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# CRITICAL STEPS AND TROUBLESHOOTING IN SAMPLE PREPARATION FOR WOOD AND PHLOEM FORMATION: FROM SAMPLING TO MICROSCOPIC OBSERVATION

# KRITIČNI KORAKI IN REŠEVANJE TEŽAV PRI PRIPRAVI VZORCEV ZA SPREMLJANJE NASTAJANJA LESA IN FLOEMA: OD VZORČENJA DO OPAZOVANJA POD MIKROSKOPOM

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***Abstract / Izvleček***

**Abstract:** We present a technical note that supplements published procedures on optimal sample preparation for performing wood and phloem formation analyses. Before beginning sampling, it is important to learn about the characteristics of the tree or shrub species to be investigated. Some tips are given how to use the Trephor tool in the best way, how to remove the outer hard bark (periderm), how microcores should be handled after removal from the tree, and how they should be oriented for embedding in paraffin, and cutting thin sections for microscopy. Possible defects that may result from improper handling are illustrated and discussed. We also present optimal images to accurately identify different cell development stages in phloem and xylem, which is particularly challenging in hardwoods and Mediterranean tree and shrub species.

**Keywords:** tissue sampling, tissue preparation, microscopic slides, microscopy, xylogenesis, phloem formation, troubleshooting

**Izvleček:** Predstavljamo tehnična navodila, ki dopolnjujejo objavljene postopke za optimalno pripravo vzorcev za izvajanje analiz nastajanja lesa in floema. Pred začetkom vzorčenja je potrebno poznati značilnosti drevesne ali grmovne vrste, ki jo želimo preiskovati. Podanih je nekaj nasvetov, kako najbolje uporabiti orodje Trephor, kako odstraniti zunanjo trdo skorjo (periderm), kako ravnati z mikro izvrtki po odvzemu iz debla in kako jih orientirati za vklapljanje v parafin in rezanje tankih rezin za mikroskopske preiskave. Prikazane in razložene so možne napake, ki lahko nastanejo zaradi neustreznega ravnanja v različnih korakih postopka. Predstavljamo tudi optimalne slike tkiv za prepoznavanje različnih razvojnih faz celic v floemu in ksilemu, ki je še posebej zahtevno pri listavcih ter sredozemskih drevesnih in grmovnih vrstah.

**Ključne besede:** odvzem vzorcev tkiva, priprava vzorcev, mikroskopski preparati, mikroskopiranje, nastajanje lesa, nastajanje floema, odpravljanje težav

## 1 INTRODUCTION
## 1 UVOD

The microcoring technique is increasingly used to obtain samples for monitoring tree responses to changing environmental conditions and assessing tree plasticity in the context of climate change. Analysis of microscopic specimens makes it possible to monitor cambial activity and wood and phloem formation in real time, and to distinguish the different phases of cell division, differentiation, and maturation and determine how and when they occur. To this end, samples (usually microcores) containing phloem, cambium, and xylem are taken weekly or biweekly from living trees, for at least one calendar year, with particular emphasis on the growing season. The most effective tool for sampling micro-

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cores is the Trephor tool (Rossi et al., 2006), which was developed for this purpose and first used in 2004. Since its first use, several protocols for sampling of microcores and sample preparation have been developed to monitor the different stages of wood and phloem formation (e.g., Rossi et al., 2006; Deslauriers et al., 2015; Prislan et al., 2014a, b, 2022; De Micco et al., 2019; Pace, 2019).

In addition, various protocols for preparing anatomical slides (including embedding, cutting and staining) have been developed and improved to achieve high-quality slides for microscopy and quantitative anatomy, including image analysis (Deslauriers et al., 2015; De Micco et al., 2019; Prislan et al., 2014a, b, 2022). Appropriate microscopy techniques are critical to accurately identify xylem and phloem cells during differentiation. Various histological and microscopic techniques are used to analyse and identify the different stages of cell division and differentiation (e.g., Balzano et al., 2021a).

Despite the availability of detailed protocols, mistakes can still happen, especially if one is a novice and there are no experts available to help with critical steps. Many technical errors can occur at all stages of the process, from sampling to quantification errors. Errors in live tissue collection can jeopardise the entire research. Repeating systematic errors at this stage runs the risk of collecting hundreds of samples over the course of a year that later prove useless for analyses.

The quality of thin sections can also be influenced by many factors: the tissue sample (microcore) itself (density, hardness, homogeneity, etc.), its storage (appropriate solution), sample preparation (possible trimming), orientation (anatomical orientation and orientation of the sample during sectioning), type and sharpness of the blade, and last but not least, the experience and skills of the operator.

Such errors may affect the measurement of cells, especially if it is done automatically, making it difficult to count cell number and measure cell dimensions, or leading to inaccurate results and interpretations.

In our experience, methods should be slightly adjusted on a case-by-case basis, as each species is different. It is also very important to document the species under study well (density, wood and phloem anatomy…) and to perform technical tests before starting the actual experiment.

