Optimization of Thin Section Preparation

for quantitative wood anatomy analysis

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6-3

TECESTEFILE

Background



WP1: Water availability and growth plasticity

Investigating quantitative wood anatomy and the hydraulic system of Norway spruce, Silver fir, and Douglas fir in response to variations in weather and climate



Climate Change

- Temperature, drought, heatwaves
- How trees respond varies
- Risk of Mortality

Norway **Spruce**

> Silver Fir

Douglas Fir

Important Species in the Black Forest









Climate Change

Need to Check: **SUITABILITY**



Study of response to variations in weather and climate

• Quantitative wood anatomy

HOW?

• Cellular level response



Methods

Expected effect of climate change in temporal dimension is tried to be explained using spatial dimension (elevation)





We can create a simulation on how these trees responds to variation in weather and Climate









Sampling collection and preparation





- Increment core
- Drying
- Pre-cutting (diamond flying cutter)
- Segmentation
- Label and storing

RECOMMENDATION

7 Optimizations in Sample Preparation



GSL-1 Microtome



- Middle of Sample holder
- Cut from pith to bark orientation

1. Sample orientation



- Tight recheck
- Alignment?

Problem: 3D effects





2. Microtome settings





- Free-play adjustment
- Tighter setting for thinner section (10 microns)



3. Blade optimization



NT cutter

Microtome blade

- Microtome blade: Higher quality, sharpness
- Marking cutting area
- Marking blades
- Utilize every part of the blade
- Cellotape
- Higher quality, speed, cheaper



4. Thicker Non-Newtonian fluid

- Filling lumen area better support
- Pushing by finger
- Smoother movement, thus better cutting quality
- Solid Liquid ?



5. Cutting thickness

- Uniform as possible
- 15 microns max,
 10 microns is the best
- Avoid blade deformation
- Avoid too much force to the sample

6. Dry/wet sectioning



- Species have different workability
- Hardness & Density
- Douglas fir Soaking in water 24h





7. Twisting

- Always happen due to blade positioning
- Thinner section easier fix
- Thicker section harder fix
- Straightening:
 - For Norway Spruce and Silver Fir on slide
 - For Douglas Fir on blade holder
- Other option:
 - Utilize water surface tension
 - Cover glasses
 - underwater





Next Step

- Cleaning starch
- Double Staining
- Rinsing
- Drying
- Fixing
- Cleaning
- Scanning

Norway Spruce – Kandel - 1980



<u>Preview</u> Data Analysis: ROXAS

Earlywood



Latewood

Up until now..

- 40 trees
- 50 years each -> 2000 tree rings
- 20 days
- 23 blades



Next..

- Reporting
- Results analysis ROXAS and RAPTOR
- Correlation weather & climate data vs wood anatomical parameters