



## Kinematic Characterization of Root Growth by Means of Stripflow

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### Abstract

Kinematic methods for studying root growth are powerful but underutilized. To carry out kinematic analysis, the Baskin laboratory, in collaboration with computer scientists, developed software called *Stripflow* that quantifies the velocity of motion of points in the root, a quantification that is required for subsequent kinematic analysis. The first half of this chapter overviews concepts that underlie kinematic analysis of root growth; the second half provides a step-by-step guide for using Stripflow.

**Key words** *Arabidopsis thaliana*, Computer vision, Deformable motion, Elongation, Velocity field

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### 1 Introduction

Plants are famously sessile yet far from motionless. Shoots reach for the sky, leaves fan out into the light, and roots tunnel through the ground. All this movement is driven by water flowing into cells. A plant cell, having left the cell cycle, might take up sufficient water to increase its volume by a hundredfold, thereby displacing its contents on the cellular scale; integrated over myriad cells, water flowing into plant cells drives extensive motion of the plant. The speed at which plants move is slow to the human eye, a pace that contributes to the popular conception of plants as doormats; nevertheless, anyone who has watched a time-lapse movie of a flower blooming or a seed germinating has seen the scale of the relevant flows.

This manifest flow is exploited by scientists to reveal underlying physiological processes. This is the essence of *kinematic* methods. Here, the physiological process of interest is growth, defined as an irreversible increase in cell volume. Growth accounts for most of the net water taken up into plant cells (to be clear, the transpiration stream balances water uptake with evaporation). While irreversible changes in volume dominate quantitatively, reversible changes also

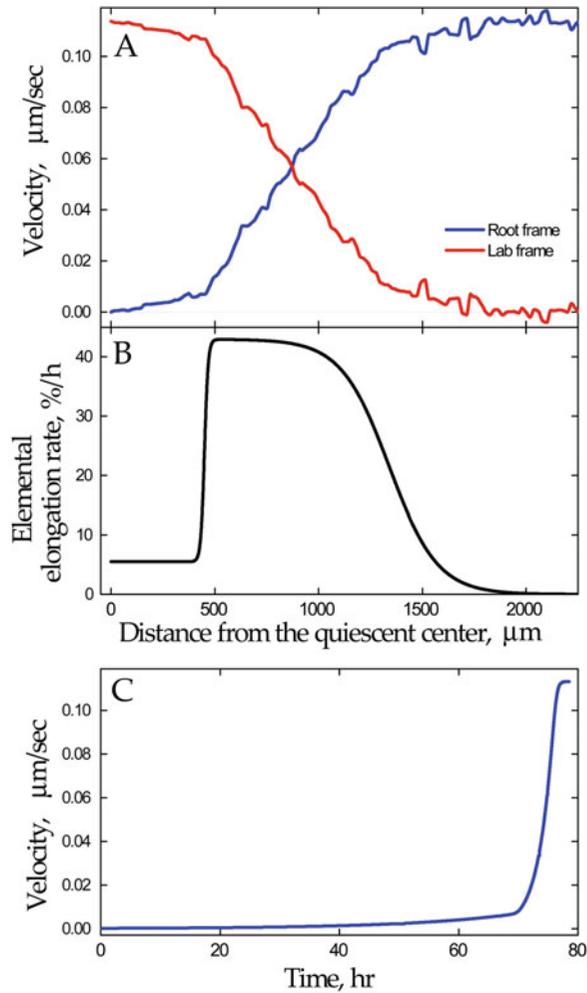
occur. Cell volume changes reversibly not only in specialized organs, such as the pulvinus, but also in cells concomitantly with growth. Reversible volume changes are likely to be significant for growth mechanistically; however, because they are difficult to isolate, here for the sake of simplicity, we attribute all volume increase to growth.

Kinematic methods for studying root growth are powerful and have been central to work in the Baskin laboratory. To pursue that work, in collaboration with computer scientists, we have developed software called *Stripflow* that quantifies the velocity of motion of all points in the root [1]. The software works well and anyone interested in doing kinematic analysis might find it useful. Accordingly, the second half of this chapter provides a step-by-step guide for running Stripflow. However, despite their utility, kinematic methods are neither common nor intuitive. Therefore, before turning to the protocol, the first half of the chapter overviews the concepts that underlie the approach.

