Light Sheet Tomography (LST) for *in situ* imaging of plant roots

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Abstract: The production of crops capable of efficient nutrient use is essential for addressing the problem of global food security. The ability of a plant's root system to interact with the soil micro-environment determines how effectively it can extract water and nutrients. In order to assess this ability and develop the fast and cost effective phenotyping techniques which are needed to establish efficient root systems, *in situ* imaging in soil is required. To date this has not been possible due to the high density of scatterers and absorbers in soil or because other growth substrates do not sufficiently model the heterogeneity of a soil's microenvironment. We present here a new form of light sheet imaging with novel transparent soil containing refractive index matched particles. This imaging method does not rely on fluorescence, but relies solely on scattering from root material. We term this form of imaging Light Sheet Tomography (LST). We have tested LST on a range of materials and plant roots in transparent soil and gel. Due to the low density of root structures, i.e. relatively large spaces between adjacent roots, long-term monitoring of lettuce root development *in situ* with subsequent quantitative analysis was achieved.

OCIS codes: (000.1430) Biology and medicine; (110.0180) Microscopy; (170.6900) Three-dimensional microscopy; (180.0180) Microscopy; (180.6900) Three-dimensional microscopy.

References and links

1. J. Lynch, "Root Architecture and Plant Productivity," Plant Physiol. **109**(1), 7–13 (1995).
2. I. De Smet, P. J. White, A. G. Bengough, L. Dupuy, B. Parizot, I. Casimiro, R. Heidstra, M. Laskowski, M. Lepetit, F. Hochholdinger, X. Draye, H. Zhang, M. R. Broadley, B. Péret, J. P. Hammond, H. Fukaki, S. Mooney, J. P. Lynch, P. Nacry, U. Schurr, L. Laplaze, P. Benfey, T. Beeckman, and M. Bennett, "Analyzing lateral root development: how to move forward," Plant Cell **24**(1), 15–20 (2012).
3. R. Tomer, K. Khairy, and P. J. Keller, "Light sheet microscopy in cell biology," in *Cell Imaging Techniques* (Humana Press 2013) ,123–137.
4. H. Downie, N. Holden, W. Otten, A. J. Spiers, T. A. Valentine, and L. X. Dupuy, "Transparent soil for imaging the rhizosphere," PLoS ONE **7**(9), e44276 (2012).
5. G. Häusler and W. Heckel, "Light sectioning with large depth and high resolution," Appl. Opt. **27**(24), 5165–5169 (1988).
6. J. Huisken, J. Swoger, F. Del Bene, J. Wittbrodt, and E. H. K. Stelzer, "Optical sectioning deep inside live embryos by selective plane illumination microscopy," Science **305**(5686), 1007–1009 (2004).
7. E. G. Reynaud, U. Krzic, K. Greger, and E. H. K. Stelzer, "Light sheet-based fluorescence microscopy: more dimensions, more photons, and less photodamage," HFSP J **2**(5), 266–275 (2008).
8. M. Minsky, "Microscopy Apparatus," U.S. patent 3013467 (1961).
9. D. M. Shotton, "Confocal scanning optical microscopy and its applications for biological specimens," J. Cell Sci. **94**, 175–206 (1989).
10. W. J. Alford, R. D. VanderNeut, and V. J. Zaleckas, "Laser scanning microscopy," Proc. IEEE **70**(6), 641–651 (1982).
11. G. Gbur and E. Wolf, "The Rayleigh range of Gaussian Schell-model beams," J. Mod. Opt. **48**, 1735–1741 (2001).

