# MATERIALS AND METHODS

**Plant culturing.** The transgenic Arabidopsis line that expresses pGATA23::GUS-GFP-NLS, pUBQ10::YFP-PIP1;4 and pUBQ10::H2B-RFP was generated from requested lines, pGATA23::GUS-GFP-NLS(De Rybel et al. 2010), pUBQ10::YFP-PIP1;4(Geldner et al. 2009) and pUBQ10::H2B-RFP (Grefen et al. 2010).

½ MS plates: 2.66 g Murashige and Skoog medium (M0222.0050, LOT no. P08381.01, Duchefa, Haarlem, Netherlands), 0.97 g MES (2-(N-morpholino)ethanesulfonic acid buffer, M3671-50G, LOT no. 120M5433, Sigma-Aldrich) and 10 g Saccharose (4621.1, LOT no. 330160546, Carl Roth, Karlsruhe, Germany) were diluted in 1 liter of sterile de-ionized water. The pH was adjusted to 5.8 using KOH. 15 g Phytagel were added to the bottle before autoclaving. Plates were poured once autoclaving was done. The seeds were surface sterilized in a sterilized solution (10 % Sodiumhypochloride, 0.1 % TRIS, in H2O) in a microtube for 10 min (shaker) and then rinsed five times with sterile water. Single seeds were sowed side by side on ½ MS plates with at least 1 cm space in between (this facilitated handling for future sample preparations). Plates were cultured vertically (slightly tilted) for 5-7 days under long day conditions (16 h/8 h day/night period) with 120-140 µmol/m²/s amount of light, at 22°C.

Light on roots: special transparent culture tubes (no. W 1607 Duchefa, Haarlem, Netherlands) were used to study the effect of light on the root system. The plants encounter conditions similar to those in the microscope chamber like roots in liquid, also the diameter of the chamber is the same. The chambers were covered by an aluminum foil, the surface of the gel around the plant was covered by small sheets of black plastic foil.

**Sample preparation.** Capillaries (3 mm in diameter, 30 mm in height (Hilgenberg GmbH, Malsfeld, Germany) and carbon rods (0.28 mm in diameter, 30 mm in height, from Conrad Electronic GmbH & Co KG, Wels, Germany) were cleaned in an ultrasound unit with 2% Hellmanex (Hellma GmbH & Co. KG, Müllheim, Germany) for 10 min, rinsed with water several times and then autoclaved. For sample preparation, the capillary was pushed 1.5 mm deep into the Phytagel to about 10 mm below the root tip and then pushed carefully over the root until the capillary reached the leaves. The capillary was pushed into Phytagel to fill the capillary. A carbon rod was inserted from the top, behind the plant. The capillary was pushed into Phytagel in order to extrude the plant up to the region of interest (0 – 4 mm above the edge of the capillary). A drop of 2 % Agarose (no. 6351.2, Carl Roth, Karlsruhe, Germany) was placed above the region of interest in order to mount the root onto the Phytagel. The plants were kept in custom made chambers until imaging, 10 mm of the thick end of a 200 µL pipet tip was glued into the lid of a 15 ml Falcon.

**Gravitropic stimulation to induce lateral root emergence.** Several studies have shown that bending the primary root induces differences in the auxin distribution of the cells outside as opposed to the cells inside the curve(Laskowski et al. 2008; Ditengou et al. 2008). This local increase in auxin can induce lateral root formation(Dubrovsky et al. 2008; Casimiro et al. 2001). It has been shown that a lateral root can be induced manually, e.g. by rotating the plant through 90°. This method to induce lateral root growth by gravity stimulation was introduced by M. Lucas et al 2008(Lucas et al. 2008). In order to test how long we need to keep the plant rotated by 90° we rotated plates with six days old plants by 90° for different periods of time. After 3 h, 6 h, 9 h and 12 h the plates were rotated back to the previous position and the number of lateral roots that appeared were counted two days later. In 42% of the plates, the primary roots show lateral emergence at the outside of the bend, after three hours of stimulation, 60% after six hours and 80% after 9 hours of stimulation. In our data sets, the first cell division occurs 7 to 14 hours after the gravity stimulation. We therefore stimulated the roots at least 6 hours before we started the recording process.

