A novel approach for the preparation of high-resolution stable isotope records from tropical tree-rings

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Introduction

Stable isotopes (carbon, oxygen and hydrogen) from tree-rings are widely used in climate and environmental studies (e.g. Schleser et al. 1999, McCarroll & Loader 2004, Leavitt et al. 2007, Saurer et al. 2008, Hennig et al. 2011, Dorado Liñán et al. 2011, Schollaen et al. 2013). Physiological processes affecting isotope fractionation such as stomatal conductance and photosynthetic rate react sensitively to climatic and environmental factors during the year. To extract valuable seasonal climatic and environmental information high-resolution stable isotope analyses of tree rings are needed. To date, the common method to prepare intra-annual isotope samples is to divide tree rings in tangential sections using a fixed-blade sledge microtome (e.g. Loader et al. 1995, Helle & Schleser 2004, Verheyden et al. 2005), a scalpel (e.g. Roden et al. 2009) or a twist drill (Fichtler et al. 2010). Thereby, the rings were subdivided into segments of equal widths along a radial direction and thickness of tangential sections varies from 20 µm to 180 µm. Accurate sample adjustment, as well as correct identification of tree-ring borders was provided by visual inspection using a binocular or microscope. Wood samples were ground to a fine powder and either cellulose was extracted or bulk wood samples were used, as recent studies on isotopic differences of bulk wood and cellulose extraction report little or no differences in the variability of the isotope signal (Borella et al. 1998, Van de Water 2002, Loader et al. 2003, Verheyden et al. 2005, Taylor et al. 2008). Important for this method is the selection of parallel rings with almost straight tree-ring borders. As the curvature of growth rings varies under natural conditions, often only a limited number of consecutive rings per sample is suitable for high-resolution intra-annual isotope measurements. Another method for high-resolution stable isotope sampling is the use of a UV-laser ablation. Wood sample material from a tree core is extracted with an UV-laser leaving 40µm-wide holes (Schulze et al. 2004). Ablated wood dust is then combusted to CO_2, at 700°C, separated from other gases and injected into an isotope ratio mass spectrometer.
Here, we tested a new UV-laser microdissection system from LEICA (www.leica-microsystems.com). Laser microdissection (LMD) is a specific form of laser-assisted microdissection that uses an UV cutting laser to isolate tissues of interest from thin sections of biological samples, which are collected by gravity below the sample. LMD is being applied widely in biomedical research and also in animal and plant research (e.g. Nelson et al. 2006, Abbott et al. 2010). We present the use of an UV-Laser Microdissection Microscope (LMD) to prepare reliable high-precisely intra-annual wood samples. The design and the handling of this UV-Laser Microdissection Microscope for dendrochronological research is described. Advantages and constraints are discussed on the basis of high resolution stable isotope analyses on woody plant material of teak (*Tectona grandis*) samples from Indonesia.

Samples and Methods

Sample preparation

The wood material selected is from living tropical teak trees (*Tectona grandis*) at Java (Indonesia) collected with increment cores of 5 mm diameter. Firstly, the cores were cut in pieces of 5 cm length corresponding with the size of the object holders of the UV-Laser Microdissection

Microscope. Secondly, transverse or cross sections of approximately 500 µm thickness from sampled cores were cut with a core microtome (Gärtner & Nievergelt 2010). To prepare the wood surface for an effective cutting process the sample lies some hours in a glycerin solution to make the surface soft. At last, the cross-sections were fixed on special object holders (Fig.1), designed at the GFZ (German Research Centre for Geosciences) and treated under the LMD.

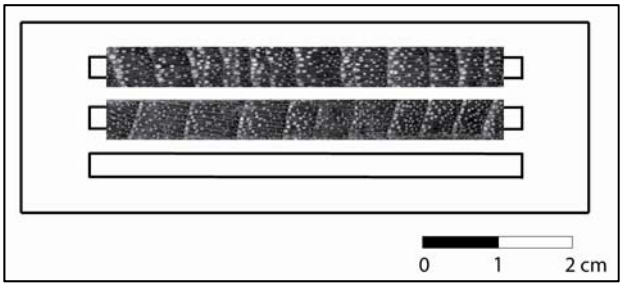

Figure 1: Object holder with wood samples. Tree-ring cross-sections are fixed between 2 metal frame slides, wherein up to three cross sections of 59 mm length can be mounted.