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12. S. Declerck, D. G. Strullu, and C. Plenchette, "Monoxenic culture of the intraradical forms of SP isolated from a tropical ecosystem: A proposed methodology for germplasm collection," Mycologia **90**(4), 579–585 (1998).
13. L. Dolan, K. Janmaat, V. Willemsen, P. Linstead, S. Poethig, K. Roberts, and B. Scheres, "Cellular organisation of the *Arabidopsis thaliana* root," Development **119**(1), 71–84 (1993).
14. D. B. Dresbøll, K. Thorup-Kristensen, B. M. McKenzie, L. X. Dupuy, and A. G. Bengough, "Timelapse scanning reveals spatial variation in tomato (Solanum lycopersicum L.) root elongation rates during partial waterlogging," Plant Soil, 1–11 (2013).
15. F. Fiorani, U. Rascher, S. Jahnke, and U. Schurr, "Imaging plants dynamics in heterogenic environments," Curr. Opin. Biotechnol. **23**(2), 227–235 (2012).

1. Introduction

Climate change, together with the declining fertility of soil, the increased cost of fertilizers and a growing world population will have a large impact on agro industries. One key challenge is the improvement of crop production with reduced dependency upon agrochemicals, and engineering more efficient root systems is essential to achieve this goal. Whether plants thrive or not when cultivated in limiting soil conditions depends largely on a number of traits that plants have evolved to optimize access to resources distributed heterogeneously in the soil. For example, root hair length and density was shown to provide significant advantages in P-depleted soils, whereas rooting depth of root branching is important to enhance water uptake efficiency [1]. To date, breeding for root traits has been problematic because root systems are difficult structures to observe. In order to be able to introduce new and more suitably adapted plant varieties, simple techniques are required to measure root traits *in situ*, in 3D, and with resolutions sufficient to observe microscopic structures such as root hairs. Unfortunately, existing methods suffer from limitations [2]. Manual sampling of roots is slow, destructive and inaccurate. X-ray micro-tomography is becoming increasingly popular in root research, but is expensive and lacking in throughput.

To address this existing need for a suitable imaging modality for *in situ* plant root phenotyping we have developed a new form of non-fluorescent light sheet imaging method. This method uses the elastic scattering of light from a sample when illuminated by a light sheet. The scattering is imaged orthogonally to the light sheet such that only scattered light arrives at the camera. The use of scattering rather than fluorescence as a contrast mechanism, as with Light Sheet Fluorescence Microscopy (LSFM) [3], makes this imaging technique similar to diffuse optical tomography but, as we use it here exclusively with samples containing a low volume density of scatterers, 3D images can be built up directly from the optical sections taken by scanning the sample through the light sheet.

We term this technique Light Sheet Tomography (LST). As an example of the utility of LST we show imaging of various fibrous structures such as paper and high density foam, both of which contain complex 3D surfaces. The use of scattering as a contrast mechanism makes LST complementary to conventional Light Sheet Fluorescence Microscopy (LSFM). LST is particularly well suited to imaging surfaces with a highly complex three dimensional topography and fibrous structures such as plant roots: taking advantage of the low scattering of recently developed transparent soil [4] and the low density of root structures. We demonstrate the application of LST to imaging roots developing *in situ* and show that the resolution obtained is sufficient to observe microscopic root traits, such as root hairs, and enough for accurate quantitative analysis. Avoiding the need for fluorescence also means plants can be rapidly phenotyped without the need for further genetic manipulation to introduce fluorescent proteins or the need for staining.

The application of light sheet imaging to root systems has many advantages over other conventional techniques. Since the axial resolution is associated with the thickness of the light sheet rather than with the numerical aperture of the imaging objective, good axial resolution can be obtained with low cost and low magnification objectives [5–7]. LST only illuminates the volume of the sample which is being imaged, with out-of-focus structures left "in the dark". As a result the light dose experienced by the root, both in terms of the duration of the illumination and the background signal is kept to a minimum. This low light dose is vital as too much light could potentially affect the growth of the roots, changing their

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behaviour and reducing the validity of any measurements. This gives LST a strong advantage over other imaging techniques such as confocal microscopy where the whole sample is illuminated regardless of how much of the sample is in focus [8,9]. Hence, long time monitoring of plant root growth can be obtained using LST. In addition, since LST can obtain a B-scan in a single exposure, the imaging speed is improved when compared with confocal microscopy, laser scanning microscopies or computed tomography approaches which require 3D reconstruction [10]. These advantages make LST an ideal tool for taking images of living plants in a low-cost high-throughput setup.

