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Minirhizotron Techniques

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I. Introduction

Special techniques are required to investigate root systems since they are hidden in the soil. Traditionally, destructive techniques like coring, trenching, and excavating have been used to access roots in situ. More recently, nondestructive techniques including rhizotrons and minirhizotrons (MRs) were developed in order to allow direct and repeated observations of the roots within the rhizosphere.

Installations using transparent "walls" to study roots in soil are termed rhizotrons (Böhm 1979). Walk-in rhizotron facilities or smaller-sized rhizotron chambers can also be used as lysimeters, and they may also include sensors that monitor soil conditions (Karnok and Kucharski 1982; Pan et al. 2001; Meier and Leuschner 2008). However, large rhizotrons have several disadvantages, with setup and operational cost being the primary ones; therefore, a very limited number of these facilities were built worldwide. In need for continuous nondestructive measurements of root traits in agricultural, silvicultural, and pristine ecosystems, the MR system was developed and has ever since gained wide acceptance. While glass plates ("root windows") have been used since the early 1900s (e.g., McDougall 1916), the MR concept was originally proposed by Bates (1937). In a work on fruit trees, he designed observation trenches in form of a walled chamber fitted with "root windows." However, what can probably be considered the first study with MRs as we know them today, using transparent tubes and an imaging device, was conducted by Waddington (1971).

MRs have helped improve our understanding of root systems, for example, in respect of standing stock, root production and longevity, root–parasite and root–hyphae interactions, and root phenology and distribution (e.g., Upchurch and Ritchie 1983; McMichael and Taylor 1987; Aerts et al. 1992; Hendrick and Pregitzer 1992; Hooker et al. 1995; Kosola et al. 1995; Eissenstat et al. 2000; Treseder et al. 2005; Vargas and Allen 2008; Ephrath and Eizenberg 2010).

Although reviews have previously discussed how to install the MR tubes and how to collect and use the obtained images (Taylor 1987; Box 1996; Hendrick and Pregitzer 1996a,b; Majdi 1996; Johnson et al. 2001; Mainiero 2006; McMichael and Zak 2006), there is an ongoing need to point out the proper use and possible pitfalls of MR systems to new users and to promote "good practice" standards. This chapter addresses five specific topics: (1) installation of MR observation tubes (MR-OTs), (2) MR image capturing systems, (3) image acquisition and analysis, (4) application of the MR technique, and (5) an outlook on recent and future developments, which could extend the range of applications of this technique.

II. Installation Protocols and Materials Used for Minirhizotron Observation Tubes

A. Installation of Minirhizotron Observation Tubes

Whichever MR system is used, it requires that transparent MR-OTs be installed in the soil. Because MR studies are conducted in a wide range of natural and artificial soil environments, soil type and species-specific factors have to be taken into account during MR-OT installation. In order to minimize soil compaction and plant damage in situ, trampling must be avoided during installation. Installing MRs before planting or when root and shoot biomass is at its annual low is recommended.

Most MR studies are conducted in rather homogeneous soils, while very stony soils are only rarely addressed because of the difficult installation process (but see Phillips et al. 2000). Holes to insert the MR-OT are usually made using an auger (Kage et al. 2000), a soil corer (Hummel et al. 1989), or a combination of both (see Johnson et al. 2001). Depending on the bulk soil density and the required depth, researchers may install tubes manually or by mechanical drilling devices (Brown and Upchurch 1987). Manual MR-OT installation is mostly conducted down to less than 100 cm depth, while greater depths are accessed by tractor-mounted or portable auger systems (Kloeppel and Gower 1995). For using a manual auger, a supporting stand should be fixed on the topsoil to guide the drill at the desired position and angle and may cause additional soil disturbance. A soil corer has the advantage of a more smooth soil interface and no additional disturbance by a supporting stand when MR-OTs are installed vertically; however, using a hammer or a ram is tedious, and the soil might become asymmetrically compacted (Figure 42.1A).

Ideally, MR tubes should be installed in such a way that they are in close contact with the soil matrix, affecting root growth only in the way other large objects such as stones do. However, it is extremely difficult to ensure complete and uniform contact of the OT surface with the soil with no gap. Contrasting considerations must be taken into account while deciding of the drill size. A tight fit will prevent the formation of a gap and will reduce the risk of tube rotation (a problem common for short MR-OT in shrinking soils; see Johnson et al. [2001] for “anchoring devices”). But it can cause soil compression that might lead to reduced root growth near the tube (McMichael and Taylor 1987). If the hole is oversized, MR-OT installation is easier and scratches at the tube surface can be avoided; however, even narrow voids between the wall of the OT and the soil will constitute a low-resistance path that can artificially increase root growth, branching, and survival (van Noordwijk et al. 1985; Volkmar 1993 and references therein; Figures 42.1B and 42.2). They are also prone to moisture condensation that may interfere with root observation (Figure 42.2). While backfilling of oversized holes with sieved soil material after tube placement makes MR-OT installation easier (Kloeppel and Gower 1995), the unnatural density and structure of the soil will most likely influence root traits. For a discussion of the appropriate installation and use of MR-OTs in wetland ecosystems see Iversen et al. (2012).

B. Angle of Installation

Commonly MR-OTs are installed either vertically (90°) or angled. Many of the angled MR-OTs are installed at 30° or 45°, but different angles are common (see Johnson et al. 2001). It was proposed that angled MRs can estimate root depth distribution of herbaceous/crop plants better than vertical tubes and to reduce the artificial funneling of roots down the root/MR tube interface (Bragg et al. 1983; Merrill et al. 1994; Pagès and Bengough 1997). It can be speculated that funneling is related to the persistence of a gap around the tube that may increase water infiltration and consist a low-resistance path that roots tend to follow due to gravitropism. However, Ephrath et al. (1999) found no preferential growth of wheat roots as a result of steep insertion angle in a sandy soil with homogeneous bulk density. No studies addressing the influence of different OT installation angles on MR results for woody species are known to these authors. Further studies with different plant soil types are needed for a conclusive answer. These authors expect differences in root-growth pattern between angled and vertically installed tubes to be soil- and species-specific but highly related to installation protocols.

