

Supplemental Material 1: LiPlaCeT User Manual.

User Manual for cell tracking analysis of time-lapse experiments using the ‘Live Plant Cell Tracking’ (LiPlaCeT) Image J plugin.

September 2021

In this manual, we provide a step-by-step workflow to identify and track individual cells and their progeny using ImageJ plugin **‘Live Plant Cell Tracking’ (LiPlaCeT)**, followed by instructions to visualize and analyze cell population dynamics using Paraview software (<https://www.paraview.org/>). The plugin is developed to work with hyper-stacks of 3D images or 3D images in time (4D) acquired with a microscope; one or two channel images can be analyzed.

LiPlaCeT plugin has been developed to facilitate cell tracking and cell lineage analysis within time-lapse experiments of 3D cell populations. Cell information, including 3D position through time, proliferation events and lineage relationships, is saved automatically in the Graphical User Interface (GUI). Annotated cell information can be transferred to Paraview, and cell genealogy tree can be constructed. Other information can be extracted such as cell cycle time, dynamics of cell lineage formation, visualization of cell growth and displacement of individual cells or the whole population and others.

Manual tracking using LiPlaCeT is an essential step to enable data analysis and visualization. The Paraview platform facilitates visualization of tracking analysis because it has many functionalities such as visualization of data in 3D and time (4D), change the view positions, rotate, translate the data, zoom in, zoom out, screen shots, movies, a variety of colormaps, time inspector to move time by time, among many others functionalities. Description of the uses of Paraview platform is also described in this manual (Section 4).

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1 Requirements and Installation

1.1 Software Requirements

Operating System: LiPlaCeT plugin has been tested in Windows 10, Linux (Ubuntu 20.04.2 LST) and MacOS

64 bits Operating System

RAM: If the user wants to track using a single hyper-stack, then the RAM memory size has to be as much as the hyper-stack size to be analyzed. If the user does not have enough RAM memory, we have also developed an approach for this case (see Section 2.10).

FIJI (<https://imagej.nih.gov/ij/download.html>)

Paraview (tested with version 5.8.1, Section 1.4 describes detailed instructions on installing Paraview)

1.2 Image Requirements

3D/4D hyper-stack (czt, (channel, slice, time)) with 1 or 2 channels.

8- or 16-bit images.

1.3 Installing LiPlaCeT plugin

- 1 Download the plugin as a JAR file and a dataset example from:
https://drive.google.com/drive/folders/1v3JwXABnBa_ewgfXxarFUGOPCnGTb8fS?usp=sharing or request the plugin from the author directly (gabriel.corkidi@ibt.unam.mx)
(LiPlaCeT_-3.0.0.jar and dataset_release.zip).
- 2 Open the FIJI distribution from ImageJ
Drag the JAR file to the FIJI window (Figure 1, red rectangle), Save Plugin at the suggested location of Image J: “plugins” folder.
- 3 Restart FIJI
- 4 Open a hyper-stack. If the plugin has been installed correctly, it should be accessed from Plugins → LiPlaCeT → GUI. If the plugin cannot be found, then something went wrong during installation. In this case, repeat steps 1-3.

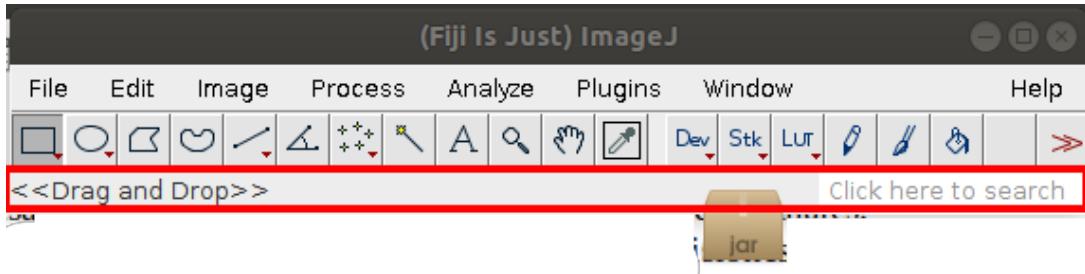


Figure 1: Installing LiPlaCeT plugin into FIJI. Drag the downloaded LiPlaCeT_-version.jar file onto the Drag and Drop bar.

1.4 Installing Paraview

The following steps allow installing Paraview:

1 Windows

Go to the download page of Paraview <https://www.paraview.org/download/>. Choose your operating system (See Figure 2 red rectangle) and Paraview version (See Figure 2 green rectangle). Note that the plugin is compatible with version 5.8.1, however it has also been compatible with other versions. Then, download the executable file (Figure 2 blue rectangle). After the download is finished, double click on the executable file to install Paraview. Follow the installation instructions.

2 Linux

Go to the download page of Paraview <https://www.paraview.org/download/>. Choose your operating system (See Figure 3 red rectangle) and Paraview version (See Figure 3 green rectangle). Then, download Paraview (Figure 3 blue rectangle) and move the downloaded file to a folder where Paraview could be easily found (Figure 4(a)). Next, extract the files. Now, Paraview can be opened using any of the following options:

Option 1: Go to the folder where the files were extracted and open the **bin** folder. Then, double click on **Paraview file** to open Paraview interface (Figure 4(b)).

Option 2: Open terminal and move to the bin folder, then type `./paraview` and press <Enter> to open Paraview interface.

Get the Software

You can either download binaries or source code archives for the latest stable or previous release or access the current development (aka nightly) distribution through Git. Specific license information can be found [here](#). This software may not be exported in violation of any U.S. export laws or regulations. For more information regarding Export Control matters please go to https://kitware.com/export_control/index.html.

Version v5.8

ParaView

Sources Windows Linux macOS

Full suite of ParaView tools, including the ParaView GUI client, pypython, pvserver, and pvbatch. Versions with MPI in the name require MS-MPI.

File	Last Modified	Size
ParaView-5.8.1-Windows-Python3.7-msvc2015-64bit.zip	Aug 4 23:09	464.1M
ParaView-5.8.1-Windows-Python3.7-msvc2015-64bit.exe	Aug 4 23:21	193.2M
ParaView-5.8.1-MPI-Windows-Python3.7-msvc2015-64bit.zip	Aug 4 21:35	468.2M
ParaView-5.8.1-MPI-Windows-Python3.7-msvc2015-64bit.exe	Aug 4 21:47	194.2M
ParaView-5.8.0-Windows-Python3.7-msvc2015-64bit.zip	Feb 18 09:10	463.0M
ParaView-5.8.0-Windows-Python3.7-msvc2015-64bit.exe	Feb 18 09:08	192.4M
ParaView-5.8.0-MPI-Windows-Python3.7-msvc2015-64bit.zip	Feb 18 09:11	467.2M
ParaView-5.8.0-MPI-Windows-Python3.7-msvc2015-64bit.exe	Feb 18 09:08	193.5M

Figure 2. Paraview download page for Windows.

Get the Software

You can either download binaries or source code archives for the latest stable or previous release or access the current development (aka nightly) distribution through Git. Specific license information can be found [here](#). This software may not be exported in violation of any U.S. export laws or regulations. For more information regarding Export Control matters please go to https://kitware.com/export_control/index.html.

Version v5.8

ParaView

Sources Windows Linux macOS

Full suite of ParaView tools, including the ParaView GUI client, pypython, pvserver, and bundled MPI.

File	Last Modified	Size
ParaView-5.8.1-MPI-Linux-Python3.7-64bit.tar.gz	Aug 4 21:57	443.1M
ParaView-5.8.1-MPI-Linux-Python2.7-64bit.tar.gz	Aug 4 21:54	434.4M
ParaView-5.8.0-MPI-Linux-Python3.7-64bit.tar.gz	Feb 18 09:09	442.6M
ParaView-5.8.0-MPI-Linux-Python2.7-64bit.tar.gz	Feb 18 09:09	433.9M

Figure 3. Paraview download page for Linux

(a)

(b)

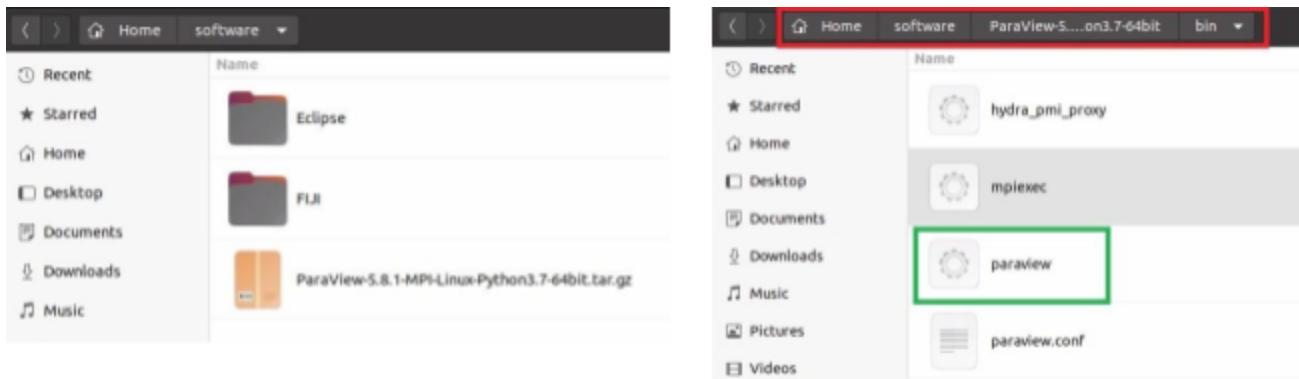


Figure 4. (a) Copying the downloaded file to a folder where it can be easily located. (b) Double click on Paraview to open it.

2 Cell tracking using LiPlaCeT Plugin

2.1 Importing image stacks in FIJI to work with LiPlaCeT plugin

The first requirement to initialize the LiPlaCeT plugin is to open a 3D/4D hyper-stack (see Section 1.2 for image requirements). Figure 5 depicts an example of a 4D hyper-stack acquired with 2 channels.



Figure 5. Example of hyper-stack that contains 2 Channels, Z-Slices and Time Frames. A developing lateral root primordium in *p35S::H2B-RFP pUBQ10::NPSN12-YFP* F1 *Arabidopsis thaliana* seedlings. Nuclei and plasma membranes are marked in red and green, respectively.

2.2 Opening LiPlaCeT Graphic User Interface (GUI)

Once an appropriate image file is opened, it is possible to open the LiPlaCeT GUI interface by going to **Plugins → LiPlaCeT → GUI**. The plugin interface appears as shown in Figure 6.

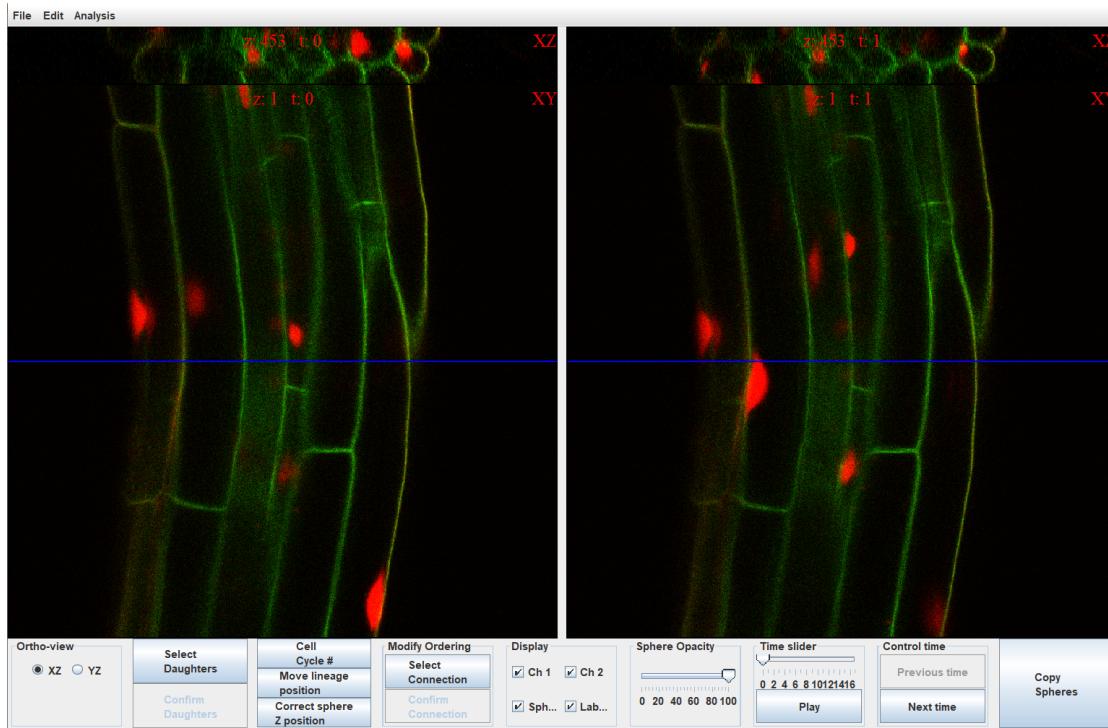


Figure 6. LiPlaCeT GUI interface with the main options used for cell tracking.

2.2.1 LiPlaCeT GUI features

The LiPlaCeT GUI, shown in Figure 6, displays two windows; the images in the upper portion display the orthogonal view from the stack in the XZ plane, which can be changed to display the YZ plane. The bottom images correspond to slices from the XY plane. Each XY, XZ, or YZ plane is indicated at the top right corner. The window at the left displays the images for time t , while the window at the right displays the images for time $t+1$. Labels are added to the images to identify the time and Z-slice number that are currently displayed. Finally, the buttons at the bottom of the interface have been designed to facilitate cell lineage tracking and their functions are described through this section.

New features are added to the plugin frequently, it is recommended to have the most recent plugin and manual version. When a newer version is available, follow plugin installation instructions and when prompted over-write the LiPlaCeT_-version.jar file.

2.3 Hyper-stack navigation

An important feature of the plugin is the ease to navigate across the hyper-stack (three orthogonal stack planes corresponding to XY, XZ and YZ and time). Navigation through slices (Z-sections) allows moving across the different images of a stack. The options to navigate across the hyper-stack are the following:

2.3.1 Moving slice with mouse wheel

Position the mouse point in the plane (XY, XZ or YZ) outside any sphere (about cell markings by spheres see the section 2.5). Then, move the **mouse scroll up or down** to move towards one image up or down, respectively. If the “display label” function is enabled, then the label (text displayed in the upper center of the image) for a slice is updated according to the new slice position. Moving through slices updates the sphere size and displays new spheres if they fall in the current slice (according to its position).

Important notes:

- (1) When left window, scrolling the mouse wheel only affects the position in this window, however scrolling the mouse wheel in the right window automatically moves the slices in both windows. This synchronized slice movement is very important to allow comparison between the cells belonging to the same slices and to easily visualize mitotic events in any plane.
- (2) At the top center position of both windows, slice numbers and time points appear and change automatically.
- (3) At the top right position of each window, the slice plane label is displayed.

2.3.2 Moving slice from view XZ or XY using blue line

The blue line indicated in the XY view displays the slice where the XZ or YZ is extracted.

This line allows easy navigating across XZ or YZ views, which is convenient because these planes usually have many slices. To this end:

To change the XZ or XZ position, locate the **mouse pointer near the blue line**. Then, press the **right button mouse** and, without releasing it, **move the line up or down**.

2.3.3 Changing orthogonal view XZ/YZ

Sometimes one plane (XZ or YZ) can be used to better visualize the orthogonal view for a target cell. The plugin allows easily changing between any of these two orthogonal views:

To change the orthogonal view, go to the bottom left corner of the LiPlaCeT GUI to locate the panel **“Ortho-view”** with radio button options. Then, select the plane to visualize (XZ or YZ).

2.3.4 Move through time frames

Moving between time points is as easy as pressing one button/key. There are three options two move across time:

Option 1: From the panel **Control time**, push the button **Previous time** or **Next time** to navigate time points of the hyper-stack.

Option 2: From the panel **Time slider**, move slider or press **Play** button.

Option 3: From the **keyboard**, press key **N/P** to move to the next or previous time point of the hyper-stack.

Important note: Moving to previous time is disabled for the initial time point (there is no previous time) while next time is disabled for the last time point of the hyperstack.

2.3.5 Zoom in/Zoom out

Small details can be difficult to observe using the full image resolution; therefore, we include an option to Zoom in/Zoom out the image.

Zoom in: locate the mouse pointer to the center of the region of interest and press the **key e** (enlarge) of the keyboard or **key +** (plus) from the keypad. Figure 7 displays an example of zooming in an image.

Zoom out: locate the mouse pointer to the center of the region of interest and press the **key d** (diminish) or the **key -** (minus) from the keypad.

Note: This functionality is only available for the XY window, which automatically updates the XZ or YZ views.

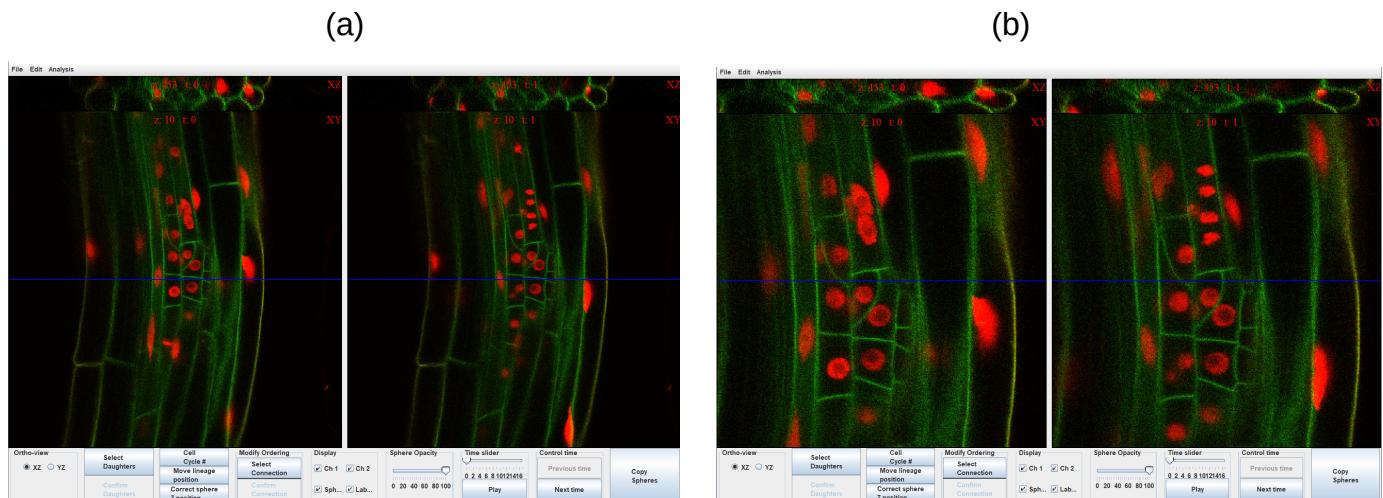


Figure 7. Zoom in/Zoom out options. (a) LiPlaCeT image panel covering the full image size (b) zoom in image to the region of interest.

2.3.6 Move through the field of view

Zoom in the image would result in a smaller field of view. The user can change the center of the field of view by locating the **mouse pointer** in the **new center** for the field of view. Then, **press the space key** of the keyboard.

2.4 Display options

The user has the following options to control the display from the interface, they appear on **Display** panel at the bottom middle of the LiPlaCeT interface.

2.4.1 Activate/deactivate channels to display

Activate/deactivate channels to display: For a two-channel image, the default parameter is to display both channels; however, sometimes it can be easier to visualize cells in a specific channel. To activate or deactivate a channel follow this step:

From the **Display** panel, **check or uncheck Ch 1** and **Ch 2** to activate or deactivate Channel 1 and Channel 2, respectively.

Note: If the two Channels are deactivated, only the created spheres (see section 2.5) are displayed. Figure 8(a) shows an example where Channel 1 and Spheres have been deactivated (in a 4D hyper-stack, this channel corresponds to cell walls), while Channel 1 corresponds to cell nuclei. Figure 8(b) displays an example where the two channels have been deactivated, therefore, only the spheres are displayed.

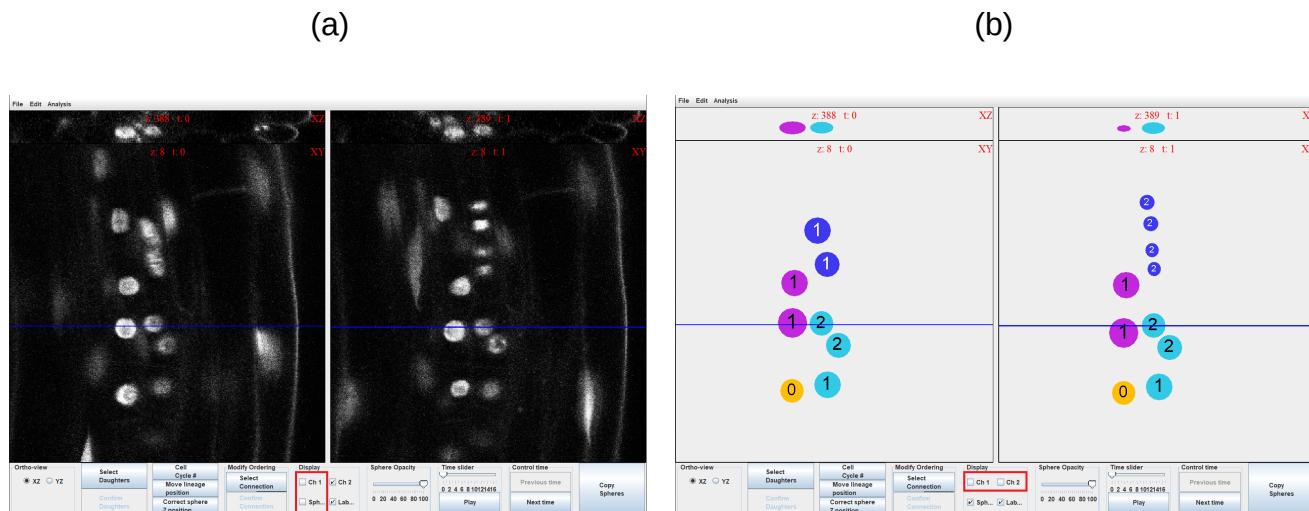


Figure 8. Channel options. (a) An example of deactivating channel 1 and spheres; and (b) an example of deactivating both channels; only spheres appear.