Most of the techniques developed for the study of xylogenesis have been applied to conifers growing in temperate climates, characterized by fairly regular cambial activity and relatively homogeneous wood structure. In Mediterranean and tropical species, whose wood is often characterized by high density and hardness, the sampling of microcores can be technically challenging. Precautions must also be taken, and existing protocols adapted when sampling shrubs.

The goal of this study is to improve the published protocols, help novices with critical steps, and focus on technical issues that are not necessarily addressed in detail in existing protocols. We want to report on the most common mistakes that are made and show possible solutions to overcome them, as well as share our own tips. We want to note some bad examples and explain their causes and solutions.

## 2 MATERIALS AND METHODS
## 2 MATERIALI IN METODE

The standard sample preparation protocol for wood and phloem formation analyses consists of: (1) collection of tissues (microcores) from living trees using a Trephor tool, (2) fixation of tissues with a solution of ethanol, formalin, and acetic acid, (3) storage in ethanol, (4) trimming of microcores, (5) dehydration and infiltration with paraffin, (6) embedding in paraffin, (7) trimming and cutting of (transverse) sections with a rotary microtome, (8) staining, (9) embedding of slides on objective glasses, (10) microscopy (different light modes), (11) image acquisition, (12) recognition of tissues and tissue parts, including determination of different stages of cell and cell wall development, (13) measurement of cells and cell parts (e.g. cell walls) – image analysis, (14) interpretation of results.

This article is a technical note describing the critical steps in the sample preparation protocol for wood and phloem formation analyses, and how to better handle the most critical steps in the process that often lead to poor quality microscopic slides and problems in measuring tissue elements (image analysis) and interpreting results.

## 3 RESULTS
## 3 REZULTATI

### 3.1 SAMPLING AND SAMPLE FIXATION – CRITICAL POINTS
### 3.1 VZORČENJE IN FIKSIRANJE VZORCEV – KRITIČNE TOČKE

Before taking tissue samples from trees, one should carefully study the characteristics of the tree species to be studied: its wood and bark anatomy (how dense and hard is the wood, peculiarities and thickness of the bark) as well as the time and duration of the growing season. Before starting the actual experiment, collect additional specimens for practice. Both novice and experienced scientists may otherwise collect a large number of unsuitable specimens, limiting the possibility of making suitable sections. During the experiment it is desirable to collect specimens throughout the calendar year to avoid missing crucial milestones, such as the beginning and end of cell production by the cambium.

The procedure for sampling and processing microcores is described in Prislan et al. (2022), and this article also includes some critical points and potential errors to avoid.

During sampling the use of a Trephor tool wounds the tissue, resulting in the production of wound tissue that is different from the tissues of interest. To avoid wounding effects, successive samples for wood and phloem formation should be taken in a spiral pattern along the stem, with sampling sites at least 10 cm apart. Two microcores are usually taken from each tree on a given day to have a reserve in case one of the two cores is not suitable. In the case of small trees or shrubs, such as grapevine (*Vitis vinifera*) (Figure 1), the sample is taken from a lower location and it is not possible to take two microcores because the sampling would cause too much damage. In this case, it is recommended to select several individuals for analysis and sample them in turn.

The quality of thin sections depends on the quality of the microcores, which is influenced by the sharpness of the Trephor cutting edge. A blunt cutting edge will cause damage to the microcores (e.g., compressed, partially crushed, broken, or twisted cores), which directly affects the quality of the thin sections (Figures 1, 2, 3).

Choose the correct size of Trephor depending on the species studied. A standard size cutting dimeter (1.9 mm) is appropriate for conifers and hardwoods with small vessels and homogeneous wood structure. A larger Trephor (cutting diameter

*Figure 1. Sampling tissues from the stem of grapevine (Vitis vinifera): removal of the outer bark (left) and taking microcores with a Trephor tool (right).*
*Slika 1. Vzorčenje tkiv iz stebla vinske trte (Vitis vinifera): odstranitev zunanje skorje (levo) ter odvzem mikro izvrtka z orodjem Trephor (desno).*

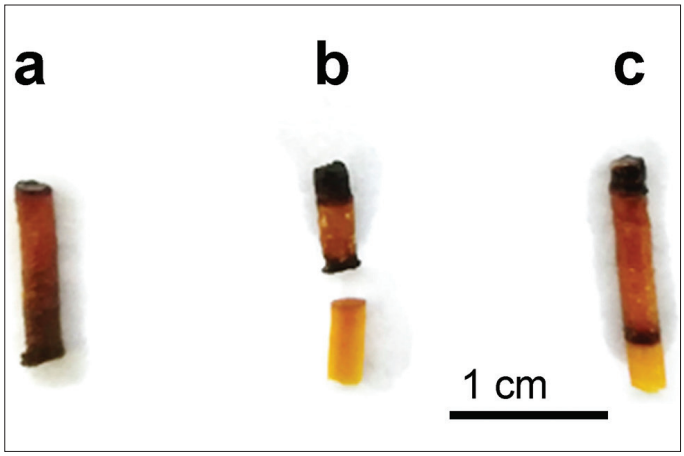


*Figure 2. Damaged microcores due to errors in sampling tissues from a living tree: (a) sample consisting only of bark (more bark tissue should be removed before sampling), (b) broken sample with crushed cambium (blunt edge of Trephor), (c) sample with too little wood tissue.*