2. Materials and methods

A low-cost light-sheet tomography system was built, as shown in Fig. 1. Standard optical elements were used and the basic total cost of the setup can be less than £1500 excluding the PC, software and laser.

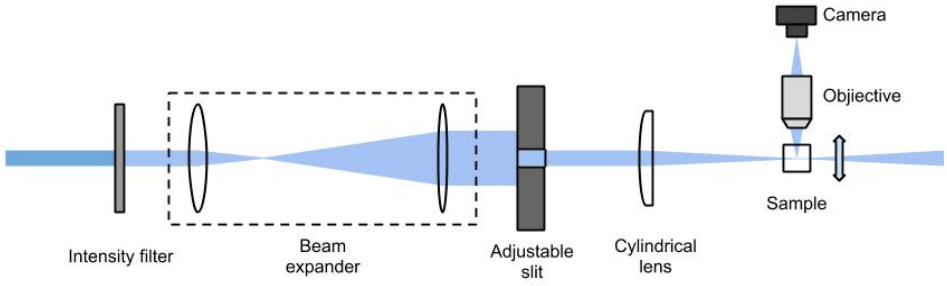

Fig. 1. Setup of the LST (top view).

A frequency-doubled solid-state laser (FCD488-020, JDSU, USA) is used as the illumination source with a wavelength of 488 nm allowing future co-registration of LST and LSFM images in a single device. The output power of the laser is 20 mW which is controlled by introducing a set of neutral density filters (NEK01, Absorptive ND Filter Kit, Thorlabs). The diameter of the laser output is 0.7 mm and a telescope consisting of two plano-convex lenses [focal length (FL) = 50 mm & FL = 500 mm, Thorlabs, UK] is used to form a beam expander to increase the diameter of the beam to 7 mm. The central element of this setup is the cylindrical lens (LJ1567L1-A, FL = 100 mm, AR-coated: 350-700 nm, Thorlabs). An adjustable mechanical slit (VA100, 0~6 mm, Thorlabs) is used before the cylindrical lens to change the diameter of the incoming beam, hence adjusting the effective numerical aperture of the illumination and adjusting the thickness of the light sheet itself. The imaging path consists of an objective (4X /0.1 or 10X /0.25, COMAR, UK) and a camera [EC1280, (1280 × 1024) pixel resolution, 6.7 µm per pixel, PROSILICA, Canada; or Neo, (2560 × 2160) pixel resolution, 6.5 µm per pixel, Andor, UK].

Recording the data of each sample was performed while moving the sample along the observation axis. For each sample, more than 100 frames were taken to build a 3D image, which gave an increment between stacks of approximately 8 µm.

3. Results and discussion

3.1 Calibration

The thickness of the light sheet depends on the numerical aperture of the illumination objective, and is additionally proportional to the width of the laser beam [11]. In this setup, the width of the laser beam was set by the adjustable slit to be 4 mm. This sets the theoretical thickness at 15.5 µm in the ideal case (with the focal length of the cylindrical lens 100 mm). The thickness can be decreased by simply changing to a short-focal-length cylindrical lens or increasing the width of the incoming beam, though this will lead to a shorter Rayleigh range and a spreading of the light sheet across the sample.

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To obtain a measure of the resolving power of LST under ideal conditions we constructed a target consisting of micro particles set in a polyacrylamide (PAA) gel. This arrangement also allowed the thickness of the light sheet to be determined experimentally. The gel was set in a small cuvette containing 2 µm polystyrene microspheres [(1.998 ± 0.022) µm, NIST certified, Thermo, USA]. The thickness of the light sheet was measured using microsphere targets. The sample was translated along the axis of observation, and images of the microspheres were captured at intervals of 0.5 µm. The size of the sheet was then estimated as the length interval for which the chosen microsphere could be detected in the image. 10 targets were used and the width of the light sheet determined to be (19.2 ± 2.0) µm. This value is larger than predicted above, most likely due to spherical aberration introduced by the cylindrical lens.