2.4.2 Activate/deactivate spheres

Our plugin allows hiding the created spheres, to this end:

From the **Display** panel, **check or uncheck** the square **Spheres**

2.4.3 Activate/deactivate labels

LiPlaCeT plugin allows hiding the label, which displays the information for current time and slice, to this end:

From the **Display** panel, **check or uncheck** the square **Labels**

2.4.4 Increase/decrease opacity

It is useful to increase or decrease opacity of the spheres to see certain details of the image hidden by the spheres. To this end:

Move the slider from the panel **Sphere Opacity** panel to the left to decrease opacity or to the right to increase opacity. Figure 9(b) shows an example where the sphere's opacity was decreased to 40.

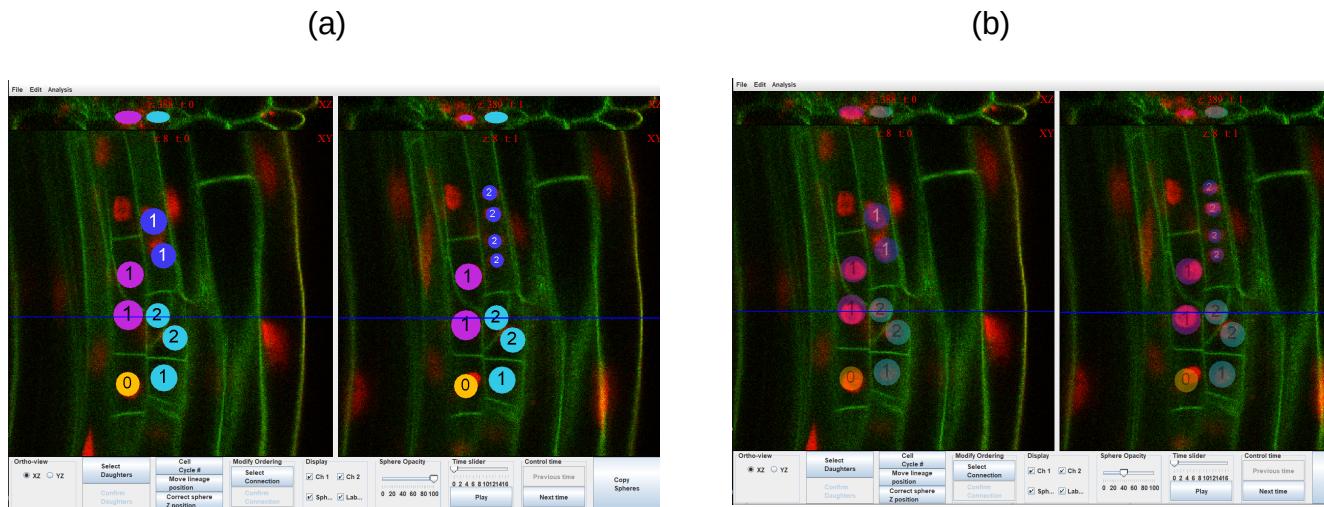


Figure 9. Modifying sphere opacity. (a) An example of displaying spheres without reducing opacity, (b) an example of reducing opacity of the spheres to 40.

2.4.5 Change channel color

Default colors for channel 1 and 2 are green and red, respectively (see Figure 10(a)). However, another channel combination can enhance the visibility of some details.

LiPlaCeT plugin allows to easily changing channel color:

Go to the menu **Edit**. Then, select **Set Color**, and choose **Channel 1/Channel 2**, a color picker window will be displayed where the user can select the new color for the channel 1 or 2, respectively.

Figure 10(b) shows an example where channel 1 color was set to blue while channel 2 was selected as yellow.

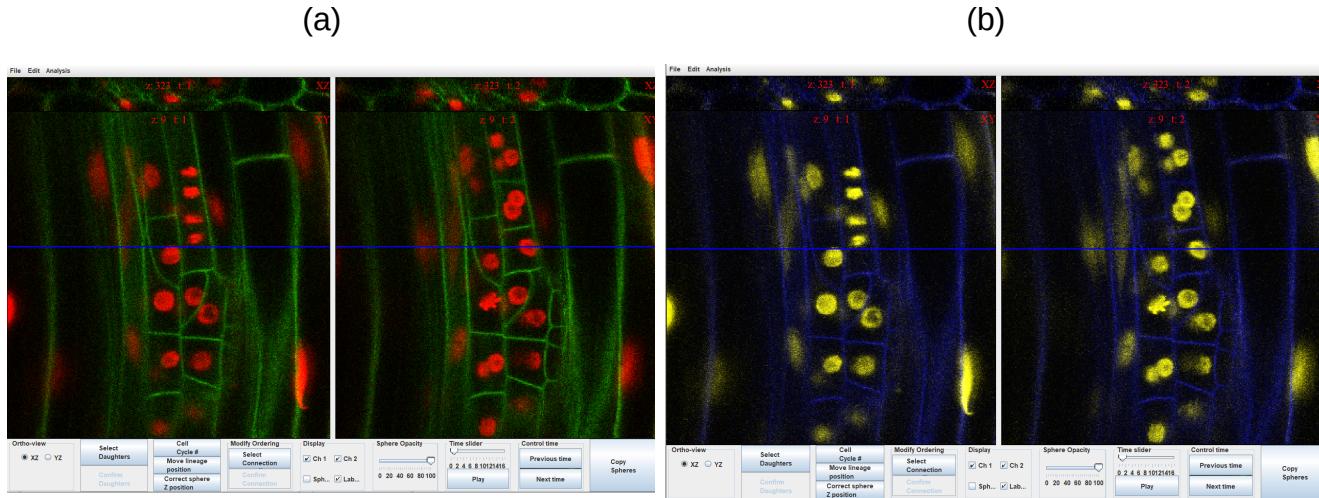


Figure 10. An example of changing color of channels (a) default colors for channel 1 and 2 are green and red, respectively, (b) changing colors of channel 1 to blue and channel 2 to yellow.

2.4.6 Change image brightness

Brightness can be changed easily:

Go to the menu **Edit**. Then, select **Set brightness** and choose **Channel 1/Channel 2**, a window will be displayed where the user must introduce the new brightness range.

Important note: The brightness values must be in the range [0,255] even for 16 bits images. To return to the original image, go again to **Edit – Set brightness**, choose **Channel 1/Channel 2** and press the button “Cancel”. Figure 11 shows an example where the brightness was modified.

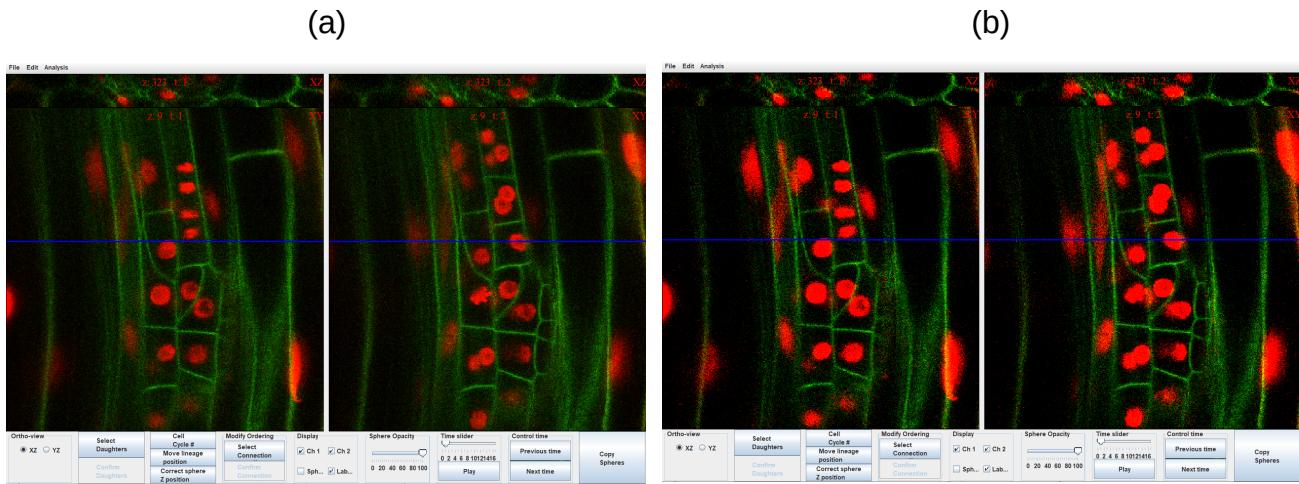


Figure 11. Examples of modification of brightness of the images. (a) hyper-stack with the original brightness and (b) brightness reduced in channel 1 to [20, 200] and channel 2 to [20, 100].

2.5 Cell tracking using LiPlaCeT plugin

In the plugin interface, each identified cell can be manually marked with a uniquely colored sphere. This permits subsequent identification of a cell lineage. The sphere also has a number, which identifies the current cell cycle.

2.5.1 Creating a sphere to identify a specific cell

Position mouse cursor over a nucleus of interest and make a left click of the mouse to identify it. Note that giving a left click in an already created sphere will not create a new sphere. Figure 12 displays some cell markings created in the GUI.

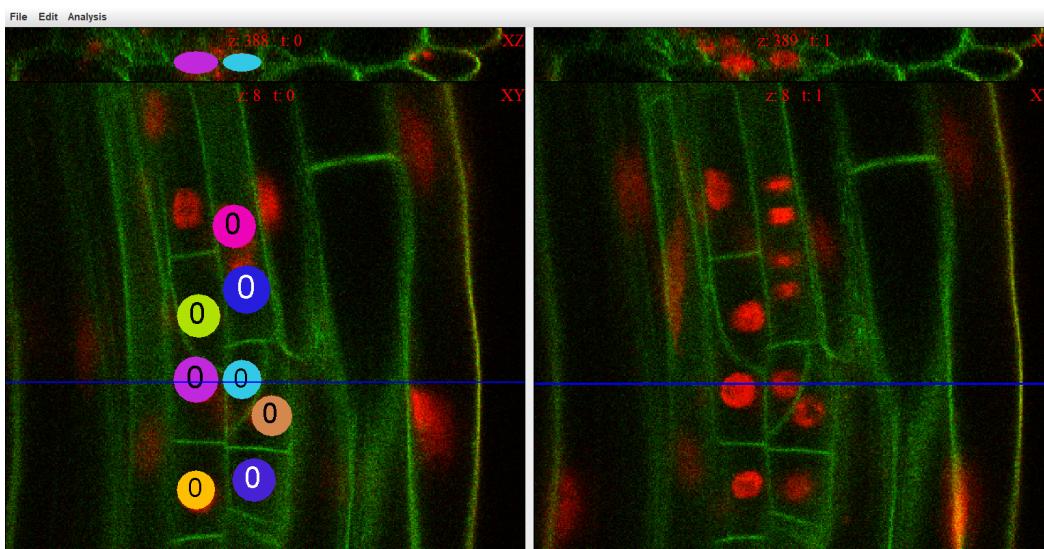


Figure 12. Creating/marketing initial cells at time $t=0$.

2.5.2 Adjust sphere size

The initial sphere size is already predefined. To adjust sphere size, position the mouse inside the sphere and move the mouse wheel up or down to increase or decrease the sphere size, respectively.

2.5.3 Eliminate a sphere

A sphere may have been created by error or it may not be necessary for the tracking. To eliminate a sphere, position the mouse inside the sphere to eliminate and right click, then the sphere would be eliminated from tracking.

2.5.4 Identifying the same cell in a subsequent time frame (copy spheres tracked at time t to time $t+1$)

If temporal resolution is short enough to allow users to visually recognize individual cells across time frames, then it is reasonable to assume that sphere position at time t must be located at similar positions at time $t+1$. To copy spheres to time $t+1$, do the following:

Press the button “**Next time**” which permits you to observe the images for **time $t+1$** in the right window. Then, press the button “**Copy spheres**” that permits all the spheres at a previous time point (left window) to appear in the right window. You can use keyboard letter **C** (copy) to perform the same command. Each new individual cell position can be manually adjusted as needed (see section 2.6)

If no division is found, go to the next time point (repeat step 2.5.4). Otherwise, go to step 2.7.1.

2.6 Adjust sphere positions to reflect new cell position in subsequent times

Nuclei in plant cells can move significantly. If from time t to time $t+1$, the nuclei (or marked cells) move, manual correction may be required. The plugin allows you to introduce corrections. This correction may also be required if during image acquisition, a drift occurred.

2.6.1 Move a single sphere (cell)

Cell position could be incorrectly located, hence it is necessary to have the option to correct sphere position. In order to do this:

Position the **mouse inside the target sphere**. Then, **drag** (left click mouse without releasing it) the **sphere to the new position**. Note that an important feature of the plugin is that **the user can** move the sphere (cell) position **using any of the three orthogonal views** (XY, XZ, YZ), therefore the user can correctly place the cell position in 3D.

2.6.2 Move spheres in view XY

To move cells in the XY view, the user has the following options:

Option 1: **move all spheres** simultaneously in XY:

Identify the panel XY where the target spheres are located (left or right window). Then, click inside the panel and **outside any sphere**. Finally, drag the mouse (left click mouse without releasing it) until the spheres reach the new position. Figure 13(b) depicts an example where the spheres (right image from the bottom) were correctly located to the new position. Copying the spheres (Section 2.5.4) resulted in an incorrect position depicted in Figure 13(a).

(a)

(b)

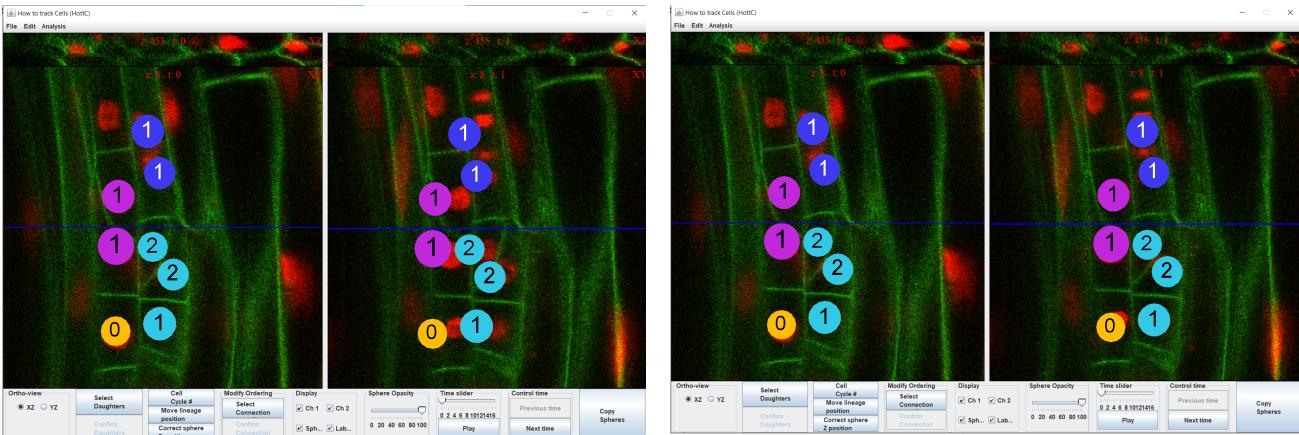


Figure 13. (a) Copying spheres to next time points using the **Copy Spheres** button may position the spheres at the right panel in the incorrect position due to several factors, for example drift; (b) Adjusting position of spheres by only dragging the mouse.

Option 2: permits **movement of only the same lineage** (spheres with same color) in XY. To achieve this:

Press the button Move Lineage Position (the button must change to green color to indicate that it is active). Then, **select** with a left click mouse any cell from a **lineage** by positioning the mouse over one cell of a lineage (sphere color must change to green). In the XY window, position the cursor outside the cells to be moved, maintain left mouse click pressed and **drag the mouse to move the spheres** corresponding to the same lineage to the desired position. If the user is satisfied with the new location, **press the Move Lineage Position button to finish the process**. The button color must change to gray to indicate that it is inactive.

Move spheres in Z-axis

Drift can also occur in the Z axis, to correct this drift the user can move cells in the Z axis. In addition, cell displacement in a growing organ may require correction in Z position. To move spheres in Z axis:

Option 1: **move all spheres in Z with keys “arrow up” and “arrow down”**.

Position the mouse cursor in a window outside any sphere and with arrows up and down change the position of spheres. Note that while the Z-position of spheres is changing, the Z position of hyperstack is maintained the same. To adjust it, move the mouse wheel.

Option 2: **move specific spheres with numeric keypad**

Push the button **Correct Sphere Z position**. Then, select one by one all the spheres whose position should be updated. Finally, indicate the desired Z section number using the numeric keypad. To confirm the selected position, press the button **Correct Sphere Z position** again.

2.6.3 Move cells of the same lineage in XY or Z axis (spheres with the same color)

Sometimes it can be useful to move only the cells corresponding to the same lineage (cells with the same color). To achieve this, do the following:

Press the button **Move Lineage Position** (located at the bottom panel of LiPlaCeT GUI, see Figure 6, the button must change to green color to indicate that it is active). Then, select click mouse **any** cell from a lineage to be moved by positioning the mouse over that sphere (sphere color must change to green). Press **key up** or **key down** to move the same lineage spheres to desired **Z position**. **Drag the mouse in the XY plane** (left click mouse without releasing it) until the spheres reach the **new position in XY**.

If the user is satisfied with the new location, push the button **Move Lineage Position**. The button color must change back to gray to indicate that it is inactive.

Building a lineage: Identify new daughter cells

An important feature of our plugin is allowing the user to manually identify mitotic events. These are important for analysis of cell cycle time, drawing a cell genealogy tree, etc. The user must perform the following steps to identify mitotic events in the GUI:

2.6.4 Identify new daughter cells (identify a cell division)

If division occurred (see Figure 14a), identify new daughter cells by the following steps: Move the sphere of the mother cell (from window corresponding to time $t+1$) to one of the daughters, and then **create a new sphere** to mark the other daughter (see Figure 14b). Then, push the button “**Select daughters**” of the interface, the button color should change to green to identify that it is currently active (See Figure 14(b) buttons). Finally, position the cursor over the sphere of each daughter cell and make a left click. This will mark the mother cell (at left window) and daughter cells (at right window) in green color (See Figure 14(c) left panel). Press the “**Confirm Daughters**” button to assign a cell division and increase cell cycle number (it will increase by one) (See Figure 14(d)).

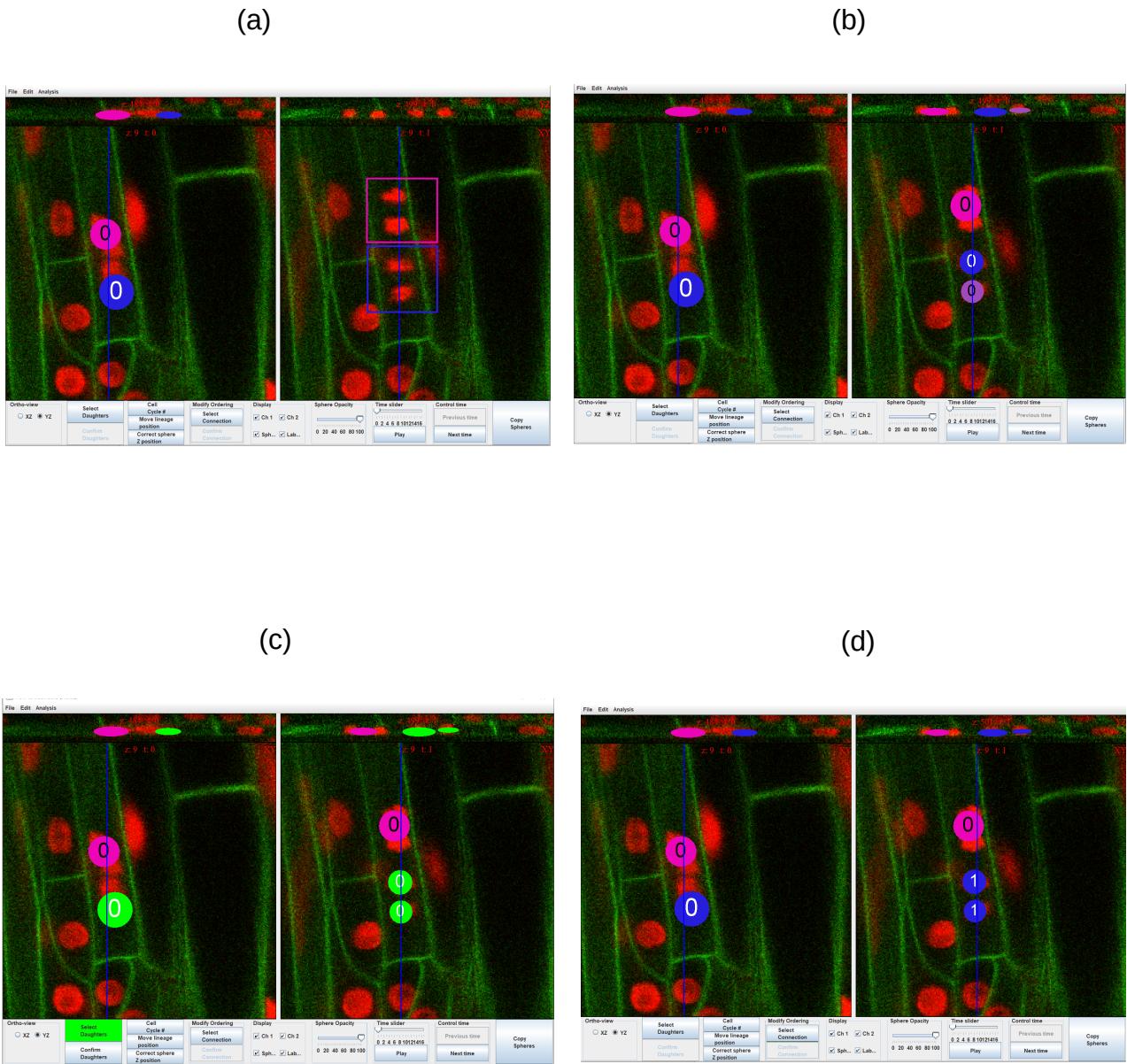


Figure 14. Building a lineage. (a) Identifying mitotic events, blue and pink spheres at the left panel are divided (blue and pink squares) at the next time point (right panel). (b) Creating spheres, spheres from the left panel are copied to the right panel, and the mother cell (blue) is moved to one daughter while another sphere is created (violet) to identify the other daughter. (c) Selecting spheres created by division using the button “Select Daughters”. The mother is automatically selected. (d) A division is identified where the blue color marks that the spheres belong to the same lineage as mother and cell cycle number is increased.