*Slika 2. Poškodovani mikro izvrtki zaradi napak pri odvzemu vzorcev iz živih dreves: (a) vzorec vsebuje samo skorjo (pred vzorčenjem bi bilo treba odstraniti več skorje), (b) zlomljen vzorec s porušenim kambijem (topo rezilo Trephorja), (c) vzorec s premalo lesnega tkiva.*

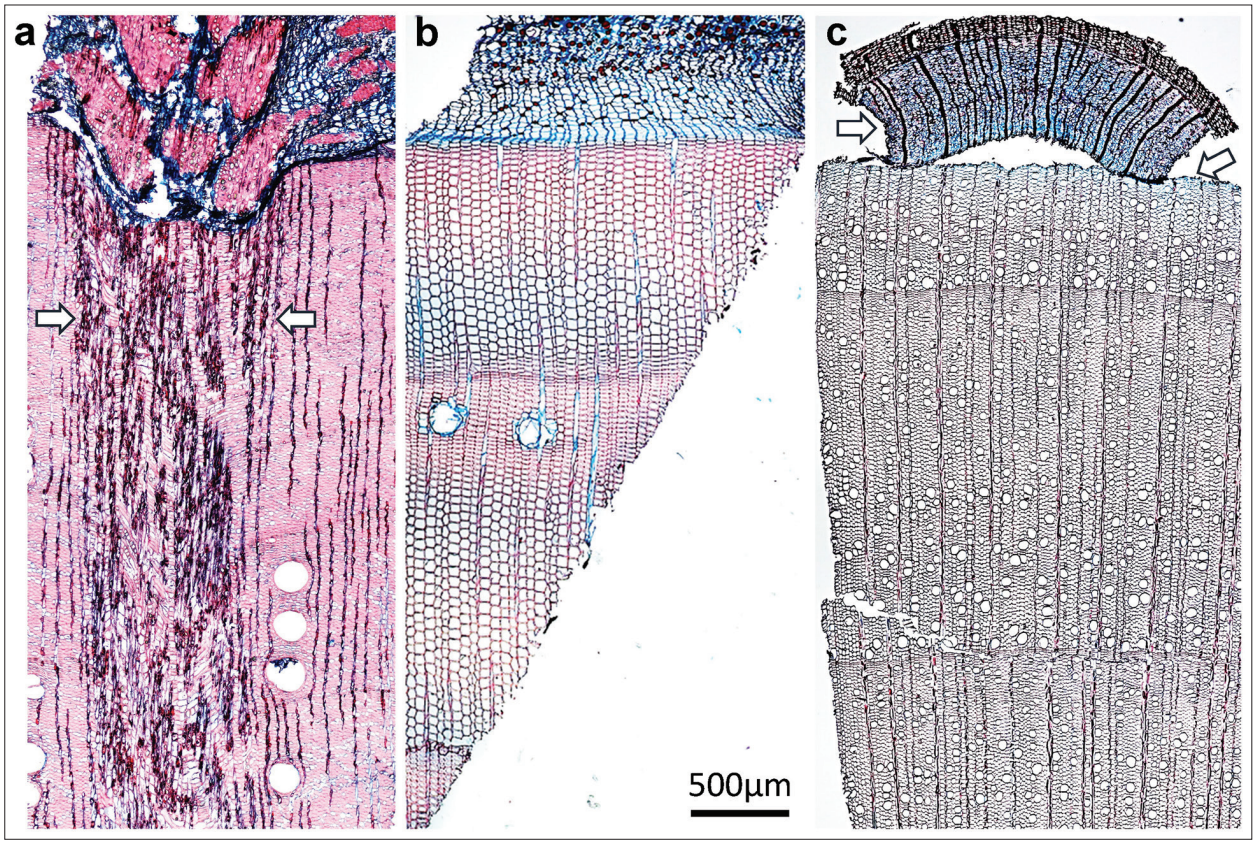


*Figure 3. Sampling errors reflected in the quality of thin sections: (a) cross-section of holm oak (Quercus ilex), whose wood and bark are mainly ray tissue (white arrows) (larger diameter Trephor should be used), (b) sample of stone pine (Pinus pinea) not taken perpendicular to the stem, (c) sample of strawberry tree (Arbutus unedo) which was not properly stored in the fixation medium (the sample was dried out, cambium is crushed, phloem is shrunken – white arrows).*

*Slika 3. Napake pri vzorčenju, ki se odražajo v kakovosti tankih rezin: (a) preparat tkiv črničevja (Quercus ilex) večinoma zavzema širok trak (beli puščici), (uporabiti bi bilo treba Trephor večjega premera), (b) vzorec pinije (Pinus pinea) ni bil odvzet pravokotno na deblo, (c) vzorec navadne jagodičnice (Arbutus unedo), po odvzemu iz drevesa ni bil pravilno shranjen v fiksacijskem mediju (vzorec se je posušil, kambij je porušen, floem se je skrčil (beli puščici)).*

> 2 mm) would help to obtain wider tissue sections for analyses. This is especially important when studying species with wider rays such as beech (*Fagus*) and oaks (*Quercus*) (Balzano et al., 2021b). Too narrow sections and large rays hinder the analyses, as in the case of *Quercus ilex* (Figure 3a).

