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## ***Interactive comment on* “Technical Note: An improved guideline for rapid and precise sample preparation of tree-ring stable isotope analysis” by K. Schollaen et al.**

### **Anonymous Referee #3**

Received and published: 23 December 2015

This paper aims to describe a new method for processing tree ring samples to cellulose for isotopic analysis. The paper is fairly well written and the method is interesting. I think that selling this method as novel may be overselling a bit because what is described here is essentially a description of further refinement of the lath processing techniques described by Li et al. (2011), Weiloch et al. (2011) and Kagawa et al. (2015). The two novel additions by this described technique are the use of a peristaltic pump during the processing and the use of a UV-laser dissection microscope (although I am not sure this last is novel given that Schollaen et al. (2014) described the use of a UV-laser microdissection system to sample tree rings. I think a little more effort needs to be taken to detail how this new method improves on earlier versions of the

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lath technique. Overall I think this is an interesting paper and presents a method that if I had the funds to procure the specialized equipment I might consider adopting. My two biggest issues with this paper are 1) That I don't feel the authors did an adequate job of representing how their method is different or better than the three other methods describing the lath technique and 2) That they only used a single species for all of their tests of their methods performance.

#### Specific Comments.

I am interested in whether the authors thought to consider comparing resinous vs non-resinous samples ? Looking at the list of species *Pinus sylvestris* and *Psudotsuga menziesii* are the only two resinous specimens presented. In other methods studies comparing cellulose isolation techniques it was noted that often the biggest differences were either between heartwood and sapwood and between resinous and non-resinous species. From the looks of the FTIR comparison the authors only used *Tectona grandis*. Why are the FTIR spectra from all ten species not shown ?

Why is *Tectona grandis* the only species used for their comparison of markings on the wood. Really what they should say is that there is no evidence for an effect from contamination in teak not all species.

Although the authors to mention the sapwood-heartwood difference it would appear they only looked at *Tectona grandis* to check this. I would like to see this test done in a more resinous species, for example, *Pinus ponderosa*, or some other conifer to see if there were any significant differences. They have the samples so I am confused again as to why this test was only done on one of their ten species.

On page 11601, I am puzzled as to how the authors assess the relative proportion of earlywood to latewood ? I also imagine this is something that would vary not only based on species but based on year given that there can be large interannual variability in latewood density and amount. Also how much variability in earlywood-to-latewood proportions is significant ?

In the last sentence on page 11601 the authors state that they calculated the potential effects of contaminants. This I would assume is based on their analysis of teak. I am concerned that this effect may not be the same for other species. Also I am confused as to why they needed to check the effect of contaminants again when apparently they already did so in a previous experiment (Page 11604 first line) ?

Page 11605 paragraph 15. I am unclear what they mean by the sentence starting "A disadvantage, like an evaporation of water resulting in enrichment. . . Is this an effect that has been noted by other researchers ? If so could this not be a bigger problem for cellulose extraction techniques than is suggested. For example what if the isotope value of the deionized water used in the lab varies from day to day or season to season. This would be a huge problem for cellulose extraction if so. Or do they just mean higher concentrations of chemicals from evaporation. Please clarify.

Page 11606 paragraph 15. Please further clarify the problem of shrinkage. I would think that shrinkage would occur no matter what size laths are used so please clarify what the issue is with thicker cross sections ? Also I would be curious if thicker cross sections increases the extraction times or leads to a gradient in purity of cellulose from the outside towards the center of the lath ?

On page 11608 you mention that you may not need to weigh samples. I am curious as to how small a ring you can sample ? I have worked on samples where some rings consisted of one or two rows of tracheids only. Would this provide sufficient sample ?

I do find this to be an interesting technique, however I am curious about the cost to set up all the specialized equipment that this method would require ? In the conclusions you mention that this is a faster, cheaper method. I would imagine this method is only cheaper if your lab already has all of the equipment. Not all of us have the resources to spend €0,000 to purchase the specialized equipment necessary to adopt this new technique even if we wanted to. I've done some asking around and the UV-laser microdissection microscope the authors are fond of mentioning costs ~ €0,000. I know it

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is not standard practice but perhaps some information on the cost of equipment could be put in the supplementary material ?

In the figure 6. I am curious how this method would perform on a species without clearly defined latewood (i.e. Pinyon pine).

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