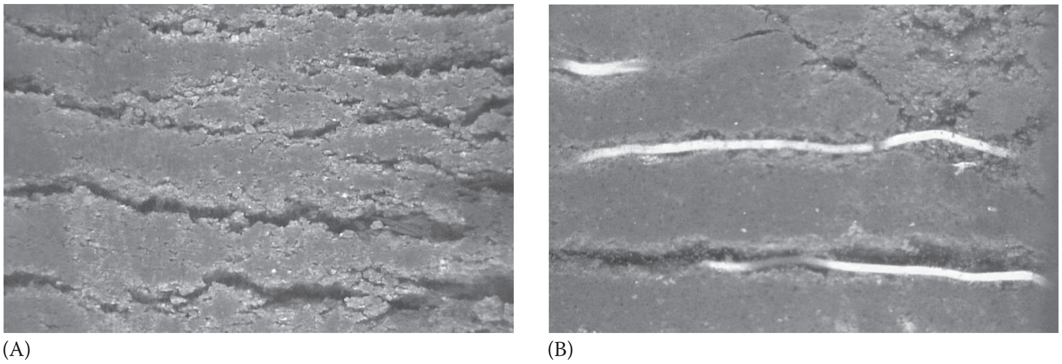

FIGURE 42.1 (A) Image of soil immediately after coring and tube installation into a sandy loam. Note the cracks in the soil profile resulting from hammering the coring tool into place. (B) Cotton roots preferentially exploiting resulting cracks in soil profile. (Images courtesy of Dennis Gitz, USDA-ARS, Lubbock, TX; pictures were taken with a camera MR, Bartz, Carpinteria, CA.)

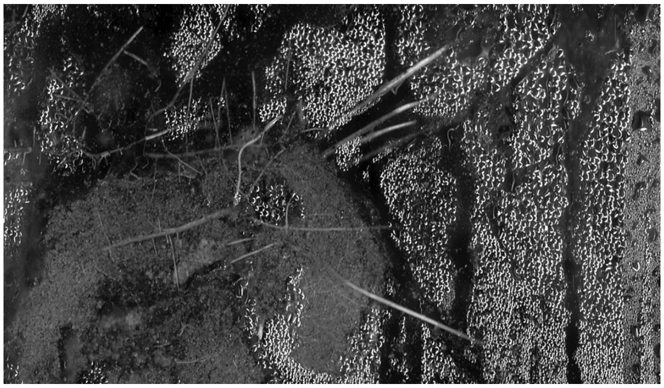

FIGURE 42.2 Picture section within an inappropriately installed MR-OT (90°) 3 months after installation (soil corer). The picture was captured with a scanner MR (CID, Cedar Rapids, IA). The roots of *Fagus sylvatica* can be seen to grow toward soil voids; many regions of the picture are obscured by condensed water in alternation to voids.

However, while vertical MR-OTs are more easily installed and depth at each recording point is more easily determined, angled tubes can reach underneath individual plants, which might be important in low plant density and/or in agricultural systems (i.e., in rows; Figure 42.3).

Installation of MR-OT in pots, lysimeters, and phytotrons with rather artificial, homogeneous soil environments seems to be less problematic because soil can be equally distributed around tubes during setup, reducing problems of air gaps and disturbance of root systems. MRs are often installed horizontally in such artificial sites (Liedgens 1998; Meier and Leuschner 2008). Horizontal tubes have the advantage of maximizing the observation area per soil depth (Smucker 1993), but a study by Dubach and Russelle (1995) revealed that there was a large variation between the root numbers on upper and lower sides of horizontally installed MR-OTs and that neither side was well correlated with the root counts on both horizontal sides.

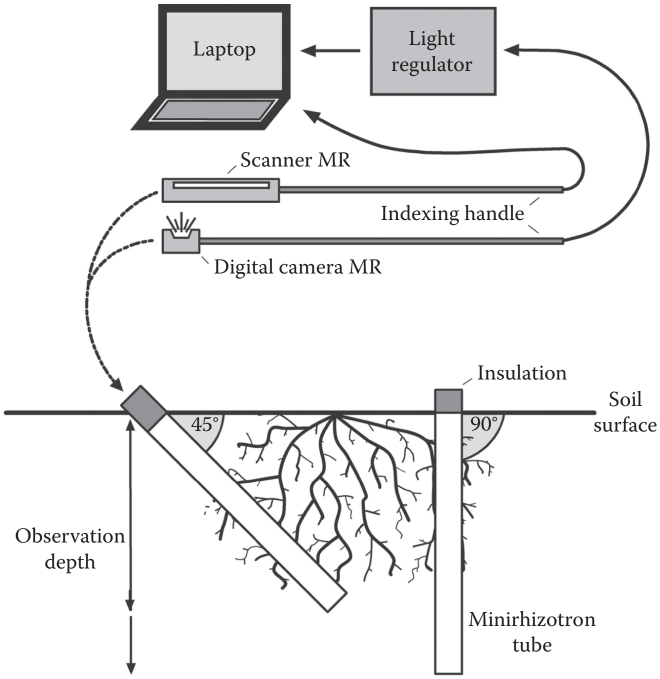

FIGURE 42.3 Setup of MR-OTs in both angled (e.g., 45°) and vertical positions (90°); aboveground light and temperature insulation and the observation depths are indicated. Images are captured by either digital camera–based or scanner-based MR systems connected to a laptop. Indexing handles allow for exact positioning of the devices in the OT; the light intensity of camera MR systems can be regulated.

C. Tube Protection from Light and Weather

Polycarbonate and PVC plugs (Box 1996; Phillips et al. 2000), rubber bungs (Majdi and Kangas 1997), or plastic end caps (Meier and Leuschner 2008) were used to seal the lower end of MR-OT that is in the ground. This is most important in moist soils in order to prevent water accumulation on the tube's inner surface, while it is of less concern in dry environments. However, the aboveground portion of MR-OT should always be insulated and covered with a lighttight cap and painted or covered with opaque tape to reduce thermal fluctuations and exclude light that can affect roots (Levan et al. 1987) and root-associated microbes (Klironomos and Allen 1995); special care has to be taken in soils that develop cracks while drying (Dubach and Russelle 1995). Especially in high-solar-radiation environments and without canopy cover, it is recommended to choose reflective colors for the tube cover and to reduce the protruding length of MR-OT to the minimum in order to avoid excessive heating (Figures 42.3 and 42.4A and B); insulation material, placed inside the protruding end of the tube, might further reduce temperature fluctuation of the soil around it. In areas with high snowfall, a support stand may be needed for angled MR-OT to prevent cracks on tubes' aboveground caused by the snow weight (Johnson et al. 2001).