### **1.1 The Kinematic Approach to Studying Root Growth**

The practical application of kinematics to plant growth can fairly be said to have been inaugurated in 1956 with publications, from three independent laboratories, on the plant root [2–4]. The root is an apt organ for kinematics because to a first approximation it can be treated as a one-dimensional system. This is reasonable given the root's cylindrical symmetry and the extent to which growth in length dominates the increase in volume. A similar one-dimensionality also applies to grass leaves, the growth of which is also well characterized kinematically (e.g., ref. 5). Kinematic treatments can be broadened to include two dimensions, as in the expansion in area within the plane of the leaf's lamina, and even three, as required for a complete characterization of a growing organ. Nevertheless, even for the root, such extensions increase complexity, mathematical as well as experimental, and will not be covered here; interested readers may dip their toes in these waters by splashing through any of the following references: [6–9].

In kinematic methods, the fundamental parameter is velocity, that is the rate and direction of movement [10–12]. For the root, movement in one dimension is represented by a velocity profile; that is, the speed at which each point in the root is moving, with the direction being taken as parallel to the root's long axis. Strictly speaking, each point within the root volume is associated with a velocity (a three-dimensional velocity *field*); however, a one-dimensional velocity *profile* is obtained by averaging velocity for sets of points that are equidistant from the root tip. This amounts to averaging over the root's cross section, reasonable because if velocity (the component parallel to the root's long axis) varied systematically over the cross section, then the root would tear or bend. Clearly, cross-sectional averaging is inappropriate during tropic bending.



**Fig. 1** Quantitative representations of root growth, obtained by Stripflow and modified numerically. **(A)** Velocity profiles. Blue line (Root frame) plots velocity with respect to the quiescent center. This coordinate transformation simplifies the mathematics and is universally adopted in kinematic treatments. The blue line is actual Stripflow output. Red line (Lab frame) plots velocity with respect to the laboratory. The maximum at zero (at the quiescent center) is equivalent to root growth rate. **(B)** The derivative of the blue velocity profile in **(A)** obtained by curve fitting [1]. **(C)** Velocity as a function of time. The blue line profile in **(A)**, assumed to represent a steady state, was used to convert distance into time [13] and replotted

In the laboratory frame of reference, the magnitude of velocity is greatest at tip of the root and falls to zero in the mature, nongrowing region of the root (Fig. 1A, red line). As expected, where there is no growth, there is no velocity. However, for kinematic methods, an alternative frame of reference is chosen, namely the root tip. In this frame, the tip of the root is the origin (position

and velocity both equal zero), and velocity rises to reach a plateau in nongrowing regions (Fig. 1A, blue line). At a first encounter, inverting the velocity profile might be counterintuitive; nevertheless, this coordinate transformation simplifies the computations and becomes easier to visualize with practice.

In Fig. 1A, an alert reader will notice that the  $x$ -axis reads distance from “the quiescent center”, not distance from “the tip”. Generally speaking, the root *tip* is a region rather than the single point implied by a coordinate origin. In *Arabidopsis thaliana*, the quiescent center comprises four cells, making it suitably “point-like”, and is usually conspicuous in images, making it an apt choice for the origin. For thicker roots, the quiescent center might comprise thousands of cells, necessitating a different choice, usually the very end of the root.

In the transformed frame (Fig. 1A, blue line), the mature region of the root has a high velocity but no growth. Doesn't this contradict what we wrote above about velocity reflecting growth? No, because growth (expansion) requires not velocity per se but rather the presence of a gradient in velocity. Imagine a single cell, for example a bacterium, that is not growing and anchored to a substrate: no velocity anywhere. Now imagine that this still nongrowing cell is sheared off the substrate by rapidly flowing growth medium: all points of the cell have the same velocity but the cell isn't growing. Now, imagine that the cell is growing: If the cell is anchored to the substrate at its mid-point, then each end will have velocities that are opposite in sign; if it is anchored at one end only, then the velocity will vary from zero at the fixed end to a maximum at the free end; finally if the growing cell is in a moving fluid, then this flow simply adds a constant velocity to all points on the cell but one end must move faster than the other end for the cell to become longer.

