X-RAY COMPUTED TOMOGRAPHY OF WESTERN RED CEDAR BARK

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Presented at the RATS session
42nd Annual Meeting of
The American Institute for Conservation of Historic and Artistic Works (AIC)
San Francisco, CA, May 27-June 1, 2014
The research I am presenting today was completed as part of the Andrew W. Mellon Foundation Post Graduate Fellowship in Object Conservation at the National Museum of the American Indian (NMAI) in Washington DC.
My research at NMAI focused on understanding the deterioration mechanisms of Western Red Cedar in the collection there. Cedar bark is well represented in the collection...
... and objects are often accompanied with small tags marked ‘Inherently Fragile. Will Have Continued Loss.’ Being an inquisitive and somewhat stubborn conservator, I decided to see if there was anything I could do to change this gloomy forecast.
After studying the material for some time I concluded there were gaps in our understanding of this material. I decided that in order to figure out a treatment protocol, I needed to understand how the material was falling apart, and to do this I needed to better understand the chemistry and microstructures of the material. Over the following year I improved my understanding of bark materials through library study of plant biology, and by attending workshops like the NMAI bark materials workshop led by conservation scientist Dr. Mary-Lou Florian, pictured here. The technical approach was complemented with field work undertaken in Sitka, Alaska, and supervised by Tlingit artist Teri Rofkar.

I should confess at the start of the presentation that whilst I have not figured out how to treat the material, I know a lot more about why it falls apart than I did when I started.
The Western Red Cedar is an evergreen tree from the Cupressaceae (Cypress) family. Trees range from about 65-70 meters tall, and 3-4 meters in diameter with the oldest known tree calculated at 1460 years old.
The natural range of the tree is in the Pacific Northwest Coast and some inland areas - British Columbia, Washington, Oregon, Northern California, Idaho and Montana. Cultural material made from cedar bark can be found in almost all of the cultures associated with these geographical areas.
Western Red Cedar is a little unusual in that it doesn’t have a true bark at maturity. The tree has a bark layer in the first few years of growth, but the wood growth rate exceeds that of bark growth rate to such an extent that it practically ‘bursts out of its clothing’ as the tree increases in girth.

The material that we see on the outside of the mature tree is actually part of the ‘secondary phloem’ layer, normally involved in the movement of nutrients around the tree, that has undergone mechanical and chemical changes to help protect the tree from the outside world.
Cells produced at a thin layer called the vascular cambium differentiate toward the outside of the tree to become phloem cells, whilst other cells differentiate toward the inside of the tree to become xylem (wood) cells (Beck 2010).

The secondary phloem layer can range between about 1/2 and 1 inch in thickness (Dallimore and Jackson 1967).
Looking at Western Red Cedar phloem under the microscope can be a little confusing, but the basic tissue structure is beautifully simple. The 480x scanning electron microscope (SEM) image above shows a small section of phloem viewed in the transverse plane.
Elongated fibers with thick cell walls form the structural scaffold of the phloem. The cross sectional shape of these cells varies from thick, almost square cells, to flattened oblong cells, arranged in axially aligned rows.
Sieve cells are distributed in rows on either side of the fiber cells. These thinner walled cells serve as the pipe work for distribution of the nutrients and metabolites, moved through pores concentrated in the overlapping ends of these long slender cells.
Thin-walled parenchyma cells are distributed between the rows of sieve cells. Cedar phloem parenchyma cells play a role in storage of nutrients in the form of starch grains visible as small round balls in the SEM image, but may also contain phenolic compounds responsible for regulation of growth, signaling, pigmentation, UV radiation screening, and defense against herbivores depending on the stage of phloem development.
When we look at cedar phloem in three dimensions, we can see that in addition to the structural arrangement described previously, nutrients are also stored and distributed through ribbon like clusters of conducting ‘ray’ cells arranged radially in the tissue.
In this image, we are looking at a small section of what would be a continuous ring around the outside of the tree. The large green section represents the wood, the red section the cambium (where new cells are produced) and the yellow, brown and orange squares the fiber, sieve, and parenchyma cells. As new phloem cells are produced at the vascular cambium layer, older phloem cells get moved toward the outside of the tree...
The relatively fast growth rate of the wood parts of the tree exerts pressure on the phloem tissue, (imagine the phloem layer as a elastic band around the increasing girth of the tree) to such an extent that the thin walled sieve and parenchyma cells in the outer phloem layers are crushed and distorted (Beck 2010). Whilst the phloem fiber cells become increasingly lignified and therefore tougher during this process, the crushed sieve cells are no longer able to move nutrients around the tree (Barton and MacDonald 1971). Some of the parenchyma cells resist compression and become filled with phenolic compounds.