D. Time Lag before First Measurement

Insertion of MR-OTs causes disturbances of the soil and root systems as discussed in Section II. A. It is unclear to date how fast different soil types and root systems return to equilibrium conditions; most researchers allow for a time period of 6–12 months, while some start their measurements immediately or within a few months (see Johnson et al. 2001). A timely start seems more unproblematic if MR-OTs are installed before planting, for example, in agricultural ecosystems or phytotrons; however, there may be a release of nutrients near recently installed tubes (Joslin and Wolfe 1999). In ecosystems with established root systems, first year's data were often found to be atypical as compared to subsequent years (Aerts et al. 1989; Burke and Raynal 1994). According to a meta-analysis by Strand et al. (2008), estimated longevity of tree fine root increased up to 40% with increasing time since OT installation (Figure 42.5). They concluded that tree root systems needed up to 3 years to return to equilibrium and that the longevity of fine roots established during the "pre-equilibrium" period was 50% shorter as compared to roots that developed in the "post-equilibration" period. The results indicate that short-term MR studies have contributed to the overestimation of fine-root turnover rates (Strand et al. 2008).

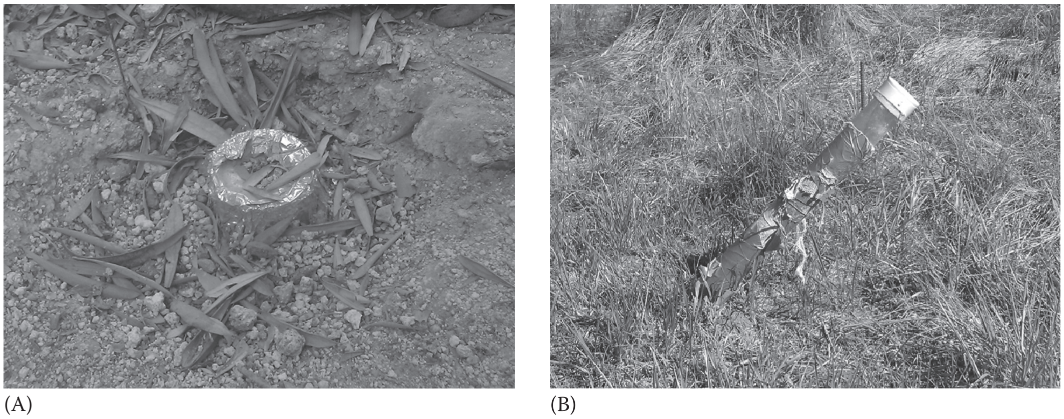
(A) (B)

FIGURE 42.4 Aboveground view on two differently installed MR-OTs. (A) A too long protruding tube installed at 45° with severed insulation; (B) a vertically installed tube. A short aboveground tube length and sufficient insulation to prevent light penetration and reduce tube heating.

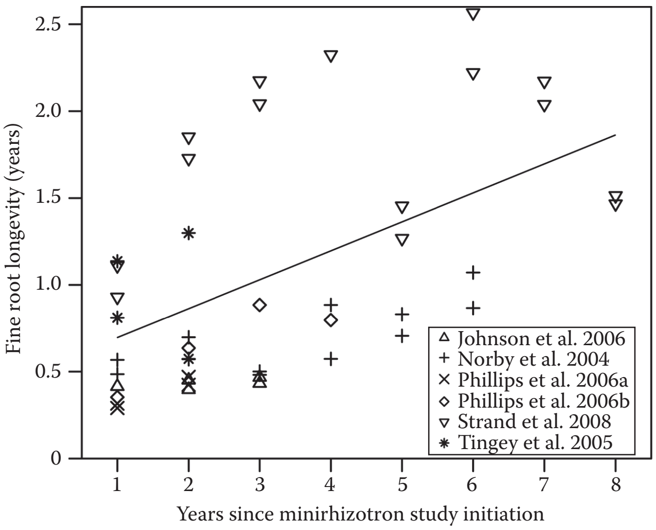

FIGURE 42.5 A meta-analysis by Strand et al. (2008) showing fine-root longevity as a function of the time since MR study initiation; six studies conducted in North American forest ecosystems are displayed. The increase in determined root longevity by time since MR-OT installation is indicated by a solid line. See Strand et al. (2008) for details.

Thus, in order to avoid having the disturbances during MR-OT installation affect the measurements of root traits, a sufficiently long soil- and species-specific equilibration time has to be taken into account. However, data collected during the “pre-equilibration” period could be used to determine the phenological pattern of root growth in general (Burke and Raynal 1994) or to determine the regrowth potential of root systems after disturbance (similar to ingrowth cores).

E. Material and Types of Minirhizotron Observation Tubes

A wide variety of materials have been used for MR-OT. Transparent and rigid MR tubes made of materials such as glass (Richards 1984; Eissenstat and Caldwell 1988; Fitter et al. 1999), polycarbonate (PC, known as Lexan; van Noordwijk et al. 1985; Box and Johnson 1987), polymethyl 2-methylpropenoate (or polymethyl methacrylate [PMMA], known as acrylic, Perspex, Plexiglas, or Acrylite; Itoh 1985; Vos and Groenwold 1987; Kloeppel and Gower 1995), and cellulose acetate butyrate (CAB or butyrate; Box et al. 1989; Hendrick and Pregitzer 1992; Wells and Eissenstat 2001; Yang et al. 2003) have been used. Inner diameter of the tubes used ranged from 13 mm (Boroscope; Upchurch and Ritchie 1983) to 64 mm (Scanner MR; Gaul et al. 2009); square tubes were used rarely (van Noordwijk et al. 1985). A future standardization of MR-OT diameters is desirable to allow for a greater flexibility in using different image capturing devices.

Rigid tubes made out of plastic have established themselves as the most common type of MR-OT because they are of greater durability than glass tubes, especially in rocky, swelling, or freezing soils, of easier use than flexible/inflated tubes (see the text in the following paragraph), and often the least expensive option. However, there are significant differences in the scratch resistance and transmissibility (e.g., for UV light) of rigid tubes; thus, on the one hand, the choice of material has to be made according to image capturing system, soil type, expected time, and intensity of use, as well as cost and availability (Wang et al. 1995; Johnson et al. 2001). On the other hand, the tube material might influence root traits. Although addressed as early as 1976 (Taylor and Böhm 1976), only a few studies have evaluated the influence of the clear materials on root growth. While Brown and Upchurch (1987) found no differences between the tube materials they tested, Withington et al. (2003) found the OT material to cause changes in root production and phenology. The production of apple roots was greatest around glass tubes, and these roots became pigmented later and lived longer than roots that grew near acrylic and CAB MR-OT. Furthermore, roots became pigmented faster next to CAB tubes than next to acrylic tubes; root survival was shorter near CAB tubes in three of four deciduous hardwood species but shorter near acrylic tubes for three conifer species. The comparison of root length density (RLD) with root standing crop from cores showed that the data gained from acrylic tubes matched more closely than the data from butyrate tubes (Withington et al. 2003). Future studies should seek to further clarify the impact and underlying mechanisms of different MR-OT materials on root traits of various species to prevent artifacts.