Figures 1, 2, and 3 show sampling and common sampling errors and their effects on thin sections. Care must be taken to remove the correct amount of bark to avoid the risk of collecting only bark tissue (Figures 2a, c). The depth of the flap to be removed must be based on the depth of the bark, leaving the uncollapsed phloem and cambium intact. If the Trephor is not sharp enough, the bark and wood may separate (Figure 2b). It is very important to pierce the stem perpendicularly in the spot where the bark has been removed (Figure 3b). Hold the Trephor securely and then hammer firmly and vigorously until the entire tip of the Trephor penetrates the tissues.

It is important to place specimens in the fixation medium (FAA) immediately after collection. Since the purpose of fixation is to immobilize proteins and cellular components to maintain the structural integrity of the tissues, it is imperative that this step is done quickly. If the sample is not stored properly, the softer tissues such as the cambium and parenchyma cells will rapidly collapse and be unusable for measurements (Figure 3c).

### 3.2 SAMPLE PREPARATION AND CUTTING – CRITICAL POINTS

### 3.2 PRIPRAVA VZORCEV IN REZANJE – KRITIČNE TOČKE

Before dehydration and infiltration with paraffin, the microcores must be perfectly oriented using a scale magnifier to identify the transverse plane and mark it with a waterproof pencil. In this way, we can easily arrange the sample in the desired orientation for cutting after the paraffin embedding.

The transverse plane often appears darker than the radial plane. If there is any doubt about the orientation, it can be determined by placing a dot in the centre of the end of the microcore. In this way, the ink will follow the fibre orientation and reach the cross section. If the sample is not well oriented on the thin sections, the tissue will appear disoriented and will not match the normal structure of the cross-section, making it impossible to identify and properly measure the cells (Figure 4a). The microcore is later placed at an angle to the mould and with the bark down so that the blade encounters the least possible resistance when cutting.

There are several problems when cutting. Because of the very heterogeneous tissue, consisting of thin- and thick-walled cells with different lumen sizes, the microcore often breaks in the area of the cambium with extremely thin-walled cells, which is then destroyed. Therefore, it is extremely difficult to produce thin sections without defects such as cracked and collapsed areas.

Perfectly sharp blades for cutting are also essential to avoid the specimen splitting, collapsing, tearing off cells and leaving a blade track (Figure 4b, c, d). This is especially true for very dense and hard tissue, where it is often necessary to change the position of the blade for each cut, so that a previously unused, perfectly sharp part of the blade can be used.

To achieve perfect cuts, it is also important to properly adjust the angle of the blade correctly. The greater the angle, the less the impact on the specimen; therefore, it is advisable to increase the angle when the bark is harder. The optimum angle is usually between 5 and 10°.

Cutting speed is also an important factor when cutting. Cutting too slowly hinders the formation of the "wax ribbon", while cutting too fast can result in highly compressed sections that are difficult to expand.

A cutting thickness of 9 µm is sufficient for studying wood and phloem formation. However, if the bark or wood is too hard, it is recommended to prepare smaller cutting blocks and make thinner sections. This will provide less resistance to the knife and allow better sections to be made. A slight variation in the thickness of the section can often solve cutting problems.

When it comes to preparing the slide, we can save space by placing as many sections as possible on it. This can only be achieved if the ribbon consisting of paraffin and thin sections is straight. Before cutting, it is useful to trim away any excess wax and tissue to maximize the number of sections that can be placed on a slide. The smaller the block area, the easier it is to make a ribbon. During sectioning, water is applied with a fine brush to con-

stantly keep both the sample and edge of the blade wet, which helps the section to slide across the blade when cutting.

The ribbons containing the cross-sections of microcore should be checked under the microscope to be sure you are selecting the best one. Although this is time consuming, it will facilitate and expedite further analyses.

Another critical step in sample preparation is the application of glycerine albumin to the slide. The key here is to apply the right amount of glycerine albumin; if we apply too much, the end result will be an opaque sample, if we apply too little, the section will be washed away from the slide in further steps. One drop from a generic disposable pipette to measure 3 ml of liquid is more or less the right amount.

Excess paraffin must be properly removed from the slides. The clearing agent (e.g., D-limonene) should be changed frequently to avoid dirty samples (Figure 4c). Also, be sure to leave the sample in the staining solution for as long as necessary (15 minutes) to avoid weak staining or overstaining. The safranin and astra blue aqueous solution is optimal for this purpose, as it allows observation in brightfield, polarized light, and epi-fluorescence modes (Balzano et al., 2021a).

