

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.

K. Schollaen et al.

karina.schollaen@gmail.com

Received and published: 20 February 2016

General response:

Dear Referees, dear Editors,

We appreciate very much the opportunity to respond to the concerns and thoughtful comments raised by the referees of our manuscript. We gratefully acknowledge that they indicate our paper may be published after minor to major revisions. In this response – prior to the editor’s decision - we provide additional input and perspective

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that make some of the referees' concerns less pressing and may dispel others altogether. In the revised MS minor comments will be considered, grammar and spelling mistakes corrected. Adequate statements of the articles put forward by the reviewers will be regarded.

Here, we detail key changes and additional data we intend to incorporate into the final manuscript to consider the major points raised by the referees. The most critical comments of the referees can be summarized in two points: 1) A certain lack of novelty is brought forward. 2) Our methods performance should have been tested on more than one species.

General response to the first issue:

Referee #1 criticized that our MS describes just "...a further modification of recently developed method...", #2 comments that "...the manuscript is somewhat limited with respect to the contribution of new ideas, data, or methods..." and #3 points out that "...this method may be overselling a bit...". However and in fact, despite these apparently pressing statements, all three referees have acknowledged that we are introducing "...three new major technical advances/findings" (#1), "a newly designed apparatus" (#2) and "...two novel additions..." (#3). In light of these somewhat contradictory statements of the referees, we see a major weakness in the presentation of our improved guideline. Independent from the different numbers of innovations identified by the referees they, unfortunately, have not recognized our MS as a "guideline". Hence, we will improve and rewrite the MS to be hopefully accepted by BG as "an improved guideline for rapid and precise sample preparation of tree ring stable isotope analysis". Why is this a guideline? Our MS fully describes, for the first time, how tree rings shall be prepared for stable isotope analysis. The guideline starts with guidance to testing and calculating for the potential impact of contaminants like chalk and pencil marks and it ends with a description on how tree-ring cellulose samples can be weighed and packed into tin or silver cups avoiding the laborious step of sample homogenization. For the intermediate step of tree-ring cellulose extraction we introduce a new device

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that improves the extraction process while allowing well established chemical protocols and published procedures to be utilized. In order to test for the completeness of the cellulose extraction process we suggest utilizing FTIR analyses proving the purity of the cellulose extracted from wood. Altogether, to our knowledge, such overall procedure, or guideline, has not been published before in entirety. If someone follows this guideline (or approach or procedure), we claim that, the isotope ratios measured on tree-ring cellulose samples processed and controlled (FTIR) this way are reliable and representative, i.e. homogenous, for any investigated tree ring. We do hope the editor will give us the chance to modify our MS accordingly. We would like to stick to the term “An improved guideline”, however, we do not insist and may change the title of the MS to “An improved approach to . . .” or “An improved procedure to . . .”

All Referees pointed out that one of the most original advances is the application of a UV-laser microscope on cellulose spline and criticized that the use of the UV laser is not discussed in further detail. Indeed, UV-laser microdissection of tree-rings or parts thereof is predestined for use within our guideline. However, details of this particular application have been published earlier (Schollaen et al. 2014) and we want to stress that our guideline can be combined with traditional methods and one does not require such expensive equipment for preparing tree-ring cellulose samples for IRMS analyses. Nonetheless, we happily for the referees' advice. We will add a paragraph illustrating and explaining the application of the tree-ring dissection technique utilizing a UV-laser microscope for obtaining stable isotope ratios from tree rings of the African baobab (*Adansonia digitata*). In doing so, we will be also addressing the 2nd major issue raised by the referees.