In mathematical terms, the derivative of the velocity profile gives the profile of growth (Fig. 1B). In the classic kinematic literature, this derivative was referred to as *relative elemental elongation rate*. Besides being ungainly on the  $y$ -axis, any elongation rate that is elemental must, by definition, be relative. Therefore, the term is usefully simplified to *elemental elongation rate*. The rate is elemental because it is obtained by differentiation. In kinematic methods, profiles of velocity and elemental elongation rate are continuous and have no reference to cells. This is appropriate because the process of cell wall deformation, which allows water to enter and cells to enlarge, while not literally infinitesimal, nevertheless occurs on a scale that is far smaller than that of an entire cell. Authors interested in growth often refer to “cell expansion” (or “cell elongation”) and thereby obscure the elemental status of the relevant machinery.

Besides revealing local growth activity, the velocity profile has other uses. It embodies the relation between position and time,

provided that the velocity profile remains constant over time [13]. This is often reasonable, particularly for roots growing in a constant environment, for example in the laboratory. If we consider a particle near the tip, we can calculate how long it will take to move a small increment of distance, say 10  $\mu\text{m}$ , given its current velocity. It arrives at that point, 10  $\mu\text{m}$  farther from the root tip, which has a slightly larger velocity. We use that velocity to find out how long it takes to move the next 10  $\mu\text{m}$ . And so on. This shows how long it takes for a particle to move through the growth zone (Fig. 1C). In the usual spatial velocity profile, the meristem occupies about 10–20% of the growth zone whereas when velocity is plotted versus time, the meristem occupies more than 90% of the time course. Material moves slowly in the meristem and consequently stays there for an appreciable period compared to the faster movement and shorter residence in the elongation zone.

Finally, the velocity profile helps us calculate the profile of deposition rate for a quantity of interest [14]. This calculation is based on the continuity principle fluid mechanics, appropriate because the growing root is in fact fundamentally a flow of water. For fluid moving in a pipe, the flux of a solute at any point along the pipe is defined as the product of the velocity of the fluid at that point and the concentration of the solute of interest at that point. With the flux known for all points all along the pipe, the derivative of flux with position gives the local rate of production (or consumption) of that solute (again assuming steady state conditions). Because of its linear nature, we may treat the root as a pipe and if we measure the local concentrations of sucrose, say, or of potassium, we can calculate the rate at which that substance is produced (or consumed) at each distance from the root tip.

Such continuity arguments apply also to cells [11, 15–17]. That is, the flux of cells at each position in the root is calculated from the profiles of velocity and cell ‘concentration’, where the latter is taken simply as one over cell length. Then, the profile of cell flux is differentiated to produce the local rate of cell deposition (this is positive or zero, cells are rarely if ever consumed). The cell deposition rate thus calculated has units of cells made per time and length; this is then converted to a typical cell division rate by multiplying by the cell length at each position. Thus, a fundamental parameter, cell division rate, is quantifiable with measurements of the profiles of velocity and cell length, both of which are straightforward to measure [18].

## **1.2 Root Kinematics in Practice**

The methodological goal is to measure the rate at which each point on the surface of the root is moving. Early methods for doing so took advantage of fairly thin roots in which the cell walls in epidermal cells are visible in micrographs. Later methods marked the root with ink or other particles, which is suitable for thicker roots. In either case, the root is photographed at several times and the

positions of identified markers measured. These methods are tedious because many particles need to be measured over several time intervals, and subjective because the user identifies the same particle in the various images and measures their position manually. Furthermore, there is a trade-off between precision and accuracy: precise measurement requires large displacements but an accurate profile requires small displacements.

A striking exception is the method developed by Erickson and Sax [2]. They modified a camera to mask the film plane, thereby exposing just a narrow slit. The slit ran parallel to the long axis of the root, centered on the root's midline, with the magnification such that the image of the root's entire growth zone was projected onto the slit. In addition, the film in the camera was driven past the slit at a constant rate. Particles on the surface of the root, which would be a spot in a regular image gave rise to streaks as time elapsed and the film moved and the root grew. By measuring the slope of the streaks at each position, Erickson and Sax recovered an essentially instantaneous rate of displacement. It is remarkable that their analogue method anticipated digital methods, such as kymographs or even optical flow, by more than forty years. Nevertheless, slopes were measured manually from the film with a protractor and accuracy was limited by drift of the root, uneven film speed, and the relatively small size of the film.

Perhaps because of the difficulty of measurement, kinematic methods for studying root growth were seldom used from the time their development in the 1950s for nearly 50 years, having to wait until methods were enhanced by the application of techniques from computer vision [19]. In this effort, pioneers were Shurr and collaborators who developed software able to obtain velocity profiles algorithmically from both leaves and roots [20, 21]. Thereafter, other groups published accounts of related software (e.g., refs. 22–24).

