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## Tree core processing for Windendro analysis

### Mounting-

- 1) Label a mounting board with the stand name, cross section #, and an arrow pointing in the direction that the bark end will face.
- 2) Slowly remove **dried** cross section from straw. A long thin metal stake (e.g. from a flag) with a little masking tape on the end works well. If there are multiple pieces, make sure you have them lined up properly before mounting.
- 3) Place wood glue in the groove of the mounting board for the length of the core. If the core has some small pieces it is better to err on the side of more glue.
- 4) Place the core into the glued groove and wrap it snugly with string. Make sure the core is aligned the way it was in the tree. You should see a shiny band alongside each core representing wood cells that were "ripped" longitudinally by your increment borer. These represent the sides of the core. On conifers, you can look at the end of each core and you'll see the vertical alignment of cells (see picture to the right).
- 5) Allow to dry for the full time recommended on the glue container.
- 6) If part of the core falls to pieces, it may still be worth mounting some or all of the remaining pieces. Use the following guidelines:
  - A. mount pieces that have >30 years on the BARK end of the core, even if the rest of the core has crumbled.
  - B. If you end up with large continuous pieces of the increment core that are separated by gaps, do the following:
    - i. If the gap is larger than ~10 years, discard the sections beyond the gap(s)
    - ii. If the gap is less than ~ 10 years, mount the section(s) beyond the gap (leaving an actual small space) and mark the location with an x on the core mount.



### Sanding-

- 1) Place the mounted core (with the string removed) horizontally in a table clamp.
- 2) Use an electric hand sander with 100-grit sand paper to flatten the surface of the core. It is only necessary to remove 1/4 to 1/3 of the core's diameter.
- 3) Re-sand with 320-grit sand paper to improve ring resolution. This only takes a couple of seconds. For some cores it may be necessary to sand further with a finer-grit paper, but this can be done quickly by hand (rather than with the electric sander). If all rings cannot be distinguished by eye after sanding with 320, move on to a finer grit and iterate this process if necessary.

**Note:** It is much faster to do steps 1 and 2 with all cores before moving on to step 3.

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## WinDENDRO Protocol: Tree Core Analysis [needs updating by Ailene]

### Getting Started-

- 1) Log-in to computer using WINDOWS not Mac OS
- 2) Open WinDENDRO from the Start menu.
- 3) A window comes up asking you to select the (image) source. Select the scanner "Epson Perfection V700/U750..." (not "WIA-Epson Perfection...")
- 4) Load "Default Core Settings V2009b.CFG" (for cores) or "Default Cookie Settings V2009b.CFG" (for sapling "cookies") under MISC Menu → LOAD CONFIGURATION
  - a. Located in **Desktop→WinDendro Folder**
  - b. Note: to save a configuration MISC→SAVE CONFIGURATION