MRs with inflatable/flexible walls have been developed to address special needs (as sampling of soil and roots) and to optimize soil–tube contact. Especially in soils that tend to shrink during drying (e.g., clayey soils), inflatable tubes were meant to solve the problem of voids that form in such cases (Merrill 1992). Materials used to make MR-OT with flexible walls include cellulose acetate (Merrill et al. 1987), polyvinyl (Merrill 1992; Merrill et al. 2005), fluoroethylene propylene (known as Teflon FEP, Kosola 1999), and rubber (Gijsman et al. 1991; López et al. 1996). As very low-cost alternative standard (opaque), drain pipes with an opening ("cut out") have also been used; soil was prevented from collapse, and roots were prevented from growing into the opening by inserting an inflatable inner tube between readings (Harun and Roslan 2003).

F. Number of Replicate Tubes

To the best of our knowledge, there is to date no study that dealt sufficiently with the required number of MR-OT. There is no doubt that this number has to take into account several aspects besides resource availability. Taylor et al. (1990) suggested that eight tubes were required in order to estimate the RLD of a plot, a number similar to that recommended for soil coring approaches. Horizontally installed tubes were suggested to reduce the number of required MR-OT (Smucker 1993); however, this seems to be due to the effect of the higher tube length per soil depth rather than of the installation angle *per se*. According to our experience, the number of tubes should reflect the variability of the soil and the root systems. If rooting patterns are known, for example, in studies of agricultural fields or orchards, where most roots can be found close to an irrigation system (Rewald et al. 2011b), fewer tubes (e.g., five to six tubes) seem to be sufficient. In ecosystems where unknown or heterogeneous root distribution is studied or experimental manipulations are conducted (e.g., drought treatments or FACE experiments), a larger quantity of MR-OT should be installed per plot (e.g., ≥12 tubes; Smucker 1993; Vogt at al. 1998; Johnson et al. 2001).

III. Image Capturing by Minirhizotron Systems

A. Image Capturing Devices

In the early MR systems, the use of simple mirrors and a light source gave reasonable correlations between MR measurements and washed root lengths (Gregory 1979), but the low image quality and its limited size generally restricted the accuracy (Keng and Kusaka 1988). Since the early 1970s, several types of image capturing devices, that is, fiber optics, endoscopes, boroscopes, root periscopes, and telescopes, have been developed to increase the quality of images and to facilitate image capturing (e.g., Sanders and Brown 1978; Richards 1984; Rush et al. 1984; Itoh 1985; van Noordwijk et al. 1985; Poelman et al. 1996). Notably, the use of miniature (color) video cameras improved the operation of MRs by using the microphone to record the camera location and other information on the audio track (Upchurch and Ritchie 1983, 1984; Johnson et al. 2001).

In the last decade, the MR technology has advanced considerably; in particular, the development of digital image capturing technologies made it possible to conduct faster and more comprehensive measurements. Although boroscope-based MRs are still being used to study very small root systems, and video camera MRs are widely used because of their availability, the two commonly used MR systems that exist today are (1) digital (video) camera–based MR and (2) scanner-based MR systems. Both systems store the images on a (mobile) computer equipped with software to capture and label pictures. They also usually have an indexed handle (e.g., Ferguson and Smucker 1989) that enables the user to take repeated pictures at the same soil location (Figure 42.3).

1. *Digital* (*video*) *camera MR*: Different sizes of cameras exist, and the diameter of the MR-OTs has to be selected according to the diameter of the camera housing. The most commonly used MR systems are produced by Bartz Technology Corporation (subsequently named "Bartz"; Carpinteria, CA), but basic camera MR systems can be easily custom made using webcams and LED lighting (e.g., Faget et al. 2010). The imaging qualities of MR cameras differ, often restraining digital zooming; but, some systems allow for optical zooming up to 100× to study root details, soil fauna, or single hyphae (Allen et al. 2007). Most camera MR systems further allow manual setting of the focus either via software- or hardware-based lens focusing and the capture of video sequences; both features enable studies of root–soil fauna interactions in soil voids (Lussenhop and Fogel 1993).

   The image recorded by the camera covers a narrow section of the tube perimeter (<2 cm wide); cameras with a wider view cannot be used because the soil–tube boundary gets blurry at the edges because of the curving tube. In order to capture a wider soil profile (on several pictures), the camera has to be rotated in each measuring depth.

   All camera MRs employ a special light source; the lighting intensity often can be changed by a control box (Figure 42.3), a feature that is rarely needed because of the exposure correction capabilities of the image capturing software. However, it is important that the light intensity will be the same over the whole viewed area. In general, the external light source and the exposed lens of MR cameras make it easier to customize the emitted/recorded wavelength by filters, for example, to take pictures of white light or green fluorescence emitted by GFP (see Section VI.A).
2. *Scanner MR*: By now, the only commercial scanner-based MR system is produced by CID BioScience Inc. (subsequently named "CID"; Camas, WA). In this device,

a modified CCD (charge-coupled device) flatbed scanner is used to capture the images. CCD-type scanners are preferred due to their larger depth of field (Dannoura et al. 2008). The scanner can take a 360° picture of the soil–tube boundary, thus recording the whole soil profile (picture size approx. 20 cm wide × 22 cm high). These bigger pictures reduce the number of pictures to be taken and annul the need for measurements at different directions. They may facilitate correct data interpretation because larger parts of the branching root system can be seen. However, in densely rooted soils, the larger picture size increases the time for data analysis significantly, and the user might consider analyzing only narrow sections of the soil profile.