During dehydration prior to embedding in Euparal, the alcohol used must not contain water, otherwise the slide will fog (Figure 4d). In addition, the Euparal embedding medium must be well dosed to avoid stains on the coverslip, which prevent observation (Figure 4e). If not enough Euparal is applied, air bubbles will form.

### 3.3 EXPECTED RESULTS
### 3.3 PRIČAKOVANI REZULTATI

Following the protocol (Prislan et al., 2022) and considering the tips suggested here should help to

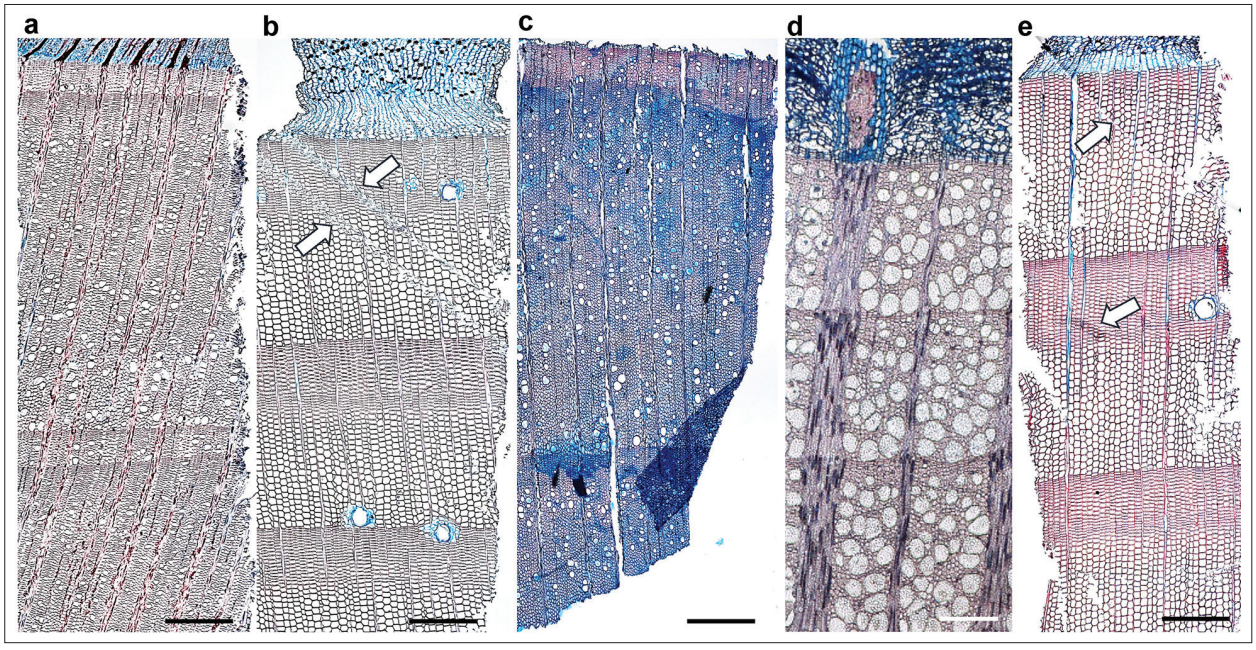


*Figure 4. Sample preparation errors: (a) microcore was not well oriented, (b) section with knife marks due to a blunt blade (white arrows), (c) section contaminated, overstained and split due to a blunt blade, (d) "foggy" slide due to presence of water in alcohol, (e) section split due to a blunt blade and traces of Euparal on the coverslip (white arrows). Black bars = 500 µm, white bar = 200 µm.*

*Slika 4. Napake pri pripravi vzorcev in preparatov: (a) mikro izvrtek ni bil dobro orientiran, (b) rezina s poškodbami zaradi topega rezila (beli puščici), (c) preparat vsebuje nečistoče, preveč obarvan in razcepljen zaradi topega rezila, (d) rezina motna zaradi nepopolne dehidracije (vode v alkoholu), (e) s poškodbami zaradi topega rezila in sledovi euparala na krovnem steklu (beli puščici). Črne merilne daljice = 500 µm, bela merilna daljica = 200 µm.*