2.6.5 Identify same cell between consecutive time points

Additional functions of the buttons “Select daughters” and “Confirm Daughters”: These buttons can also be used to indicate the same cells between time points. If at time $t+1$

a new cell is marked by sphere with one color, but at time t it was not marked and later introduced, to indicate that this is the same cell, activate the button “Select daughters”, click over this cell in left and right window and then push the button “Confirm Daughters”. Correct cell cycle number as described in section 2.6.3.

2.6.6 Correction of the cell cycle number if needed

If by any reason a correction of cell cycle number is required (e.g. at a time point when cells are a part of a lineage, but no previous time points are available to trace the lineage to their mother cell) perform the following:

- i Push the button **Cell Cycle #** from the interface, the button color must change to green to identify that it is active.
- ii **Select the target sphere** by left click of mouse to change cell cycle number.
- iii **Press key M** (more) or + (plus) to **increase** cell cycle number. **Press key L** (less) or - (minus) to **decrease** cell cycle number.
- iv Repeat step (ii-iii) for all the cells that need to have their cell cycle number corrected.
- v **To finish** the process, push the button **Cell Cycle #** of the interface, the button color must change to gray to identify that it is inactive. Figure 15(b) depicts the correction of cell cycle number for cyan spheres.

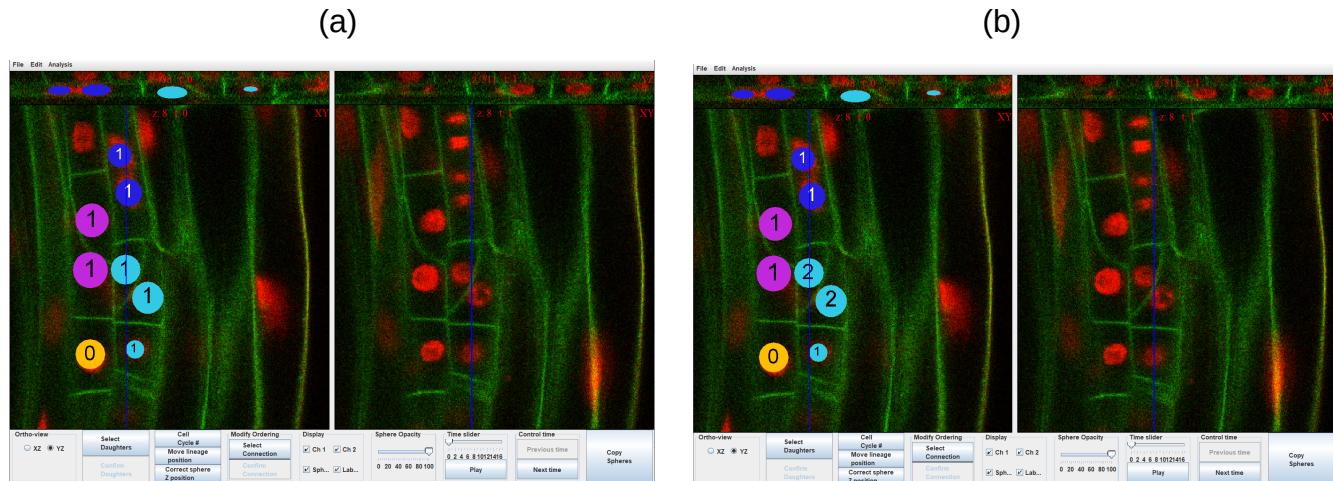


Figure 15. (a) Identifying incorrect cell cycle number and (b) correction of the number.

2.6.7 Identifying cells belonging to same lineage at time $t=0$

At the initial time point it may be possible that an expert can identify cells that have already initialized their lineage in previous times (however the user can not identify

their divisions because the acquisition started at time $t=0$), there is an option to identify cells belonging to the same lineage at time $t=0$.

Create the same lineage for cells: Press the button **Select Daughters** of the interface, the button color should change to green to identify that it is currently active. Select the spheres belonging to the same lineage, the selected cells would change their color to green. Once that all the spheres in the same lineage have been selected the user must push the button **Confirm Daughters** to create a new lineage. The selected spheres would be assigned to the same sphere color to identify that they belong to the same lineage (see Figure 16), and the cell cycle would increase by one.

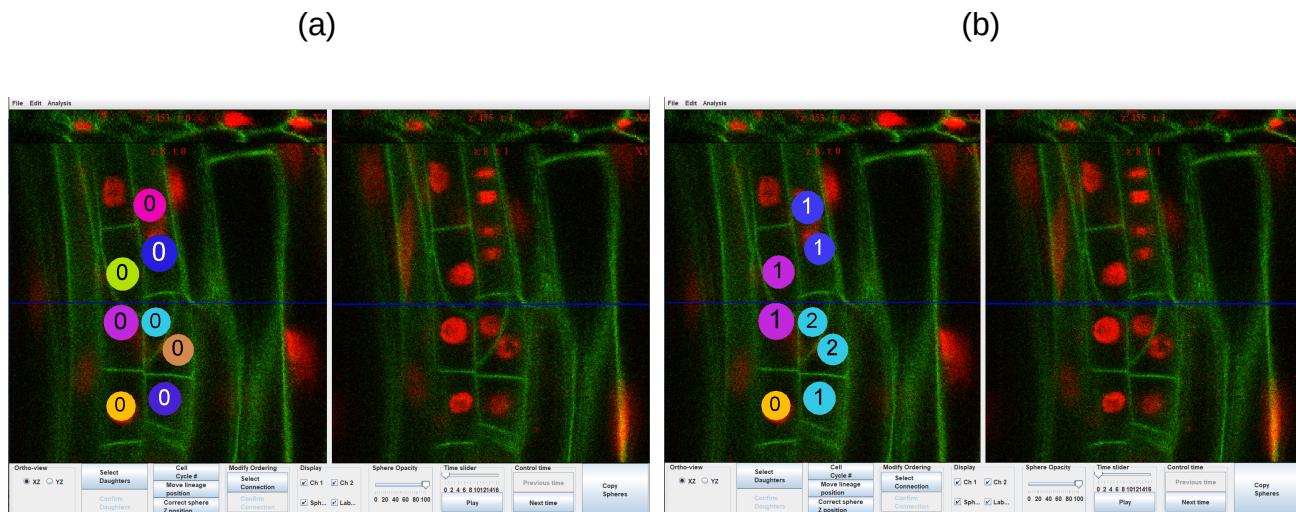


Figure 16. Creating the same lineage at initial time. (a) creating initial spheres at the first acquisition time, (b) four different initial lineages were detected where each color identifies a different lineage.

2.7 Example of tracking cells according to their relative position. Case study: ID assignment within a cell file in Arabidopsis RAM

2.7.1 Track cells

In the provided example, we analyze cell proliferation dynamics in an endodermis cell file within the root apical meristem (RAM) of an *A. thaliana* seedling primary root. To study the dynamics of this cell population, it is important to gather information from each cell such as position, length, time and position of proliferation events, and lineage history. However, to understand the complex dynamics of this transit amplifying region, other parameters, which are relative to the neighboring cells and the root apex as a whole 3D structure, can be instrumental to shed light on the short and long-range regulatory processes that result in root growth responsive to genetic, chemical and environmental queues. To this end, we measure parameters useful to calculate relative values such as cell growth rates across the whole tissue, cell displacement rate relative to the most apical cell of the file, and whole-tissue proliferation activity through time. To this end, analysis of cell proliferation requires tracking cells and their lineage information as previously described (section 2.7). To track cells in the context of the growing root, an additional descriptor is necessary in order to describe cell position as distance relative to a coordinate hallmark (in this example, the most distal root cell in a file). LiPlaCet sorts the spheres such that the sorting describes their distance to the root tip and growth direction of the root apex.

2.7.2 Sorting cells for initial time $t=0$

2.7.2.1 *Creating initial cells*

The first sphere created must be the one at the tip as depicted in Figure 17(a), and then the user must identify the second cell in the row, which corresponds to the yellow sphere in the example shown in Figure 17(b). Finally, the user creates the third sphere in the row, which is marked with pink color (see Figure 17(c)). Note that the spheres were created in ascending order to distance to the root tip. In this example only one row was tracked but the user can track many rows at the same time.

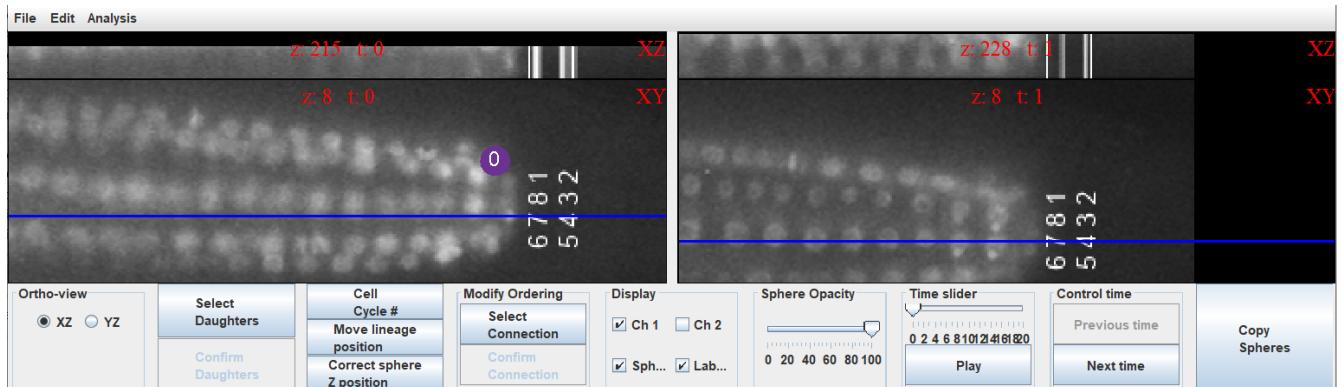
2.7.2.2 *Changing sphere label*

The sphere number by default corresponds to cell cycle number, in order to **visualize the sorting information** in the sphere go to: **Edit**, then select **Set Sphere Label**, and select **Sorting Information**, the sorting information is displayed at the center of the sphere as depicted in Figure 18(a). The first number corresponds to the cell id, while the second number corresponds to its neighboring cell with closest distance to the root tip.

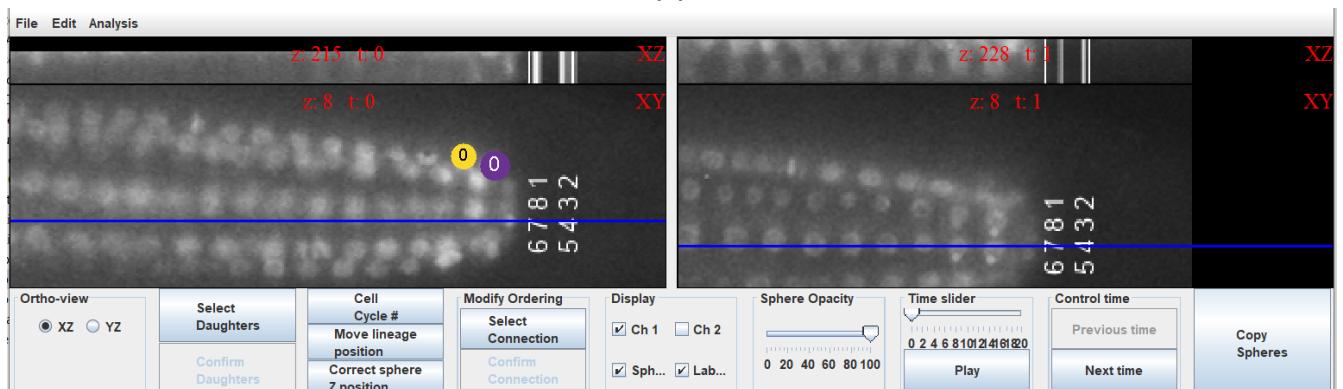
2.7.2.3 Updating sphere sorting information

Updating sphere sorting information: Note that Figure 18(a) has cell id equal to -1 for all cells indicating that sorting information has not been update. In order to update the sorting information go to **Analysis**, then select **Update IDs sorting**. The sphere id and the neighboring sphere id is updated (see Figure 18(b)). A neighboring sphere id equal to -1 means that this sphere is the tip of the root.

(a)



(b)

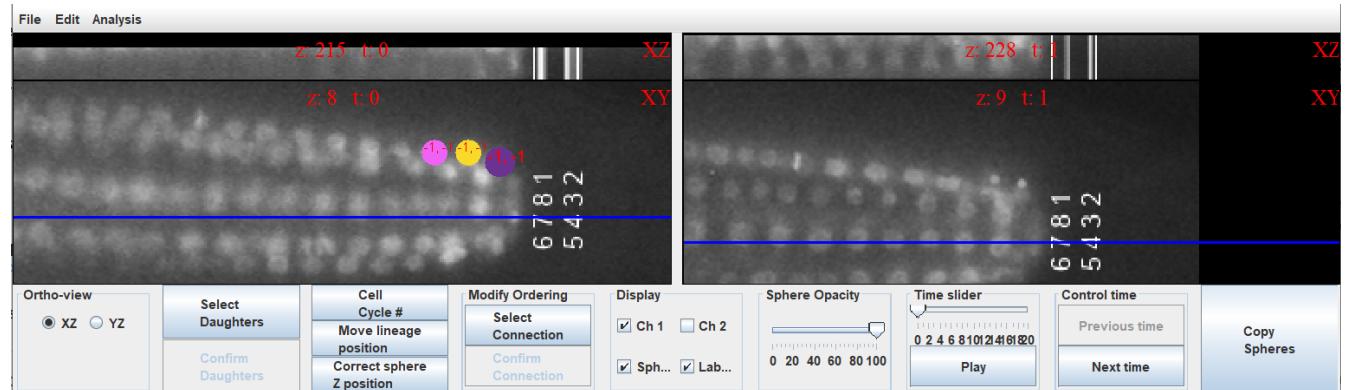


(c)



Figure 17. Creating sorted cells: (a) violet cell corresponds to the tip of the root, (b) the next cell from the row to be tracked corresponds to the yellow sphere, (b) while pink sphere corresponds to the third cell in the cell file.

(a)



(b)

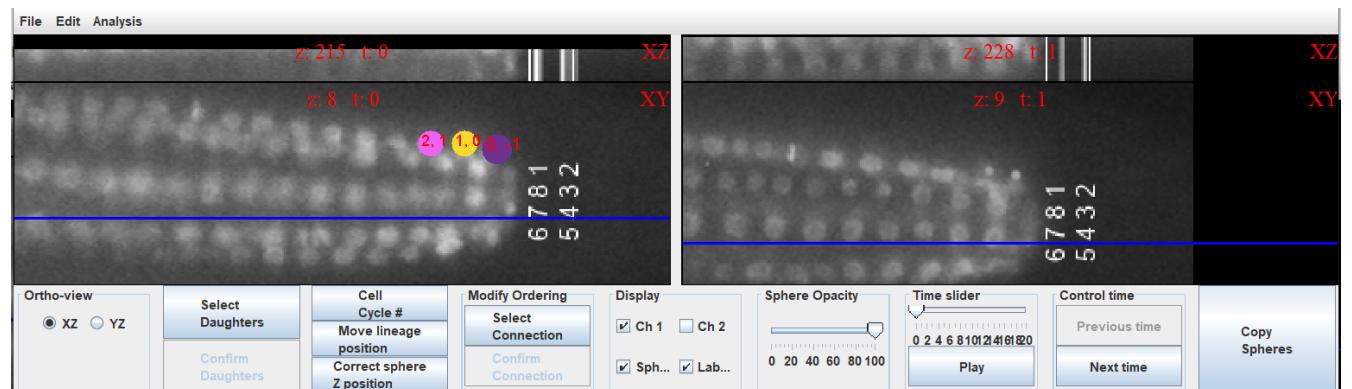


Figure 18. Updating sorting information: (a) creating the spheres for row 1, but the sorting has not been updated. (b) updating the sorting information, the first number corresponds to the cell id while the second number corresponds to its neighboring cell that is closest to the root tip.

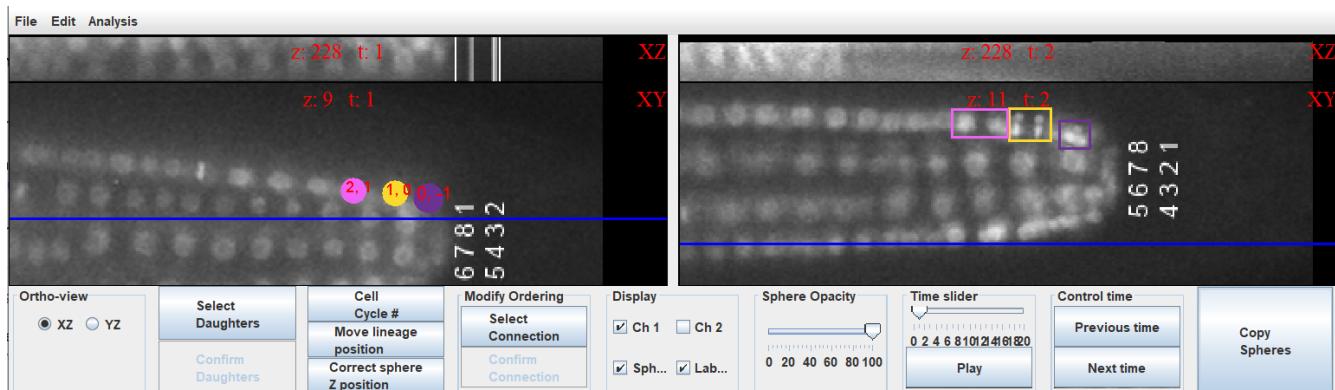
2.7.3 Creating sorting for $t > 0$

Sorting at time t , requires the previous time ($t-1$) already been sorted. For example, we can initialize the sorting as described in section 2.8.2.1 to continue with $t=1$ as explained in this subsection.

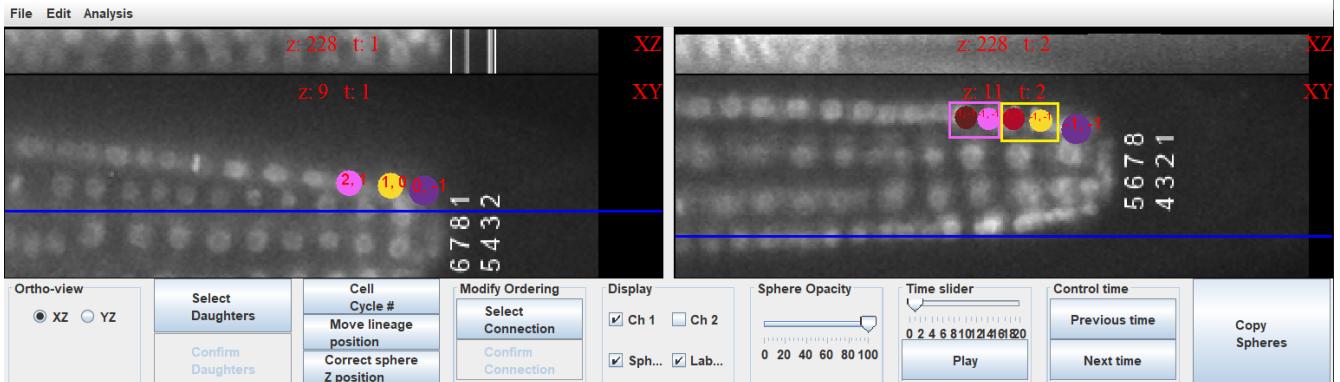
2.7.3.1 Track cells with sorting information

To track cells, identify each division and sort the spheres according to the following rule. If cell division is detected (see Figure 19(a)), then the user must **create a mitotic event** as explained in Section 2.7.1. In case of transverse cell division, the mother nucleus **sphere** must be located at a closer distance to the root tip than the created spheres which must be located at a farthest distance from the tip. Figure 19(b) depicts an example of creating the correct order for a mitotic event where the mother cell for yellow and pink nuclei at the right panel are located at a closest distance to the tip (violet sphere) than the daughters spheres identified with red and brown spheres which are located farther from the tip. Now that spheres are sorted according to distance to the root tip, the user can **create the mitotic event** to identify that yellow and red spheres belong to the same lineage while pink and brown spheres belong to other lineage as explained in Section 2.7.1(see Figure 19(c)). Finally, update the sorting information from spheres by going to: **Analysis** and then selecting **Update IDs sorting**. Figure 19(d) shows the result of updating the sorting information, ID and neighboring cell is automatically and correctly updated for each sphere because we are following the rule previously described. Note that for sphere id 4 its neighboring sphere is 3, while for sphere 3 its neighboring sphere is 2, and so on until sphere id 0 with neighboring sphere -1, indicating that sphere id 0 marks the most distal cell of the cell file in root apical meristem.

