



STSM Report

Harmonizing sample preparation to create a network of Mediterranean conifers cell size chronologies

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Introduction

In recent years, tree-ring anatomical structure (conduits size, density, cell wall thickness, ray abundance, etc.) has been recognized as a novel source of valuable ecological information. However, despite the high potential interest, with conifer species these kind of investigations have been significantly constrained by some methodological limitations and time-consuming procedures for data collection. Recent technological and software improvements permit to significantly increase the number of samples processed throughout the various steps of the data acquisition at trees, number of rings (years) and number of anatomical features (i.e. cells) level. This opens the door towards completely new approaches of whole-sample anatomical studies in conifers, as it has already been shown for angiosperm trees and herbaceous plants (Fonti et al. 2009; Olano et al. 2013; von Arx et al. 2012; Wegner et al. 2013).

Nonetheless some methodological constraints still remain and are related to prepare high quality samples for a fruitful data collection. The conduits size of conifers is much smaller than in angiosperms; this hampers a rather fast sample preparation and image acquisition and requires the use

of the microtome for cutting thin sections and the microscope for collecting images. It is well-known that a pre-requisite to obtain reliable and flawless results are the high quality of thin sections and images. Time invested in these preparation steps usually pay back several times by reducing efforts for manual editing.

Aim of the STSM

The aim of the STSM was twofold: harmonized the methodologies for wood sample preparation and data acquisition and implementing a cell-chronology network of long-lived conifers in the Mediterranean.

Description of the work carried out during the STSM

The activities were split in two: samples preparation and data acquisition.

Regarding the samples preparation I transferred the experience of the University of Padova dendrolab to the CSIC lab. Actually on many papers and books it is easy to find the classical protocol for preparing, cutting, staining the different wood types (Gärtner and Schweingruber 2013), however, along the process there are several small tips that could help a lot to save time and to increase the final sample quality. Finally it is generally much easier to learn something watching and then doing rather than reading the same information and then trying to transfer them in practice.

We concentrated on conifer wood because our future collaboration will be involved on *Pinus* and *Juniper*. The samples were prepared following the standard protocol for wood anatomical analysis involving: i) the cores (both 5 and 10 mm in diameter) were cut into small (3-5 cm) pieces and boiled in water until the wood were fully soaked; ii) thin sections (ca. 20 μm) were cut with the rotary microtome; iii) these sections were then stained with safranin and astrablue and permanently fixed with Canada balsam.

The rotary microtome at CSIC is the Leica 2255, extremely similar to our Leica 2245. This microtome is fully automated but it also permits the manual use. Starting to cut two different problems, visible on the image on the right, emerged: i) the blade holder cannot move laterally and does not fit the common thin microtome blades (the one that I suggest) and ii) the