These chemical and mechanical changes contribute to the tissue’s function in providing protection against UV radiation, weathering, grazing from animals, and attack from insects and other forms of bio deterioration (Esau 1977, Franceschi et al., 1998). Cedar bark objects made from phloem tissue near the outside of the tree will have stronger fibres, more crushed cells between these fibres, potentially making it come apart more easily, and increased levels of phenols, potentially making them more resistant to UV and bio deterioration.
One of the better understood mechanisms of deterioration for Western Red Cedar phloem is related to the close association of the thick-walled fibre cells and the thin-walled, more delicate parenchyma and sieve cells. Fractures in one or more of the less robust parenchyma or sieve cells contribute to stress concentrations that, as the cell wall fails...
... are transmitted axially along the row of cells, eventually contributing to portions of tissue cleaving away. This arrangement of weak and strong cells is also the reason why cedar splits easily along the width of the tissue, a trait useful for preparing the material for production of cultural material.
My early research also examined the role of pectin in deterioration of culturally processed phloem tissue. Pectic materials exist as a gel like polysaccharide distributed in some cell walls and throughout the intercellular spaces in Western Red Cedar phloem. Pectin in the living plant is negatively charged and tends to attract Ca2+ and other positively charged ions (Cooper 1997) such that it is sometimes referred to as calcium pectate (more on this later).

While little has been written about the role of pectin in culturally processed cedar bark, empirical observation indicates that pectin gels of different concentration eventually recrystallise under ambient museum conditions. 

The image on the screen shows the extent of crystallisation of small gel disks after about one month in the climatically controlled NMAI conservation lab. It seems likely therefore that the dehydration of pectic materials in dry cedar bark will impact cellular cohesion to some extent. In other words, the cellular ‘glue’ has dried out.
I wanted to visualise the changes that occur across the width of the phloem, but given the disrupted nature of some cells in this tissue, found that physical preparation of consistent optical microscopy slides was extremely difficult.

The Western Red Cedar phloem photomicrograph above, given to me by Dr. Mary-Lou Florian, is one of the best images I have seen of how swollen cells impact the surrounding tissue. In this extremely well prepared slide, the swollen cell (possibly a radially arranged parenchyma cell) has disrupted what would have been at one time a continuous row of fiber cells.
Some of my NMAI colleagues had been experimenting with computed tomography (CT) scanning as a means to better understand what goes on in the visually inaccessible parts of objects. I felt that this non-destructive technique had potential for allowing me to more accurately understand what was going on within the phloem structure.

The resolution on the Smithsonian Institutes medical scale CT scanner was much too low to resolve the small tissue structures I was interested in. This led me to Micro Photonics, a commercial imaging lab in Allentown Pennsylvania.
The micro CT scanner used in this study is the Bruker Skyscan 1172, pictured here in the Micro Photonics lab. The sample requires no special preparation before being mounted by gently clamping on a spindle in the scanning chamber.

I won’t go into too much detail about how CT scanning works in this paper as I’m sure many of you already understand this, but essentially the scanning device produces hundreds of two-dimensional ‘slices’ though the sample, and collates these using computer software to produce a three dimensional model. The model can then be used to analyse the two and three-dimensional morphological parameters of the specimen.
The video above is a three-dimensional rendering of the cedar bark sample, composed from two-dimensional X-ray images, stacked and arranged using *Bruker Skyscan CT Vol* software.

The sample can be virtually sectioned in any plane, and while this is useful for visualising the sample shape and volume, the 1172 MicroCT can’t resolve the extremely fine cellular structure of the phloem tissue in any useful detail.
A second piece of software, *Skyscan Dataviewer*, was used to produce this video.

Here, we are looking at a cross sectional view of the phloem structure, oriented with the inner phloem near the top of the screen, and the outer phloem toward the bottom. The software allows the user to cycle through hundreds of two-dimensional cross section images produced by the scanner, revealing tissue structure patterns.

The large bright spot that appears in the tissue closest to the vascular cambium (near the top of the screen) at about 30 seconds was somewhat unexpected but interesting none the less. This bright speck, and others like it, were identified as bio-mineral crystals, but I will expand on this later.

As the video progresses, we see that the tissue in the lower section of the screen is disrupted at various points throughout. I believe that these disruptions are a result of compression of the outer phloem and swelling of phenol filled parenchyma cells described previously.

The video illustrates that even across a very narrow cross section of phloem tissue, we see considerable variation between relatively well-organised tissue near the vascular cambium, and the disrupted tissue less than 5 mm toward the outside of the phloem layer.

This video helps us visualise how objects made from more disrupted Western Red Cedar outer phloem might be less stable during handling and treatment than inner phloem objects.
I wanted to see if I could put some numbers to the visual patterns I was seeing in these videos, and asked the team at Micro Photonics to run some analysis. Analysis was performed using Bruker CT Analyzer software which uses built-in algorithms to calculate various morphometric parameters.