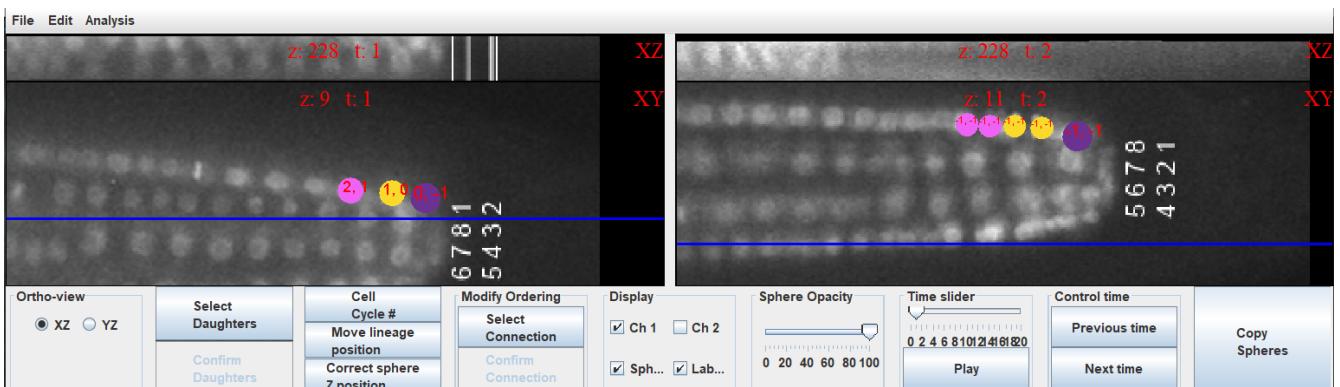
(a)



(b)



(c)



(d)

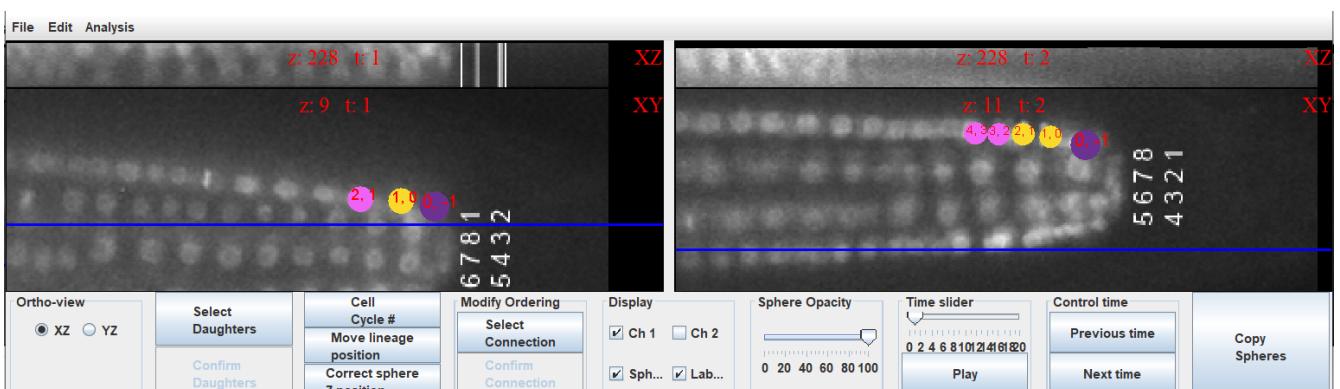
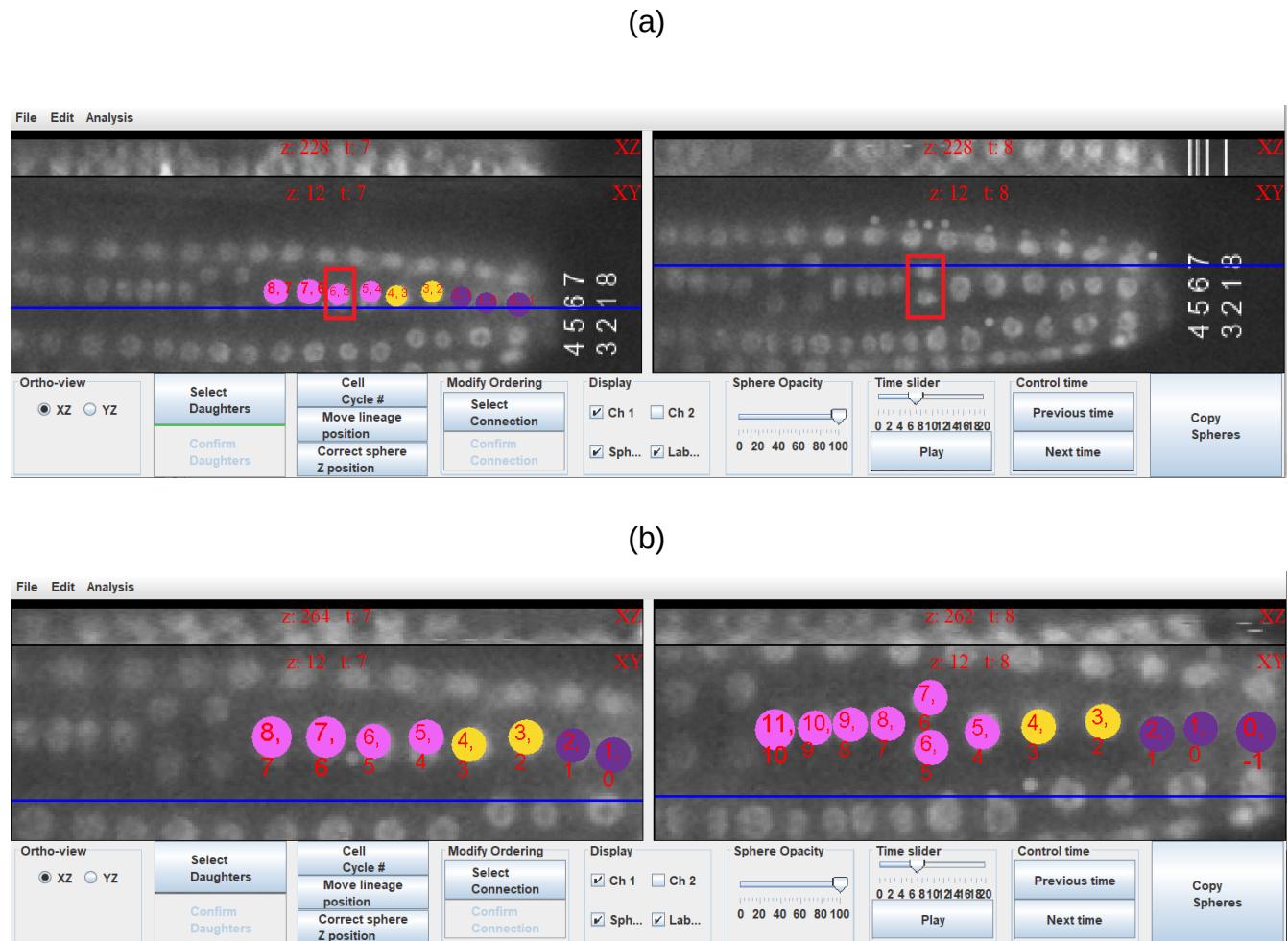


Figure 19. Example of sorting of spheres on images of the root apical meristem for $t > 0$. (a) cell division is identified for pink and yellow spheres from the left panel with pink and yellow rectangle in the right panel, respectively. (b) creating mother and daughter spheres for yellow and pink division events. Mother cell must be closer to the tip than the daughter sphere. (c)Creating the mitotic event where each color indicates that spheres belong to the same

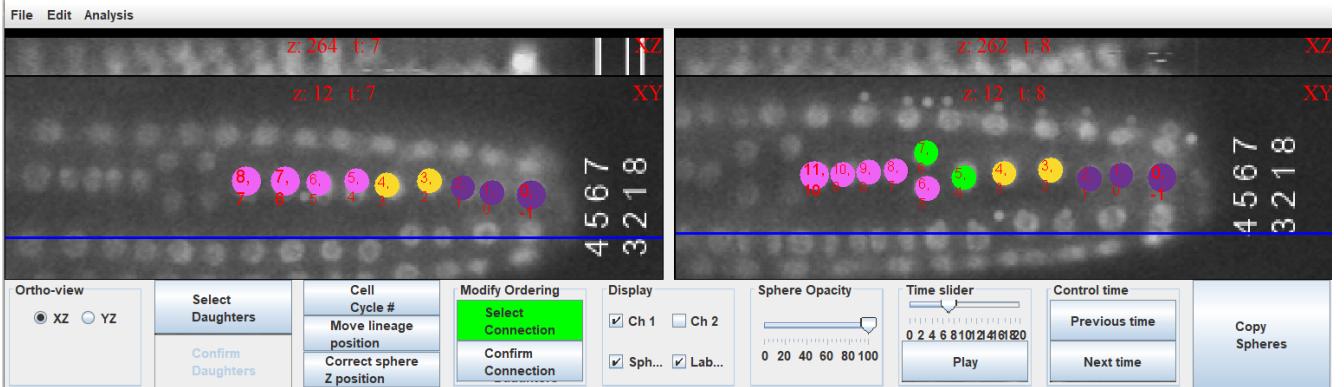
lineage. (d) updating sorting information in decreasing order to distance to the tip of the root.

2.7.4 Updating sorting for longitudinal divisions

Cell division usually takes place in the same direction as the root growth, however in some cases this is not the case and division occurs perpendicular to the root growth axis. Figure 20(a) depicts an example in red square of a longitudinal cell division (perpendicular to the root growth axis). Creating the sorting information as described in sections 2.8.1 – 2.8.3 will lead to sphere id 7 to have a neighboring cell to the one with id 6, however this is not correct since the neighboring cell from sphere id 7 is the sphere with id 5 (see Figure 20(b)). To correct this sorting, click the button **Select Connection** from the **Modify Ordering** panel, then select the sphere to be corrected, then select the new neighbor as depicted in Figure 20(c). Finally, press the button **Confirm Connection**. The neighboring information for cell id 7 is updated to sphere id 5 (see Figure 20(d)).



(c)



(d)

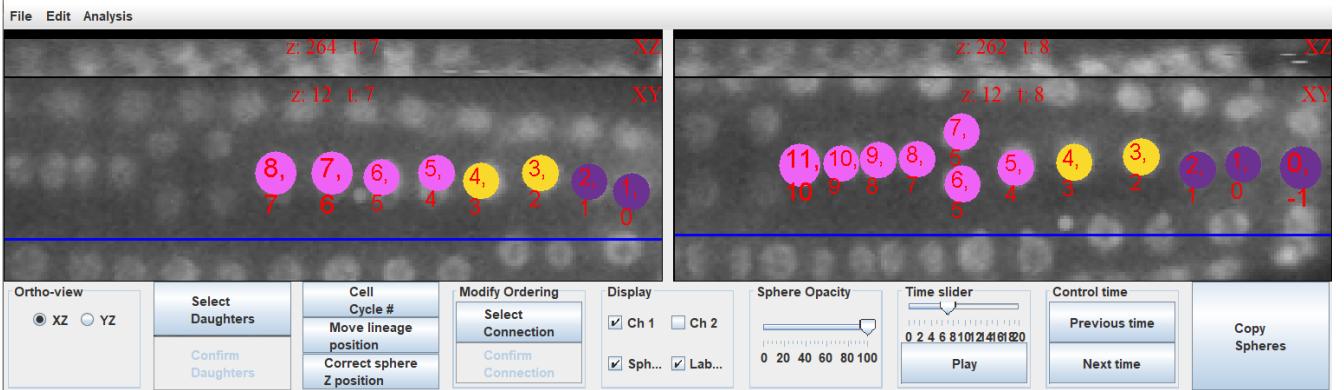


Figure 20. Updating sorting information for longitudinal divisions (perpendicular to the root growth axis). (a) identification of a cell division (left panel red rectangle) that will divide longitudinally (right panel red rectangle). (b) using the approach described in Sections 2.8.1 – 2.8.3 gives an incorrect neighbor id for sphere 7. (c) Modifying the ordering for sphere id 7 such that its neighbor corresponds to sphere id 5. (d) sphere id updated correctly for sphere id 7.

2.8 Saving/opening a tracking state

Saving the current progress of tracking is very important to perform the tracking in several sessions, if needed. The plugin allows to easily save the tracking information.

Note that a sphere should appear in at least two consecutive time points; otherwise the file cannot be saved.

2.8.1 Saving current tracking state

Go to the **File** menu. Then, select **Save state**, a window will be displayed where the user must select the folder where the file will be saved and indicate the file name. Finally, **press** the button **Save** to save the state.

2.8.2 Opening a saved state

Go to the **File** menu. Then, select **Open state**, a window will be displayed where the user must select the folder and file to be opened. Finally, **press** the button **Open** to load the state.

2.9 Undo action

A very useful feature of LiPlaCeT plugin is allowing to undo undesired actions performed by the user. The currently undo actions allowed are: creating spheres, deleting spheres, moving spheres, copy spheres and creating a division event.

2.10 Working with big volumes

The LiPlaCeT plugin requires the hyperstack to fit in the RAM memory. However, this sometimes may not be possible due to large files. The plugin is designed to handle these cases. Follow these steps:

- 1 **Create sub-hyperstacks** such that they fit in the RAM memory and there is one time overlap between them. Thus, create sub-hyperstacks in an incremental way, e.g. from time t_0 to time t_1 , from time t_1 to time t_2 , etc.
- 2 **Perform tracking for the first sub-hyperstack** from time t_0 to t_1 , once that tracking has been finished
 - 2.i Save complete tracking information of the current sub-hyperstack by using the option: **File → Save state**
 - 2.ii Save tracking information only of the last time point of the current sub-hyperstack by using the option: **File → Save state last time point**.
- 3 **Perform tracking for the second sub-hyperstack** from time t_1 to time t_2 and follow these steps:

Using the LiPlaCeT plugin, open the second sub-hyperstack and next open the last time point of the previous sub-hyperstack (saved using option explained in step 2.ii). This operation permits all the tracking information from the last time point of the previous sub-hyperstack to appear in time t_0 of the second

sub-hyperstack. Continue tracking until time t_2 . Once tracking has been finished, save tracking as explained in step 2.ii.

- 4 **Repeat step 3 for all the sub-hyperstacks** in an increasing order.
- 5 **Merge tracking information** obtained from sub-hyperstacks. The last step is to merge all the tracking information generated. To do this:
Go to Plugins → LiPlaCeT → Merge Tracking
A window will be open where the user must select the files to be merged. Then, press Open.
A *.txt with name “Merge_track_yyyy-MM-dd_HH_mm_ss.txt” is generated at the folder where the sub-tracking files were saved.
- 6 **Analysis of tracking** of the merged file:
Open the first sub-hyperstack. Then, open the merged file and generate files as explained in Section 3 and 4.

3 Creating 2D cell genealogy plot and tracking statistics in LiPlaCeT

An important tool for visualization in 2D a cell genealogy is a plot that includes a tree structure of a sequence of mitotic events in a lineage mapped to time scale. To this end:

Go to the Analysis menu of LiPlaCeT. Then, select the submenu **Tree plot** and click in **Save tree plot as png**

Important note: The *.png file is saved in the same folder where *.tif file was saved. This *.png file is saved with the following name “TreePlot_”+DATE+”_”+TIME For each individual cell lineage two files are generated: (1)The tree plot representation for the cell lineage is shown in Figure 21. The nodes (circles) represent cell mitotic events. Note that the new mitotic event has 2 descendants with a number. This number is a cell identification number (cell ID number) and (2) a *.csv file that contains five columns: **A cell ID** number: This should correspond to the number displayed in the tree plot, **Cell_Cycle_No**: The cell cycle number of a cell, **the cell cycle duration** defined as time from cell birth to the next cell division, time-point-birth and time-point-division. Note that a division time equal to 0 means that division time could not be calculated with the information provided (for example, for incomplete cycles of the last cells in a lineage). Additionally, two csv files are creating displaying relevant information of the tree: (1) number_cells.csv contains information on the number of cells that each tree has in a given time point and (2) track_statistics.csv contains statistics for each lineage such as total length of the track (total displacement), total number of spots for the track, number of division (splits), mean division time and standard deviation of division time.

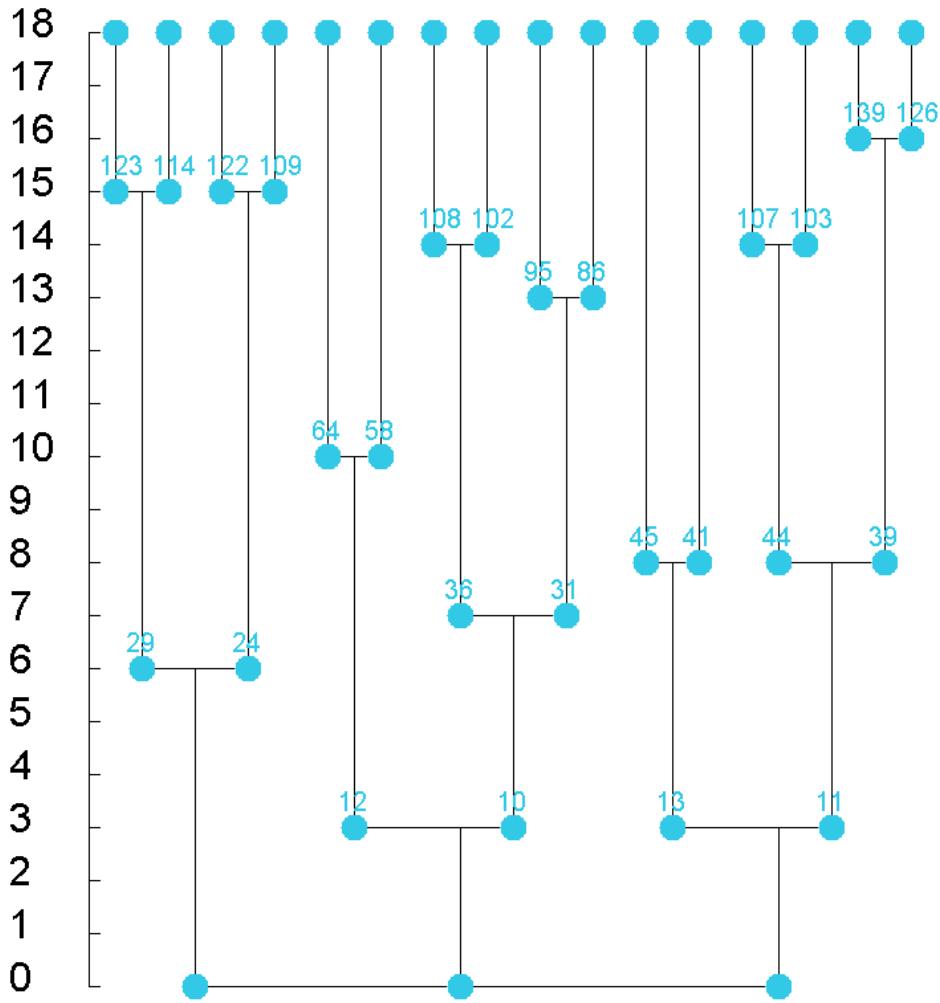


Figure 21. An example of a tree plot of a cell lineage. Three cells are identified at time t_0 indicating that this lineage had already initialized the cell division from the lateral root founder cell before the first data acquisition (see Figure 15).

4 Visualization, using Paraview, of information generated with LiPlaCeT regarding cell tracking, cell lineage and cell proliferation

4.1 Transferring data from LiPlaCeT to Paraview

Paraview has several options to visualize data. Programming the routines for visualization using Python is the most widely used tool. However, this requires programming skills that are not easily learned. Another approach is generating a VTK

file, which is a file format compatible with Paraview. The plugin allows you to create this file, and the user only needs to open it in Paraview.

4.1.1 Generating VTK files for Paraview

To generate the *.vtk from the LiPlaCeT Plugin:

Go to **Analysis** menu and select the submenu **Generate VTK**, two options are displayed:

Option 1: Unique file for all lineage: this option generates a VTK file in which tracking information of all cell lineages is saved in a single file.

Option 2: Separate files for each cell lineage: this option generates a VTK file for each cell lineage, therefore the user can select specific lineages to be visualized.

Important note: For each time point, three files are generated in which tracking information is saved. **Sphere_*.vtk** file contains the sphere position and different parameters describing a cell lineage to be used for colormap. **Trajectory_*.vtk** file includes information on the cells trajectories. **Vectors_*.vtk** file contains information on the 3D vector orientation of a mitotic event.

The files will be generated in a folder created in the same path where the 3D/4D image stack is saved. The folder name has the format “ParaviewVTK_”+DATE+”_”+TIME.

If **Option 2** is selected, then new folders will be created with the format “track_”+ID (each ID refers to a separate cell lineage).

4.1.2 Visualizing VTK files as spheres in Paraview

This subsection explains how to visualize important features from the tracking such as mitotic events, cell cycle number and cells belonging to the same lineage. To visualize the tracking data in Paraview do the following:

1. Open Paraview, and then to open files:

Option 1: Go to **File** menu and select **Open**

Option 2: Click the **Open** button (See Figure 22(a) red square)

- 2 Go to the folder where the VTK files were saved and select the sphere files (See Figure 22(b) red rectangle), then press OK.

Note: The files will be opened using temporal information by opening all the files as a group.

- 3 Then, press the button **Apply** (See Figure 22(c)) for the files to be read by Paraview.

- 4 In order to visualize the data as a Glyph, follow these steps:

Select the file **Sphere_TP_*** from the **Pipeline Browser** (left portion of the interface).

Then, go to the **Filters** menu (top portion of the interface), select the submenu **Common** and click in **Glyph** (See Figure 22(d)).

- 5 In the Pipeline Browser, there is a tab “Properties”, which displays several options for the **Glyph** filter (See Figure 23).

Glyph Source panel-Glyph type: Select the option **Sphere** (See Figure 23 red rectangle).

Orientation panel-Orientation array: Select the option **no orientation array** (See Figure 23 blue rectangle).

Scale panel-Scale array: Select the option **no scale array** (See Figure 23 green rectangle).

Scale panel-Scale factor: Indicate 5 (See Figure 23 green rectangle).

Masking panel-Glyph mode: Select the option **All points** (See Figure 23 purple rectangle)

Finally, press the button **Apply**. If the spheres are very big or small, change scale factor to a bigger or a smaller number and press **Apply**.

- 6 The data should be displayed in the Paraview Layout.

If the data are not visualized, probably this is because it is out of range. Press the button view Z (Figure 24 purple square) to bring the data to the center.

The **time inspector** (Figure 24 red rectangle) allows the user to navigate across different time points.

The **Coloring panel** of **Properties tab** allows setting the values to be color mapped.

Note that this representation assigns numbers to colors. Select the following options: It can be useful to use a different feature to be displayed with the spheres color, it can be changed using the button **Choose preset** in the **Properties tab** (Figure 24, green square) from the **Coloring panel**. See Section 4.3.2, 4.3.3 and 4.3.4 for details in these coloring options. The different features to select are the following:

Constant_color: Cells belonging to the same lineage will have the same color.