Before the scanner can be used, white balancing has to be done within a calibration tube. The maximum scanner resolution is 1200 dpi enabling moderate and post–image capturing digital zooming on picture details; however, in routine use, lower resolutions (approx. 300–600 dpi) will likely be chosen to reduce measuring time. Homogeneous lighting and automatic focusing are intrinsic features of scanner MR; the autofocus is convenient during standard use but limits observations in soil voids. The inner diameter of MR-OT for the currently available scanner MR must be 64 mm, thus "regular" tubes (as used by camera MR systems) cannot be used due to smaller diameters.

Each MR system has its own advantages and disadvantages; most pronounced differences exist in picture size and resolution. Future users should choose a device that suits their research needs, but the availability/costs of OTs should also be considered.

B. Temperature Increase by the Minirhizotron Lighting System

The lighting systems of MR image capturing devices can cause an increase in soil temperature up to 3.5°C (Van Rees 1998). It has been argued that short temperature increases from MR systems are insignificant when considering the surface soil temperature fluctuations in the field (McMichael and Burke 1996). However, diurnal fluctuations in soil temperatures at greater soil depth are much smaller; therefore, the effect of short-term temperature increases caused by stationary light systems on root growth and development may be more pronounced at depth than at the surface, especially in the winter when soil temperatures are low (Gaul et al. 2008). Because of the possible effects of increased soil temperatures from MR light on root growth and development as well as on soil fauna activity, lighting intensity and exposure time should be reduced as much as possible.

IV. Image Acquisition and Analysis

A. Frequency of Image Capturing

One of the main advantages of the MR technique is the possibility to conduct continuous measurements. Tingey et al. (2003) demonstrated that MR sampling frequencies had major effects on estimated fine-root production and mortality in both evergreen and deciduous tree species. Because fine roots are short-lived and particularly prone to herbivory, they may appear and disappear between image capturing events (Hendrick and Pregitzer 1996a,b; Eissenstat and Yanai 1997; Vogt et al. 1998), leading to underestimation of fine-root production and mortality (Johnson et al. 2001). Most MR studies choose capturing frequencies between 2 and 4 weeks with lower sampling rates during expected root dormancy (e.g., winter). However, the main factor that will determine the frequency of image collection in MR studies is the studied root trait (Taylor 1987). For example, in studies of root turnover rates, the image capturing rate will depend on an approximated mortality rate, while for documentation of standing stocks or rooting depth, lower sampling frequencies can be chosen (see Mainiero [2006] for temperature dependence).

B. Effect of Imaging Direction

Potential errors in MR studies might be caused by the spatial orientation of image capturing. A study by Dubach and Russelle (1995) found a large variation in root numbers on different sides of horizontally installed MR-OT. Furthermore, root distribution is highly influenced by the irrigation regime; for example, in agricultural systems, roots are especially concentrated close to water emitters (e.g., Shani et al. 1995; Rewald et al. 2011b). While methodological studies are virtually absent, the need for careful selection of the imaging direction is obvious, unless 360° are scanned.

C. Image Analysis

The first comprehensive analyses of MR pictures were based on manual tracing of roots on transparent sheets (Cheng et al. 1991). For more detailed analyses, special computer programs are used. Taking into account that the number of images taken on a single MR experiment will be in the magnitude of thousands, image file handling is of great importance. Most MR image analysis programs require files to be named in a way that allows the program to distinguish between different experiments, tubes, depths, and dates. The most commonly used naming convention is ICAP (Bartz, Carpinteria, CA); the names are either given automatically (e.g., BTC I-CAP Image Capturing System; Bartz, Carpinteria, CA) or manually.

Various commercial and freeware computer programs are used to analyze MR images, examples are Rootfly (Birchfield and Wells 2006), RooTracker (Duke University, Durham, NC), Root Measurement System (Ingram and Leers 2001), and WinRHIZO Tron (Régent Instruments, Quebec, Canada). In all programs, the user must trace the roots manually; the process involves marking roots on a computer screen by moving the mouse along the roots and setting nodes and diameters. The overlaid marks can be copied to the consecutive MR picture taken later at the same position so differences between the two pictures can be determined, for example, increases in length, in diameter, or the death of root segments. Commonly, a function that allows recording of root segment–specific information like color and mycorrhizal status is also available. A common shortcoming of many analysis software types is that each root segment in the branching root system is labeled as single roots; a feature that allows grouping of roots according to orders is rare (WinRhizo Tron 2011a; Regent, Canada). Only recently, CID Bio-Science Inc., Camas, WA, developed a program (CI-690) that allows the user to trace roots using a touch screen input; although this is an interesting approach believed to make root tracing easier, no published report of end-user experience is available at the time of publication.

As mentioned previously, a major difference between the camera MR and the scanner MR system is the image size. Larger images (i.e., especially higher images, paralleling several images taken with camera MR systems) might allow for more accurate measurements since certain errors that result from difficulties in tracing roots in small images, for example, measuring the same root twice in different frames when analyzing overlapping roots in multiframe pictures, can be avoided.

D. Automated Image Analysis

Since manual analysis of MR images is very time consuming, its automation is sought for a long time (e.g., Richner et al. 2000). Although still in early stages, some computer programs allow for automatic detection of roots in MR images. The methods are generally based on image thresholding and region- or contour-based techniques to distinguish roots from the soil background, which often includes extraneous objects (Erz et al. 2005; Zeng et al. 2010). However, until now automatic root detection is limited since a low contrast between roots and background often results in systematically lower RLD compared to manual analyses (Vamerali et al. 1999). For root–soil systems with a high contrast, Zeng et al. developed a system that can detect, label, and measure individual roots, thereby setting the stage for automated tracking of roots through time (Zeng et al. 2008). Future approaches will likely involve the use of advanced imaging techniques like combinations of visual light and near-infrared reflectance to distinguish automatically between living and dead roots, organic matter, and mineral soil (Nakaji et al. 2008; Lei and Bauhus 2010 and references within).

V. Applications of the Minirhizotron Technique

If installed and analyzed carefully, the most serious limitations to the MR technique seem to be the initial costs of hard- and software and the time lag until soil and root dynamics come back to steady-state conditions after tube installation. Furthermore, while labor costs for tube installation and picture capturing are moderate, image analysis can become very time consuming and sufficient resources have to be allocated for this purpose (e.g., Coupe et al. 2009).