UV-Laser Microdissection Microscope

The UV-Laser Microdissection Microscope from LEICA (LMD 7000) enables the analyses of cells or tissues and is frequently used in biomedical research. Biological samples are viewed under an upright microscope, dissected via an UV-laser coming from above and collected into tubes by gravity (Fig. 2).

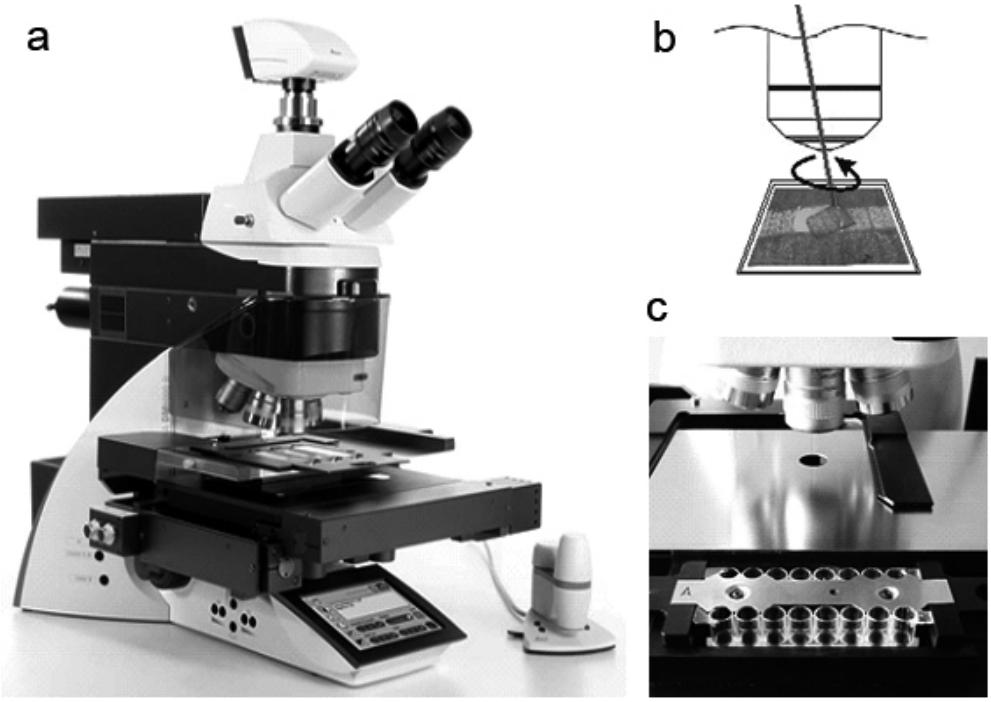

Figure 2: Leica LMD 7000, Research microscope with an UV-Microdissection Laser (a). Marked wood areas are cut by a moving UV-laser (b) and fell down by gravity into tin or silver capsules standing in a collection device (c).

This UV-laser is able to cut fast and easy dissections of tree-rings or/and parts thereof with contact- and contamination-free specimen collection. Cutting lines can be drawn accurate by mouse or with a pen-screen and dissectioning of areas of any size or shape is possible. This

allows to exclude irrelevant areas as resin ducts or rays. Furthermore, it is possible to cut serial section or even to pool sample material, e.g. if the mass of the cut area is not sufficient for stable isotope measurements. The laser moves via optics and the cross section sample stays fixed. The dissected wood samples can be collected directly in thin or silver capsules for carbon and oxygen isotope analyses with conventional IRMS systems. The whole drawing and cutting process can be automatically documented (e.g. images, videos, database) for quality control.

Sample collection

The cross sections of approximately 500 µm thickness from teak cores are fixed in object holders and visualized under the microscope (magnification 5x, 10x). Several annual tree rings were graphically subdivided into segments, with the number of segments per year varying depending on the tree-ring width and sample mass for following stable isotope measurements (range from 10 to 40 segments per year). The scheme of the intra-annual sampling process is illustrated in Figure 3. Every drawn segment is dissected with the UV-laser and collected by gravity in single silver capsules standing in the collection holder. The laser dissection process from wood segment selection to the cutting process was documented with an image-database (IM500).
Finally, the dissected and collected wood segments were given in the Isotope Ratio Mass Spectrometer (IRMS) for $\delta^{18}O$ measurements. The mass of dissected samples varies from 80 µg to 220 µg.