### Scanning & Path Creation-

- 1) Place a core on the scanner, with the core down.
  - a. *If scanning multiple cores at once* – line both cores up right next to one another with pith and bark ends of core together. Use the wooden scanning aids to make sure the cores are lined up straight on the scanner (this make analysis easier). If you are scanning core 1 and 2 together, place core 1 on the right of the scanner (closer to the computer).
  - b. *If core is scratched, has tiny rings or is otherwise difficult to read, you can mildly wet the core with a sponge before scanning, or touch up with 300, 600, and 1200 grit sandpaper.*
- 2) When you are ready to analyze the core, click on the **Scanner icon** (or select **Image Menu→Acquire Image**). Every time you acquire a new image, a window will pop up asking you to save the changes on the present image. Make sure to save!
- 3) "Epson Scan" and "Preview" windows will pop up! Make sure the scanner is set to "Photograph" under document type and is in color. Click **Preview** button, then select the area you want to scan. Select the area around the core (the image is saved as a TIF, so the smaller the image, the smaller the size of the file and the faster it will scan. Do make sure to leave some extra room though).
- 4) Set the resolution to what you would like. We have been using around 800-1200 dpi, but it depends on how fine the tree rings are. Finer rings typically demand higher resolution. Saplings will probably require higher resolution than tree cores. Higher resolution means it takes longer to scan! If scanning a core in which it is especially difficult to distinguish rings, you may also want to play with the brightness and contrast settings by clicking on the "**Brightness...**" button.
- 5) Scan image!
- 6) As soon as a scan is complete, save the image (in the **Image** menu → **Save as...**). This is important in case WinDENDRO crashes or if you have to close the program and start over—at least you won't have to scan the image again!\* Name the file as the Site-Core-#. Save the image file as "Tree ID – Core ID.tiff" (eg "A10-all.tiff") under **Desktop→WinDendro→[year collected] collected→Tree Cores** (or **Sapling Cross sections**) and select the stand folder. For tree numbers 1-9, add a zero so that the numbers will sort correctly (ie. A1 becomes A01).
- 7) You will need to zoom out (little z in the lower left-hand corner of screen) before you can conduct the analysis. If you realize that the core is not straight, you can change the path creation parameters to analyze the core in segments rather than one long line. Go to 'Path' and then 'Creation Parameters' and choose the last option which has a plus sign with 2 lines coming out of it.
- 8) Click the "+" icon on the pith side of the core first, then beyond the bark side of the core. This creates the "path." *For multiple cores repeat 7 and 8.*
- 9) The identification window comes up when you have completed drawing the path; enter the site, tree & core ID (i.e. P096-2) and the year the core was taken. If this is the first core you are analyzing since opening Windendro, a window will pop up telling you "Data are ready to be saved. No data file is open. Would you like to..." Click **Open** and select the appropriate data file from **Desktop→WinDendro→WinDendro→[year collected] collected→Tree Cores (or Sapling Cross sections)** Folder (if not than data will be saved on previously loaded data file). Make sure to change data files if you change sites/sides of the mountain. Change files BEFORE you scan in the new core, but AFTER saving the image you're working on (otherwise you will save data to the

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incorrect data file). To do this, go to **Data→Close File** to exit the file you are currently writing to and then **Data→Open File** or **New File** to switch between files or add a new file.

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## Analyzing the Core 1) Check & edit Rings 2) Remove Gaps

- 1) As a general rule, try to work from the bark end to the pith end. Edit the rings. **Delete rings** by clicking on them. **Add rings** by clicking where they should be added. You can also **move rings** or change the **orientation** of a ring by holding down the SHIFT button while clicking on the ring.
  - c. **Important:** Most recent ring (2012 or the year that core was collected) starts where the bark meets the wood.
  - d. **Note:** Although our climate record only goes back to ~1900, please identify rings as far back in the core as possible (i.e. the whole core, unless there is a big rotten spot or something) because this helps with crossdating.
  - e. **Helpful hint:** A good way to double check your ring identification is to click on **Window → Tile**. This makes a graph pop up with the ring width on the y axis, and allows you to more easily see if you missed identifying any rings (i.e. ring widths are exceptionally wide) or if you “double” identified a ring (i.e. ring widths are zero). This is also the window that is used to cross-date cores. See ‘Crossdating Protocol’ for more information on this.
  - f. **Helpful hint:** If winDENDRO is automatically detecting too many or too few rings, you can adjust the auto-detect sensitivity. In the white sidebar with the red and blue squiggly lines, you can drag the black line so it just touches the peaks of the blue line. The peaks of the blue lines are areas of greater density and should represent rings. This only affects the current visible portion of the image, so you can adjust smaller or larger portions of the core by zooming in and out on the image.
- 2) After you have edited all the rings to your satisfaction, go back and **remove the gaps**. Removing the gaps can be done by going to **Path menu→Gap→Define**. Starting at the top of the gap, click on the left side of the path, then the right side of the path (**do not** click and drag or double click). A line should appear there. Then click on the lower right side of the path, where the gap ends, and then the lower left side of the path (see p. 81 of the WinDENDRO manual for more info).
- 3) If you want to start your analysis over at any point, click on **Path menu→Delete Active Path**. This will delete the path you are currently working on, but you can toggle between paths by hitting *tab* on the keyboard.
- 4) Occasionally, if a core was broken into pieces, there will be a segment of core that is backwards (i.e. it got flipped around by mistake). You will know this because the pattern of light vs. dark cells in the rings will look backwards. If you come across this when you are analyzing a core, then analyze the whole core, including the flipped section, as you would an intact core. Then, make a note of the years that need to be flipped in an excel file. In the past, this has been done in an Excel file called “2008-[current year] Tree Ring Notes (or Sapling Notes) (read before analysis!).”
- 5) Once you’ve finished, **save the image AGAIN (save it over itself) with the ring analyses included** and then scan a new core!
  - When acquiring next image Windendro automatically asks to save the last image. At this point, you should resave the image with the analysis over the image you originally created (without the analysis). Make sure that you have saved the image WITH the ring analysis (this is necessary for cross-dating)