Figure 24 depicts an example of selecting Constant_color, the value range of color maps is from 0 to 3, indicating that 4 different lineages were tracked. The blue color is assigned to lineage 0 while red is assigned to the lineage 3. **Mitosis**

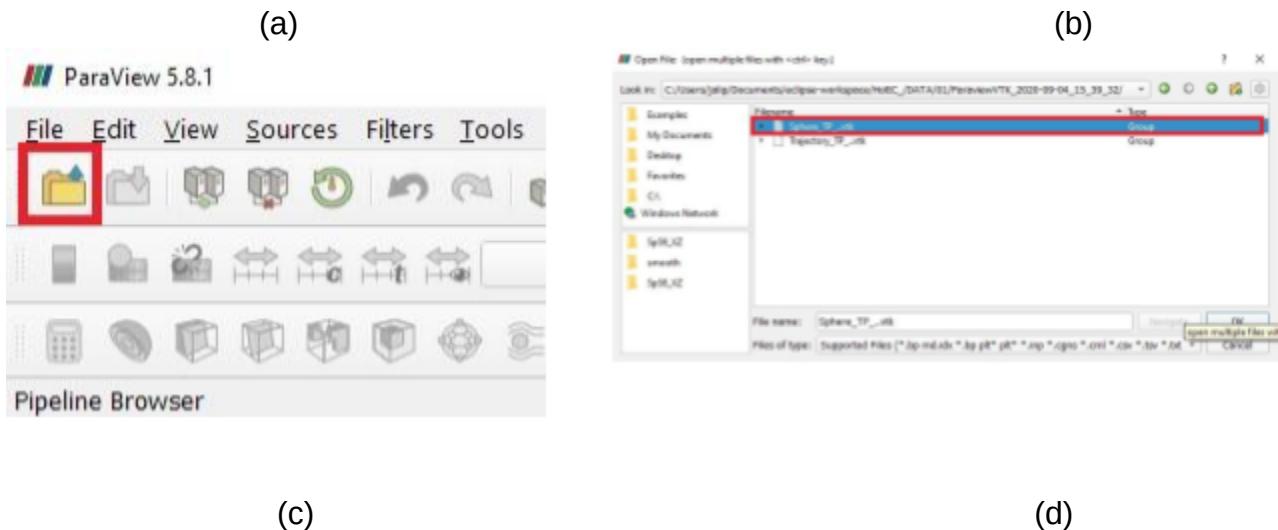
Event_color: This option allows identifying cells that will pass the mitosis in the next time point. The color assigned to value 1 identifies cells that will divide while color assigned to value 0 identifies cells that will not divide. Figure 25 displays an example of mitotic event visualization where maroon (correspond to value 1) spheres identify the cells that will divide in the next time point.

Cycle_No_Color: To visualize cells that pass a specific cell cycle number (e.g. the 1st, 2nd, etc. after the beginning of the experiment) go to the last time point using a time inspector (top portion of the interface). Press the **Rescale Data Range** button (orange rectangle in Figure 24). Figure 26 displays color coded cell cycle numbers with a scale at the right portion of the Paraview main window.

Velocity_color: To visualize the velocity of displacement for each cell and computed as $|P(\text{cell}_{(i,t-1)} - P(\text{cell}_{(i,t)})|/dt$ where $P(\text{cell}_{(i,t)})$ correspond to the position 3D of cell_i at time t and $P(\text{cell}_{(i,t-1)})$ correspond to the position 3D for mother of cell_i at time $t-1$, and dt is the elapsed time between acquisitions (currently assumed to be 1). Figure 27 displays color coded velocity with a scale at the right, red spheres have higher velocities while blue corresponds to lower.

Displacement_cumulative_color: To visualize the total displacement for each cell in the track and computed as the $D(\text{cell}(i,t), \text{cell}(i,t-1)) + D(\text{cell}(i,t-1), \text{cell}(i,t-2)) + \dots +$

$D(\text{cell}(i,t-(k+1)), \text{cell}(i,t-k))$ where $D(\text{cell}(i,t), \text{cell}(i,t-1))$ correspond to the distance between the position of $\text{cell}(i,t)$ and its mother $\text{cell}(i,t-1)$. Figure 28 displays color coded displacement_cumulative_color where red indicated cells that are moved a larger distance from its initial position (due to division or cell movement) while blue corresponds to cells with small movement from its initial position.



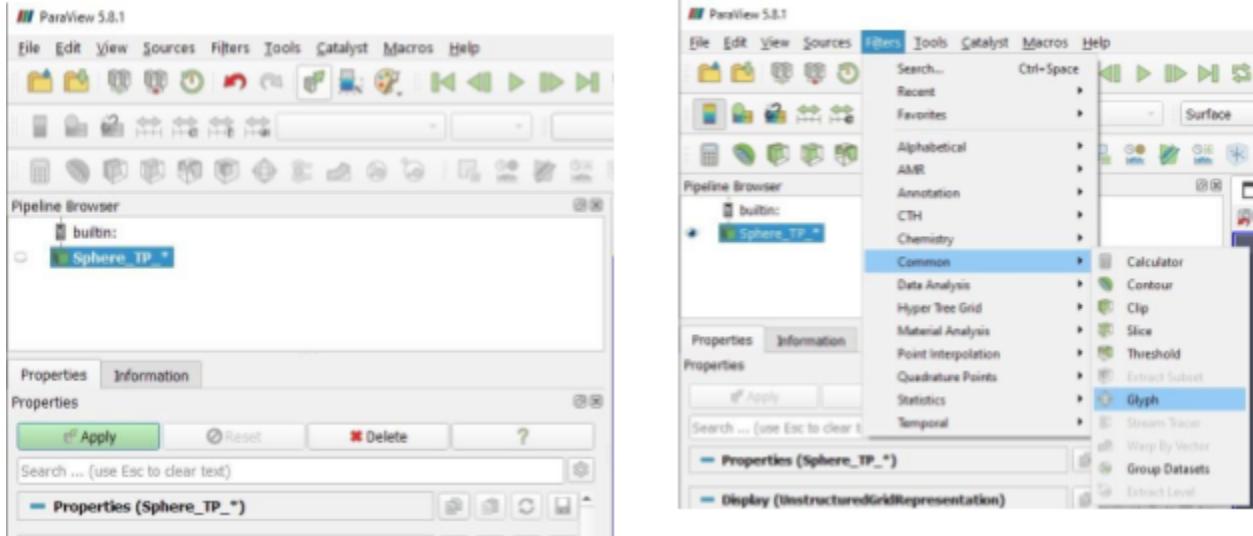


Figure 22. Paraview interface. (a) button used to open files; (b) selecting the spheres file (Spheres_TP_*.vtk) which contains the tracking information, (c) **Apply button** to open files in Paraview, (d) Select Glyph filter for visualization.

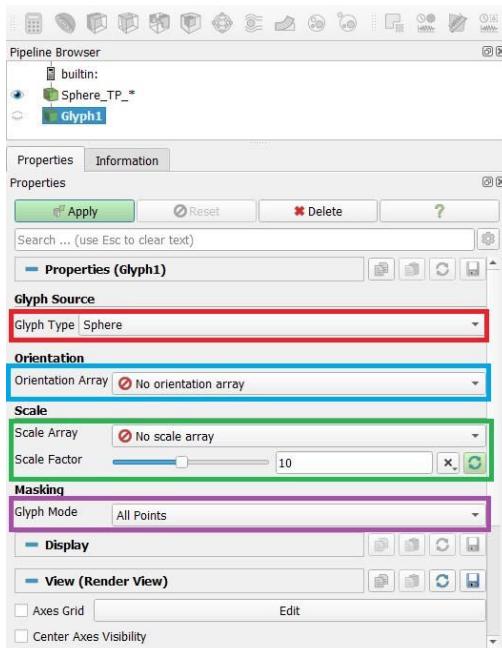


Figure 23. Changing the parameters in the **Properties** tab.

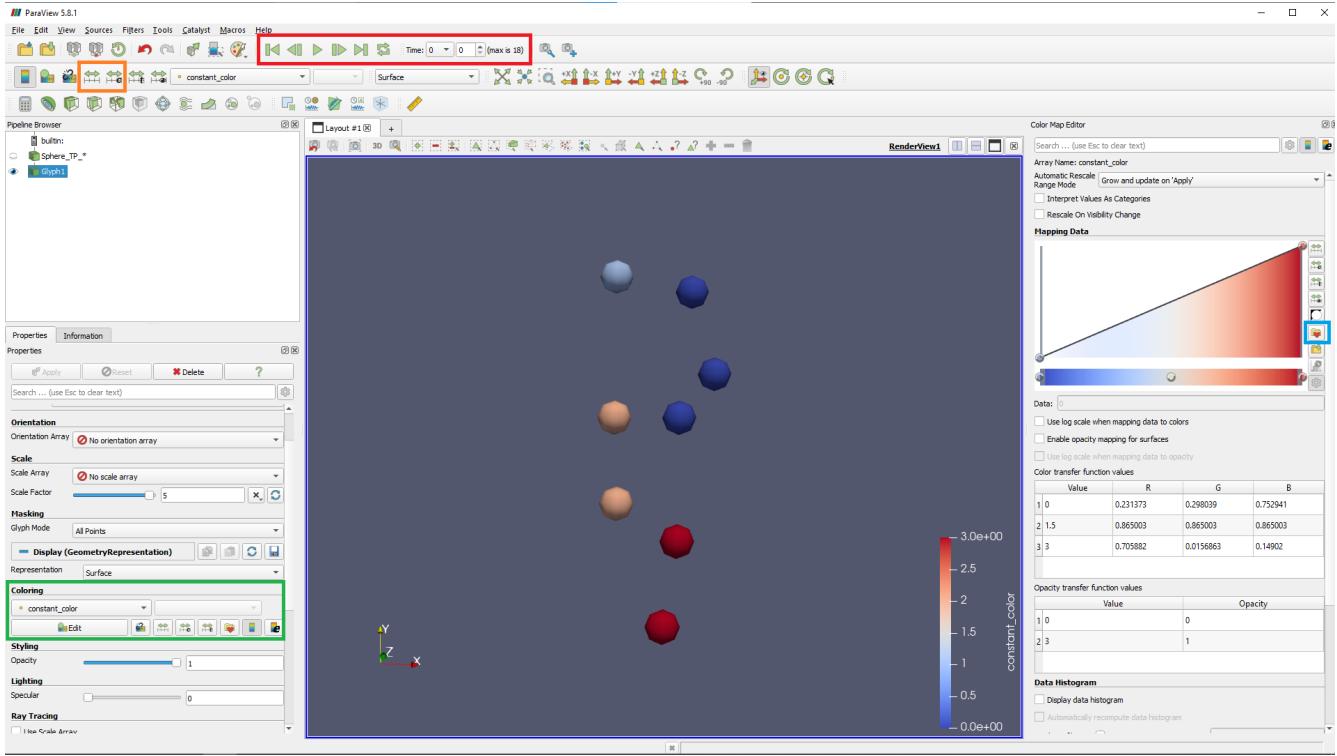


Figure 24. Visualization of spheres after application of Glyph filter. Green square allows you to choose the values for the colormap. Orange rectangle allows rescaling the range of values to be displayed by the colormap. Red rectangle allows to move across time.

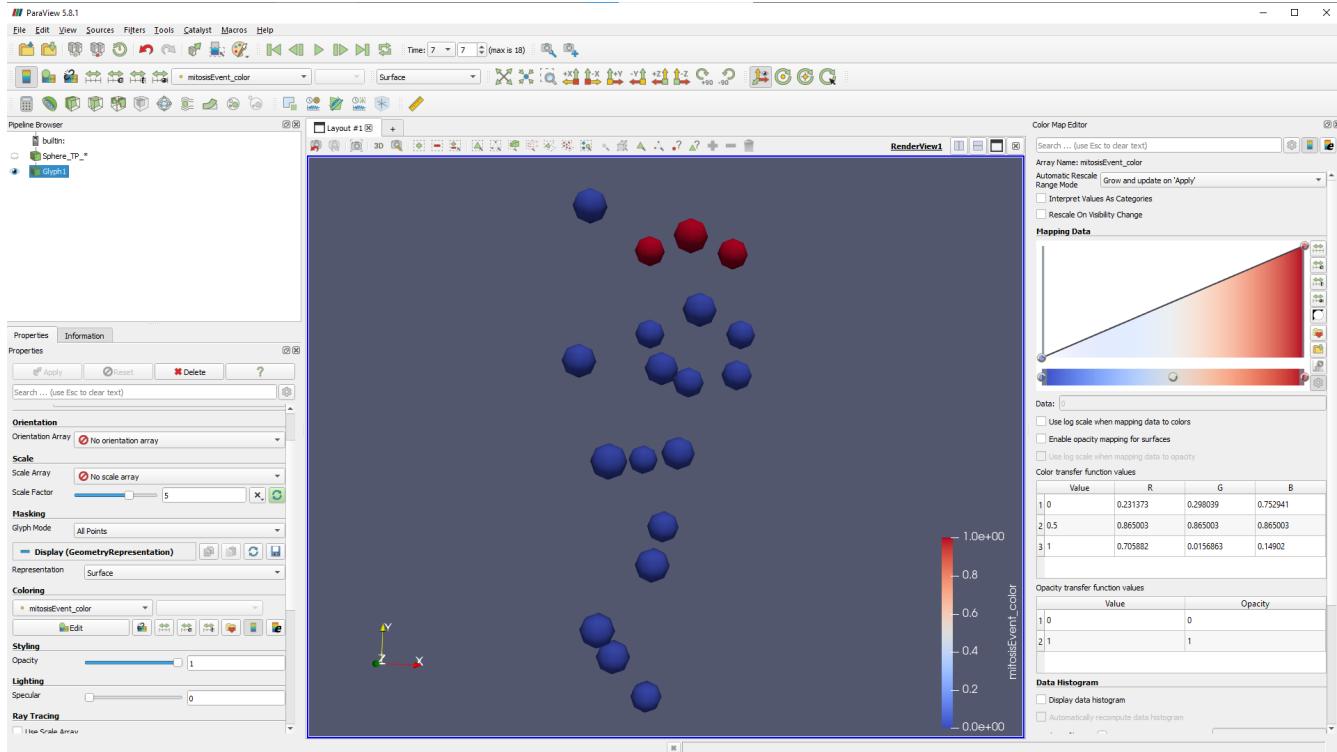


Figure 25. Visualization of cells that will divide in the next time point.

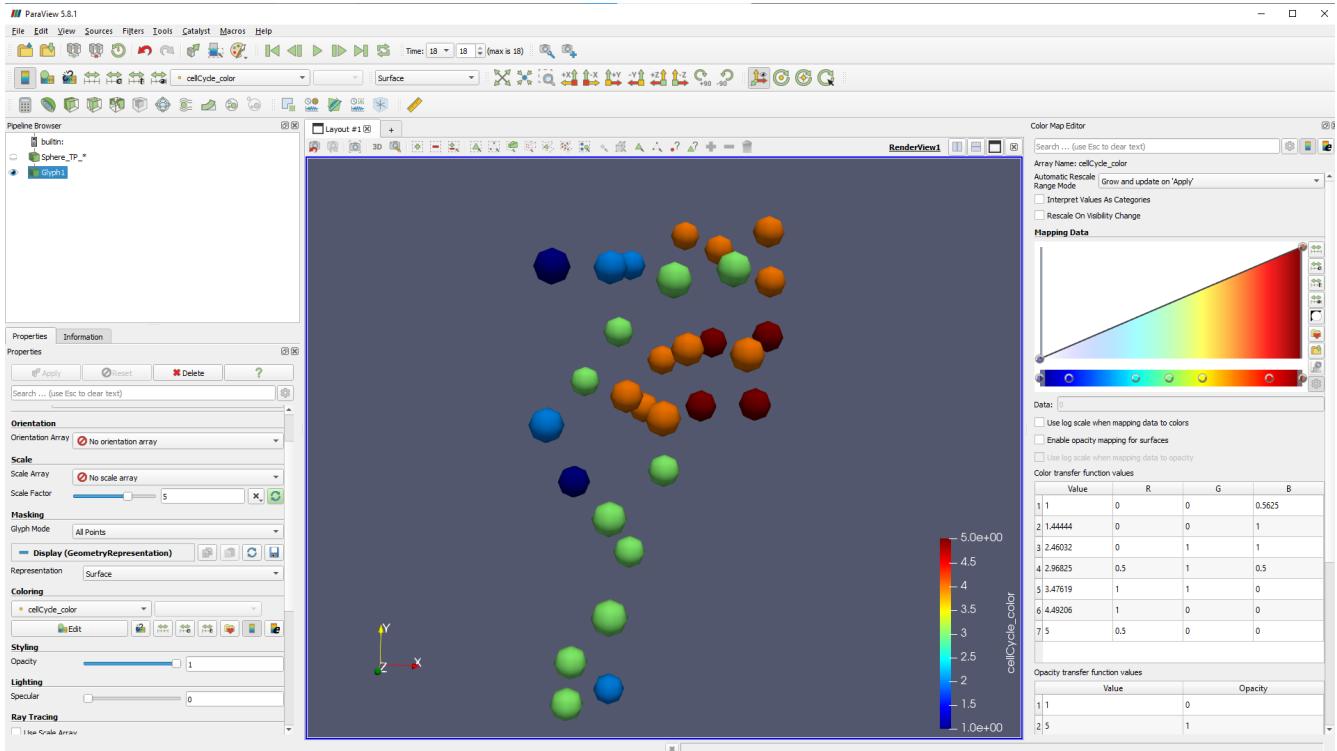


Figure 26. Heat map of the cell cycle number.

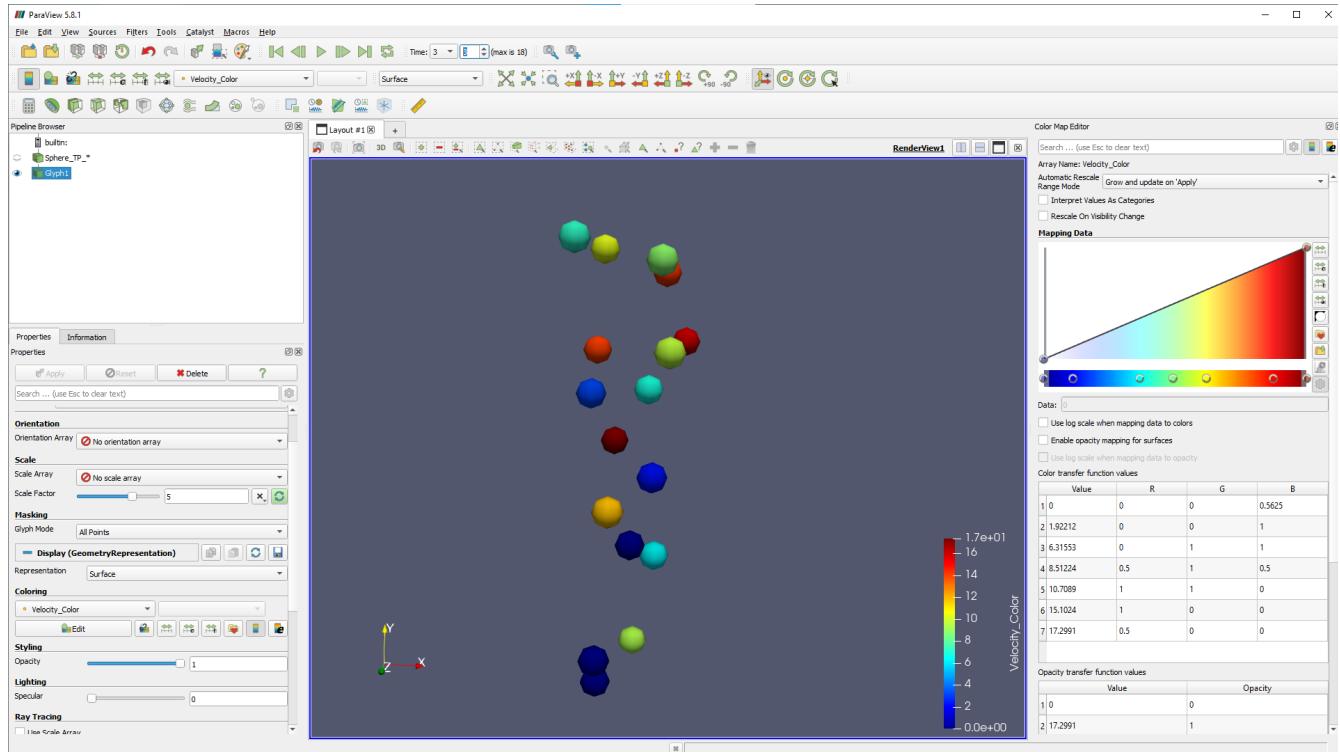


Figure 27: Heat map of velocity.