A. Minirhizotrons and Measurements of Standing Stock and Root Depth Distribution

MRs have been used extensively in assessing RLD (Bland and Dugas 1988; Franco and Abrisqueta 1997) and rooting depth (Hendrick and Pregitzer 1992; Majdi et al. 1992; Baumann et al. 2005). Fewer studies have related the measured RLD to root biomass (Johnson et al. 2001; Brown et al. 2009). The most commonly used method in root research is destructive soil coring (e.g., Rose et al. 2011); unlike MRs, soil cores reveal root masses directly without the need for calibration (Ephrath et al. 1999). Furthermore, roots from soil cores can often be reliably sorted into live and dead masses according to visual/mechanical or chemical criteria (Rewald et al. 2011c). Many studies found correlations between MR data and root biomass determined by soil coring, although the level of correlation varied between studies and species (e.g., Box and Ramseur 1993; Murphy and Smucker 1995; Jose et al. 2001; Gaul et al. 2009). At high soil bulk density, for example, rigid tube measurements consistently overestimated actual rooting density (as determined by soil coring) of both wheat and beans, while in the case of flexible MR-OT, the two measurements did not differ significantly (De Ruijter et al. 1996). Thus, soil coring might be preferred to study effects of different bulk densities instead of rigid MR-OT.

Contradictory reports were published concerning the underestimation of rooting frequency in the surface soil layers. While many studies on crops concluded that MRs underestimate RLD especially in the surface soil layer (Gregory 1979; Bragg et al. 1983; Upchurch and Ritchie 1983; Parker et al. 1991; Samson and Sinclair 1994), others did not. For example, Jose et al. (2001) found that the root biomass of *Zea mays* was slightly underestimated by the MR technique in the top 30 cm of the soil but for two tree species no significant difference occurred in surface or deeper soil layers. In general, there are more studies reporting underestimation of crop rooting density than that of trees by MRs (e.g., Rytter and Hanson 1996; Franco and Abrisqueta 1997; Ephrath et al. 1999; Jose et al. 2001; Gaul et al. 2009). These conflicting reports may be the result of a number of factors that can influence MR data such as species, soil type and density, tube installation technique, replicate numbers, and sampling errors. While in short-term experiments with slow-growing species or in dense soils, the soil cores could be more suitable to quantify fine-root abundance and distribution, the MR technique is a reliable method with a minimal site disturbance.

Two methods are used to relate the MR RLD data to the soil volume and to convert the RLD to biomass (see Johnson et al. [2001] for details). Merrill and Upchurch (1994) and Merrill et al. (1994) used the number of roots that intersect the MR picture to calculate the expected root length within the soil volume taken by the MR-OT. In the other approach, assumptions regarding depth of view must be made when root length or root surface area is converted to biomass per unit of soil volume. Typically, the values used for depth of field range from 2 to 3 mm (Sanders and Brown 1978; Itoh 1985; Steele et al. 1997; Brown et al. 2009). Specific root length (SRL) data are used in order to convert RLD to biomass values. Since SRL varies with diameter or root branching order, it should be determined for the different root classes by destructive sampling (Rewald et al. 2011a). These volumetric data sets can also be expressed on a ground surface area basis by relating the volume to the length of the MR-OT. In homogeneous rooting systems, a direct "biomass calibration" of MR data may be done with root biomass density data obtained from nearby soil cores (Ephrath et al. 1999; Johnson et al. 2001).

B. Minirhizotrons for Estimation of Root Production and Demography

A number of independent methods are available for estimating fine-root dynamics and turnover (Tierney and Fahey 2001; Majdi et al. 2005). Besides MRs, methods to determine fine-root production and turnover include (1) indirect mass-related techniques (by coring), (2) experimental setups including ingrowth cores and miniature root-growth chambers, and (3) changes in carbon isotopic ratios (Powell and Day 1991; Hendrick and Pregitzer 1992, 1993; Majdi 1996; Gaudinski et al. 2000; Rewald and Leuschner 2009). However, sequential coring will only reflect root growth and death during the period prior to sampling, while MRs, provided that root system was allowed to return to equilibrium growth after soil disturbance (see, e.g., Gaul et al. 2009), can determine short-term changes in root dynamics. Thus, the MR technique is suggested to provide more realistic results of fine-root dynamics than sequential coring (Publicover and Vogt 1993; Hendricks et al. 2006; Majdi et al. 2007). Two major limitations of the ingrowth core method compared to MR are (1) that no information on time of root ingrowth or mortality is obtained when the amount of living and dead roots is measured and (2) that the homogenized/replaced soil of the reconstructed ingrowth core will present a physical and chemical artificial and less competitive soil environment, which will be colonized at different rates than other parts of the rooting volume (Majdi 1996). Furthermore, destructive methods cause repeated and prolonged soil disturbance, so they are not suitable for long-term research on plots of limited size.

The reliability of the MR method depends inter alia on the accuracy of assessing the physiological status of roots, that is, whether roots are dead or alive. For using Kaplan–Meier statistics for root longevity calculations (Majdi et al. 2001; Tierney and Fahey 2001; Pritchard et al. 2008a; Strand et al. 2008), the observed roots have to be pooled into two groups: roots that are alive and roots that died during the observation period. However, to date three different criteria have been used for determining the death of roots in MR studies: color changes (Cheng et al. 1991; Hendrick and Pregitzer 1992), signs of disintegration (Repo et al. 2008), and disappearance (Comas et al. 2000).

When roots become dark brown or black, they are considered dead. However, root color may darken as a result of secondary growth or suberization, and this can complicate visual estimates of the physiological status (Figure 42.6). Furthermore, under visible light, it is sometimes difficult to distinguish between roots and organic debris, which might influence the determination of root disintegration. To aid in separation of live and dead roots, some MR systems included both visible and ultraviolet (UV) lights (Bartz, Carpinteria, CA). Under UV light, live roots are supposed to fluoresce more strongly than dead roots (Dyer and Brown 1983). However, Wang et al. (1995) found that some dead root still fluoresced under UV light; consequently, no significant differences between estimates of live root proportions by visible or UV light were found.

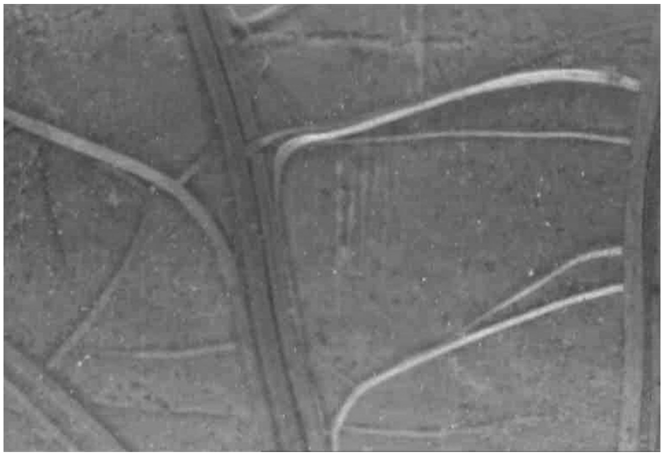

FIGURE 42.6 Root branches of *Tamarix aphylla*. Older roots (dark) can be clearly distinguished from newer (white) roots. The picture was taken with a camera MR, Bartz, Carpinteria, CA.