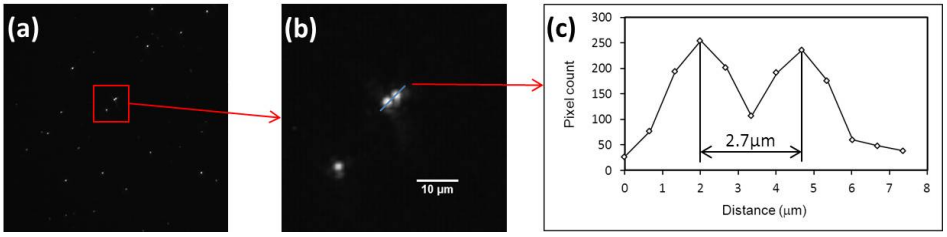

Fig. 2. (a) Image of 2 µm calibrated colloids in PAA under 10X objective. (b) Amplification of the image (a). (c) Profile of two spheres on the image.

A measure of the resolution in the x-y plane was obtained by analyzing the local maxima of pixel intensity generated by the microspheres [Fig. 2(a)]. An estimate of the resolution of the system was determined from the smallest distance between microspheres for which distinct peaks are resolvable [Fig. 2(b)]. The distance was assessed using a line scan bisecting the center of a pair of apparently adjacent microspheres [Fig. 2(c)]. The result indicates that our LST set-up can achieve a transverse resolution better than 3µm, significantly smaller than the smallest feature that needs to be resolved for basic plant root phenotyping.

To address the imaging depth of the system, a target consisting of a suture (0.10 ~0.149 mm diameter, W8830, blue, Johnson & Johnson) in transparent soil was examined. The space between grains in the transparent soil was saturated with 20% (w/v) sorbitol to provide refractive index (RI) matching. Images of the suture at different depths (2 mm ~5 mm, every 0.5 mm) were taken and the diameter of the suture on the images was calculated. The result showed that at depth from 2 mm to 4 mm, the apparent diameter of the images raised slightly from approximately 160 µm to 180 µm. However, image quality reduced more significantly when imaging deeper than 4 mm. In this case the transparency of the medium directly affects output image quality by degrading both the lateral resolution and the quality of light sheet, leading to a poorer axial resolution. In fact it is imperfections in the refractive index matching in the soil which set the limit on the resolution of the system. Hence sample preparation is critical both in the initial preparation of the sample and in maintaining the index matching as the plant grows.

3.2 Test samples

The performance of the LST system was assessed using two test materials with complex structures: fibrous structures and structures with complex surface topography. To obtain the images, we set the (flat) samples at 45° to the illumination plane and the observing axis hence ensuring optical sectioning of the sample with an illumination depth of $\sqrt{2}$ times the thickness of the light sheet as observed by the camera. All the images were taken with a 10X objective. 3D data sets were constructed by recording a video while moving the sample towards the objective at 10 μms^{-1}. A 3D visualization of the sample was created with the ImageJ plugin "3D viewer".

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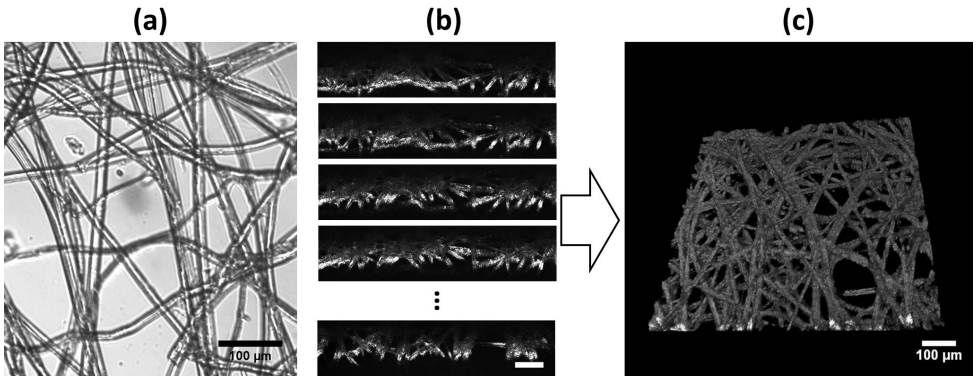