General response to the second issue:

In particular referee #1 claims our methods performance should have been tested on more than one species and the other reviews support this criticism of ref. #1. As outlined below in our detailed response we are willing to provide additional data on oxygen isotopes on teak, as well as further FTIR analyses proving the purity of other

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tree species than teak. However, besides introducing a new device for particularly careful cellulose extraction on potentially very thin wood cross sections we do not recommend any changes to the “classical” procedure of chemical cellulose extraction. As a matter of fact, different chemical prescriptions of cellulose extraction from wood exist (e.g. Loader et al. 1997, Rinne et al. 2005, Brendel et al. 2000). The vast majority of extraction methods uses NaOH and NaClO₂ as reagents and only Brendel et al. 2000 suggested a hydrolysis procedure with acetic acid. In the guideline presented in our MS we do not propose any new chemical treatment, i.e. we have used well tested option the “classical” NaOH and NaClO₂ treatment. Its validity has been proven in international inter-laboratory comparisons (e.g. Boettger et al. 2007). We claim that there is negligible chance of failure applying the well tested and established chemical procedures with a newly designed Teflon device that allows more convenient, accurately and, particularly, more gentle sample handling than similar extraction approaches published earlier (e.g. Kagawa et al. 2015). The device introduced by us is versatile. On the one hand, it allows extraction of up to 150 cm of wood increment equaling 1500 tree rings of 1mm width in average. On the other hand, it can be adapted down to 1/6 in size to minimize the use of chemicals if number of samples is low. Furthermore, the Teflon device can even be used with the chemical protocol proposed by Brendel et al. 2000 or potentially any other future chemical procedure. Note, independent from the chemical protocol used, our guideline suggests testing the purity of tree-ring extracted cellulose by FTIR analysis. Nonetheless, we do accept and will address the referees’ complaints. We are happily willing to provide additional FTIR spectra on the various tree species we have investigated in this study. We will be able to show that there is no significant difference between the different devices because the protocol of chemical treatment is the same. We suggest to display these FTIR spectra in the supplementary material section. Furthermore, we are willing to provide oxygen isotope data for teak trees derived from extraction with the classical devices (Wieloch et al. 2009) as well as with the new device. With our “guideline” study we do not intend to prove that testing the purity of cellulose is obsolete for any future study. On the contrary, we do

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suggest testing the purity of extracted cellulose by FTIR analysis whenever an operator suspects incomplete removal of resins or lignins. Some of the tree species studied here have a wide geographical and altitudinal distribution and relatively broad genetic variability. Hence, they may reflect a broad variety of chemical wood components that cannot be captured in a single study like ours. We propose that the purity of cellulose extraction from new sites/regions or new species may be tested, at least occasionally, prior to mass spectrometric analysis of stable isotopes.

Comments to Referee #1:

Comment 1: This paper describes further modification of recently developed method for tree-ring cellulose extraction (Li et al. 2011, Kagawa et al. 2015), where cellulose is extracted from tree-ring spline prior to tree-ring separation (cross-section method). Three new major technical advances/findings are reported in this paper: 1) Potential application of UV-laser microdissection microscope to tree-ring cellulose spline. 2) Semi-automated chemical extraction applied to the cross-section method. 3) They evaluated the effects of contaminants (pencil marks, chalk and corn starch) on the oxygen and carbon isotope values for wood samples. This paper confirms previously reported findings, such as (1) Cellulose spline from various tree species can be extracted without losing its tree-ring structure, (2) stable carbon isotope ratios, and (3) chemical purity of cellulose prepared from teak corresponds to that of the “classical method” (however, such checks were not performed for other nine species). I found this work still in its preliminary stage and a little more experimental effort would make a better publication, especially if authors checked the validity of the method with other nine species. Another concern is, they tried the method only on one species (teak) and on one element (carbon). For example, checking oxygen isotopes of teak and chemical purity of cellulose for other nine tree species with FTIR does not take too much time (should take about 2 weeks). Previous studies have already confirmed that both oxygen and carbon isotopes, and chemical purity match between the classic and cross-

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section method for several tree species. The word “guideline” is defined as “a principle put forward to set standards”. To be a “guideline”, I think at least they should confirm that the method works universally, in terms of chemical purity and stable carbon and oxygen isotope ratios, by testing more than several species.