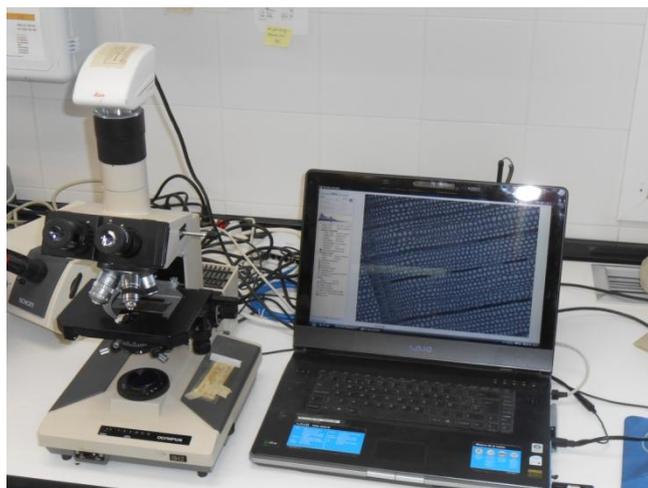


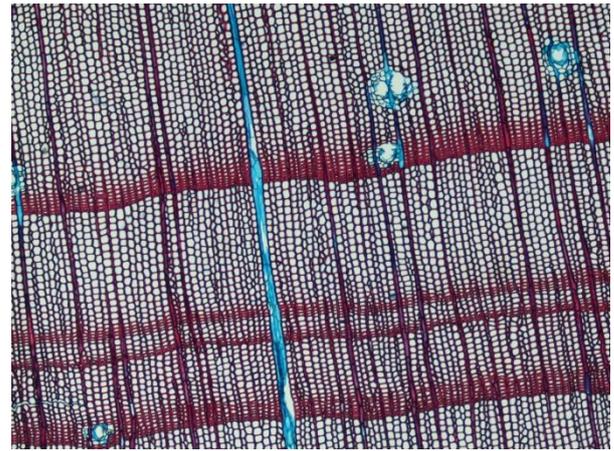
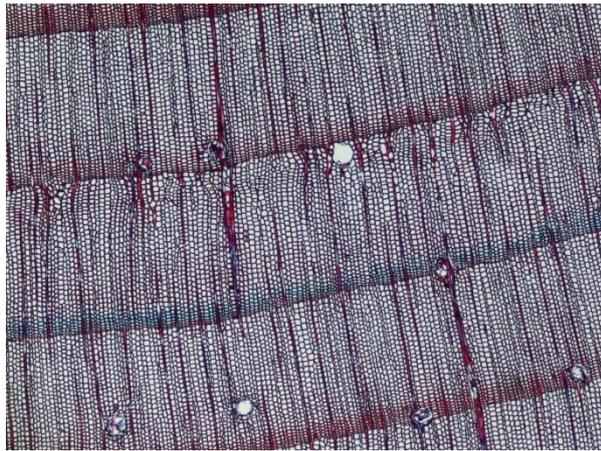
sample holder cannot freely rotate but it is fixed and permit to cut a core piece just horizontally.

If the objective is just to cut small wood pieces for anatomical analysis these should not be major issues but, cutting long (3-5 cm) pieces in my experience it is better to have almost no limit on freely rotate and/or translate the sample. Wood is far from being homogeneous and sometimes just changing the cutting angle or the position can dramatically change the quality of our slices. Even with these issues it was possible to cut and produce high-quality slices (example in following figure) however I suggest Julio Camarero to strongly consider the possibility to buy a new blade and a new sample holders.



The following step was the data acquisition. Firstly, image acquisition was performed classically with a digital camera (Leica DFC 290) mounted on a light microscope (Olympus BH2) (Figure below); images were captured at both 40× and 100× which represent the most common magnifications adopted to process long tree-ring sequences.





Then we moved to the image processing: after installing all the software, multiple overlapping images were taken from each sample and stitched together using PTGui v8.3 (New House Internet Services B.V., Rotterdam, NL) to obtain high-resolution images of the entire thin section as a low-compression JPG file. Images were then automatically analyzed using ROXAS v1.6, and I shown how to manually edit the final result. ROXAS is an image analysis tool specifically designed to quantify the xylem structures in cross-sectional view of trees (angiosperms and conifers), shrubs and herbaceous plants. It is built around the image processing and analysis capabilities of Image-Pro Plus \geq v6.1 (Media Cybernetics, USA) and also interacts with MS Excel \geq XP for data output. Data output is saved into well-organized spreadsheets comprising statistics on the level of the entire sample, each annual ring and individual conduits (von Arx and Carrer 2014).

Description of the main results obtained

To develop an extensive networks of tree-ring parameters we need to share common standards for data acquisition. Nowadays this is obvious for many tree-ring parameters however quantitative wood anatomy just recently has shown its potential and starting with a common acquisition protocol will be fundamental for future collaborations.

With this STSM we defined a common standard between Padova and Saragoza and two "twin" labs could share from now on most of the wood anatomy measurements.

Furthermore we organize a students exchange. Within few months a MS will visit Saragoza lab, his master thesis work deals with quantitative wood anatomy on several tree species, mainly juniper and pinus, growing in a dry site in Spain. Dr. Camarero has already sent the cores to Padova, we are processing them, the next step will be to measure and analyze the samples. For this last part the students will spend few weeks in Saragoza. A second student, in this case a PhD one, will spend a

period in Spain for sampling and measuring several pine sites trying to build long cell-lumen chronologies.

This report may be posted on the Action website.

The confirmation letter by the host institution of successful execution of the STSM is attached separately.

References

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