A challenge faced by any method for quantifying the velocity profile algorithmically is that the root tip, which contains the meristem, has a shallow gradient in velocity but a large absolute velocity; whereas, in contrast, the elongation zone has a steep velocity gradient but a low to vanishing absolute velocity (Fig. 1A, red line). Routines that are optimal in one regime tend to do poorly in the other. In some cases, researchers have been interested in following a single root over a prolonged time (e.g., refs. 21, 25) and have not required particularly high spatial resolution, focusing on the elongation zone. However, spatial resolution in the meristem is particularly important where the intent is to use the velocity profile to quantify the deposition of cells or other material.

### **1.3 Root Kinematics Using Stripflow**

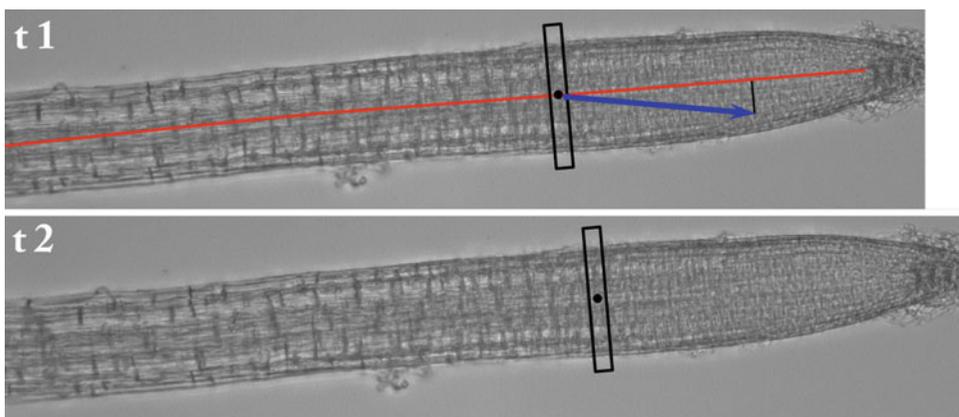
The Baskin laboratory entered the fray in 2003 with the publication of *RootFlowRT* [26]. While *RootFlowRT* would run on most computer platforms, the input was a stack of nine images, separated by

about 1 min each. Recently, the lab introduced Stripflow [1] which requires only two images as input. Stripflow has been found to be convenient and reliable and is freely available to the community (<https://github.com/TobiasBaskin/Stripflow-release>) although it runs only in MATLAB. We feel that Stripflow will be useful to those interested in characterizing root growth and here we provide a step-by-step guide for running the software.

#### 1.4 Conceptual Overview of Stripflow

Stripflow gets its name by leveraging the prior knowledge that movement at nearby points is similar. The input is a pair of images of the growing root separated by a time interval long enough for the root tip to move 10 to 20 pixels. In its first step, Stripflow uses a Fourier-based method to find the average displacement between the two images to the nearest pixel. This speeds up subsequent processing. Next, starting at the first point of the root's midline, Stripflow places a strip (hence the name) over the root, centered at that point. The strip is as wide as the root and the length of the strip, selected by the user, is typically about 15% of its width (Fig. 2). Stripflow will then search the relevant area of the second image to find the best match for the strip. The component of the displacement of the strip that is parallel to the local midline is taken as the velocity for that point on the midline. The strip is then centered on the next pixel of the midline and the process repeated. In this way, the velocity profile is built up (Fig. 1A).

Along with the distribution files for running Stripflow (<https://github.com/TobiasBaskin/Stripflow-release>), we provide files with a detailed account of the underlying image analysis, notes for running the program, and a set of test files with output so that the user can verify the installation if need be.



**Fig. 2** Illustration of part of the velocity field estimation used in Stripflow. An image of the root is shown at two times. A strip (black rectangle) is centered on a pixel of the midline (red line) and the best matching strip is found in the second image. The vector from the point at  $t_1$  to the point at  $t_2$  (blue arrow) is projected onto the midline using the tangent at that center pixel. This is done for each pixel on the midline

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## 2 Materials

### 2.1 Plant Species

Stripflow was developed for *A. thaliana* roots. Any species with roots thin enough to transmit light should be suitable. The use of thicker (opaque) roots will require testing and possible modification.