### Additional Notes:

-Refer to the winDENDRO manual for troubleshooting.

-Each core has its own text file associated with the image and analysis. The .tif file has the scanned image and the analysis, whereas the .txt file just has the ring widths. It is important to save the .txt file as something you find easily and associate with the analysis. Example: all cores from same stand in same .txt file. Alternatively, you can save each core in its own .txt file.

-If you did not select exactly on the pith or if your core lacks the actual pith than the earliest year in your data should be ignored.

*\*In order to acquire an image saved on a disk, click on Image/origin and change to disk. Then go to Image/Acquire Image and select the image you would like.*

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*\*\* For example, if I save an image of core AR07 – G16 – 1 only than I save file as "AR07 – G16 – 1.tiff" BUT If I am saving both core 1 & 2 in the same image than save as "AR07 – G16 – all.tiff"*

## **Cross-Dating Protocols for Adult Tree Cores**

### **Visual Cross-dating in WinDENDRO**

- 1) Open the image of the core you'd like to analyze (**Image→Acquire Image**).
- 2) Open a data file (you should be prompted to do this). For 2008 data, we used the file "Official 2008 Tree Core Data REGULAR.txt" in Mt Rainier/WinDendro/2008 collected/Tree Cores. A similar file should be created for 2009.
- 3) **Window→Tile** (a window with graph of ring widths by year should come up below image) so that you can see the ring widths of both cores.
- 4) Make sure that all rings are correctly added on each core in image.
- 5) Look at graph of ring widths. If two cores are present in the image (from same tree), do they seem to line up as you would expect? (i.e. do they have wide ring widths in the same years and narrow ring width in the same years?) If not, look carefully at ring width patterns in the graph, starting with the most recent ring, and find the year(s) when the two lines seem to get out of sync. Inspect the cores at these years, and look for possible mistakes (i.e. missing rings, or false rings). It helps to look for especially sharp valleys and peaks, as rings may need to be inserted or added here. Make any necessary adjustments by inserting or deleting rings in the image. Cores collected early (June-July), especially from high elevation trees, may not have a visible ring for the year they were collected. If they do, it should all be large celled/light colored.
- 6) If you are working on a core from a stand that already has reference data available, load reference data now (Graphic-Load Reference data... then choose files from: Pinus contorta!/crossdating/side of mountain). It is helpful to load averaged data for all trees of that species at that stand (should be made already) and a sampling of individual trees. This will give you an idea of the general trends and the variance at each year. The graph will display a correlation value between the active path and active reference data. The reference data can show you if your core is off from the stand by a year in certain places, and can be used to determine false and missing rings in your core. Attempt to line up the cores with greater than 50 correlation to the reference data, and until 1800. Don't spend too much time trying to crossdate before 1800, this is often difficult and the data is not as useful. Once you are satisfied you have both cores adequately analyzed, choose one core from the tree (the longest record, or least damaged core if one is really rotten) to make "Reference Data." Click on the path in the core image to activate it (the line in the graph window will be continuous rather than dashed if it is active). Select **Graphic → Create Reference Data from → Active Path**. Save Reference data as the Species\_Tree\_Core# (i.e. TSME\_G01\_1). For 2008 data, I saved reference data in the folder Mt Rainier/WinDendro/2008 collected/Tree Cores/Core Images/Crossdating.
- 7) Open another core image and start the process again. (Prior to opening the next image, you will be asked to save the one you are working on. Resave it with the same name.)
- 8) If you are working on cores from a stand that do not already have reference data. Create reference data for 4 to 5 cores before starting to crossdate. Start by trying to crossdate the most straightforward cores from the stand (undamaged, rings clear). Once you have several cores crossdated successfully, you can create averaged reference data to crossdate new cores against (Graphic-Create Reference Data From-Merging reference data). As you analyze additional cores, be sure to add them to the merged data. Do this by deleting all the reference data on your graphic, loading every core file one by one, and then merging. If you finish all the trees from a stand, save the merged data as Species\_StandLetterREF (ie G\_TSME\_GREF\_1)

Note: Making reference data from the cores you are working on is also very helpful in crossdating between cores from the same tree!