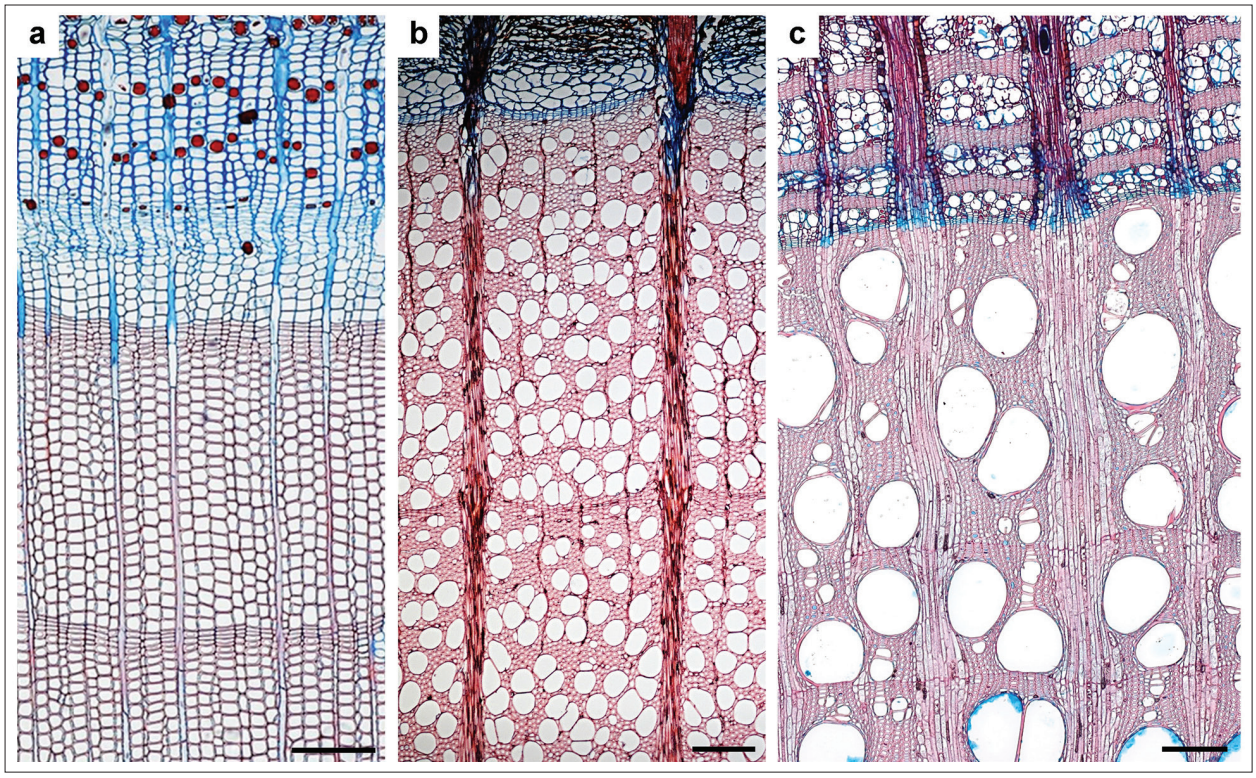


*Figure 5. Examples of high-quality cross-sections for quantitative image analyses of wood, cambium and phloem of: (a) Pinus halepensis, (b) Fagus sylvatica, and (c) Vitis vinifera. Scale bars = 200 µm.*
*Slika 5. Primeri visokokakovostnih prečnih prerezov za kvantitativno analizo slik tkiv lesa, kambija in floema pri vrstah: (a) Pinus halepensis, (b) Fagus sylvatica in (c) Vitis vinifera. Merilna daljica = 200 µm.*

obtain high-quality slides and images needed for subsequent analyses (Figure 5).

For successful analysis of wood and phloem formation, it is critical to obtain high-quality images to accurately identify the different cell development stages in phloem and xylem, which is particularly challenging in Mediterranean and tropical hardwoods. For the analyses, we need to identify the following:

(1) cambial cells and their division;

(2) postcambial cell development with the cell enlargement phase;

(3) secondary cell wall deposition and lignification; and

(4) cell maturation ending with autolysis of the protoplast and complete lignification of the cell walls.

Polarized light is commonly used to distinguish between primary and secondary cell walls and cellulose deposition (Figure 6a, b). It helps to distinguish between enlarging cells that contain only a primary cell wall and those that are in the phase of cell wall thickening with the appearance of a birefringent secondary wall.

Lignified and unlignified cell walls and tissues can be observed using epifluorescence techniques. Fluorescence imaging of sections stained with an aqueous solution of safranin and astra blue (or other combination of stains) can clearly highlight the contrast between lignified and unlignified cell walls to clearly show the boundary between postcambial cells with primary cell walls and those in the phase of secondary cell wall deposition, as well as the progression of lignin deposition in the areas of secondary wall thickening and maturation phases (Figure 6c). Fluorescence also highlight the presence of cytoplasm in the cell lumen, facilitating differentiation between mature and non-mature cells.