Four regions of interest were defined by virtually ‘shrink wrapping’ four naturally occurring sections of the sample labeled in the image above. The cell wall thickness, and volume of negative space within each region were calculated using built-in algorithms under the software 3D Analysis function, and are compared in the following tables.
This table shows the results of measurements of average cell wall thickness across the 4 regions shown on the previous slide.

<table>
<thead>
<tr>
<th></th>
<th>Region 1</th>
<th>Region 2</th>
<th>Region 3</th>
<th>Region 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bark Structure Volume (excluding air space):</td>
<td>3.45717 mm³</td>
<td>2.23383 mm³</td>
<td>2.7576 mm³</td>
<td>2.63842 mm³</td>
</tr>
<tr>
<td>Average Cell Wall Thickness:</td>
<td>0.01523 mm ± 0.00538 mm</td>
<td>0.01442 mm ± 0.00476 mm</td>
<td>0.01434 mm ± 0.00471 mm</td>
<td>0.02152 mm ± 0.01113 mm</td>
</tr>
</tbody>
</table>
As we can see from the table, there is little detectible variation in the average cell wall thickness between regions 1-3.
The cells in region 4, (the area closest to the vascular cambium) have a significantly higher average thickness than those in regions 1-3. This is most likely influenced by the high number of cells in this region with not yet fully developed intracellular spaces.
This table shows the results from measurements of negative spaces within the four phloem regions.

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<tr>
<td><strong>Total Structure Volume</strong></td>
<td>7.25978 mm³</td>
<td>4.53243 mm³</td>
<td>5.74543 mm³</td>
<td>4.35312 mm³</td>
</tr>
<tr>
<td><strong>Volume (including negative space)</strong></td>
<td>3.50052 mm³</td>
<td>2.11017 mm³</td>
<td>2.771 mm³</td>
<td>1.51355 mm³</td>
</tr>
<tr>
<td><strong>Negative Space Percent</strong></td>
<td>48.22 %</td>
<td>46.56 %</td>
<td>48.23 %</td>
<td>34.77 %</td>
</tr>
<tr>
<td><strong>Average Width of Tissue Negative Spaces</strong></td>
<td>0.01950 mm ± 0.000863 mm</td>
<td>0.01817 mm ± 0.000664 mm</td>
<td>0.01835 mm ± 0.000631 mm</td>
<td>0.01691 mm ± 0.000593 mm</td>
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The table shows that, although regions 1-3 have very similar amounts of negative space as a percentage of the total tissue volume...
...the average size of each discreet area of negative space in region 1 is larger...
...than in regions 2 and 3, and significantly larger than in region 4.

The larger areas of negative space within region 1 may make this material less suitable for the production of fine grade cultural material than that in regions 2-4. In simple terms the bark in region 1 has bigger holes in it.

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One of the more unexpected results of CT scanning this material was the visualisation of relatively dense material distributed throughout the bark.

The model above has been produced using the same Skyscan CT Vol software described previously, with the settings adjusted to represent only the most dense material within the tissue.

The video on this slide goes some way to illustrating the number and distribution of dense materials (represented by white specks), which turn out to be bio-minerals, within the bark structure. Bio-mineralization is the precipitation of minerals as a result of the metabolic functioning of a living organism, and is widely practiced by plants. Plant-tissue biominerals are overwhelmingly of three chemical types: calcium oxalate, silica, and calcium carbonate. Of these types, calcium oxalate is thought to be the most prevalent and widespread.
This video was produced using the Skyscan Data Viewer software described previously.

In this video we are looking at a transverse view of the phloem tissue with colours assigned to the different pixel density values. In this case, blue and white pixels represent the most dense material within the tissue, while yellow and red colours the least dense material.

As we cycle through the two-dimensional images, distributions of blue/white coloured dense material appear to flow from the tissue nearest the vascular cambium toward to the tissue closer to the outside of the tree, giving us a much clearer idea of how the bio-minerals are distributed throughout the phloem. Of course the material is not ‘flowing’, but if we translate this into a three-dimensional volume, it would be distributed in gently sloping radially-arranged channels (running from the inside to the outside of the plant) in much the same orientation as the phloem rays.

The video also indicates flushes of dense material in association with areas of disruption. It is unclear, but interesting to speculate about whether this material is contributing to dehiscence in these areas (splitting at maturity along a built-in line of weakness in a plant structure in order to release its contents).
The sample material was examined using scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) to help characterise the relatively dense material within the phloem structure.

As we can see in the series of images above, white specks of material in the intercellular spaces agree with the distribution of dense material observed in the previous video.
Using EDS mapping, we see calcium detected in correlation with the white material. The association of Ca2+ ions with negatively charged pectin makes it difficult to distinguish between the distribution of calcium pectate and calcium oxalate using EDS mapping alone.
When we look at the white material in more detail we can see that what appear to be amorphous globs are in fact made up of clusters of small shard-like crystals. The individual crystals in this image measure in the region of 1-2 microns in diameter. Again using EDS mapping, we can see that the rhombohedral and tetragonal crystals map closely for calcium and oxygen, suggesting that they are likely calcium oxalate.