Response: We thank the reviewer for these general remarks on our manuscript and his critical comments. We understand that the validation of the method with other species is much appreciated. However, we think that including tests of all nine species, shown in the manuscript, will not make a better publication. As the reviewer mentioned previous studies have already confirmed that the chemical purity matches between the classical and cross-section method for several different tree species (Kagawa et al. 2015). We did not intend with our manuscript to analyse again what was already checked by Kagawa et al. (2015). As outlined above (general response to major issues) and in the revised manuscript our guideline suggests tests whenever the doubts on cellulose purity arise (e.g. cellulose not white after extraction etc.) Rather, we picked up the outcome of the previous publications and focused on our original advances, such as an improved semi-automatic cellulose extraction system and the application of the UV-laser microscope. Further, we focused on presenting a general “guideline” that stretches from pre-analyses considerations (evaluation of the effects of contaminants such as pencil marks, chalk and corn starch), wood sample preparation through semi-automated chemical extraction of cellulose from tree-ring cross-sections to tree-ring dissection for high-precision isotope ratio mass spectrometry. For further explanations we would like to refer to our general response above.

Changes in the manuscript: We will re-write our MS to be more concise in what our guideline facilitates and more clearly point to potential constraints. Furthermore, we will add oxygen isotope data of teak to the revised MS. If requested by the editor, we also offer testing more tree species on purity of the cellulose extraction (FTIR). However, as outlined above we do believe that this would be unnecessary additional workload that would not absolve future studies on new species or sites from ensuring and testing for

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complete cellulose extraction.

Comment 2: One of the most original aspects of this study is the (potential) application of UV-laser to tree-ring cellulose splines. UV-laser has a great potential because it may make treering separation process automatic in future. Thanks to recent breakthrough in cellulose extraction, this process became much more efficient and instead, tree-ring separation, weighing and packing have become the major time limiting process now. UV-laser has a potential of automating this bottleneck process, however, the paper does not report about the application of UV-laser to cellulose spline. Contrary to what readers would expect from the title, authors do not state clearly how “improved” their sample preparation method is, compared to previously published cross-section method (Li et al. 2011, Kagawa et al. 2015). Especially, it is not clear how much advances authors have made in terms of how “rapid” (how much improvement does semi-automated extraction make?) and how “precise” (analytical resolution for manual separation is about 0.2mm, what about UV-laser?). Considering the fact that the journal “Biogeosciences” has higher impact factor than “Chemical Geology”, where the two preceding papers (Li et al 2011 and Kagawa et al. 2015) appear, authors should present significant advances from preceding works to warrant publication of their work in this journal. I found this paper focuseing too much on what has already been checked in preceding works and too little on their original advances, such as application of UV-laser microscope on cellulose spline and automation of cellulose extraction. I therefore find this manuscript acceptable with major revision, provided authors can present such significant technical advances from previously published cross-section method and provide additional experimental data to prove that their method is universally applicable to major tree species used in dendrochronology. Otherwise, I think the manuscript should be submitted to other journal, such as “Rapid communications in mass spectrometry” or “Dendrochronologia”, where previous works on cellulose extraction method appear.

Response: We agree with the reviewer that an example of the application of the UV-laser microscope on cellulose cross-section will improve the original advances of our

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presented “guideline”. Regarding details on the UV-laser microdissection technique we like refer to Schollaen et al. 2014.

Changes in the manuscript: We will add an example of applying UV-laser microscope dissection on a cellulose cross-section from African Baobab (*Adansonia digitata*) wood. This will be the first time that cellulose splines are used with an UV-laser microscope. Further, we will add some more sentences to clearly highlight the improvement of our method in terms of how "rapid" and "precise" it is.