The plant is placed vertically in a medium-filled chamber while the leaves remain in the air. The root grows on the surface of a Phytagel cylinder cast in a glass capillary. Prior to the onset of image acquisition the Phytagel cylinder is extruded from the capillary. This cylinder is rigidified by an embedded carbon rod. A perfusion system exchanges the entire medium in the plant chamber every 15 min and could apply any solvent agent in between subsequent recordings. The perfusion speed can be adjusted. The plant is inserted from above but is held from below. Thus, the opening remains accessible for diurnal illumination of the leaves, by. The light stems from a standard fluorescent lamp. A flexible glass fiber (1/2 inch in diameter) guides the light towards a location above the plant. A shutter between the lamp and the fiber is operated by the data acquisition software of the LSFM to turn the light off whenever a stack of fluorescence images is recorded, a standard time switch for the lamp generates the diurnal cycle (supplement figure). The spectral properties of the simulated sun light could be adjusted by using colored filters. The intensity can be adjusted by changing the distance between the fiber and the lamp. The optimal intensity for Arabidopsis is 120-140 µmol/m²/s.

A great value of our attention is on high quality standards that we applied to our work. We are confident that our data is derived from plants that experienced close-to-natural growth conditions(Maizel et al. 2011), but we constantly perform quality controls.

**Time-lapse Imaging.** The Arabidopsis seedling stably expresses pGATA23::GUS-GFP-NLS, pUBQ10::YFP-PIP1;4 and pUBI10::H2B-RFP. Two channels were observed, GFP and YFP were excited with the light from a 488nm diode laser, fluorescence emission was filtered with a 525/50 band-pass filter. RFP was excited with the light from a 561nm diode-pumped solid state (DPSS) laser, the fluorescence emission was filtered with a 607/70 band-pass filter. Image stacks consisting of 233 planes with an axial pitch of 0.645 µm were recorded every 5 min for 45 h. A Carl Zeiss N-Achroplan 40x/0.75 W objective lens was used in the detection path and a Carl Zeiss EC Plan-Neofluar 5x/0.16 served in the illumination path. An Andor Clara camera (6.45 µm pixel pitch) was driven in a binning (2x2) mode. Camera exposure time was between 30 ms and 100 ms, the laser intensity was set between 0.12 mW and 0.6 mW depending on the respective fluorescence intensity. Please find individual settings information in supplemental material. The microscope chamber was perfused continuously (0.55 ml/min) with fresh ½ MS medium (no sugar). Plants were kept for a maximum of 120 hours in the microscope chamber in long day conditions (16 h/8 h day/night period) with 120-140 µmol/m2/s amount of light, at 23°C.

**Image processing.** All image data is stored in a 16-bit lossless Tagged Image File Format (TIFF). Time series three-dimensional drift registration in Fiji (ImageJ). The complete dataset was loaded as “virtual hyperstack” via the “Bio-Formats Importer” plugin (<http://fiji.sc/Bio-Formats>; http://www.openmicroscopy.org/site/support/bio-formats4/users/imagej/load-images.html). The “virtual hyperstack” was registered using the Fiji plugin “Correct 3D drift” (http://fiji.sc/wiki/index.php/Correct\_3D\_drift). The plugin registers the time points using the phase correlation algorithm implemented in ImgLib1(Pietzsch et al. 2012) (<http://fiji.sc/Imglib>) based on the intensity of the membrane channel. The result was saved as single TIFF files, i.e. each z-plane is saved in a single TIFF-file, which generates several hundred thousand images. A Mathematica program converted all files into z-stacks of TIFF files (Supplement x). The data set was then cropped to the region of the LRP along the x-, y- and z-directions.

**Lineage tracking.** Cell lineage tracking was performed manually for each dataset by means of the program “TrackGen” written in *Mathematica*. A folder containing a time series of image stacks of time points needs to be specified in the program. “TrackGen” then provides an interactive visual interface for easy navigation in time and space in the dataset. For a time series the image stack of the current time point is displayed in the program’s visualization pane. Upon a change of the time point control, the program automatically loads the image stack of the requested time point. In the program, the three-dimensional position of the first and last occurrences of each cell nucleus that contributes to the LRP was identified manually. “TrackGen” allows to manually track the lineage of individual cell nuclei in time series datasets and offers comprehensible visualization of all identified nuclei. For an identified cell nucleus, the program creates a new object and associates to it a) a unique identification number, b) the spatial coordinates of the nucleus in x, y and z, c) the time point of occurrence, d) the cell nucleus lineage in form of a list holding the identification numbers of all progenitor cell nuclei. All data was then exported and saved in a XLSX file.

