continues.

A student of Dr. Yohe's, Steve Teteak, is studying medieval glass beads of exquisite craftsmanship. These beads are precious and difficult to study because of their small size and they are hard to work with being made of glass. I have scanned beads for Mr. Teteak to help him examine the internal structure of the beads to gain insight of how these ancient craftsmen made this beads, about which little is known.

Another application that has found use of the microCT is a Department of Geology student to to examine rocks on earth to develop methods that will elucidate information about extraterrestrial rocks. This work is led by Dr. Katie O'Sullivan and two of her undergraduate students, Stewart Harvin and Virginia Rodriguez. In this study, quantitative textural analysis on Mojave Desert basalt is providing information on the degree of fractionation, accumulation, and coarsening of crystals in a flow. This analysis has the potential to indicate if the rock was contaminated with sediment as it flowed across the planetary surface. The broader goal of this work is to develop methods that can be to analyze extra-terrestrial materials because meteorite samples are hard to get and usually small. The non-destructive microCT approach is ideal for analysis of these types of samples. This work will help elucidate the properties of natural Earth surfaces including understanding their history, both of which are DoD priorities (Army BAA Earth Sciences section 2.1 and 2.2).

The presence of the microCT at CSUB has attracted the attention of researchers from outside of CSUB. Many researchers (students and PIs) have visited or sent samples from the following institutions and companies:

California State University, San Marcos (Dr. Diego Sustaita; see Fig. 4)
Mazzei Injector Corporation, Bakersfield, California (Mr. Angelo Mazzei)
Occidental College, Los Angeles, California (Dr. Gretchen North)
Pepperdine University, Malibu, California (Dr. Stephen Davis and undergraduate student Natalie Aguirre)
Reed College, Portland, Oregon (Dr. Aaron Ramirez)
University of Alberta, Edmonton, Canada (Dr. Uwe Hacke and Ph.D. students Killian Fleurial and Ryan Stanfield)

University of California, Davis (Post-Doctoral Scholar Dr. Anna Davidson)
University of California, Riverside (Ph.D. student Eleneis Avila Lovera)
University of California, Santa Barbara (Ph.D. student Randall Long)
University of California, Santa Cruz (Ph.D. student Helen Holmund)

Figure 4. 3D reconstruction of harvest mouse for Diego Sustaita who is studying structure and function of animals.

Impacts of teaching and student learning

Research done with the microCT has had a strong impact on teaching and student learning at CSUB and beyond (see number of students above; Fig. 5). One activity in this context is that PI Pratt had a student use the system to study pine tree drought resistance in his Plant Physiological Ecology class in the fall 2017.

A number of student led presentations have included microCT data collected at CSUB (* = undergraduate student and ** = MS student):

**Baer, A. Thesis defense presentation entitled "Xylem functional traits at different tree positions within *Populus trichocarpa*", CSUB, March 20, 2018.
*Rodriguez, V. Africa Array Forum, Houston, TX, Oct 23rd, 2017.
*Harvin, S. CSUB Student Research Scholars Competition March 9th, 2018- Stewart only
*Harvin, S. and *Rodriguez, V. CSUB Student Research Poster Competition, April 6th, 2018.
*Harvin, S. and *Rodriguez, V. San Joaquin Geological Society CSUB poster night, April 10th 2018.
*Harvin, S. V. Geological Society of American Joint Rocky Mountain/Cordilleran Annual Section Meeting, Flagstaff, AZ, May 15th-17th, 2018.

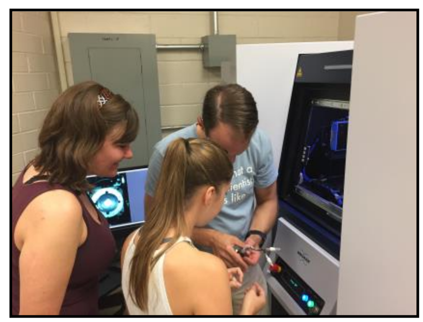

Figure 5. PI Pratt working with graduate student Helen Holmund and undergraduate Natalie Aguirre in setting up a scan.

Publications and presentations

In addition to the above student presentations, there have been two presentations by PI Pratt and senior scientist Jacobsen at an international microCT conference:

Jacobsen, AL, Pratt, RB, Davidson, AM. Quantification of ancient and modern wood microstructure using microCT, microCT user meeting in Ghent Belgium, 16-19 April 2018.
Pratt, RB, Jacobsen, AL. Examining water flow pathways in woody plants: A new microCT-based in vivo method using an iodine contrast agent. microCT user meeting in Ghent Belgium, 16-19 April 2018.

Two peer-reviewed publications have been produced using the microCT (* = undergraduate student):

Jacobsen AL, *Valdovinos-Ayala J, and Pratt RB. 2018. Functional lifespans of xylem vessels: Development, hydraulic function, and post-function of vessels in several species of woody plants. American Journal of Botany 105: 142–150.

Pratt RB, Jacobsen AL. 2018. Identifying which conduits are moving water in woody plants: a new HRCT-based method. Tree physiology in press.

Literature Cited

Jacobsen AL, *Valdovinos-Ayala J, and Pratt RB. 2018. Functional lifespans of xylem vessels: Development, hydraulic function, and post-function of vessels in several species of woody plants. American Journal of Botany 105: 142–150.

Metscher BD. 2009. MicroCT for developmental biology: A versatile tool for high-contrast 3D imaging at histological resolutions. Developmental dynamics 238: 632-640.

Pratt RB, Jacobsen AL. 2018. Identifying which conduits are moving water in woody plants: a new HRCT-based method. Tree physiology in press. https://doi.org/10.1093/treephys/tpy034