Fig. 3. Images of lens tissue. (a) Bright-field image of the paper under 10X magnification showing 2D structure of lens tissue. (b) Individual sections of the sample illuminated by the light sheet. The laser was incident from the "bottom" of the images. Images were cropped to 1280 × 300 pixels to highlight the details. Scale bars are 100 µm. (c) 3D image of the paper. (Media 1)

The first test material consisted of samples of lens cleaning tissue. The tissue paper is typical of the kind low density material that LST is particularly suited to imaging, and has fibers with diameter of approximately 20µm [measured using bright-field imaging, as shown in Fig. 3(a)], which is about twice the size of the average cell in a root hair. The results show that imaging using LST produced good contrast throughout the whole thickness of the sample (~120µm) [Fig. 3(b)]. We have also used dense foam to illustrate imaging of a highly absorbing sample with complex surface topography. Since light absorption of these foams was much higher than for the lens tissue, the power of the laser was increased to 1.40mW. On these samples, LST produced good contrast up to a depth of 200µm in dense foam [Fig. 4(b)], and 3D reconstruction of the data showed the system is suitable for imaging the topography of highly uneven surfaces.

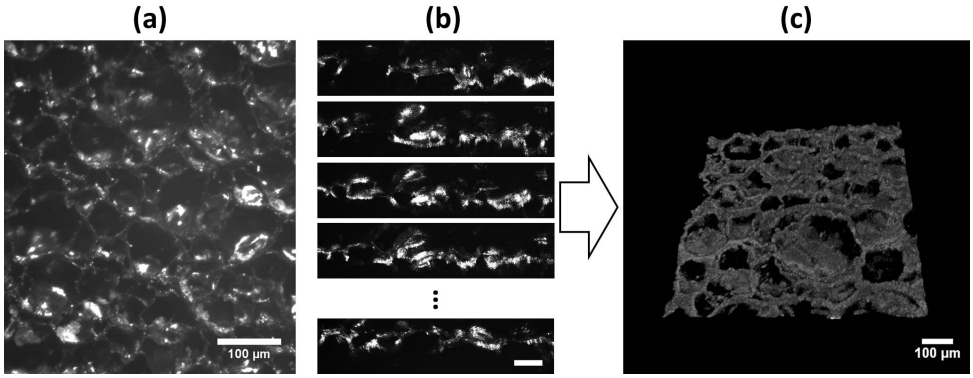

Fig. 4. Images of dense foam. (a) Bright-field image of the dense foam under 10X magnifications. (b) Different stacks of the sample by laser-sheet section. Images were cropped to 1280×300 pixels. Scale bar 100 µm. (c) 3D image of the sample. (Media 2)

3.3 Analysis of root structures in transparent soils

Wild type *Arabidopsis thaliana*, ecotype C24, was used for root imaging *in situ*. The seeds were sterilized on filter paper by ethanol washes. Seedlings were germinated in Petri dishes with MSR nutrient media [12] solidified with 6 gL^{-1} phytagel (Sigma P8169) before being transferred to transparent soil. Plants were incubated and grown at 20°C with 16 hours light: 8 hours darkness. Growth period was 1 ~ 2 weeks post-germination. Transparent soil [4] was

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used as the particulate growth substrate in glass cuvettes (internal dimension 10 mm, volume 3.5 ml). Immediately before imaging, MSR nutrient media with 20% (w/v) sorbitol was added to saturate the substrate to allow transparency, as shown in Fig. 5(b). Thus images can be taken directly without the need to remove the sample from the soil.