### 2.2 Plant Growth

Germinate *A. thaliana* seeds on agar-solidified nutrient medium (*see Note 1*). For the highest quality imaging, place seeds inside the agar so that the roots grow within the medium, rather than on the surface (*see Note 2*). When this is not possible, imaging can be improved by placing a few drops of growth medium on the agar medium and adding a coverslip over the root (or roots) to be imaged (*see Note 3*).

### 2.3 Microscope

1. An ordinary compound microscope (e.g., Olympus CH2) with either a 4× or a 10× objective. The tube lens for projecting the image onto the camera should be selected based on imaging parameters (*see Note 4*). Useful tube lens magnifications are 1.67×, 2.5×, and 5×.
2. Lighting. To avoid photobiological effects, particularly for long-term imaging, place a visible-light absorbing-IR transmitting filter in the light path. Alternatively, the microscope's light source can be replaced by one or more IR-emitting diodes.
3. Camera. Select a camera that has neither lens nor IR absorbing filter. The more pixels on the chip, the better the resolution. Neither high light sensitivity nor rapid image acquisition is needed. The software to control the camera should allow time lapse imaging and deactivation of automatic contrast and brightness adjustment (*see Note 5*).

### 2.4 Computer

1. Download Stripflow (*see Note 6*). The latest release is at <https://github.com/TobiasBaskin/Stripflow-release>. In addition to the code, there are information files, including a readme that is essential to be familiar with, and test files in case it is helpful to have a “positive control”.
2. Copy downloaded files to a secure place and duplicate them in a working directory.
3. Read the “readme” file for further information and any modifications implemented after this article went to press.
4. Install software for making simple measurements on images. We recommend ImageJ (<https://imagej.net/Welcome>). In the measurement software, set the coordinate frame so that the lower left-hand corner is  $x = 0$  and  $y = 0$ . In ImageJ, this is done by invoking “Analyze . . . Set measurements” and checking the “invert  $y$ -coordinates” box.

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### 3 Method

**Steps 1** through **5** relate to image acquisition and working with living roots. **Step 6** and following refer to image analysis, which is done subsequently, at any time.

1. Launch the image acquisition software and set it up to capture and save a pair of images separated by the desired time interval (*see Note 7*).
2. Bring a plate of plants to the microscope and place on the stage with the agar medium facing the objective lens (*see Note 8*).
3. Focus on a root, move the tip to the edge of the frame, and adjust the focus (*see Note 9*).
4. Acquire a pair of images (*see Note 10*).
5. For single stack mode, find a new root and repeat **steps 3** and **4** (*see Note 11*). For multistack mode, displace the stage by a known amount (*see Note 12*). This amount must be less than the field of view (*see Note 13*). Acquire a pair of images. Continue until reaching the region of the root where root hairs are fully elongated. Find another root and repeat (*see Note 11*).
6. Determine the number of pixels per  $\mu\text{m}$  for the imaging conditions (*see Note 14*).
7. Crop the images to remove areas without root. Leave about the width of the root on each side (*see Note 15*).
8. Save cropped images as .tif files, which is the file format Stripflow requires.
9. Obtain the coordinates for midline points (*see Note 16*). Open the cropped image in ImageJ or related software. Start about 20 pixels in from the edge. Typically five to seven points are sufficient. If the root is highly curved, more might be needed; less if it is straight (*see Note 17*). Midpoint coordinates are obtained for the first image of a pair only. When using Stripflow in multistack mode, the magnification is typically high enough that two or three points suffice (i.e., the end points and if needed a center point); however, midpoints are needed for each image pair in the multiframe stack.
10. Obtain the coordinates for the quiescent center, or other suitable reference point for the origin of the velocity profile. The reference point can be the end point of the midline.
11. Measure the working diameter of the root at the left and right sides of the image. These data are used to create a rough motion mask. If there is debris beside the root, the diameter values can be set small enough to exclude it. Generally, the

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growth direction | 1
                 | 0
                 | 1
pixels per micron | 1.85
                 | 2
midline coordinates | stack1
                  | 30 512, 380 510, 1092 542, 1486 540, 1910 458, 2256 342, 2414 282
                  | dlr 235 235 ← root diameter, left & right
                  | endm
sec between images | 45
output file name   | 9_8_Contr5-int1.txt
image file names   | Sep8-Con-R5-1.tif
                  | Sep8-Con-R5-2.tif
                  | 2414 282 ← quiescent center coordinates

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**Fig. 3** The input.txt file used for Stripflow in single-stack mode, with annotation. Lines without annotation reflect information needed for the multistack mode and should be left as they are

diameter values are set to span 20 to 30 pixels more than the root.