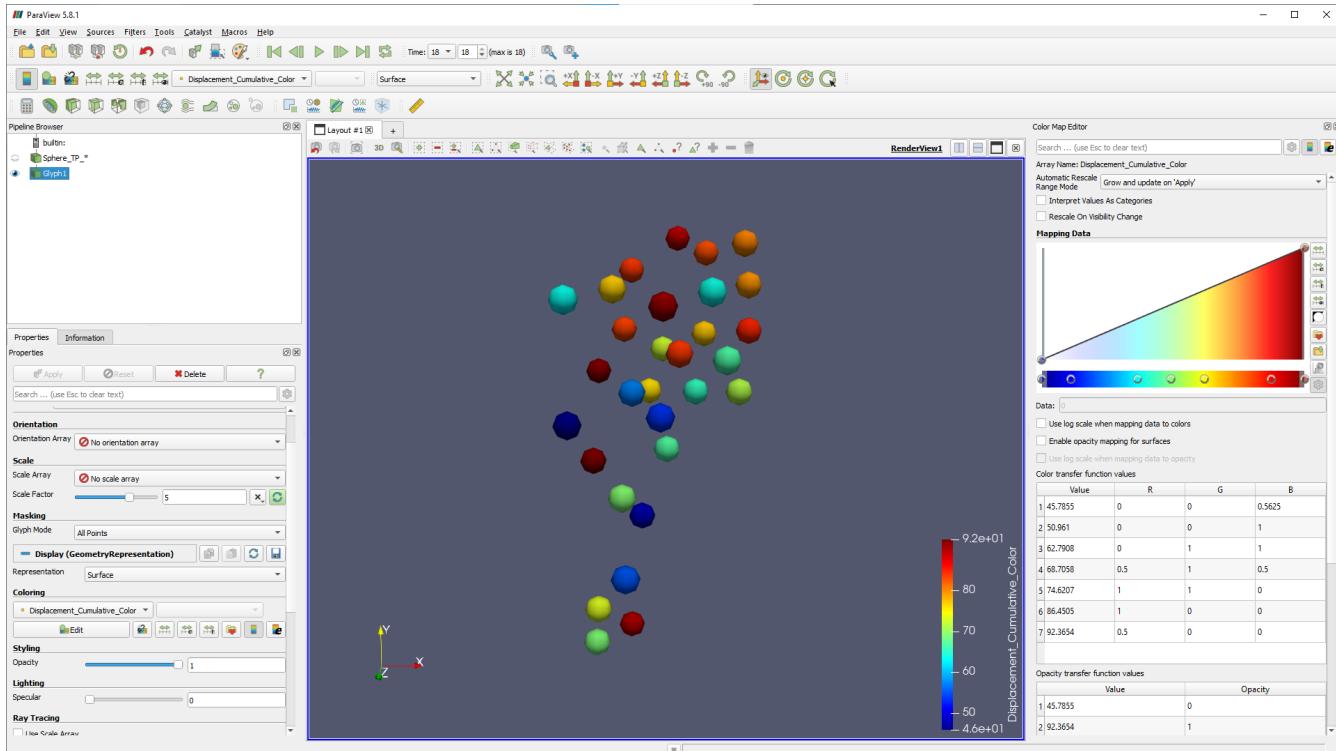


Figure 28 heat map of total cell displacement cumulative

4.2 Cell tracking visualization and generation of database with cell information

4.2.1 Visualizing Trajectory files for individual cells

Trajectory file contains information on the movement for each nucleus (trajectories) and position of nuclei of daughter cells after mitosis. This can be visualized using the generated files for the trajectories.

Open Paraview software and then open the vtk files using any of the following options:

Option 1: Go to **File** menu and select **Open**.

Option 2: Click the **Open** button (See Figure 22(a) red square).

A window will be displayed permitting to open the folder where the **Trajectory_TP_*.vtk** files were saved with the option to select the Trajectory files, then press OK.

Important note: The files will be opened using temporal information by opening all the files as a group. Then, press the **Apply** button (See Figure 22(c)) for the files to be read by Paraview. Figure 29a displays the trajectory across time for each cell with different colors, the trajectories positions are obtained directly from the position of the spot at the LiPlaCeT GUI.

Smoothing the trajectories: Smoothing the trajectories sometimes can be better for a specific problem, hence we have included an option to smooth the trajectories. Our smoothing approach consists on tracking the spot position from their birth at time t_i up to a mitotic event (if there is no mitotic even then it is tracked until the last time point) at time t_f , then averaging all the positions and setting the spot position from t_i to t_f equal to the average. This ensures that the spot will have the same position until there is a mitotic event. In order to set the smoothing option, go to **Analysis -> Set Smooth Tracking ->** and select **Smooth**. Then, generate the vtk files. Figure 29b displays an example of smoothing the trajectories, the direction of mitotic event are easily visualized.

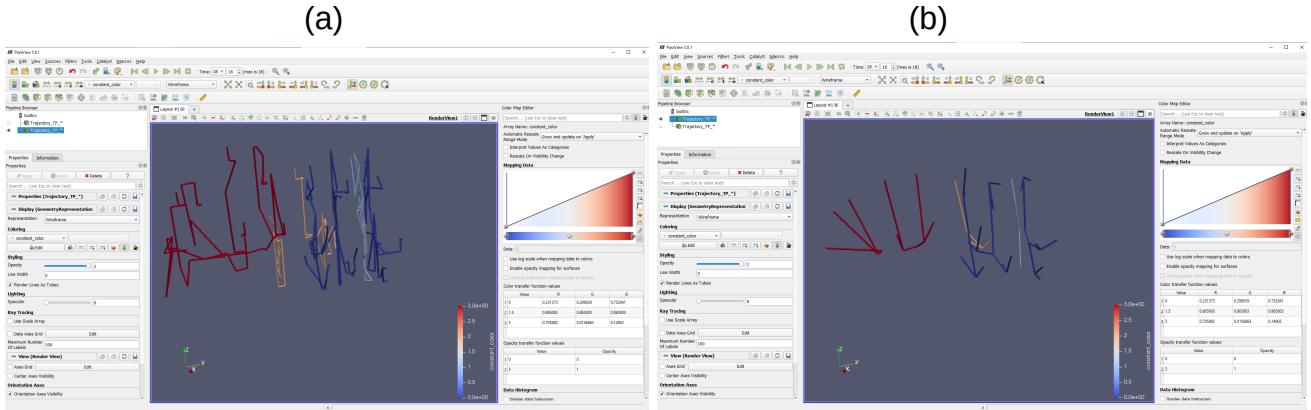


Figure 29. Paraview trajectories for each cell. (a) displaying the trajectories as obtained from LiPlaCeT, (b) smoothing the trajectories.

4.2.2 Visualizing the direction of cell displacement after mitotic events

Vector files contain information of the mitotic events direction. This also gives information on the direction of cell growth. Every time a cell passes a mitotic event, vectors are generated displaying the direction in which new nuclei will be located. The process to visualize this information is similar to the sphere visualization (Section 4.1.2).

- 1 **Open the data in paraview:** Open the Paraview software, then:

Option 1: Go to **File** menu and select **Open**.

Option 2: Click the **Open** button (See Figure 22(a) red square).

Go to the folder were the **Vectors_TP_*.vtk** were saved and select them, then click **OK**.

Note: The files will be opened using temporal information by opening all the files as a group. Then, press the button **Apply** for the files to be read by Paraview.

- 2 **Visualize the data as a Glyph**, following these steps:

Select the file **Vectors_TP_*** from the **Pipeline Browser** (left portion of the interface). Then, go to the **Filters** menu (top portion of the interface), select the submenu **Common** and click in **Glyph** (See Figure 22(d)).

In the Pipeline Browser, there is a tab “Properties”, which displays several options for the **Glyph** filter (See Figure 30).

Glyph Source panel-Glyph type: Select the option **Arrow** (See Figure 30 red rectangle).

Orientation panel-Orientation array: Select the option **Direction_Division** (See Figure 30 blue rectangle). If this option is not available, then use the time inspector to move to the next time point (See Figure 24 red rectangle). Move the time until the option **Direction_Division** is visible (it would be visible at a point when a mitotic event was detected).

Scale panel-Scale array: Select the option **Direction_Division** (See Figure 30 green rectangle).

Scale panel-Vector scale mode: Select the option **Scale by Magnitude** (See Figure 30 green rectangle).

Scale panel-Scale factor: Set this value to 1 (See Figure 30 green rectangle).

Masking panel-Glyph mode: Select the option **All points** (See Figure 30 purple rectangle)

Finally, press the button **Apply**.

The data should be displayed in the Paraview Layout (See Figure 31), which represent the following information:

Vectors only appear at every mitotic event (See Figure 31 small vectors finishing at the spheres) and an average vector of all mitotic events is also calculated and displayed at the middle of all the vectors (See Figure 31 at the bigger vector which does not finish at a sphere).

- 3 A csv file (Direction_Data_TP_*.csv) is generated for each time point where all the mitotic events are identified, and the position of nuclei of daughter cells respective to the position of nucleus of mother cells is used to calculate mitosis direction. The csv file format is:

Time Point, Direction X, Direction Y, Direction Z, R, Azimuth Elevation.

Time Point: the time point at which the mitotic event takes place.

Direction X: Direction of the mitotic event in the X coordinate.

Direction Y: Direction of the mitotic event in the Y coordinate.

Direction Z: Direction of the mitotic event in the Z coordinate.

R: Spherical coordinate R.

Azimuth: Spherical coordinate Azimuth (degrees).

Elevation: Spherical coordinate Elevation (degrees).

An additional csv file (Direction_Data_Average.csv) has the information with the average direction of mitotic events for a certain number of Time Points.

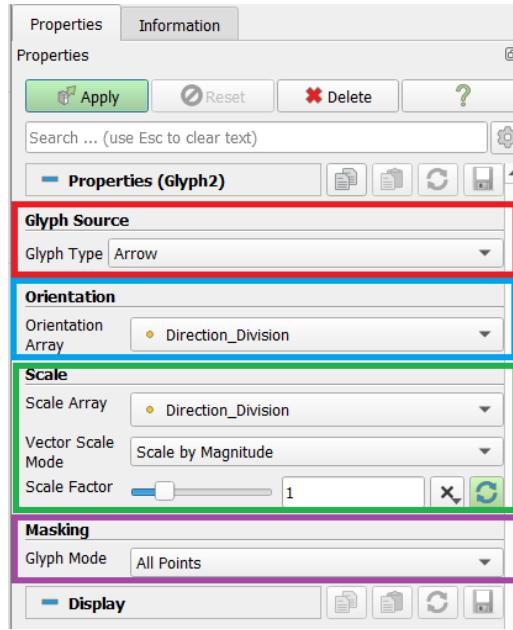


Figure 30. Paraview Glyph options for vtk vectors. Changing the parameters in the properties tab.

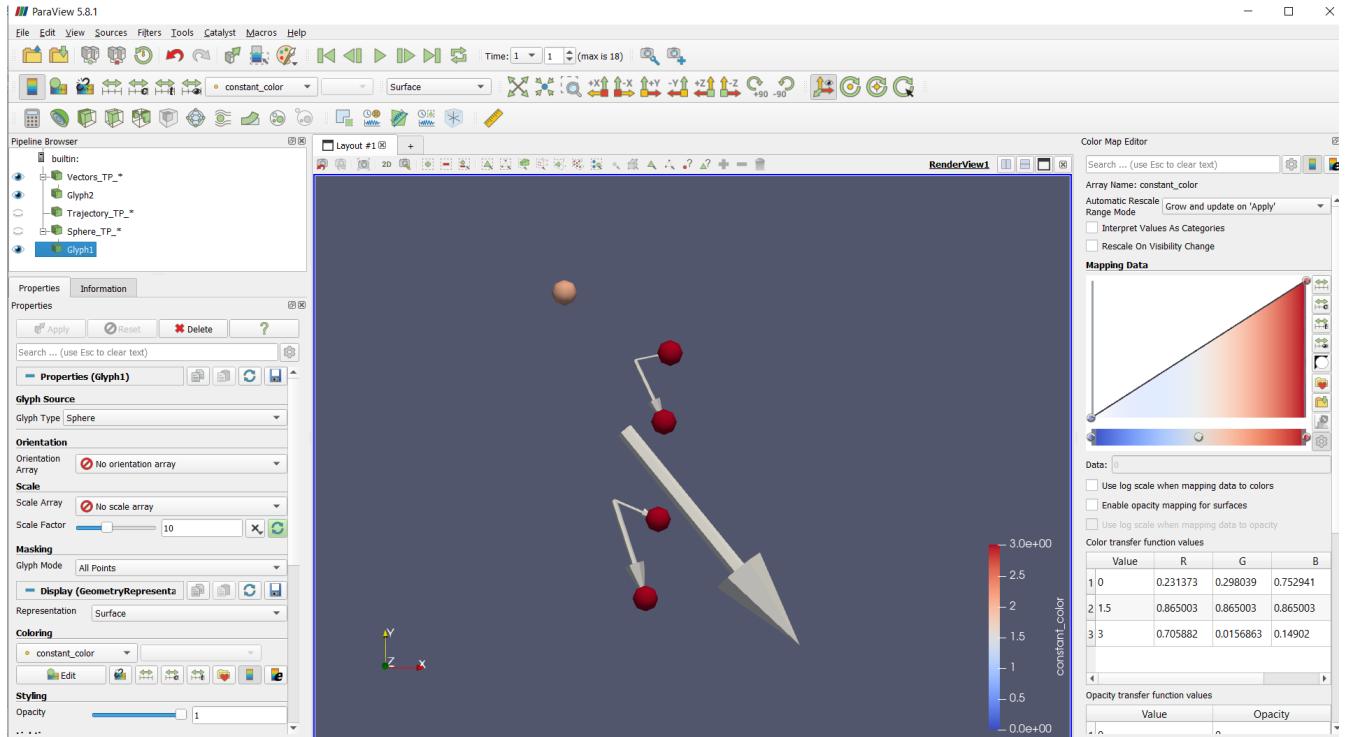


Figure 31. Vectors displaying mitotic event direction. The bigger vector at the middle is the average direction from all mitotic events.

4.2.3 Visualizing spheres for individual lineages

This option allows visualizing only a specific cell lineage. First, create the individual files as explained in Section 4.1.1 Option 2, then perform the steps explained in Section 4.1.2, 4.2.1 and 4.2.2, using the generated individual folders.

4.3 Analyzing cell dynamics

If cells are tracked according to their relative cell position as explained in Section 2.7, additional descriptors to those described in Section 4.1.2 can be obtained from the track such as distance to the firsts cell of the file, cell displacement rate and cell elongation rate.

4.3.1 Displaying 3D vector maps of tracked cells

Our plugin allows visualizing cell dynamics in Paraview. To this end, generate *.vtk files and visualize the files as explained in Sections 4.1.1 and 4.1.2. Figure 32 displays an example of tracking 8 “rows” from *A. thaliana* root, each tracked row is identified by selecting a solid color in the tab Coloring from Properties (see Figure 32 red square). LiPlaCeT by default creates three different Coloring options for a tracking, which are constant_color, Mitosis Event_color and cell_cycle_color (see Section 4.3.2, 4.3.3 and 4.3.4 for details in these coloring options). Additionally, if the tracking has the cells sorted as explained in Section 2.8, one can compute more descriptors that can be displayed in the Coloring tab. Figure 33 displays the additional descriptors that can be displayed, specifically, cell_displacement_rate, Distance_to_apical_nucleus, cell_growth_rate and distance_between_nuclei. These descriptors will be explained in the following subsections.

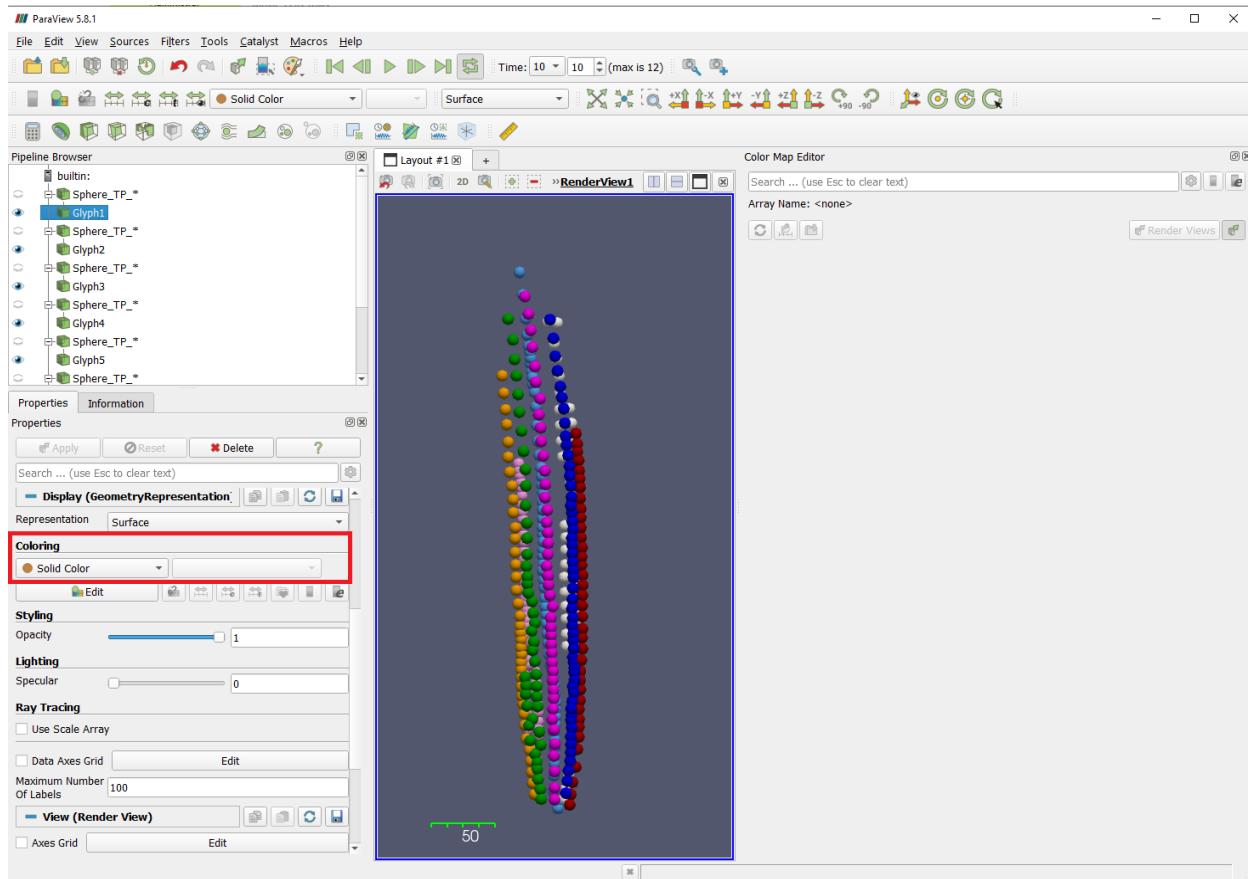


Figure 32. Displaying cell tracking as spheres where each color identifies a row of cells that were tracked.

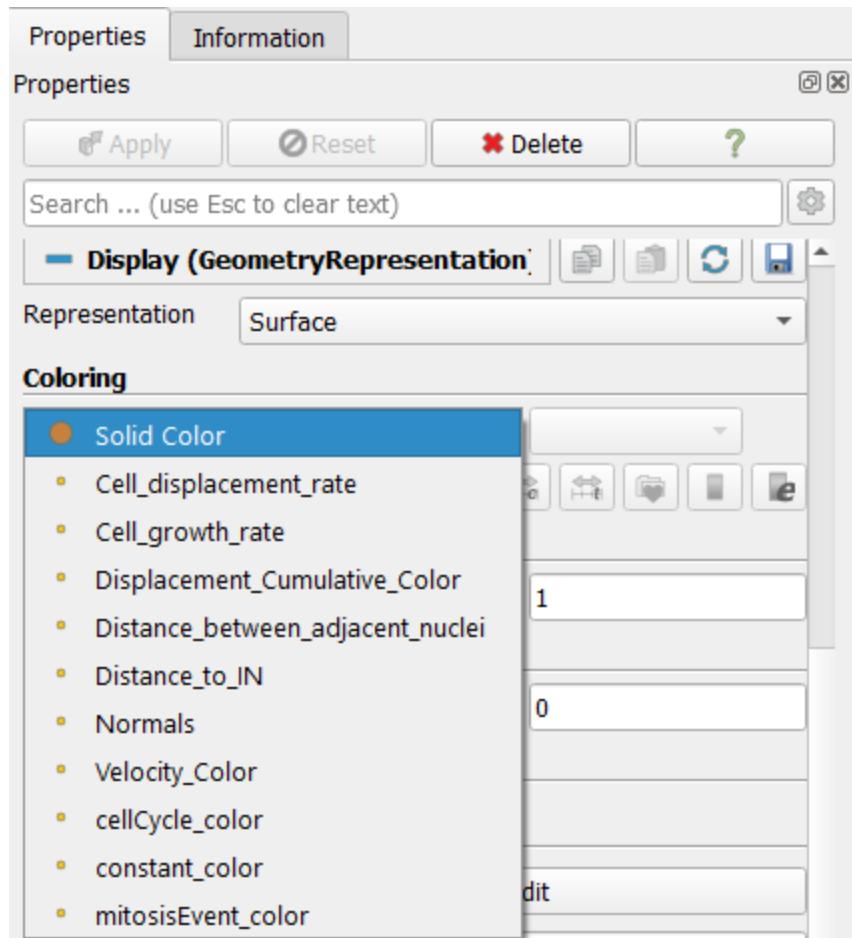


Figure 33. Coloring options for *.vtk files with sorting information (see Section 2.8).

4.3.2 Displaying 3D vector maps of proliferation events

If the user selects `mitosisEvent_color` from the Coloring options (Figure 33), then the color identifies the cell that will have evidence of division by the next time point. Figure 34 displays an example of selecting `mitosis_Event_color` (see Figure 34 red rectangle), the nuclei that will divide in the next time point appear as maroon (corresponds to value 1) spheres while the rest of them appear in blue (corresponds to value 0) spheres.