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## Checking Your Visual Cross-dating of Adult Tree Cores with dplR

1. After you have visually checked your cross-dating, you should validate your work statistically to make sure you haven't missed anything. This can be done using the dplR package for R. See the following reference for details:  
Bunn, A. 2010. Statistical and visual crossdating in R using the dplR library. *Dendrochronologia* 28: 251–258.
2. Before you use dplR, you need to get the .txt data file ready with the ring widths and other information from WinDendro. This can be done in Excel or R; I usually import the .txt file into Excel (Open a blank excel file, go to Data → Get External Data→ Import Text File). The first thing to do is get rid of all rows that are copies of the same core, and use only the most recent row (see the "analysis date" column in the data file) for each core (every time you open an image to analyze/edit it in WinDendro, a new line of data is added to the data file, potentially yielding many replicate lines for a single core).
3. After you have removed extra rows, and have only one row of data per core (there should be 2 cores per tree, though), then look at the "2008-2010 Tree Ring Notes (read before analysis!)" file (or similar file in which you noted sections of core that need to be flipped, or other important notes from your analysis in WinDendro. Make any changes that need to be made in your data file (e.g. flip years of data that need to be flipped).
4. After your data file is in good shape, then you can import the file into R and use dplR to validate your visual cross-dating.
5. Crossdate the cores by species within a stand (i.e. crossdate all the ABAM at AE10 together; then all the TSME at AE10, etc). So far we have only cross-dated cores against one another, not against a known chronology (I'm not sure how to deal with cross-dating a few "new" cores in a stand where all the other cores have already been crossdated- look at the Bunn 2010 paper for hints.)
6. Before cross-dating you will also need to remove the earliest year of growth in the core and the most recent year of growth, as these are generally incomplete/inaccurate (again, this can be done in excel or R).
7. Here is a sample of Ailene's R code for doing this in 2010, along with comments and followed by a for loop that will print the diagnostic plots for all cores, by species per stand, as an example:

```
#Crossdating for 2010 Manuscript
coredata<-read.csv("cores0809.csv", header=TRUE) #This data file contains visually cross
dated ring widths, with earliest and latest year removed, contains all species cored in 2008 and
2009 from south side stands (9) on Mt Rainier, tags and species names are included.
dim(coredata)
head(coredata)
#Crossdating
#####PARADISE#####
#####Tsme#####
para.tsme.dat<-para.dat[para.dat[,2]=="Tsme",]
dim(para.tsme.dat)
para.tsme.rwl<-t(para.tsme.dat[,8:224])
dim(para.tsme.rwl)
para.tsme.rwl.df<-as.data.frame(para.tsme.rwl)
dim(para.tsme.rwl.df)
rownames(para.tsme.rwl.df)<-c(2007:1791)
colnames(para.tsme.rwl.df)<-para.tsme.dat[,5]
para.tsme.stats=corr.rwl.seg(para.tsme.rwl.df)
para.tsme.stats
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#For this species, several cores were flagged as being poorly correlated with other conspecific
trees in the stand.
#One of flagged cores is P292, at segments: "1900.1950, 1925.1975" Now, look at this core in
greater detail to see which years are off and if cross-dating will help.
flagged=para.tsme.rwl.df$"P292"
names(flagged)=rownames(para.tsme.rwl.df)
para.tsme.rwl.df$"P292"=NULL
quartz()
seg.50<-corr.series(seg(rwl=para.tsme.rwl.df,series=flagged,seg.length=50, ,bin.floor=0)
quartz()
