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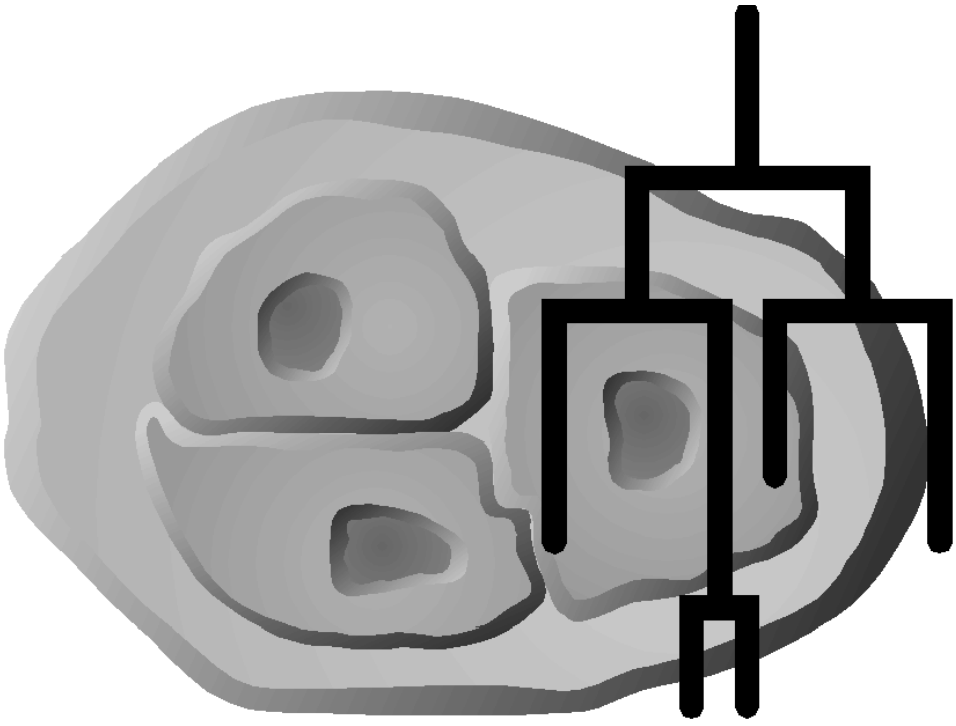
REALITY MOTION SYSTEMS GMBH

SIMI°BioCell

Users Manual



SIMI°BioCell

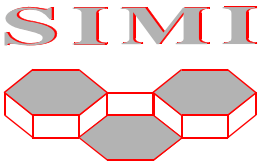
Users Manual

for Version 2.23

ACKNOWLEDGEMENTS

Special thanks to Prof. Ralf Schnabel and Titus Kaletta from the Max-Planck-Institute for Biochemistry.

Serving our customers with an individual system tailored to their special needs...



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Publisher

SIMI Reality Motion Systems GmbH
Pappelgasse 5
Postfach 1518
D-85705 Unterschleissheim
Germany
Phone: +49-89-321 45 9-0
Fax : +49-89-321 45 9-16
E-Mail: simi@simi.net
Web: www.simi.net

Manual

Titus Kaletta (kaletta@alpha.bio.nat.tu-bs.de)
Toni Zeitler (zeitler@simi.net)

Introduction

*Welcome to the fascinating possibilities of Cell Lineage Analysis…and thank you for choosing **SIMI°BioCell**. It will be a powerful tool to explore the embryo.*

The primary purpose of this Users Manual is to explain how to use **SIMI°BioCell**. It contains the detailed information you need to use this software to YOUR fullest ability. The manual's format facilitates its use as a reference volume, but you may find that at least one sequential, recreational-type reading will provide a broader foundation upon which to understand the items you may want to investigate in depth later.

It is important to understand at the outset that **SIMI°BioCell** is not like any other program. The interface is very intuitive, the controls and tools look and act similar to those you have used in other IBM compatible software, and the software keeps you informed every step of the way. You should read this manual to tap the full capability of **SIMI°BioCell.**.

In **1994**, a co-operation between us and Prof. Ralf Schnabel (Max-Planck-Institute for Biochemistry, Munich) started to develop a software to investigate embryogenesis. The technical part was **SIMI°Motion**, the software for two- or three-dimensional motion analysis, the biological part was a worm with a transparent embryogenesis and the medium was a time-lapsed video recording acquired by a 4d-microscope. The result was **SIMI°BioCell** that is able to track the cells, their division pattern and their specification as tissues during the development into a worm.