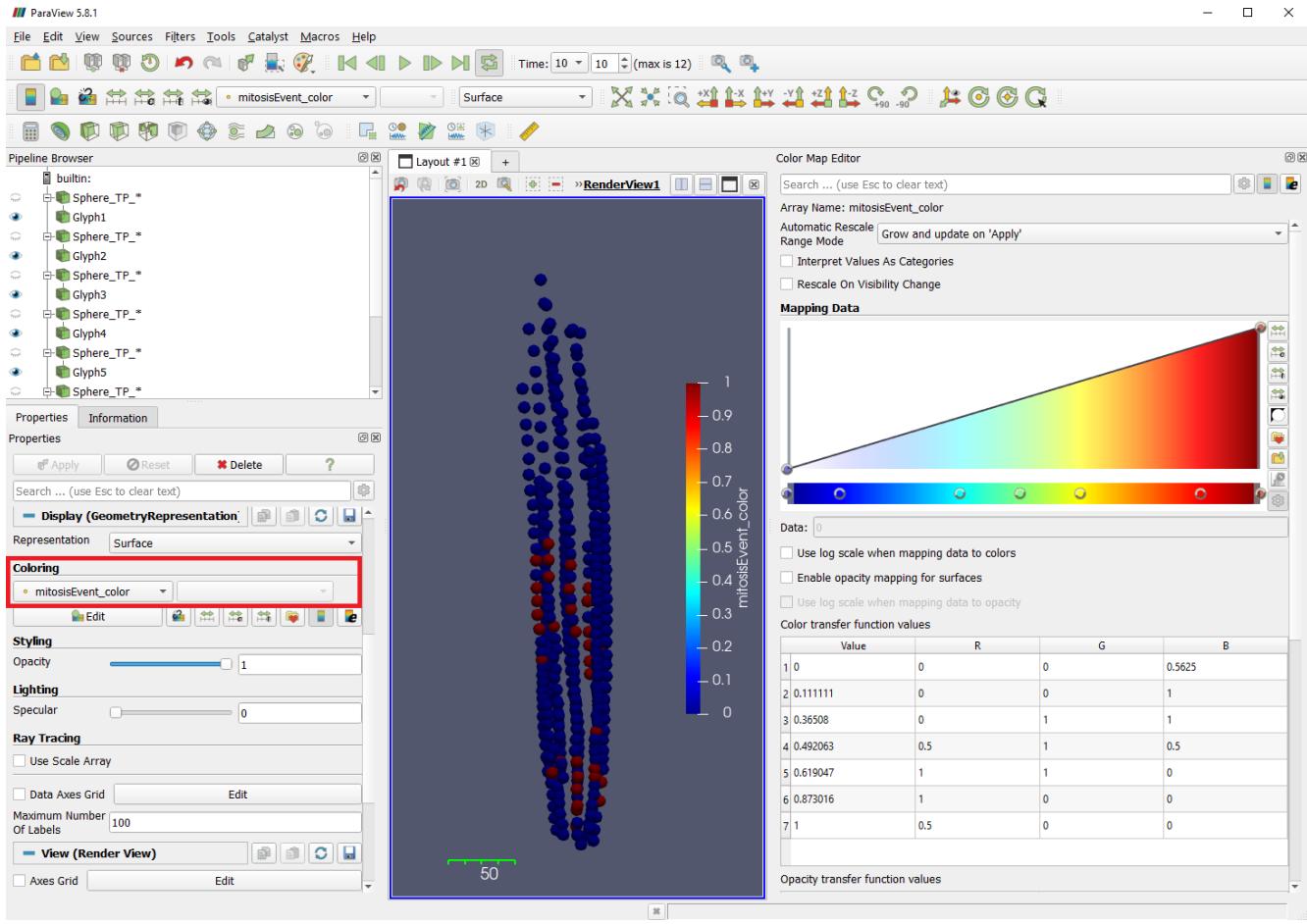


Figure 34. Selecting mitosisEvent_color in Coloring option (red square) to display the cells that will divide (spheres in maroon) in the next time point.

4.3.3 Displaying 3D vector maps of cell lineage

If the user selects constant_color from the Coloring options (Figure 33), then the color identifies the cells that belong to the same lineage. This means that all the cells with the same color arise from the same precursor cell. Figure 35 displays an example of selecting constant_color (see Figure 35 red rectangle), three different rows from the cortex of *A. thaliana* root apical meristem were tracked.

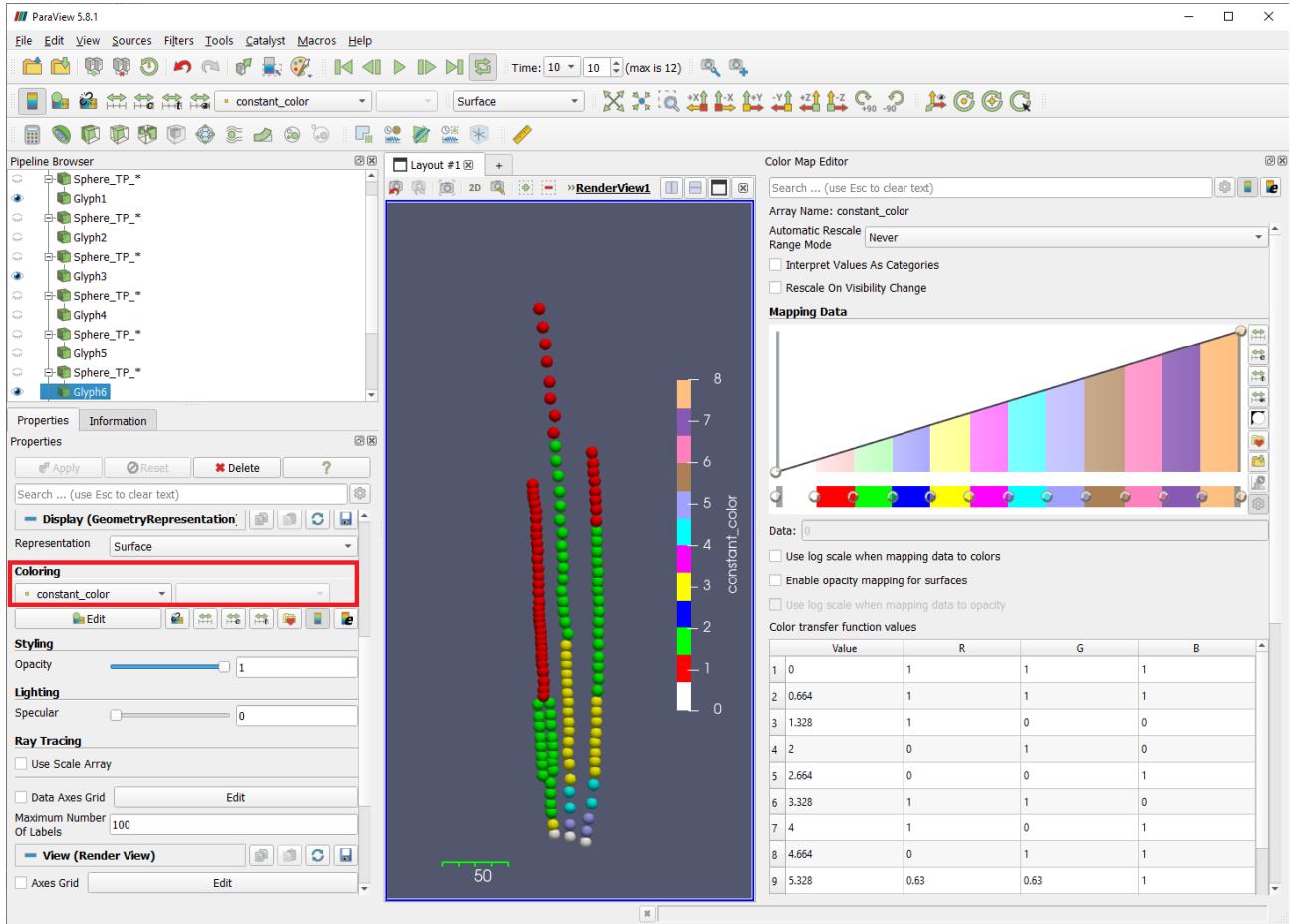


Figure 35. Selecting constant_color in Coloring option (red square) to display the cells that belong to the same lineage, three rows from the cortex of *A. thaliana* root apical meristem were tracked.

4.3.4 Displaying 3D vector maps of cell cycle

If the user selects cellCycle_color from the Coloring options (Figure 33), then the color identifies the current value of the cell cycle for each nuclei. When a cell is identified as a precursor (mother) cell, its cell cycle is set to zero. If a cell divides into two daughters, the daughter cell's cycle number is set to be equal to the mother cell cycle number increased by one. Figure 36 displays an example of selecting cellCycle_color (see Figure 36 red rectangle). At the distal root portion, the cell cycle number is low (blue) because the mother cell can be generated at the tip.

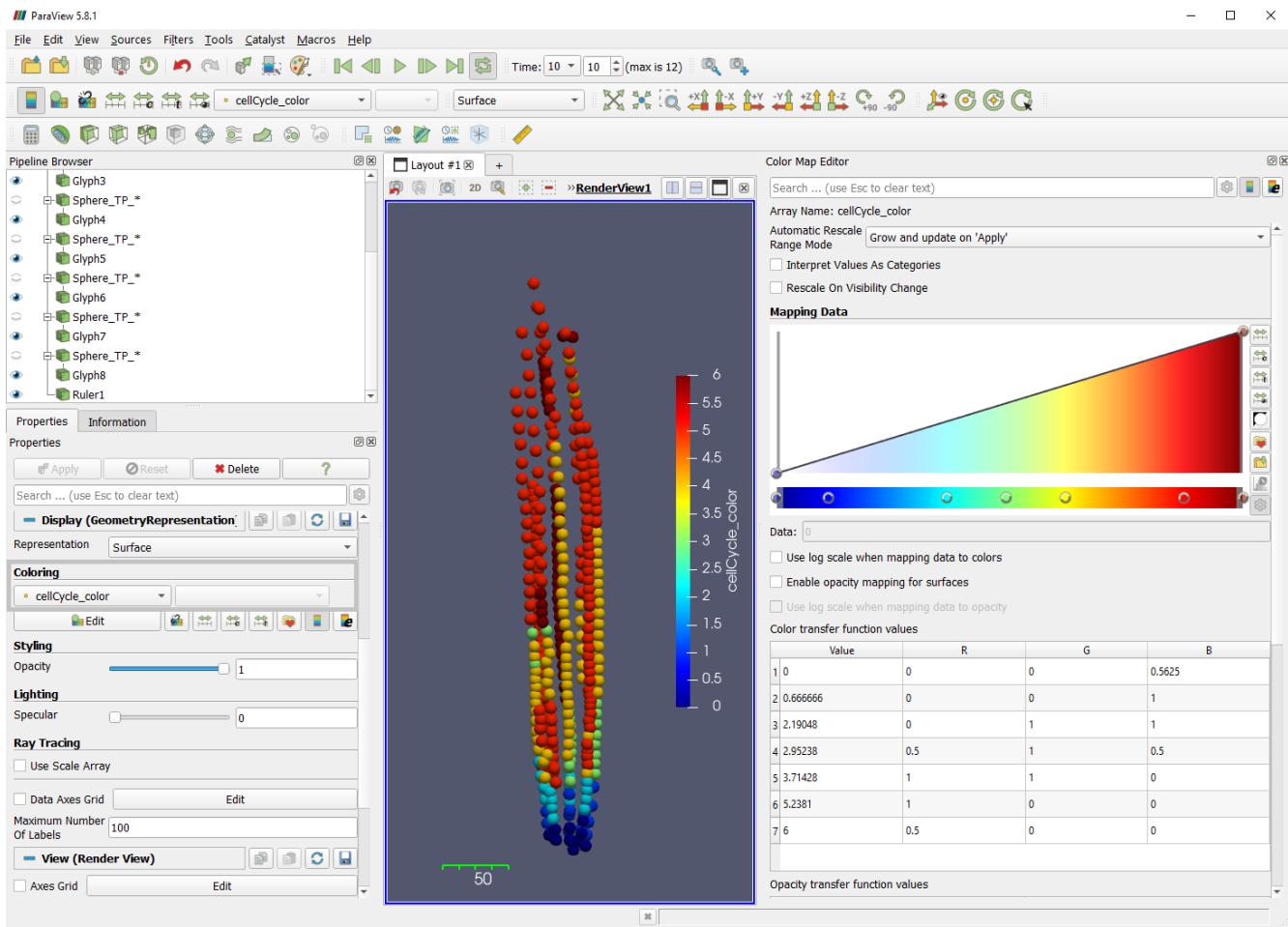


Figure 36. Selecting `cellCycle_color` in Coloring option (red square) to display the cell cycle number for each cell.

4.3.5 Calculating cell descriptors based on nuclear position and time information recorded for each tracked cell through time

The analyses described in this manual include approximations of cell length, relative cell position within the root apical meristem, and cell growth and displacement dynamics through time. To this end, we analyze the relative positions of nuclei within a cell file. As LiPlaCeT users can identify each cell by its nuclear position (as in example presented here), in this section we will refer to each identifier for a nucleus, instead of a cell, in order to clarify the methodology, value and limitations of the parameters described.

4.3.5.1 Estimating distance between adjacent nuclei

When only nuclei are fluorescently marked, an approximation of cell length profiles is calculated by measuring the distance between adjacent nuclei. Given a **nucleus i** in time t and its adjacent **nucleus i-1** (for any nucleus the sorting information allows to

easily identify its adjacent nucleus). For calculation of the distance between nuclei **i** and **i-1**, the first step is to identify the user-defined **nucleus i** and its adjacent nucleus, which corresponds to **nucleus i-1**. Then, the distance between them corresponds to the Euclidean distance between their positions (Figure 37). The accuracy of this parameter as an approximation of cell length is limited by the variability of intracellular position of each nucleus. However, the inherent error of this parameter is compensated across subsequent calculations of the same parameter through the same cell file. As a result, although the accuracy of this parameter is limited for evaluation of individual cell length, it provides valuable information regarding cell length patterns across a 3D cell population.

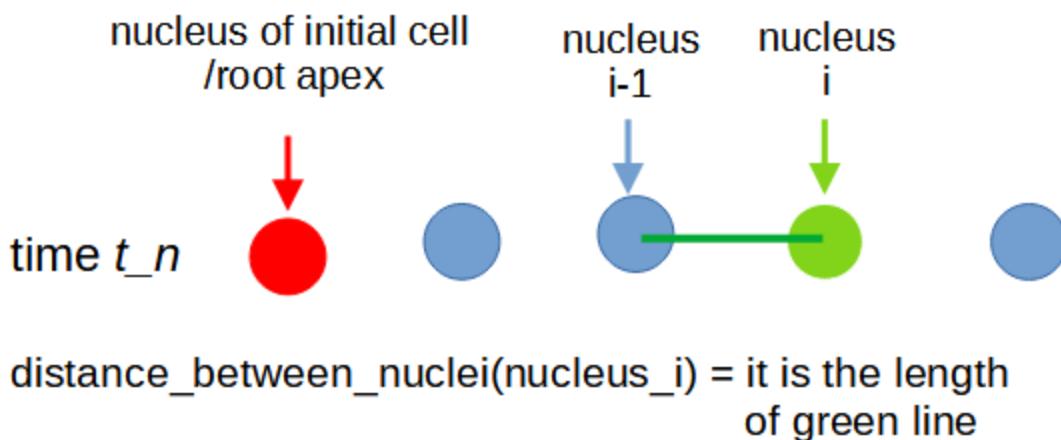


Figure 37. Schematic description of the methodology used to calculate the distance between adjacent nuclei. Distance between nuclei (distance **d**) is calculated for each nucleus within a cell file, with the exception of the nucleus contained in the initial cell located at the most apical end of the file (red circle). Given a user identified nucleus **i** (green circle), the adjacent nucleus in the apical direction is designed as nucleus **i-1**, and the Euclidean distance between them is calculated.

4.3.5.2 *Displaying 3D vector maps of distance between nuclei*

In order to display patterns of changes in distance between nuclei along the root in Paraview, go to the Coloring tab (see Figure 34 red rectangle) from Properties, and select **Distance_between_adjacent_nuclei** (see Figure 33). This procedure changes the descriptor to display in Paraview. Figure 38 depicts an example of choosing “distance between nuclei” from the Coloring option. Note that distance between nuclei can reach values up to 17 micrometers for the nuclei analyzed. At the first position of

the file, the value given is zero because there is no adjacent nucleus for the first cell in the apical direction.

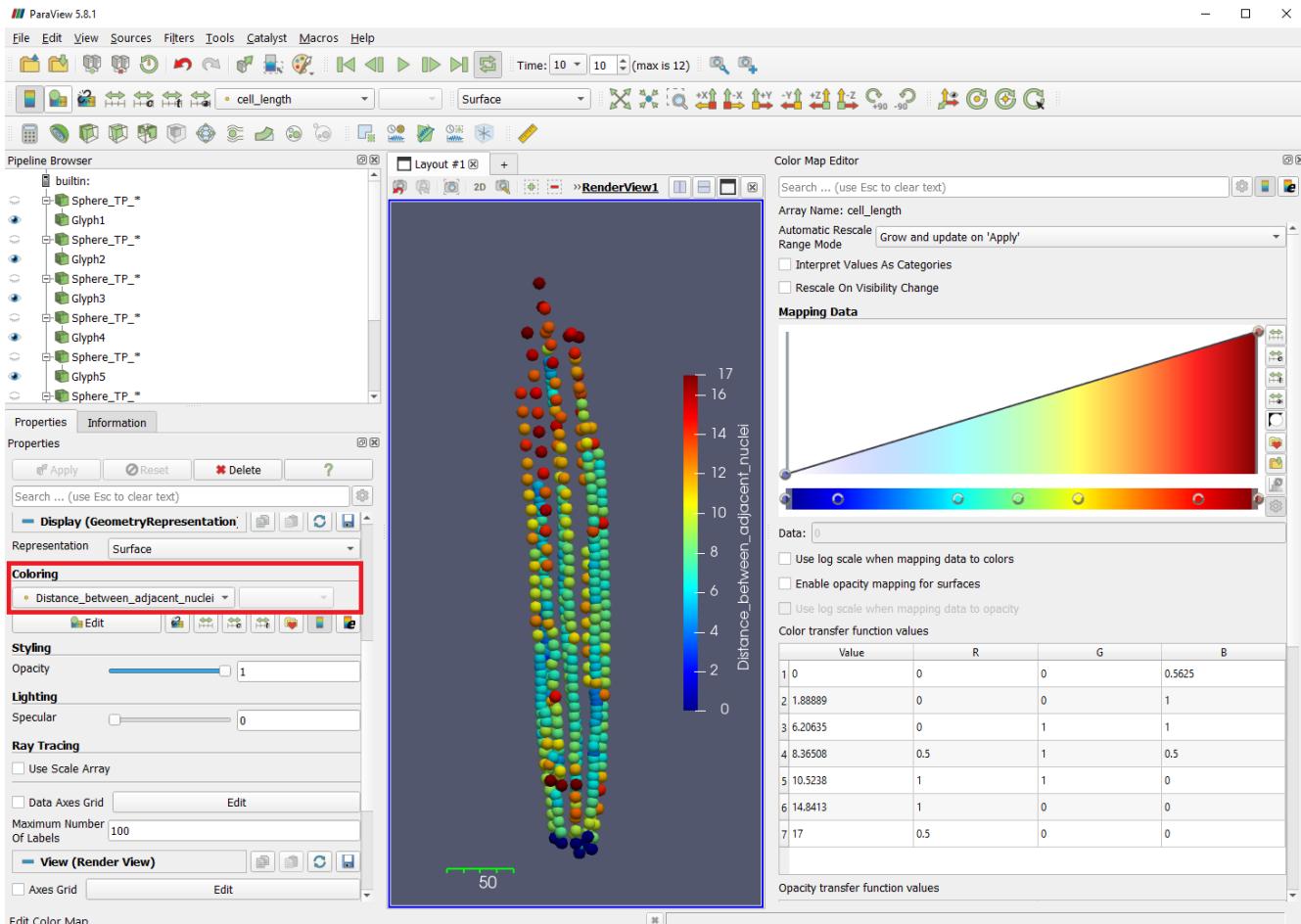


Figure 38. Selecting the option “distance_between_adjacent_nuclei” from the Coloring tab.

4.3.5.3 Estimating distance from a nucleus to the nucleus of the most distal cell

Sorting information as described in Section 4.2 is very important to do a correct estimation of the distance from any cell to an initial cell of a file within the root apical meristem. Given a **cell i**, we can identify its adjacent cell in the apical position (**cell adjacent 1**). As described in section 4.3.5.2, we will refer to this value as **nucleus i** and **nucleus adjacent 1**, in order to clarify this particular descriptor, and calculate the distance between nuclei (distance **d**). Next, given **nucleus adjacent 1**, we can identify its adjacent nucleus (**nucleus adjacent 2**) and calculate the distance

between them. We can iterate this process up to arrive to the nucleus located at the most distal position, the initial nucleus (IN). The distance to IN for **nucleus i** is the sum of all the distances between nuclei calculated (Figure 39).

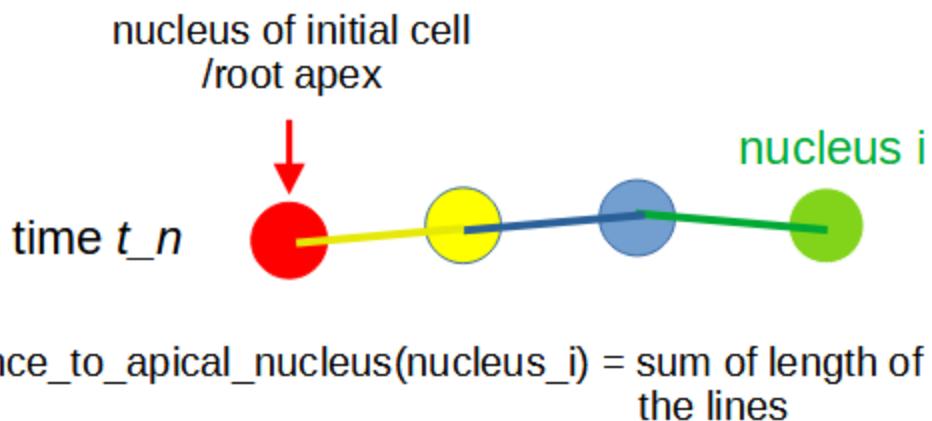


Figure 39. Schematic of a methodology to estimate the distance to the nucleus of the initial cell (NI). To calculate the distance to the NI for **nucleus i**, first, the distance between the nucleus of **cell i** (green circle) and its adjacent nucleus in the apical direction (blue circle) is calculated. Next, the distance between nuclei is calculated for the nucleus represented by a blue circle and its adjacent nucleus is identified (yellow circle). Finally, the distance between the nucleus represented by the yellow circle and its adjacent nucleus, in this case corresponding to the NI (red circle), is calculated. The process stops because the red mark corresponds to the nucleus of the first cell of the file. The distance to the NI for **nucleus i** is the sum of the lengths of green, blue and yellow lines.

4.3.5.4 Displaying 3D vector maps of Internuclear Distance to the IN

In order to display the cell distance to the apex in Paraview, go to the Coloring tab (see Figure 34 red rectangle) from Properties, and select `distance_to_IN` (see Figure 33). This procedure changes the measure to display in Paraview. Figure 40 shows an example of selecting `nuclar_distance_to_IN` in the Coloring option. The root apex is at the bottom; hence nuclei close to the apex have small distances, which are identified with blue color. As nuclei are positioned far away from the most distal cell, the distances increase to reach a maximum value (maroon color) at the top of the image, which corresponds to the nuclei that are more distant from the most distal cell, the values for this descriptor have a distance of approximately 450 micrometers.