Moreover, the disappearance of roots from the MT picture might not always be a result of root death and decay, but disappeared roots may remain alive and only vanished from the field of view because of a change in growth angle or superposition by other roots, biofilms, hyphae, or moving soil particles. The criteria used for classifying roots to "dead" or "alive" fractions as well as the methods used for calculations may lead to significant differences in estimated root turnover rates (see Satomura et al. 2007 and references therein).

Furthermore, the discrepancies in estimated fine-root turnover rates between different approaches are illustrated by several studies based on changes in carbon isotopic ratios, which have reported higher turnover rates than usually measured by MRs (Gaudinski et al. 2000; Trumbore et al. 2006). The model of Guo et al. (2008) indicated that median-based longevity estimates made by MR studies underestimated actual longevity, whereas simulated mean residence time of carbon from isotopic studies overestimated longevity. Longevity distributions of fine roots are often positively skewed, indicating different fine-root longevities within one root system (Tierney and Fahey 2002; Joslin et al. 2006; Trumbore et al. 2006). The model of Guo et al. (2008) considers the heterogeneity of root systems by assuming that the most dynamic pool is dominated by first-order (i.e., the root tip) and second-order roots, while longer-lived roots are mostly roots of higher orders (Eissenstat et al. 2000; Wells et al. 2002; Guo et al. 2008). Because isotopic studies are based on residence time of root mass, they are biased by larger and older roots that are less numerous but contain more carbon and live longer. On the other hand, since MR estimates are rather number based, they are biased by the frequent first- and second-order roots, which have the fastest growth and turnover but contain less carbon. Pritchard and Strand (2008) suggested conducting root survival analyses based on volume, instead of the individual roots themselves, to improve the quantification of turnover of fine-root mass in MR studies. Furthermore, a longer duration of MR studies and the classification of root orders during picture analysis might increase data accuracy. In the absence of one standard method to determine root longevity, combined approaches including MRs and coring are widely recommended (Nadelhoffer 2000; Hertel and Leuschner 2002; Hendricks et al. 2006).

C. Minirhizotrons for Studying Root Morphology

MRs have been used for assessing root morphology (Upchurch 1985; Withington et al. 2003; Basile et al. 2007; Figure 42.7). The most common morphological parameter assessed is root diameter; fewer studies have addressed root pigmentation and branching. However, care has to be taken when choosing the material of MR-OT; Withington et al. (2003) showed that the root morphology of different tree species was significantly different between plastic and glass MR-OT. Compared to glass tubes, the mean root diameter of roots observed through plastic tubes was higher in two of six species, and the time from birth to pigmentation was significantly decreased against CAB tubes in four out of six tree species.

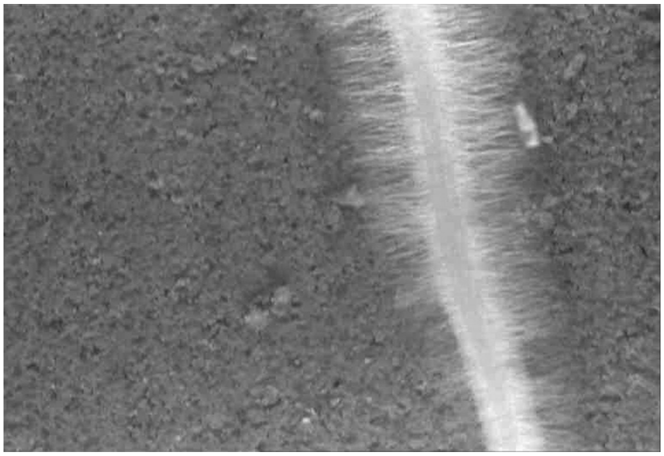

FIGURE 42.7 Root of *Cakile maritima* featuring root hairs. Although no soil voids can be detected, root hairs seem to concentrate at the tube–soil interface. The picture was taken with a camera MR, Bartz, Carpinteria, CA.

D. Minirhizotrons for Studying Belowground Interactions

MRs have so far been used for studying belowground interactions between roots and their mycorrhizal partners, roots and soil fauna/plant parasites, and competitive interactions.

Until now, most of the studies aimed at understanding the dynamics of mycorrhizal colonization have used soil cores (Mukerji et al. 2006). Fungal structures down to single hyphae can be studied with magnifying MR image capturing devices (Figure 42.8), allowing density estimates of ectomycorrhizae, rhizomorphs, and colonies of saprophytic fungi (Treseder et al. 2005; Pritchard et al. 2008b; Hasselquist et al. 2010). Direct observation of invertebrates in MRs is appealing because the methods used to extract invertebrates from soil select mobile, desiccation-resistant species. However, unresolved issues are high light or UV intensities, which might drive away invertebrates from the viewing area, and increased soil temperatures, which could affect soil fauna activity (Snider et al. 1990; Lussenhop and Fogel 1993). Observations of mycorrhizae or soil fauna by means of MRs are still rare, and future studies should seek to take full advantage of this direct, in situ observation technique.