Images of *A. thaliana* roots were obtained approximately 10 days after germination. Image sequences were taken perpendicular to the face of the glass cuvettes. Images could be obtained throughout the depth of the roots [Fig. 5(c)-5(f)] and had resolution sufficient to resolve anatomic features such as root hairs [Fig. 5(e)] or epidermis and cortex cells [Fig. 5(d), 5(e)]. Better sharpness was obtained when the light sheet was closer to the camera [Fig. 5(d)]. 3D images of *A. thaliana* roots [Fig. 5(g)], revealed the structure of epidermal tissue and its spiral arrangement of cells. These results demonstrate the ability of LST to quantify microscopic root traits *in situ* and in a substrate that mimics soil physical structure. The root morphologies observed with LST in transparent soil [Fig. 5] are atypical of those observed when plants are grown in gels where roots grow longer and straighter [4] but match those seen for *ex vivo* root samples from real soil.

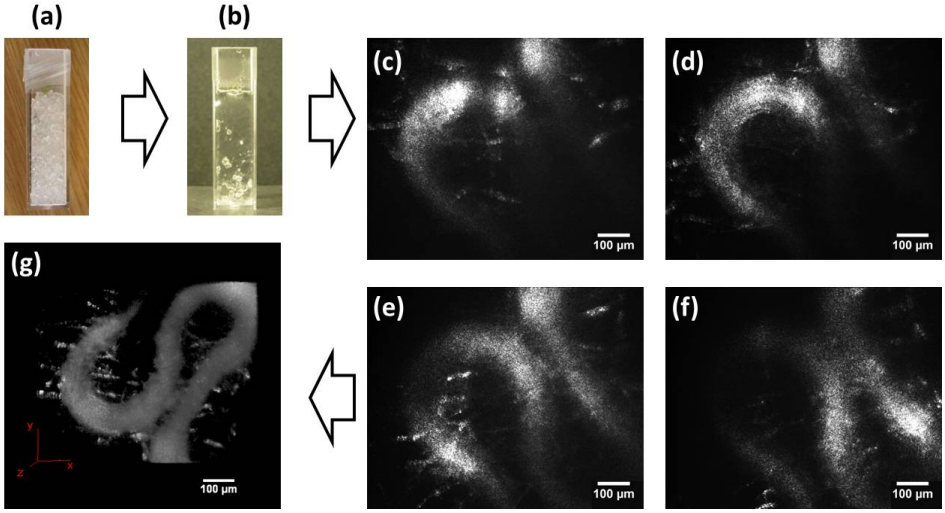

Fig. 5. Root of a live *A. thaliana*. (a) Plant growing in transparent soil. (b) Sample after addition of RI matched solution. (c-f) Images slices at different depth obtained using LST demonstrate it allows detection of root hairs (e) and coarse anatomic features (c,d,f). LST is also suitable to image *A. thaliana* root 3D structure (g). (Media 3)

3.4 Time-lapse imaging of root growth

Time lapse imaging of root growth was carried out with *Latuca sativa* (lettuce, var. capitata, Seed Parade, UK) roots because roots of *Latuca sativa* have higher elongation rate. Lettuce plants both in Phytagel and transparent soil are studied because either media has its advantage: plants in Phytagel can provide better image quality due to uniform media while transparent soil is more similar to real soil condition for plants.

On the second day of germination, the plants were transferred to cuvettes containing $6gL^{-1}$ phytagel with half-strength M&S basal medium (Sigma). For time-lapse imaging, transparent soil saturated with 85% Percoll® for RI matching. Unlike sorbitol solution, Percoll® is a colloid solution and therefore has limited effects on root growth. The cuvettes were incubated at the same condition with *A. thaliana* plant for approximately 4 days before imaging.

Automation of image acquisition of lettuce root development was achieved by introducing high resolution motorized translation stages (M111.12S, PI, Germany) and high quantum efficiency sCMOS Neo camera into the system. Since lettuce roots are bigger than

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A. thaliana root, a 4X objective was used to achieve a larger field of view of (4.16 × 3.51) mm with the sCMOS camera. Thus the NA of incoming light was adjusted to 0.009 to provide an appropriate Rayleigh range with 35 μm waist thickness.