Comment 3: Title: Unless authors can specify improvement in rapidity and precision of their method, I think current title is too broad and should be more specific to better reflect the contents of this paper. The title of this paper says “an improved guideline”, however, I think trying the method only on one species and one element (carbon) does not give enough supporting data and is still not universal enough to call it a “guideline”. Authors should prove that the method is equally applicable to other major tree species used in dendrochronology by providing more experimental data. Authors did confirm cellulose spline can be extracted from nine tree species (Pine, Larch Spruce, Juniper, Fir, Oak, Cedar, Baobab and Beech) with well-preserved tree-ring structure. However, as for chemical purity and carbon isotope ratio of the cellulose prepared, authors checked only with one species (teak). Checking oxygen isotopes of teak tree rings, for example, could have been easily done without taking too much time, and running chemical purity test (FTIR) for the nine tree species can be done within one or two days. Due to the lack of supporting data, I think this work is still in its preliminary stage and it would make a better publication if authors checked the validity of the method with other nine species. Why you did not do these experiments?

Response: This comment repeats concerns that were raised in the general remarks. Therefore, we would like to mainly refer to our responses to comment 1 and to our general response given in the beginning. Rapidity and precision may not be as striking advantages as the versatility and handiness of the introduced device, that can be used with a variety of published chemical protocols, which eventually define the speed of the

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extraction process much more than the device itself. We believe that the title is not too broad, because our MS does not only describe a new device, but provides guidance on how tree rings can be well prepared for stable isotope analysis. The guideline starts explaining how potential impact of contaminants can be estimated and it ends with a description on how tree-ring cellulose samples can be weighed and packed into tin or silver cups avoiding the laborious step of sample homogenization.

Changes in the manuscript: We will re-write our MS to better present this guideline.

Comment 4: Materials and method: P.11593, L.6-7 Here, authors use 10 different tree species for cellulose extraction, but they only measure carbon isotopes of teak. Why you did not measure oxygen isotopes of teak and chemical purity of cellulose from other nine species? It does not take much time to do this experiment.

Response: We will add oxygen isotope data of teak to the revised MS. If requested by the editor, we will perform additional FTIR tests on more species.

Comment 5: P11596L3 “3.5 classical cellulose extraction: : :” Did authors compare weight recovery of cellulose (cellulose weight / original wood weight) between classical and cross-section method? Were they similar?

Response: Cellulose yields (not concentrations) have been determined. No significant differences in cellulose yield have been observed when comparing the “classical” approach (Wieloch et al. 2009) with our new device. Please note, the chemical treatment (Boettger et al. 2007) is has not been modified, a newly designed device has been used, only. Moreover, please note that other studies on extracting cellulose from wood laths did not observe significant differences in cellulose yield either (c.f. reference list)!!

Comment 6: Results P11602L12 “5.2 Classical vs cross-section cellulose extraction method” Add data for oxygen isotopes of teak tree rings. I think it will be d18O analysis of about 200 samples for both classical and cross-section methods and should take too long to do this. L 5.3 “Purity of cellulose cross-sections”: Add FTIR data in this section

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for other nine species to prove that the cross-section method can universally produce cellulose from sufficient chemical purity.

Response: This comment repeats concerns that were raised in the general remarks. We will add oxygen isotope data of teak to the revised MS. If requested by the editor, we will perform additional FTIR tests on more species.

Comment 7: In conclusion, you use too much space for writing the findings that were already reported in previous studies, or otherwise self-evident. Please delete such description, i.e. cellulose extraction not being time-limiting, or pooling not necessarily required etc. And use more space for writing your original findings, i.e. what this study clarified for the first time. For example, you can compare your semi-automated extraction method with previous ones (Li et al . 2011, Kagawa et al. 2015) and point out how much improvement you have achieved in terms of time (?? times as many samples processed per man-hour compared to the Teflon-container method?), cost (how much the whole system costs), and user-friendliness (less exposure to toxic gas?, perhaps your method is more successful on thinner cross-sections and fragile species/samples?). I think such information will better meet the readers' interests.

Response: Thank you very much for your constructive comments. We will add the information you suggested and rephrase the appropriate parts in the manuscript.

Further minor comments, in which we generally agree, will be taken into account when redrafting the revised version of the manuscript.

On behalf of the authors; Yours sincerely

Karina Schollaen

Interactive comment on Biogeosciences Discuss., 12, 11587, 2015.

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