A 2003 study that examined distribution of calcium oxalate in different conifers (Hudgins et al 2003) agrees with the distribution in our sample, with calcium oxalate most abundant between radial walls of all cell types, and generally embedded in and enveloped by cell wall material. The study also concludes that there are higher concentrations of crystals in the middle and outer phloem than in the inner phloem tissue, and that some members of the Curprassaceae family have 10-20 times the amount of CaOx as are found in other conifers.

The large volume and widespread distribution of calcium oxalate in Western Red Cedar phloem may well be a contributing factor in the deterioration by cell wall abrasion of cultural material made from this tissue. Some of the abrasive action is likely mitigated in the living plant as the crystals are distributed within a pectic gel matrix, but as the pectic material crystallises in harvested and processed phloem, the lubricating action of the pectin is greatly reduced. The higher distribution in middle and outer phloem tissue may indicate increased abrasive action in cultural material made from these parts of the phloem.
When we look at the more well-defined crystals using the SEM we can see that these crystals are much larger -- between 10 and 20 microns in diameter -- and have a less regular shape than the smaller calcium oxalate crystals. Using EDS we can see that the crystals are formed from aluminum (green), titanium (yellow), potassium (purple) and silicon (blue) and are likely silicate biominerals formed in combination with metal ions absorbed from the surrounding soil.

While these crystals are much less abundant than the calcium oxalate crystals, their relatively large size could be enough to induce localised stresses necessary to initiate the axial cleavage mechanism described previously, contributing to delimitation of layers of phloem tissue.
I had hoped to use the Bruker analytical software to map the number and distribution of crystals within the phloem tissue. While this ‘bright speck analysis’ functionality exists within the software, the resolution of the scans was too low to accurately detect the extremely small individual calcium oxalate crystals. The 1-2 micron diameter crystals are smaller than the individual 3.77 micron pixels size selected for these scans.

I am currently making arrangements to have cedar sample analysed at Skyscans Laboratory in Belgium where they have a relatively new unit -- the 2011 x-ray nanotomograph -- with spatial resolution in the range of hundreds of nanometers (1million nm = 1 mm).

Some research into the size and scale of pertinent features of sample material can be helpful in selecting the appropriate scanning technique- CT, Micro-CT, to Nano-CT.
Whilst some of these findings may seem abstract, they have implications for the conservation of Western Red Cedar bark material objects. Hilary Stewart (Stewart 1984) mentions a process of ‘grading’ cedar bark after harvest by splitting the thickness of the harvested phloem. Further investigation of this process may help refine the currently used terms ‘inner’ and ‘outer’ phloem.

Cedar bark objects in the collection of NMAI range from material that appears to be made from very fine, well ordered phloem consistent with that observed near the cambium, while other objects appear to be made from a ‘rougner’ grade of phloem material.

Further research is required to gain a better understanding of how much of the phloem harvested from the tree is used to produce cultural material. How do harvesters and makers determine the inner/outer tissue boundaries? How are different parts of the phloem tissue used for different applications?
This research concludes that cultural material made from phloem tissue close to the cambium in the living tree may have quite different properties to material formed from phloem tissue in relatively close proximity (Hudgins et al. 2003). Disruption of sieve and parenchyma cells, observed in the outer but not the inner phloem, will likely impact the structural stability of cultural materials made from these tissues. Likewise abrasion associated with increased accumulation of calcium oxalate crystals in outer phloem tissue may result in increased cell wall damage during manipulation of some bark objects during treatment and handling for exhibit and storage. The higher concentration of extractable phenols in Western Red Cedar outer bark versus inner bark indicate that inner bark material objects may be more susceptible to damage from UV and biological deterioration than those made from outer bark tissue (Franceschi et al., 1998, Lattanzio et al., 2006). This needs to be considered in relation to storage and display of these types of objects.

Whilst I agree that these conclusions do not necessarily solve the problems that I initially set out to, I believe they bring us one step closer to understanding where to begin. Beyond my current research commitments, I would like to correlate what I have learned about this unique material with condition issues observed in museum collections, and eventually draw up a list diagnostic features and treatment protocols for the different types of phloem tissue.
Thank You!

My sincere thanks to the following people, all of whom have been invaluable in putting this research together.

Marian Kaminitz
Emily Kaplan
Kelly McHugh
Susan Heald
Lauren Horelick
Mary-Lou Florian
Terri Rofkar
Michelle Austin Dennehy

and

The Smithsonian Institute
The National Museum of the American Indian
The Andrew W. Mellon Foundation
The Margaret A. Cargill Foundation