Before imaging, the cuvette was mounted onto the motorized stage with a piece of thick paper attached to it. The paper was working as an obstructer which allows the upper part of the plant to get light for photosynthesis while the root part was still kept in the dark to allow the gathering of high contrast images. A fiber coupled incandescent lamp (OSL1-EC, Thorlabs) provided light for plant growth. Sections from along the Z axis at intervals of 6.5 μm were taken every 15 minutes for 18 hours meaning 72 sets of images in a sequence were collected for a single plant. The number of steps along the Z axis varied with the orientation of the root: from 100 frames to 150 frames.

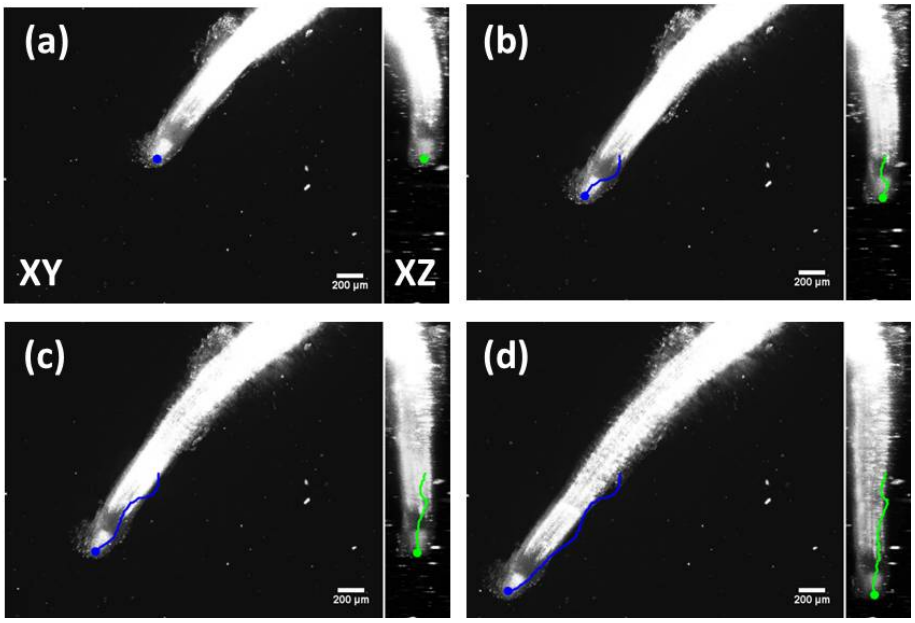

Fig. 6. Projected images of lettuce root at different time point. (a)~(d) are images taken at respectively 0h, 6h,12h and 18h after the start of the experiment. The XY plane was obtained from Z projection of the image stacks whilst the XZ plane was reconstructed from 150-image sequences. Tracking of the root tip was achieved with the manual tracking plugin in ImageJ. (Media 4)

Plant growth can be observed by taking frames of each time points to shows the maximum intensity projection in XY and XZ projection of the root, as shown in Fig. 6 (Media 4). When roots were grown in gel, features of the root anatomy such as root hairs and root cap were clearly visible in both projections. Very little movement was observed in the region of root hairs which confirm division and cell elongation zone is between root cap and root hair [13].

Tracking of the root tip was achieved manually. The position of the root tip was identified visually and marked manually so that the position of the root could be recorded at each time frame using ImageJ. The list of three dimensional coordinates was then used to determine the tip velocity in 3D space. The root tip velocity measured using this approach indicated that root elongate at $80\mu mh^{-1}$ on average. The elongation rate is therefore slower than that observed in natural environment (1-2 cm per day). This is mainly due to a lack of aeration of the media.