ccf.50=ccf.series.rwl(rwl=para.tsme.rwl.df,series=flagged,seg.length=50, bin.floor=0)
#Crossdating won't help for P292 (based on the dot plots; see Bunn 2010 for more details)
##Another flagged core: P311:"1800.1850, 1850.1900, 1875.1925, 1900.1950, 1925.1975"
flagged3=para.tsme.rwl.df$"P311"
names(flagged3)=rownames(para.tsme.rwl.df)
para.tsme.rwl.df$"P311"=NULL
quartz()
seg.20<-corr.series(seg(rwl=para.tsme.rwl.df,series=flagged3,seg.length=20, bin.floor=0)
quartz()
ccf.20=ccf.series.rwl(rwl=para.tsme.rwl.df,series=flagged3,seg.length=20, bin.floor=0)
#added&took away some rings in Windendro, based on the flagged years and dot plot.
#Here is more R code, that is a for loop for crossdating, so it will involve less copying and
pasting than the above!
coredata<-read.csv("SouthsidecoresXDatTest.csv", header=TRUE) # All cores cross dated,
earliest and latest year removed, contains all species cored in 2008 and 2009 from south side
stands (9), generally 2 cores per tree. check cross-dating by core (not by tree).
library(dplyr)
dim(coredata)
head(coredata)
species<-unique(coredata$Species)
stands<-unique(coredata$Stand)
treeid<-coredata$Tag
#Crossdating for loop: this prints the stats for flagged cores and images for ALL cores (i couldn't
figure out how to only print for flagged cores)to help you diagnose cores that are poorly
crossdated and should be checked in windendro
for (i in 1:length(species)){
  spdat<-coredata[coredata$Species==species[i],]
  for (j in 1:length(stands)){
    print(stands[j]);print(species[i])
    spstdat<-spdat[spdat$Stand==stands[j],]
    spstdat.rwl<-t(spstdat[,10:226])
    rownames(spstdat.rwl)<-c(2007:1791)
    colnames(spstdat.rwl)<-spstdat$Tag
    spstdat.rwl.df<-as.data.frame(spstdat.rwl)
    quartz()
    spst.stats<-
    corr.rwl(seg(spstdat.rwl,seg.length=50,main=paste(stands[j],species[i]))#seg.length can
be changed to analyze cores on a finer scale (right now set at 50 years)
    print(spst.stats$flags)
    flagged<-spst.stats$flags
    for (k in 1:dim(spstdat.rwl)[2]){

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spstdat.rwl<-t(spstdat[,10:226])
rownames(spstdat.rwl)<-c(2007:1791)
colnames(spstdat.rwl)<-spstdat$Tag
spstdat.rwl.df<-as.data.frame(spstdat.rwl)
rownames(spstdat.rwl.df)<-c(2007:1791)
colnames(spstdat.rwl.df)<-spstdat$Tag
flagtree<-colnames(spstdat.rwl.df)[k]
flagged=spstdat.rwl.df[,k]
names(flagged)=rownames(spstdat.rwl.df)
spstdat.rwl.df[,k]=NULL
#quartz(width=12,height=5)
#seg.20<-
corr.series.seg(rwl=spstdat.rwl.df,series=flagged,seg.length=20,bin.floor=0,
main=paste(flagtree))#you may want to look at these line plots, but they use up too many
windows if you are doing it for a whole stand
quartz(width=12,height=5)
ccf.20=ccf.series.rwl(rwl=spstdat.rwl.df,series=flagged,seg.length=20,bin.floor=0,main=pas
te(colnames(spstdat.rwl.df)[k]))
}}}
```

8. After you have identified cores that are flagged in dpIR, use the dot plots to assess if cross-dating will actually help (i.e. if shifting the core by a year at some point will improve its correlation with other trees in the stand). If it seems that it will, then look at the core image again in windendro, focusing on the “problem” years flagged in dpIR. If it seems likely, while looking at the core, that there is a false ring or missing ring that could account for the problem, make the required correction and save the new data file.
9. After all cores have been checked and validated with dpIR/WinDendro, then save the new Windendro data file (with any updates that you made based on cross-dating). You should again clean up the data file (i.e. remove duplicate rows for the same core, remove columns that are not necessary, remove earliest width, fix any problem segments that were flipped, etc). Then, the data file is ready to be analyzed!