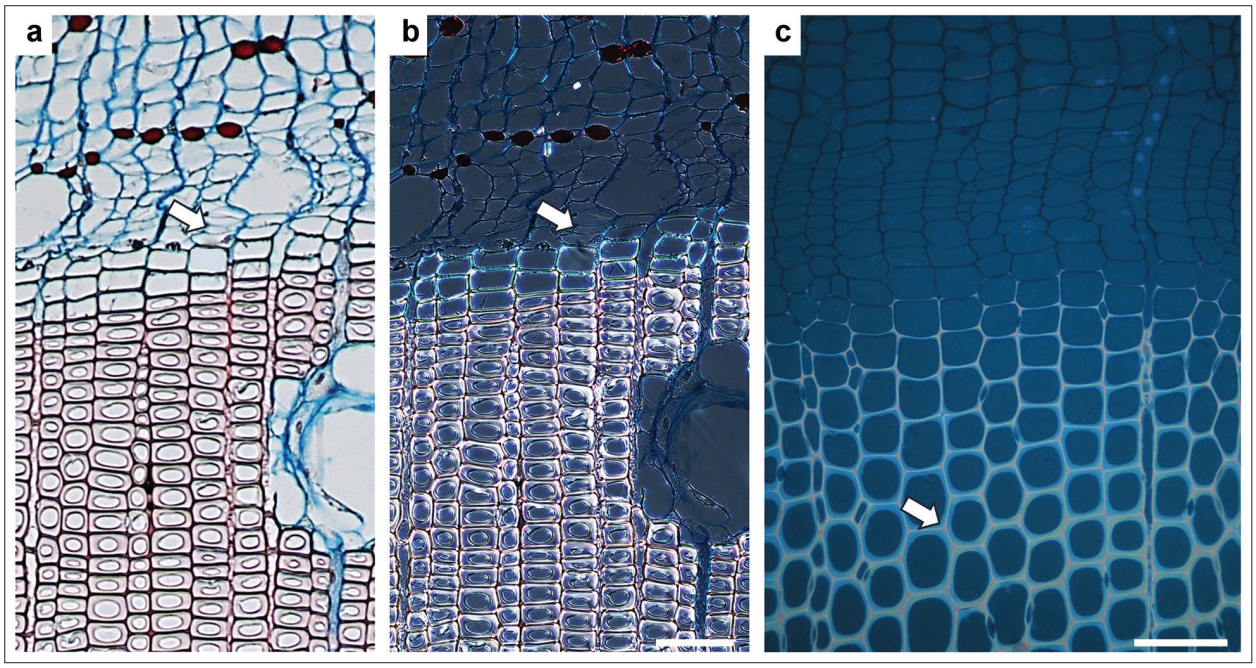


*Figure 6. Cross-section of phloem, cambium and xylem of Pinus halepensis: (a) under a light microscope, bright field mode, safranin-astra blue staining, (b) under polarized light allowing to distinguish between enlarging tracheids (white arrow) containing only a primary cell wall and tracheids in the phase of cell wall thickening, which contain a birefringent, shiny secondary wall, (c) progress of lignin deposition in the (shiny) areas of the middle lamella and secondary wall, starting from the middle lamella in the cell corners (white arrow). Scale bars = 500 µm.*

*Slika 6. Prečni prerez floema, kambija in ksilema alepskega bora (Pinus halepensis): (a) s svetlobnim mikroskopom, svetlo polje, obarvanje s safranin-astra modrim, (b) s polarizirano svetlobo, ki omogoča razlikovanje med traheidami v fazi rasti (bela puščica), ki vsebujejo le primarno celično steno, in traheidami v fazi odlaganja celične stene, ki vsebujejo optično aktivno sekundarno celično steno, ki se sveti, (c) potek odlaganja lignina (svetleča mesta) v srednji lameli in sekundarni steni, z začetkom v vogalih celic (bela puščica). Merilne daljice = 500 µm.*

## 4 CONCLUSIONS
## 4 ZAKLJUČKI

Monitoring xylogenesis and phloem formation provides useful information about xylem and phloem formation in real time, but requires specific tools, techniques, and skills for successful sampling, preparation of high-quality sections, and the ability to recognize the cells and the different stages of cell differentiation. The aforementioned skills are preferably acquired with the help of experts. If this is not possible, experience using existing protocols and technical references must be gained to master the critical steps. Many errors can occur at all stages of the process, from sampling to sectioning and quantification errors, especially if one is a novice in this field. This article is designed to share our experiences and help novices to not get discouraged and avoid the most common mistakes. First, we recommend acquiring knowledge of the species one intends to study. In our experience, protocol procedures should be adapted to it. Practicing on test samples can also help to gain the necessary experience without compromising the actual samples used in the research. It is hoped that the tips we have provided in these technical notes will help beginners to deal with some of the critical steps in the procedure that are not addressed in existing protocols.

## 5 SUMMARY
## 5 POVZETEK

Tehnika odvzema mikro izvrtkov in priprava preparatov za mikroskopijo se danes najpogosteje uporablja za spremljanje kambijeve aktivnosti ter nastajanja lesa in floema, med drugim tudi za oceno plastičnosti dreves v kontekstu podnebnih sprememb. Mikroizvrtke iz debel dreves jemljemo z orodjem Trephor (Rossi et al., 2006), ki je bilo prvič uporabljeno leta 2004. Razvitih je bilo več protokolov za vzorčenje in pripravo vzorcev mikroizvrtkov za mikroskopske analize kambija, ksilema in floema (na primer Deslauriers et al., 2015; Prislan et al., 2014a, b, 2022; De Micco et al., 2019; Pace, 2019; Balzano et al., 2021a). Namen tega prispevka je nadgraditi objavljene protokole, da bi začetnikom pomagali pri premagovanju kritičnih korakov med postopkom, ki v obstoječih protokolih niso nujno podrobno obravnavani. Predstavljamo najpogostejše napake in na podlagi naših izkušenj podajamo nasvete, kako se jim izogniti.