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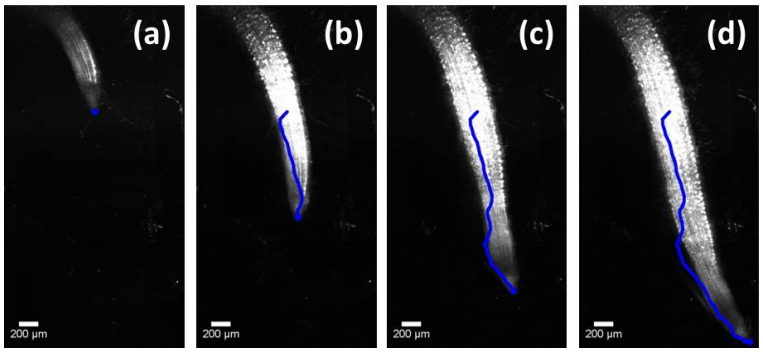

Fig. 7. XY plane projected images of lettuce root at different time points. (a)-(d) are images taken at respectively 0h, 5h, 10h and 15h after the start of the experiment. (Media 5)

For comparison, lettuce plants grown in transparent soil were analyzed using the protocol described above. Images were captured with an interval of 15 minutes for 24 hours. Figure 7 shows the projections of root at time point 0, 5, 10 and 15 hours, respectively. The root tip grew out of range after 15 hours. Images of subsequent time points are included in Media 5. In transparent soils, the quality of the light sheet is reduced due to inhomogeneous RI of the media cause by imperfect matching of the liquid and the particles, but also by the presence of air trapped in small cavities. The matching liquid based on Percoll® introduces also scattering in the liquid phase. The combination of these factors causes a severe reduction in the axial resolution of the images. However the image quality was still sufficient to extract quantitative information on root growth, with the root tip speed being 190 μms^{-1}, significantly larger than that in Phytagel.

4. Conclusion

A better understanding of root-soil interactions is essential to breed new crop varieties with improved nutrient acquisition efficiency. Currently, root-soil interactions studies are extremely limited by the lack of techniques to image plant roots *in situ*. Natural soils are opaque and light imaging is achievable only at the surface of soil samples [14]. There has been considerable effort to develop imaging such as X-ray micro-tomography, MRI or Neutron imaging [15] to image roots in soil, but the cost of instrumentation is high, the throughput is usually limited, and images do not allow detection of root anatomical traits.

Using standard optical components, a low-cost light-sheet tomography (LST) setup with the ability to take *in situ* 3D images of plant roots has been demonstrated. A distinct advantage of the method is the very low light dose to which the roots are subjected whilst being able to maintain sufficient resolution to image the 3D microstructure of the root system. Results show that LST is a promising new technique for the study of root-soil interactions. With the use of newly developed transparent soils, it is possible to track root movements *in situ* during 18 hours or longer. Although the instrument presented did not achieved those obtained with microscopes such as LSFM, the important anatomical features needed for phenotyping such as root hairs and tissue structure could be observed. Another advantage of LST is that image formation does not rely on fluorescence and genetically engineered lines expressing reporter genes. The technique is therefore widely applicable to most crop species. The simplicity and low cost of LST is also essential. In the future, portable instruments could be developed using the same concept and applied in glasshouse screens.

Though the use of scattering as a contrast mechanism means LST will not usually be able to achieve a resolution as good as that of LSFM. However it yields similar information in a much simpler and cheaper device, without the need for labeling or staining of the sample. In fact it is very complementary to LSFM: any LSFM system could easily be adapted to use for LST through the replacement of color filters with neutral density filters and appropriate

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sample preparation. It is envisaged that the range of uses for LST could also be extended through the use of optical clearing techniques similar to those we use for rendering the soil transparent in this letter. In suitable samples it is still able to produce images with a resolution of less than 3 microns with only basic image reconstruction and without any need for image processing. This resolution, the overall quality of the images and the suitability for more densely scattering samples could be increased by the introduction of techniques such as structured illumination and de-convolution.

The lateral resolution obtainable with the setup presented in this study was shown to be 2.7 μm or greater with the main limitation on resolution being the accuracy of the refractive index matching in the sample.

We have shown the feasibility of the system for plant root phenotyping when combined with novel transparent soils by obtaining images of plant roots *in situ*. Clear 3D images were obtained without using fluorescence. Long term monitoring of lettuce root during 18 hours and tracking of root cap movement were achieved, yielding quantitative information on root growth rate.

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