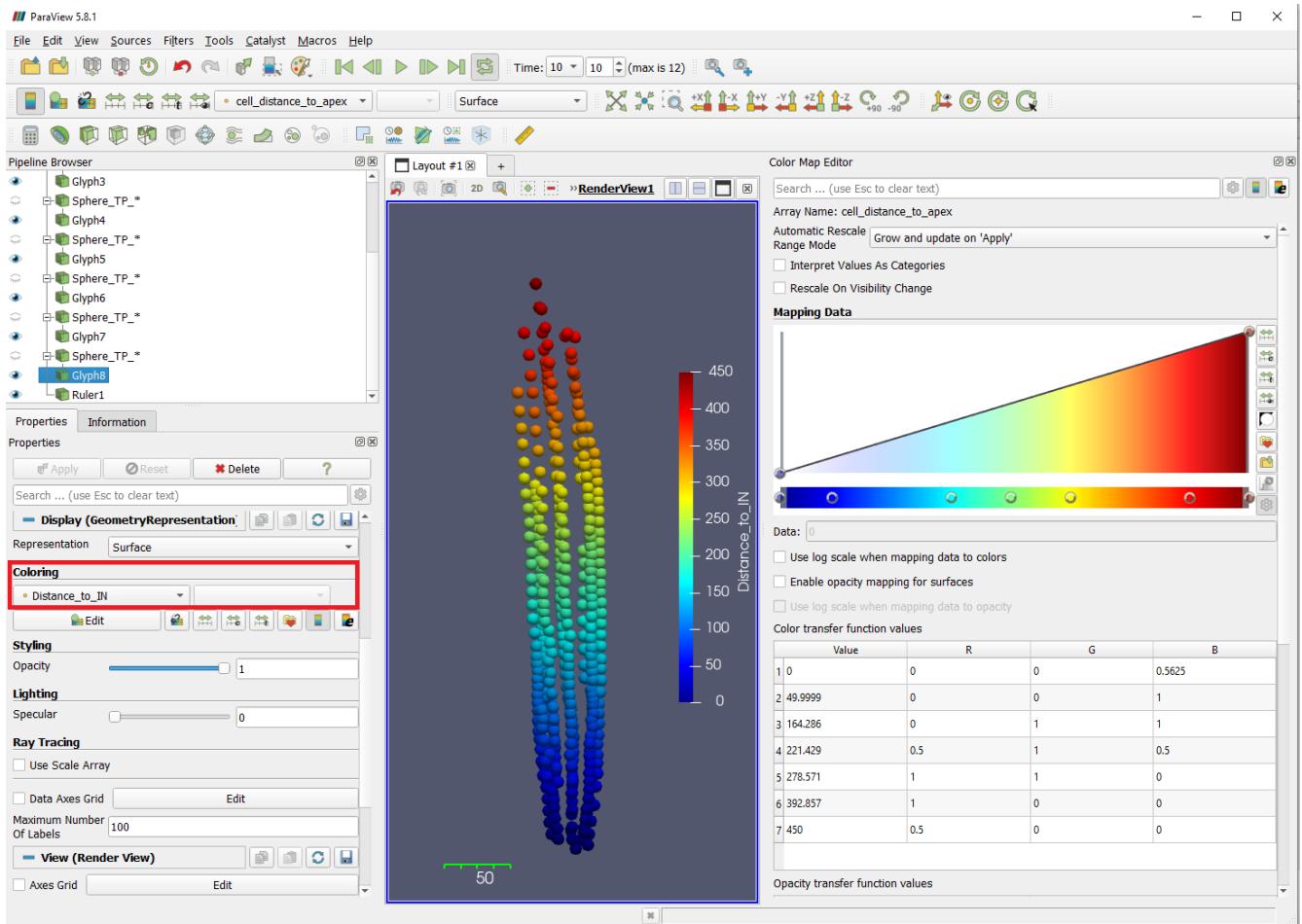


Figure 40. Distance to most distal cell, located at the bottom. From bottom to top the cell distance to apex is increasing.

4.3.5.5 Estimating Cell Growth Rate

Rate at which the estimated cell length changes over a period of time. Figure 41 depicts an example of calculating cell growth rate. It is calculated by the difference of the distance between nucleus i and its adjacent one (di) at time t_{n+1} (d_2) and di at time t_n (d_1) divided by the elapsed time between acquisitions t_n and t_{n+1} ($\Delta t = t_{n+1} - t_n$).

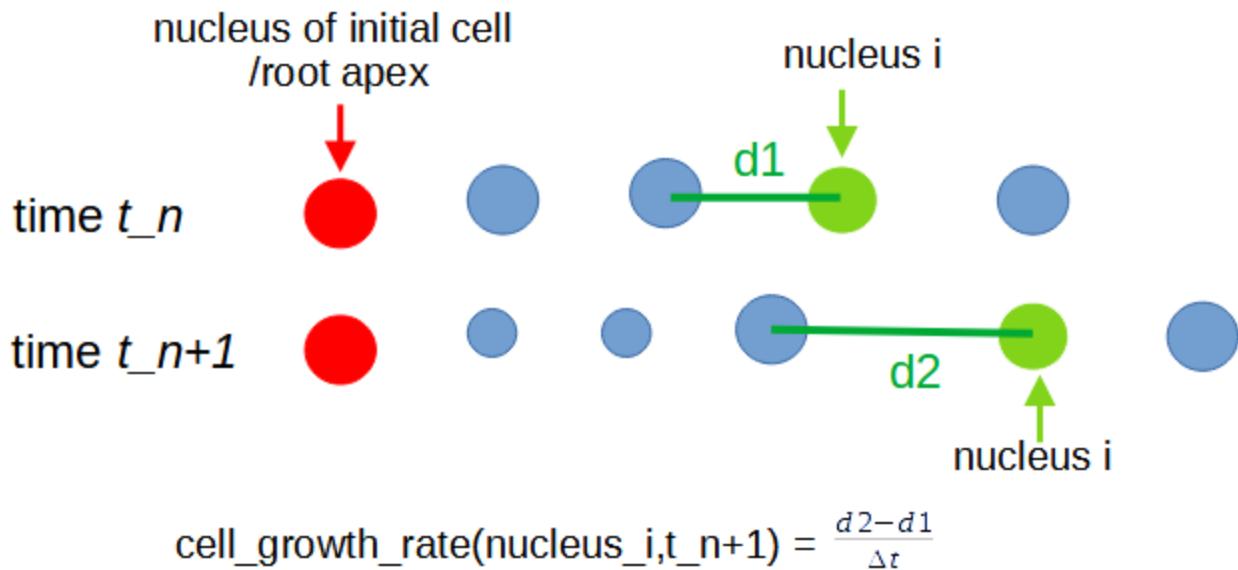


Figure 41. Cell growth rate for **cell i** at time t_{n+1} is calculated by the difference of distance between adjacent nuclei for nucleus i (d_i) at time t_{n+1} (d_1) and d_i at time t_n (d_2) divided by the elapsed time between the two acquisitions.

4.3.5.6 Displaying 3D vector maps of Cell Growth Rate

In order to display the cell growth rate in Paraview, go to the Coloring tab (see Figure 34 red rectangle) from Properties, and select Cell_growth_rate (see Figure 33). This procedure changes the parameter to display in Paraview. Figure 42 depicts an example of cell growth rate for *A. thaliana*. Note that cell growth rate has positive values if cell i at time t_{n+1} increases its size, while it can have negative values if it decreases its size (usually if the cell is divided). The values of cell growth rate can be as low as -2 or as high as 2. Lower and negative values are usually from the middle of the image to the bottom, meaning that there are divisions in this region while higher values are from the middle of the image to the top, meaning that cells are growing.

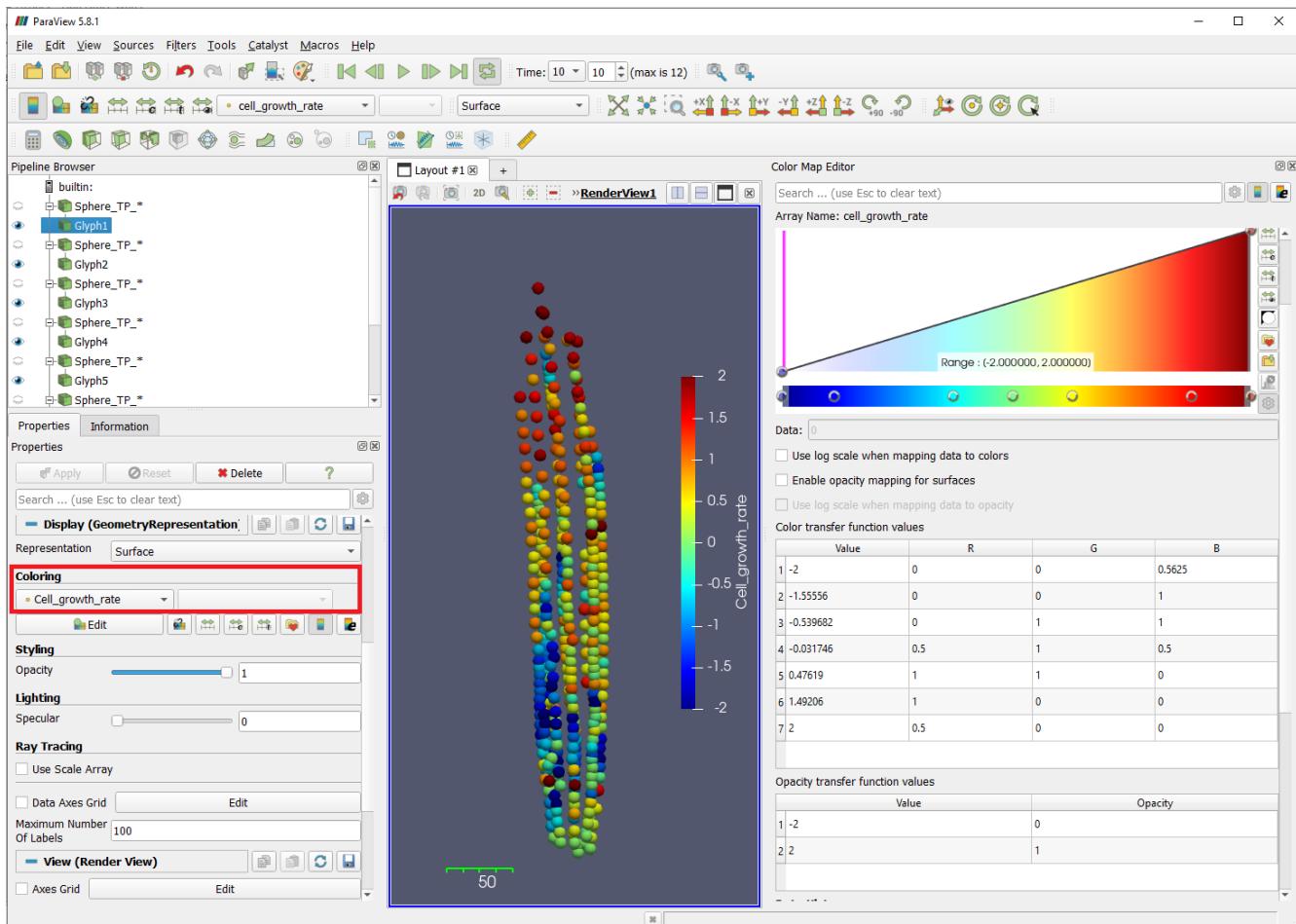


Figure 42. An example of cell growth rate for cells of the root apical meristem in *A. thaliana*.

4.3.5.7 Estimating Cell Displacement Rate

Rate at which the estimated cell distance to apex changes over a period of time.

Figure 43 depicts an example of calculating cell displacement rate, it is calculated as the difference (d_2) of the cell distance to apex for **cell i** at time t_{n+1} and cell distance to the nucleus of most distal cell (d_1) for **cell i** at time t_n divided by the elapsed time between acquisitions t_n and t_{n+1} ($\Delta t = t_{n+1} - t_n$).

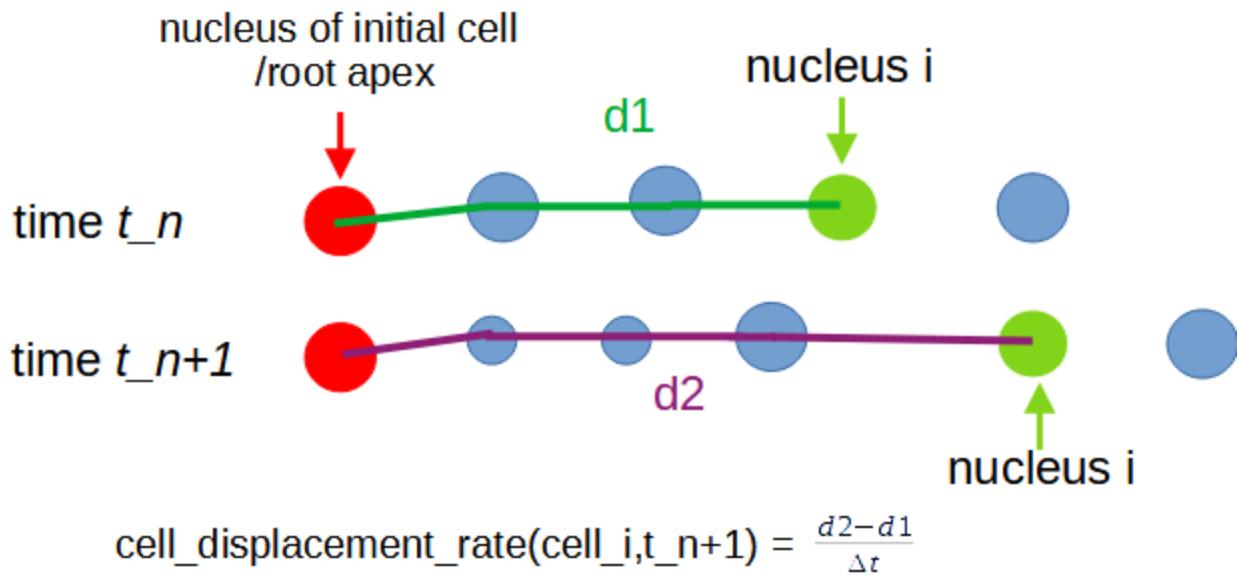


Figure 43. Cell displacement rate for **cell i** at time t_{n+1} is calculated by the difference of cell distance to apex of **cell i** at time t_{n+1} and time t_n divided by the elapsed time between the two acquisitions.

4.3.5.8 Displaying 3D vector maps of Cell Displacement Rate

In order to display the cell displacement rate in Paraview, go to the Coloring tab (see Figure 34 red rectangle) from Properties, and select Cell_displacement_rate (see Figure 33). This procedure changes the parameter to display in Paraview. Figure 44 depicts an example of cell displacement rate for *A. thaliana*. Note that cell displacement rate is lower at the tip while the displacement rate increases up to 25 microns/hour at the most distant cells of the root apex.

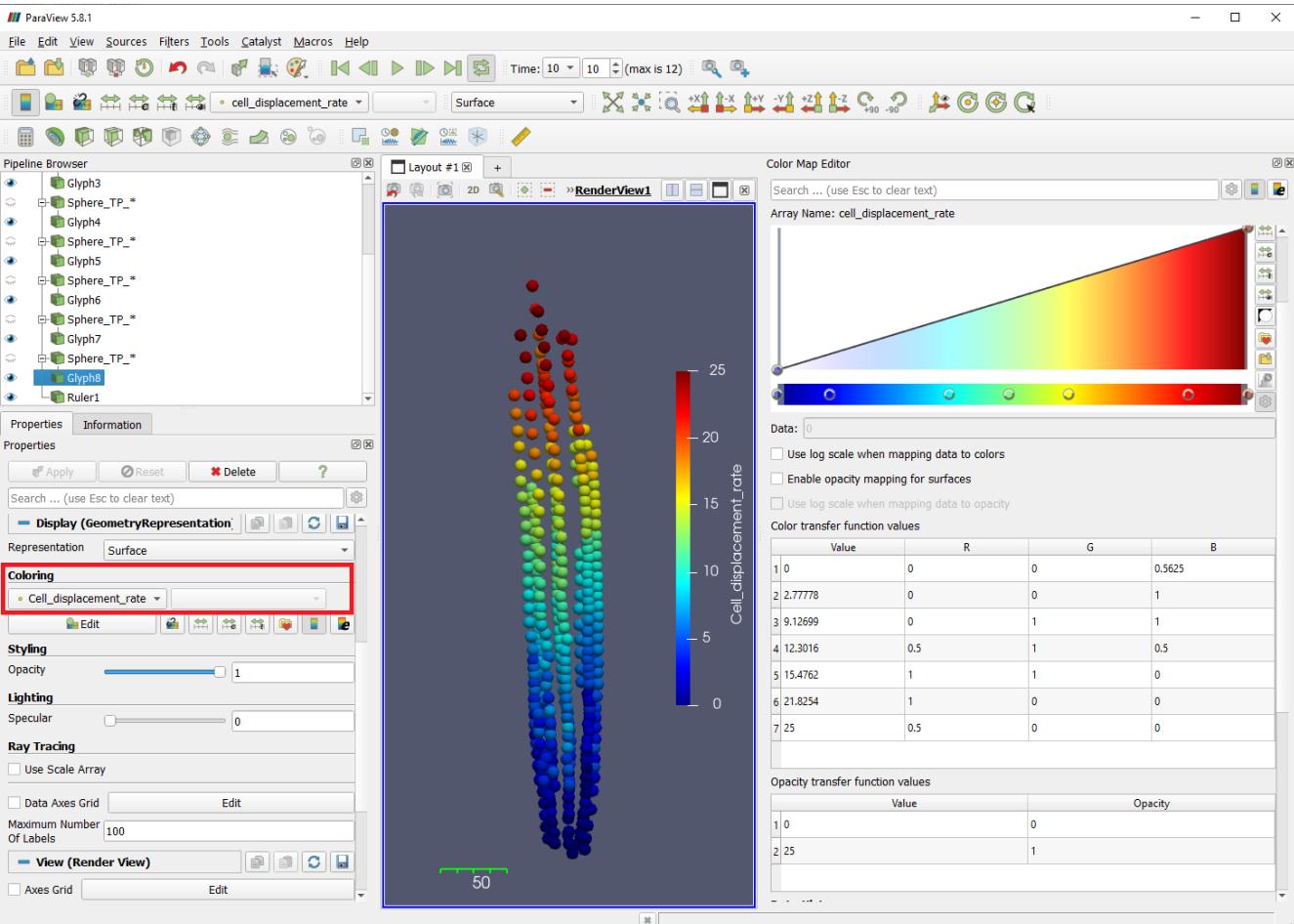


Figure 44. An example of cell displacement rate of cells within the root apical meristem of *A. thaliana*.

4.3.5.9 Saving cell dynamics to file

Generating the *.vtk files as explained in Section 4.1.1, also creates an additional file (Data_cell_dynamics.csv) which saves the cell dynamics information (if sorting information is available, see Section 2.8). The *.csv file format is:

Time Point, Distance_to_apical_nucleus, cell_displacement_rate,
cell_growth_rate, distance_between_nuclei

Time Point: the time point at which the cell is located

Distance_to_apical_nucleus: Parameter as explained in Section 4.3.5.3

cell_displacement_rate: Parameter as explained in Section 4.3.5.7

cell_growth_rate: Parameter as explained in Section 4.3.5.5

distance_between_nuclei: Parameter as explained in Section 4.3.5.1

Keyboard commands

- e** enlarge or zoom in
- d** diminish or zoom out
- m** more, increase cell cycle number
- l** less, decrease cell cycle number
- c** copy to the next time point

ANNOTATION

Left mouse click (any view)	Creates a spot at the mouse location (only if the mouse location is not inside any spot)
Right mouse click (inside a spot)	Removes the spot
Mouse wheel up (inside a spot)	Increases the radius of the spot
Mouse wheel down (inside a spot)	Decreases the radius of the spot
Left mouse drag (inside a spot)	Move the spot position
Left mouse drag in XY panel (outside spheres)	Move (translate) all the spheres in the XY view
Keyboard key ↑ (inside any panel)	increases the z values of all the spots by 1 (at the given time point)
Keyboard key ↓ (inside any panel)	decreases the z values of all the spots by 1 (at the given time point)
Create a link between mother and daughter	<ol style="list-style-type: none">4 Click button “Select Daughter”5 Click inside the mother at the left panel (Sphere color should change to green to indicate that it is selected)6 Click inside the daughter at the right panel (Sphere color should change to green to indicate that it is selected)7 Click button “Confirm Daughters”. (Spot color for the daughter must change to the mother's color to indicate same lineage)

Create a link for a division (Spots at right panels created using button "Copy Spheres")	<ol style="list-style-type: none"> 1. Click button "Select Daughter" 2. Select the daughters at the right panels (Daughters spot's color and mother's color should change to green to indicate that it is selected) 3. Click button "Confirm Daughters". (Spot color for the daughters must change to the mother's color to indicate same lineage)
Create a link for a division	<ol style="list-style-type: none"> 1. Click button "Select Daughter" 2. Click inside the mother at the left panel (Sphere color should change to green to indicate that it is selected) 3. Click inside the daughters at the right panel (Sphere's color should change to green to indicate that it is selected) 4. Click button "Confirm Daughters". (Spot color for the daughters must change to the mother's color to indicate same lineage)
Button "Copy Spheres"	Copy spots created at the left panel to the right panel with automatic link

STACK NAVIGATION

Mouse wheel up (outside any sphere)	Move the slice from the current view one slice up
Mouse wheel down (outside any sphere)	Move the slice from the current view one slice down
N	Move to next time point
P	Move to previous time point
Button Next time	Move to next time point
Button Previous time	Move to previous time point
Right click -drag blue line	Move slice up or down faster
Panel Ortho-view Radii button (XZ, YZ)	Allows to select the ortho-view to display