**Lineage reconstruction.** The lineage of all cell nuclei indentified with “TrackGen” was reconstructed using a second program “TrackAlyzer” written in *Mathematica*. For the reconstruction, “TrackAlyzer” loads the XLSX file created with “TrackGen” and reconstructs the complete lineage of the cell nuclei. In the program, the data structure used to represent the lineage information of a cell nucleus is a directed graph (tree). A vertex represents a nucleus object and holds all associated information. We established a compressed graph representation for cell lineages, in which vertices in the graph hold the associated information of either the first or the last occurrence of cell nucleus. Edges connect the vertices of daughter and their progenitor cell nucleus object. Edge weights represent the time span information between any two nuclei. “TrackAlyzer” provides a comprehensive interface for visualizing and studying the cell lineages in three dimensions as a function of time. All properties of lineages, single cell nuclei and cell divisions within can be inspected and emphasized individually. After inspection of the data, “TrackAlyzer” was used to group cell lineages that belong to the same cell file and assign them an identification number and color. We term the cell file that contributes most of the cell mass the master cell file and labelled it #0. Cell files to the left were assigned negative indices (#-1, #-2, #-3), whereas cell files to the right were assigned positive indices (#+1, #+2, #+3). These indices express the position of a cell file relative to the master cell file. The graph representation of cell lineages and all assigned properties were saved in the *Mathematica* serialized package format (MX) optimized for fast loading and accessibility.

**Data interpolation.** In order to compare and visualize the datasets, we performed several processing steps on the raw lineage data. Since we identified the spatial coordinates of the first and last occurrence of a cell nucleus, we added linear interpolants in between. The spatial coordinates were determined by linear interpolation. For added nuclei objects, we adopted the lineage information of the first occurrence. The additional information was exported to a file in the XLSX format.

**Data standardization.** The LRPs do not have a particular orientation when imaged in the microscope. Therefore, nuclei positions are arbitrarily located in three dimensional space. In order to standardize our datasets we performed an affine transformation of the nuclei coordinates consisting of a translation and a rotation. The transformation ensures the orientation of the LRP in a new orthonormal base that is spanned by the principal component vectors of the cell nuclei coordinates that exist in the last time point of a dataset. The first principal component points along the longitudinal axis of the main root and represents the length axis of the LRP. The second principal component represents the height axis of the LRP and the third component points along the width axis of the LRP. For the translation, we determined the center of the LRP at each time point by computing the central position of the cell nuclei positions and smoothed the resulting list of center coordinates by applying a mean filter that had a range of ten time points. The translation is implemented by subtracting the computed mean nuclei position . We then performed a rotation transformation of the nuclei coordinates. According to Euler’s rotation theorem to describe extrinsic elemental rotations in three-dimensional Euclidean space , we define to be the rotation with the angle of degrees around the z-axis, to be the rotation with the angle of degrees around the y-axis, and to be the rotation with the angle of degrees around the x-axis. The rotation operation is commonly formulated as a matrix and the angle is expressed as counterclockwise rotation about a given angle around the axis. The sequence of extrinsic elemental rotations corresponds to a three-dimensional rotation group and can be expressed as in the x-y-z-convention (Goldstein reference) as the composition

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These extrinsic elemental rotations are generated with the *Mathematica* built-in functions “RotationTransform” and “Composition”. The composed transformation matrix is then applied to the coordinates of all cell nuclei of the dataset. The Euler angles , , were determined manually. The obtained standardized data was then exported to a file in XLSX format and was used for all successive processing and analysis.

**Data synchronization**. The lapse of time between gravity stimulation and the onset of imaging differs between the six recorded datasets. Therefore, we compensated for the temporal difference between the recordings by carrying out two kinds of data synchronization. For temporal synchronization of the datasets, we added the time span between gravity stimulation and the onset of imaging. A second approach was to synchronize on the number of cells.

**Computation of quantitative LRP properties**

**Growth rate.** To estimate cellular growth rate and doubling time for the datasets, we performed data fitting on the total number of cells for all dataset assuming an exponential growth model with the parametric form

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where *N(t)* is the number of cells at time point *t, a, b* and *c* are parameters. The parameter *b* is the growth rate in number of cells per time step. The parameter *c* is the initial number of cells at time point *t0*. The fitting is based on a nonlinear model fitting and was performed with the *Mathematica* function “NonlinearModelFit”. Additionally, the square roots of the total cell numbers at time point *t* were chosen as weights. The confidence level was set to 5%.

**Doubling time.** Computation of the cell doubling time for all datasets based on the growth rate determined by fitting an exponential growth model. The estimate for parameter b is multiplied by 100 to obtain the growth rate in percent. The doubling time is determined as . In all experiments a single time step corresponds to five minutes.