12. Edit the input.txt file (*see Note 18*). This is a text file that Stripflow uses to read information specific to each run (Fig. 3). Many lines are the same for all runs, or groups of related runs. Thus, the file is usually edited, rather than made from scratch. The readme file provided with the software explains the syntax in detail. Generally the following lines need to be edited for each pair of images: coordinates for midline and quiescent center, right and left diameters, input image file names, and output file name. Sometimes, magnification, seconds between images, and growth direction will need to be edited. This example (Fig. 3) illustrates the input.txt file for single-stack mode. For multistack mode, the file has additional lines, which are specified in the readme file.
13. Make a working directory that contains the input.txt file and a directory named tiff, into which place the two images to be used as input (*see Note 19*).
14. Open MATLAB and navigate to the directory containing the Stripflow scripts.
15. Open the appropriate read input file script. For single-stack mode, open “read\_singlestack\_input\_v1\_0.m”. For multistack mode, open “read\_input\_v1\_0.m”.
16. If necessary, adjust the strip width, given in line 5 of the script. This is the value in pixels that defines the length of strip (i.e., the dimension parallel to the long axis of the root). The larger the value, the more smoothing there will be; the smaller the value, the more noise. Users will need to explore setting this parameter for their system.

17. Edit the path (line 8, begins “folder = ...”) to specify the working directory (where the input.txt file and tiff folder are located).
18. Run (*see* **Note 20**).
19. While Stripflow runs, it will display an image of the root with the midline points and quiescent center (or selected origin) superimposed. It is a good idea to check this to make sure that the midline points were entered correctly. When the run is finished, a plot of the velocity profile will be displayed (*see* **Note 21**).
20. Check the output. Stripflow saves an image of the root with the superimposed midline and origin points, an image of the velocity profile plot (*see* **Note 22**), and a text file with the velocity profile as  $x, y$  coordinates (position in microns, and velocity in microns/s).
21. Further handling of velocity data is beyond the scope of this protocol. However, commonly a key step is to curve fit the velocity profile for differentiation. One approach to do so is to use piecewise polynomials (e.g., ref. 15); another is to fit a single function (e.g., ref. 27).

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## 4 Notes

1. For *A. thaliana*, we find optimal root elongation with a modified Hoagland’s solution containing the following: 4 mM  $\text{KNO}_3$ , 1 mM  $\text{Ca}(\text{NO}_3)_2$ , 2 mM  $\text{KH}_2\text{PO}_4$ , 0.3 mM  $\text{MgSO}_4$ , 89  $\mu\text{M}$  Fe citrate, 46  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 10  $\mu\text{M}$   $\text{MnCl}_2$ , 0.76  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.3  $\mu\text{M}$   $\text{CuSO}_4$ , 0.1  $\mu\text{M}$   $\text{MoO}_3$ , 1% sucrose, and 1% agar. This is autoclaved but the pH is not adjusted. We recommend Difco Bacto Agar insofar as roots grow more slowly on other sources of agar, as well as on other types of gelling agent, that we tested.
2. We recommend this arrangement because when roots grow on the surface of the agar, they are shrouded by a film of water, which reduces the area available for imaging.
3. When putting on the coverslip, care must be taken to avoid air bubbles forming at the surface of root, which interfere with imaging.
4. To determine appropriate magnification, we recommend pilot runs with representative roots. At low magnification, the entire growth zone is included in the image. This allows Stripflow to be run in single-stack mode, simplifying the acquisition procedure. However, the resolution might be insufficient for reliable velocity profiles. This depends in part on the number of pixels in the camera. Increased magnification increases resolution but

requires Stripflow to be run in multistack mode to obtain a complete velocity profile. In this case, the stage is moved and successive imaging is done to span the growth zone. Besides increasing the number of images required, the movement of the stage must be known. Stage movement can be known either by virtue of using a motorized stage or by imaging a background image at a distant focal plane.