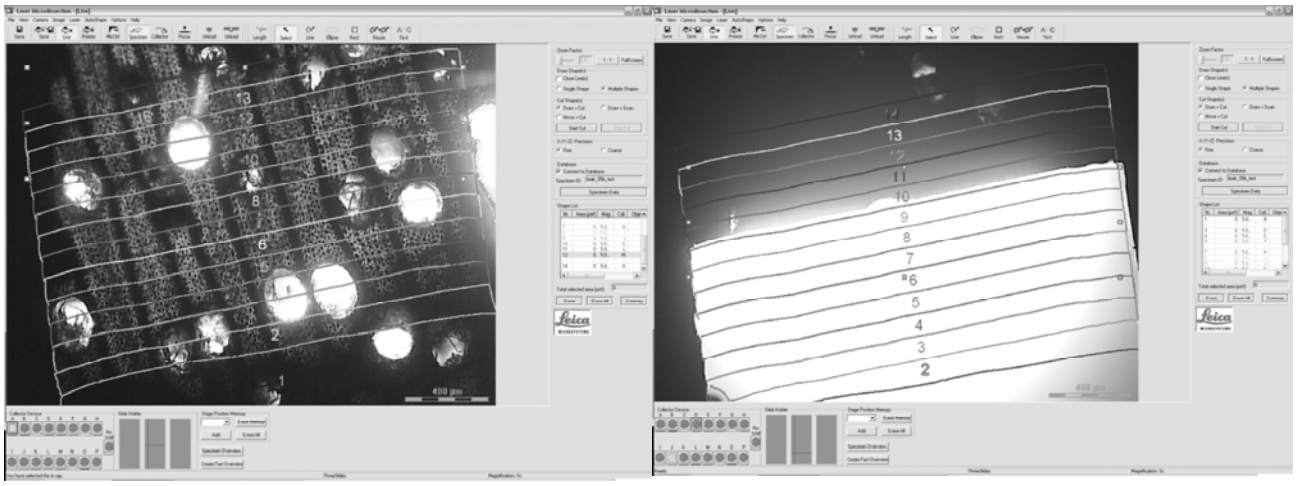

Figure 3: Left part: Selected segments (numbered 1,2,3, etc.) within one teak tree-ring for cutting process with an UV-Laser Microdissection Microscope (LMD 7000, Leica). Right part: Cutting process with the first 10 parts already cut and fallen down in separate collector devices.

Results and Discussion

High-resolution intra-annual $\delta^{18}O$ variations

The $\delta^{18}O$ profile shows a clear annual cycle. Wood formation starts with a parenchyma band showing $\delta^{18}O$ values that are similar to the $\delta^{18}O$ values at the end of the previous ring. Then the $\delta^{18}O$ values are rising up to a seasonal maximum appearing early in the growing season. This $\delta^{18}O$ maximum is followed by a decline to a seasonal minimum typically in the 2nd third of each tree ring before $\delta^{18}O$ is marginally rising again in the last third of the growing season. The annual $\delta^{18}O$ pattern follows the annual cycle in rainfall amount and its corresponding isotope signature at this site (Schollaen et al. 2013). High tree-ring $\delta^{18}O$ values during the start of the growing season represent the $\delta^{18}O$ signature in precipitation of the prior dry season. Low tree-ring $\delta^{18}O$ values reveal the $\delta^{18}O$ signature in precipitation of the main rainy season. To our knowledge, this is the

first time that intra-annual $\delta^{18}O$ values in tropical trees can reflect the rainfall pattern over an entire year with distinct rainy and dry season signals.
As AD 1987 shows, less than 10 data points per tree ring are mostly not sufficient to produce reliable intra-annual variations. Minimum 10 data points per tree ring are recommended to illustrate high-resolution intra-annual stable isotope records.
This result shows the crucial value of the new dissection method as it allows for new approaches towards high-resolution isotope chronologies.