Belowground resource competition, mediated through root–root interaction, is of wide importance in plant ecosystems (Rewald and Leuschner 2009). Since MRs can estimate root biomass and distribution, they were suggested to be able to assess the degree of competition (Jose et al. 2001; Båth et al. 2008). However, until now studies are restricted to tree–crop interactions (e.g., Campbell et al. 1994; Gillespie et al. 2000) due to difficulties in distinguishing roots of different species in situ (but see Section VI.A). In addition to root

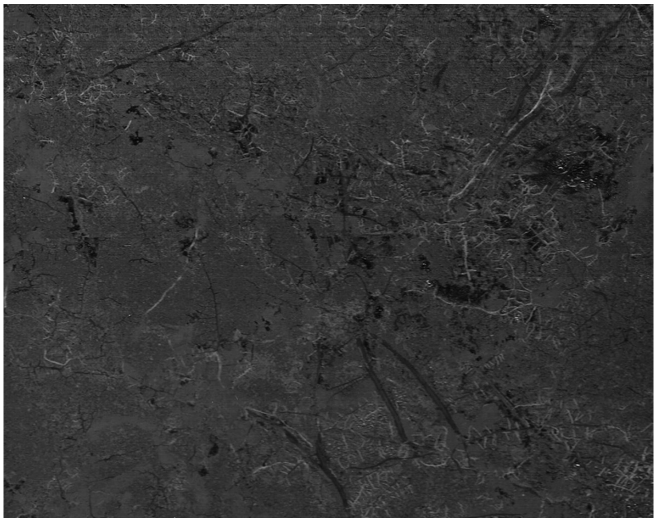

FIGURE 42.8 Roots of *Fagus sylvatica* and hyphae on a sufficiently installed MR-OT surface (i.e., only marginal soil voids); three root orders can be distinguished. The image was captured with a scanner MR system, CID, Cedar Rapids, IA. (Image courtesy of M. Lukac and D.L. Godbold, Bangor FACE, Bangor, U.K.)

FIGURE 42.9 **(See color insert.)** *Solanum lycopersicum* roots infested with *Orobanche aegyptiaca* tubercles. The picture was taken with a magnifying camera MR, Bartz, Carpinteria, CA.

competition, belowground parasitic interactions can also be studies by means of MRs. Eizenberg et al. (2005) successfully used MRs for in situ monitoring of the early stages of the root parasite *Orobanche* development (Figure 42.9) and for detecting herbicide effect on the underground stages of the parasitic interaction.

VI. Recent and Future Developments in Minirhizotron Systems

A. Distinguishing between Species

Understanding of plant interactions is greatly limited by the inability to identify and quantify roots associated with different species; thus, developing such capabilities would greatly improve the MR technique. Discrimination of root fragments by visual morphology inspection is difficult and time consuming even for experienced taxonomists (Hertel and Leuschner 2006; Li et al. 2006), especially if picture resolution and visual view are limited. To overcome this limitation, Faget et al. recently used transgenic plants, expressing green fluorescent protein (GFP), combined with a modified MR imaging system (Faget et al. 2009, 2010). By illuminating the roots with white light, they could see all roots, but when switching to the appropriate UV excitation wavelength and detecting the appropriate fluorescence, only the roots that contain the GFP showed in the picture. This allowed the users to distinguish between transgenic and nontransgenic roots (Figure 42.10). Other promising technologies, aiming to distinguish between root species identities, are the near-infrared reflectance spectroscopy (NIRS) and the Fourier transform infrared spectroscopy (FTIR), which provide information about the presence, character, and number of functional chemical groups (Nakaji et al. 2008; Naumann et al. 2010); similar technologies are envisaged to be operative in MR-OTs.

FIGURE 42.10 **(See color insert.)** The fluorescent green roots belong to a genetically transformed *Zea mays* genotype expressing the GFP. The dark nonfluorescent roots belong to a non-GFP maize variety and can be clearly distinguished from the GFP roots. The picture was taken with a custom-made UV-MR camera system. (see Faget et al. [2009] for details; Image courtesy of M. Faget, M. Liedgens, P. Stamp, P. Flutsch and J.M. Herrera, Zurich, Switzerland.)

B. Automatic Imaging Systems

Automatic image capturing systems would allow increased sampling frequency. Smucker et al. (1987) have designed an automatic device (to move a camera along the MR tube one screen distance at a time) many years ago, but it was not widely used. Recently, Allen et al. (2007) equipped MR tubes with a custom-made robotic automated MR system; however, only few automatic scanning systems are commercially available to date (e.g., CID, Cedar Rapids, IA and RhizoSystems, Idlyllwild, CA), allowing for continuous observation of root growth in surface soil layers. Having multiple automatic MR that respond to remote commands or environmental triggers (e.g., rainfall) will allow simultaneous data collection at multiple points in space and time and reduce the risk of missing short-lived roots.

C. Measurement of Environmental Parameters

The combination of the current MR techniques with other optical measurements could allow for measurements of soil traits (e.g., soil water content and temperature, pH values, and nutrients) that influence root growth. Standardization in MR-OT diameter and a careful selection of tube materials (e.g., low ion content) could allow for their multiple use for other techniques such as frequency domain reflectometry (FDR) and capacitance or neutron probes for determining soil water contents (e.g., Andrén et al. 1991; Kirkham et al. 1998). While a dual use of tubes would directly allow determining the influence of soil moisture on the observed rooting pattern, probe diameters and tube requirements are often not matched with MR imaging devices, preventing wide use.

Another promising technology that could be combined with MR systems are optical sensors ("optodes"). Optodes are growing in popularity due to the low-cost and long-term stability but are currently used in "rhizoboxes" only (Gansert and Blossfeld 2008). The fundamental principle is based on the ability of selected substances (embedded in foils) to act as dynamic luminescence quenchers. In the case of oxygen, if a ruthenium complex is illuminated with blue light, it will be excited and emit a red luminescent light with an intensity, or lifetime, that depends on the ambient oxygen concentration. The emitted light can be recorded with a photo sensor. Currently, optodes exist for O_2, CO_2, pH, Ca, P, and N determinations (e.g., Grunth et al. 2008; Strömberg 2008). Transparent, planar optode foil on the surface of MR-OT would provide valuable information about root/soil interactions including root exudation and presents an alternative to electrode-based sensors or other more soil disturbing analytical instrumentation.

VII. Concluding Remarks

MR systems have proven to be very useful for studying rhizosphere processes like fine-root growth and turnover, root morphology, and belowground interactions. MRs are composed of two constituents: clear OTs, which are installed in matching soil cores, and an image capturing device. Installed properly and after an equilibration period, they allow for studying the rhizosphere in a continuous, nondestructive manner. Although MR systems have provided many insights into rhizosphere processes, our review outlined wide variations in tube installation procedures, image acquisition, and data processing techniques. Since the MR image capturing devices improved constantly during the last decades, the main issues that have to be taken under consideration by future methodological studies and when using the MR system are the MR-OT installation (e.g., installation procedure *per se*, angle, tube material), the time lag between installation and the start of the study, the frequency of measurements, and the image analysis. To facilitate the utilization of the MR technique, extensive future research is needed on automatic image analysis and on imaging techniques beyond visible light.

We have no doubt that as root research continues to gain importance as a research field, this will result in future large-scale development of the MR technique.

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