5. The free software Micromanager is excellent and able to control many available cameras (available at <https://micro-manager.org>).
6. Stripflow runs in MATLAB (available to purchase at <https://www.mathworks.com/products/matlab.html>).
7. Often, this means setting up a time-lapse sequence for a total of two frames. The optimal time interval depends on the rate of root growth, image quality, and the number of pixels in the root image. This will need to be determined empirically by the user for their conditions. As the time interval gets too small the noise will increase. As the time interval gets too long, the velocity profile will become overly smooth. With roots growing  $\sim 400 \mu\text{m}/\text{h}$ , we find a time interval of 30 s reliable. Fortunately, the sensitivity is not acute. We recommend users start with 30 s and compare both shorter (20 s, 15 s) and longer (45 s, 60 s) intervals.
8. Placing the microscope horizontally will avoid gravitropism. But where this is not possible, using a conventionally oriented (upright or inverted) stand is fine, provided that the plate with seedlings is kept horizontal on the stage for less than the time required for growth rate to change based on gravitropism. For *A. thaliana* roots, this is more than 10 min.
9. It is best if the root runs horizontally across the image. If the root image is at more than about a  $20^\circ$  angle to the horizontal, rotate the camera to better align the root. Roots imaged so that growth is vertical (i.e., along the  $y$ -axis of the image) will fail to be analyzed correctly. However, roots may move either from left to right or right to left in the two frames.
10. If using Stripflow in single-stack mode, then it can be helpful to extend time lapse imaging, obtain several velocity profiles for successive times, and average them. For example, acquire eight images separated by 15 s (which requires 1 min, 45 s) and obtain velocity profiles for the following frame pairs: (1, 3), (2, 4), (5, 7), and (6, 8). Then take the average of these four velocity profiles. If you are willing to use some but not all of the images twice, then also obtain profiles for two additional frame pairs (3, 5) and (4, 6). In our experience, averaging three profiles is usually sufficient to tame the noise, but individual mileage will vary.

11. When imaging on a vertically oriented (upright or inverted) microscope, we recommend keeping the imaging time per plate to less than 10 min. This will minimize complications from gravitropism. To image more roots during a given section, we prepare several plates, take one out of the growth chamber, image, return it to the chamber, take out its neighbor, and so on.
12. Stripflow includes functionality to obtain the displacement amount from user-acquired background images. To use this mode, after acquiring a pair of images at a given position in the root, the user focuses on something in the “background.” For example, a film can be placed on the top of the Petri dish that has a complex image printed on it. The background image must be in a focal plane distinct from the root. Importantly, the root itself must not be used because it changes between image pairs due to growth. When the use of a background is invoked, Stripflow registers background images from each adjacent image pair and thus obtains the stage displacement. Note that we have used this mode rarely and some coding or debugging might be required.
13. We recommend the displacement be about four-fifths of the field of view.
14. We recommend imaging a stage micrometer with the same instrument settings and measuring directly.
15. Cropping is not strictly necessary but makes Stripflow run faster. It is essential that each image pair is cropped identically and it is helpful to crop identically all images in a set of velocity profiles to be averaged (as in **Note 10**). Identical cropping can be accomplished readily in imageJ by opening the images to be cropped and then clicking on “images to stack”. Then choose the rectangle tool and draw an ROI that surrounds the root, leaving some empty space on either side. Then click on “crop”. Finally, click on “save as . . . image sequence”. This will let you choose file names and also file format. Note that Stripflow requires the image files to be in the .tif format. In addition, growth of the root needs more or less parallel to the  $x$ -axis (*see Note 9*).
16. A limitation of Stripflow is that midline points need to be provided. In early attempts we found that algorithmic determination of the midline failed frequently enough to be inconvenient. In subsequent releases we hope to provide automated midline detection. For now, the user must enter them by hand.
17. In ImageJ, one way to do this is as follows. Select the “segmented line” tool and adjust the preferences to show a spline fit, rather than linear segments between points (under “Edit. . . Options. . . Profile Plot Options”, check “sub-pixel

- resolution”). Then, record the anchor points in the log. We have a macro for recording anchor points, available on request.
18. If desired, the file name of the input.txt file can be changed. To do so, edit line 8 in the read. .input script.
  19. This is the basic directory architecture for running Stripflow. The program scripts are elsewhere. We recommend making a unique working directory for each pair of images to be used as input for generating a velocity profile.
  20. Common errors involve path and file names. If the program does not run, check that the path is specified exactly (*see* Subheading 3, step 17) and that the input image names listed in the input.txt file match the actual image file names exactly.
  21. Expect a run time of around 10 s. The exact time will depend on the size of the image and computer processing power.
  22. The velocity plot also records the strip width and the tip velocity. The latter is the value used to invert the profile from laboratory to root-centric reference frames. This value represents the rate of “root growth”.

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