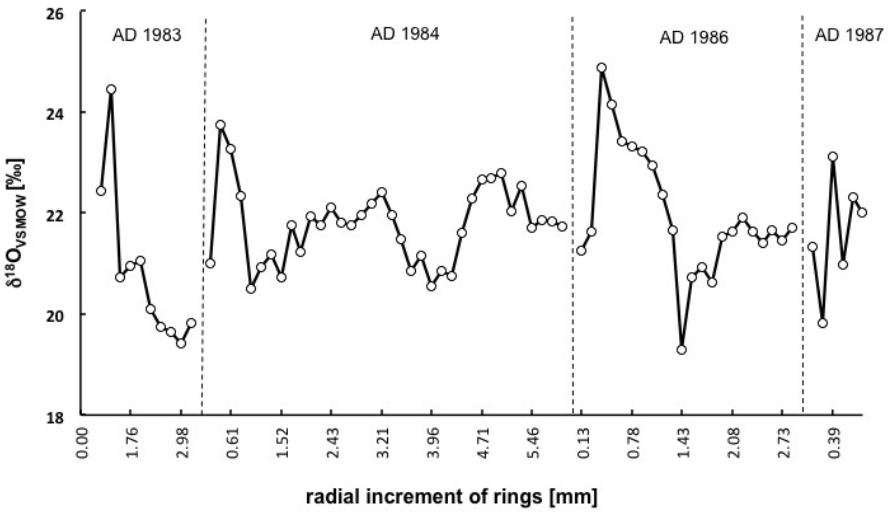

Figure 4: Intra-annual $\delta^{18}O$ variations from tree core 15b collected at Java, Indonesia, plotted vs. radial increment of the particular tree ring. The data points represent the number of segments dissected per tree ring. This isotopic cycle is hypothesized to reflect the annual cycle in rainfall amount and humidity at this location (Schollaen et al. 2013).

Evaluation of the laser microdissection from Leica (LMD 7000)

The automatic high-precision laser dissection tool is a fast and reliable method for preparing high-resolution wood samples. The LMD can be used for inter-and intra-annual carbon and oxygen stable isotopes investigations on tree rings. Our current measurement scheme contains 3 steps:

1. Preparation of thin cross sections (max. 1000 µm thickness),
2. UV-laser microdissection of inter-or intra-annual wood sections and
3. Stable carbon and oxygen isotope analysis via Isotope Ratio Mass Spectrometry (IRMS).

There are several advantages using the LMD: Relevant cells/tissues can be selected on screen by pen, where else non-relevant tissues (e.g. resin ducts) can be removed. Any size and area can be dissected by the UV-laser and the complete selected sample is recovered. In addition, samples can be pooled and if desired cellulose extraction is possible after the cutting process. The use of thin cross sections leaves the core intact and provides the opportunity for other dendrochronological investigations. Furthermore, there is a detailed documentation of the dissecting and cutting process. The laser dissection method avoids several disadvantages of the laser ablation method where spot size is limited, the loss of particles is >60µm (dust filter) and no online monitoring of the laser ablation process is possible (Schulze et al. 2004).
Limitation of the LMD system is a non-automatic z-focus. When the selected shape does not fall down after the cutting process, a new cutting process must be started manually. The LEICA system uses high-precision optics to steer the laser beam using prisms along the desired cut lines

on the tissue. This involves the limitation that the laser can only cut drawn lines/areas marked on the visible screen. If larger areas should be dissected a new shape must be drawn and a new cutting process must be started. However, pooling of selected specimen solve this limitation.

Conclusion and Applications

The automatic UV-laser dissection system (LMD7000, LEICA Microsystems) allows the user to cut samples of wood at unprecedented precision. Here, we tested the LMD tool as a fast and reliable method for preparing high-resolution intra-annual wood samples from tropical trees for standard stable isotope measurements. The new technique facilitates inter-and intra-annual tree-ring analysis and opens various possibilities for wood anatomical and plant physiological studies. For instance, detailed assessment of carbon and oxygen isotope variability in wooden parts such as resin ducts, rays, fibres vessels or parenchyma cells are possible. Plant structure in relation to plant functioning by combining wood anatomy and stable isotope analysis can be evaluated. Furthermore, the UV-laser microdissection system facilitates the establishment of long and continuous high-resolution isotope chronologies for high quality climate reconstructions. Multidisciplinary analyses (e.g. wood density, wood chemistry analyses) on the same wood sample are now possible with a complete recovery of the sample due to the use of thin cross sections.

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