**LRP height.** We term the distance between the LRP tip and the parent root surface the height of the LRP. To determine the height as a function of time, we take the minimum and maximum distance along the y-axis in the master cell file. For that we use the standardized data as described above.

**LRP width.** The width of the LRP was calculated by taking the maximum distance between any two cell nuclei along the z-direction.

**LRP length.** In order to compute the length of the LRP along the x-axis, we first split the nuclei positions into two intervals based on the computed LRP height. The first interval ranges from the minimum along the y-axis up to 50% of the height. The second interval ranges from 50% of the height to the maximum along the y-axis (100% of the height). Volume: In order to estimate the volume of the primordium as a function of time, we compute the three dimensional convex hull based on the cell nuclei positions at each time point as described elsewhere (http://wias-berlin.de/software/tetgen/). The computation is based on the *Mathematica* package “TetGenLink”, which creates TetGen objects for each time point using the interpolated cell nuclei positions. The TetGen objects are then tetrahedralized and the volume is computed as the sum of all tetrahedra.

**Contribution of cells. Using the interpolated nuclei positions, we determined the contribution of cell files to the lateral root in terms of number of cells. When comparing the contributions of cell files between different datasets, we assigned distinct integer identification numbers. The cell file contributing most of the cell mass is termed the master cell file and is assigned the file #0. Cell files to the left of the master cell file (in the radial view) are assigned negative numbers, whereas cell files to the right are assigned positive numbers. Additionally, we investigated the contribution of founder cells to the LRP by determining the total cell numbers that belong to the each cell lineage.**

**Computation of cell layer assignment. We further investigated the corresponding cell layers for each dataset. We phrase an automatic layer generation, which creates up to four layers for each dataset over time. The visualization was realized with “OpenSceneGraph” (**[**http://www.openscenegraph.org/**](http://www.openscenegraph.org/)**). (Missing information: how are divisions classified?!). Our automatic cell layer assignment algorithm is based on the approach that each cell layer represents a three-dimensional surface, which we realized with a Delaunay triangulation of the cell nuclei positions. We used the cell layer surface in order to compute normal vectors for each cell nucleus. We classify cell divisions based on the orientation of the cell division axes of a dividing cell nucleus into three different classes. Anticlinal cell divisions are parallel to the long axis of the parent root. Periclinal cell divisions are oriented perpendicular to the surface of the parent root. If the angle between normal vector and the axes of cell division is greater than a chosen threshold, the cell division is classified to be periclinal. Otherwise the cell division is classified to be anticlinal. We validated the classification algorithm manually for each dataset and corrected misclassified divisions. We then inserted a third type of division which we term radial. We classify a cell division to be radial if it is oriented perpendicular to the long axis of the parent root. In order to complete the cell layer assignment, we use the information about a cell’s layer history to decide, to which layer the cell is assigned if the previous angle query is true. We further state that a new cell layer is generated if the parent cell divides periclinal. If it is a periclinal division, then the layer is extended by an additional cell nucleus.**

**Spatial and temporal patterns of cell division. To study the cell lineage information, we designed a structure map, which enables both a total overview of all existing lineages trees as well as a detailed highlighting and investigation of local structures in these trees. The structure map has a matrix-like structure, in which each entry is called a tile and includes a visualized unique lineage tree with cell file and data information. The map features an interactive color-coded feedback of descriptor information such as number of nodes, number of leaves, number of anticlinal, periclinal and radial divisions. The applied color map provides an immediate identification of similar appearances of such properties over all data sets and lineage trees.**

**Assignment of cell division type.** Cell division classification: angle measured in independent cell files reveal three types of division (anticlinal, periclinal, radial) that soon are no more clearly detectable as such because of the radialization process. Cell division classification with better division angle measurement that takes the dome shape surface bending into account.

**Computation of cell layer assignment.** After classification of the cell division types, we applied a layer assignment algorithm written in *Mathematica* to assign all cell nuclei to cell layers. We initialize the cell nuclei in the beginning to be members of cell layer #0. The progeny of each cell division that is classified to be periclinal is assigned as a member of two new cell layers. We, therefore, add trailing numbers to indicate the cell layer affiliation. The cell nucleus that is further apart in terms of Euclidean distance from the main root surface is assigned the indicator #2 whereas the closer cell nucleus is assigned the indicator #1. E.g., if a cell in layer #0 divides periclinal, the progeny will be assigned to cell layer #01 and #02, respectively depending on the Euclidean distance to the lateral root base. Thereby, we obtain all cell nuclei assigned to a cell layer.