Najnovejši protokol Prislan et al. (2022) priporočamo kot referenco, kamor dodajamo naše nasvete. Pred začetkom novega projekta predlagamo, da skrbno preučite značilnosti drevesne vrste, ki jo želite preučiti (anatomija lesa in skorje, čas in trajanje rastne sezone ipd.). Po naših izkušnjah je treba protokol prilagoditi glede na značilnosti preiskovane vrste. Predlagamo tudi odvzem nekaj dodatnih vzorcev za preizkus metode. Da ne bi zamudili ključnih mejnikov (npr. začetek in konec kambijeve aktivnosti), je zaželen odvzem vzorcev preko celotnega koledarskega leta. Da bi se izognili učinku poškodovanj, moramo mikro izvrtke odvzemati spiralno vzdolž debla, pri čemer morajo biti mesta vzorčenja med seboj oddaljena vsaj 10 cm. Običajno se z vsakega drevesa na določen dan odvzameta dva mikro izvrtka, da imamo enega za rezervo. Pri majhnih drevesih ali grmih, kot je na primer vinska trta (slika 1), se prvi vzorec odvzame spodaj in ni priporočljivo odvzeti dveh mikroizvrtkov na vzorčenje, ker bi to povzročilo prevelike poškodbe. V tem primeru lahko za analizo izberemo več osebkov in jih vzorčimo izmenoma.

Rezilo Trephorja mora biti ostro. Topo rezilo bi povzročilo poškodbe na mikro izvrtku, kar bi posredno vplivalo tudi na kakovost tankih rezin (preparatov) (slike 1, 2, 3). Glede na vrsto, ki jo proučujemo, izberemo pravo velikost Trephorja. Večji Trephor (premer rezila > 2 mm) omogoča, da odvzamemo širši pas tkiva za analize, kar je pomembno zlasti pri preučevanju vrst s širšimi trakovi. Odstraniti moramo ustrezno količino zunanje skorje, da se izognemo tveganju, da bi mikro izvrtek vseboval predvsem skorjo in premalo drugih tkiv (lesa) (sliki 2a, c). Vbod s Trephorjem opravimo pravokotno na deblo na mestu, kjer je bil odstranjen zunanji del skorje (slika 3b). Pomembno je, da vzorce takoj po odvzemu vložimo v fiksacijsko sredstvo (FAA), da preprečimo poškodbe mehkejših tkiv (slika 3c).

Pred nadaljnjo obdelavo morajo biti mikro izvrtki pravilno orientirani, sicer bo tkivo na preparatih deorientirano, kar bo onemogočilo prepoznavanje in merjenje celic (slika 4a). Bistveno je tudi, da pri rezanju uporabimo popolnoma ostra rezila, da na preparatu ne bi prišlo do razpok, kolapsa, »izpuljenih« celic ter da preparat ne bi vseboval sledi rezila, ki jih vidimo kot pas raztrganih celic (slike 4b, c, d). Pri rezanju je pomembno, da nastavimo pravilen kot rezil (med 5° in 10°) in prilagodimo hitrost rezanja. Težave pri rezanju lahko pogosto rešimo že z manjšo spremembo debeline rezine.

Še en kritičen korak pri pripravi vzorca je nanos glicerin albumina na objektno steklo, ki »prilepi« preparat na steklo. Topila za zadnje korake pri dehidraciji tkiv je treba pogosto menjavati, da se izognemo nečistočam v preparatu (slika 4c). Pri dehidraciji uporabljeni alkohol ne sme vsebovati vode, sicer se vklopni medij zamegli (slika 4d). Poleg tega je treba vklopni medij Euparal primerno dozirati, da se izognemo nastanku madežev na objektnem ali pokrivnem steklu (slika 4e) in da se v preparat ne »ujamejo« zračni mehurčki. Z upoštevanjem protokola (Prislan et al., 2022) in tukaj predlaganih nasvetov bi morali nastati visokokakovostni preparati in slike, primerne za kvantitativno analizo slike (slika 5).

Pri analizah nastajanja lesa in skorje moramo prepoznati različne vrste celic in tkiv v lesu in skorji (floemu) ter njihovo razvojno stopnjo. Za razlikovanje med primarnimi in sekundarnimi celičnimi stenami običajno uporabljamo mikroskopijo s polarizirano svetlobo (slika6 a, b). Z epi-fluorescenčno mikroskopijo pa lahko poudarimo kontrast med lignificiranimi in nelignificiranimi celičnimi stenami, da prepoznamo potek odlaganja lignina na območjih srednje lamele in sekundarne stene ter nastanek popolnoma diferenciranih zrelih celic (slika 6c). Upamo, da bodo ob upoštevanju predstavljenih na-

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