In the meantime **SIMI°Motion** offers a wide range of components to assemble an individual system. Our modules from "filters" and "automatic marker recognition" up to "highspeed video" have been designed from the practice in close Cupertino with our customers.

In the course of time the **SIMI°Evolution** even developed new species :

- **SIMI°BioCell** looks into the microscope.
- **SIMI°Scout** the latest creature in the line offers analysis of behaviour, interaction and communication. In the field of sports a new dimension in game and tactical analysis was born.

Would you like to join the **SIMI°Evolution**, even taking over an active part to develop the variety of species that analyse dynamic processes? Feel free to contact us, we are looking forward to your suggestions.

Contents

Chapter 1

Installing software for Windows

Installing **SIMI°BioCell** is as easy as you might think of!

You need...

- an IBM compatible PC with at least 8 MB memory and 3 MB free space on your harddisk.
- a laser videodisc player (e.g. Sony LVD-8000P) connected to a serial port of your PC as source for the video data and a video board with live video display capability (e.g. ///FAST Screen Machine).
- or as alternative Microsoft Video for Windows, a video capture board with compression (e.g. M-JPEG) and any video source for the video data.
- Microsoft Windows 3.1

To install...

- Just execute "install.exe" on the installation disk.
- Enter the destination directory in which you want **SIMI°BioCell** being installed.
- Press the OK button! Icons for the program are created automatically.
- Don't forget to connect the dongle at the parallel or serial port!

Chapter 2

Getting Started with SIMI°BioCell

In this session you will learn how to composite a video recording and SIMI°BioCell, the basic handling of this software and how to use it as a convenient lineage tool.

This chapter is thought for users who never have worked with this software so far. It accompanies you from inserting the disk, creating a new project to finish your first lineage session. A complete description of all functions you will find in the Chapter 4 **List of Functions**.

1 Starting SIMI°BioCell

Assuming you have already installed the SIMI°BioCell software and are set up with the hardware devices, you are ready to start your first lineage session.

Start SIMI°BioCell by clicking on

SIMI°BioCell

- Insert the disk.
- Double-click on the SIMI°BioCell icon.

You will see SIMI°BioCell on-screen (Fig. 2.1). To find out what you are looking at, read on.

2 What is on the screen

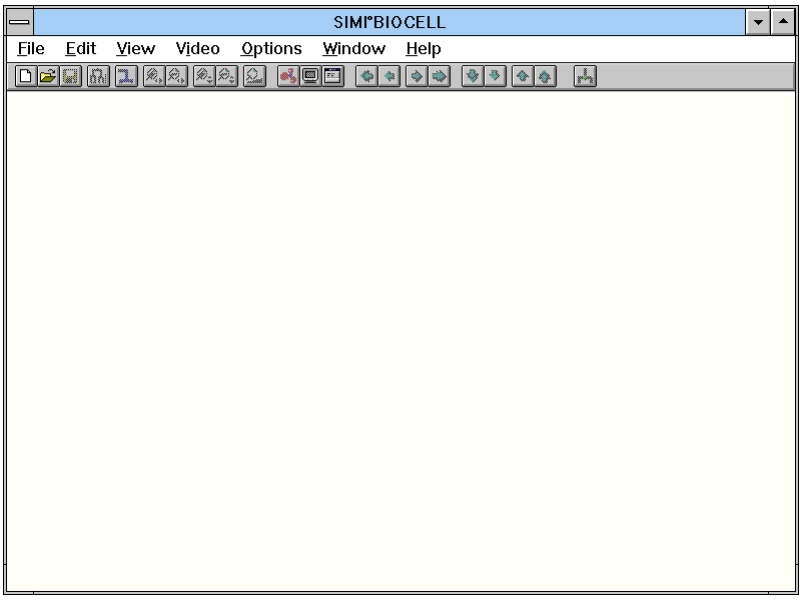


Figure 2.1 **SIMI°BioCell** screen.

The following section details the SIMI°BioCell window.

2.1 Title Bar

The top of SIMI°BioCell's window is the title bar, a standard Windows feature. Here, SIMI°BioCell displays its own name. In addition, you see the title of the document.

2.2 Menu Bar

You find the titles of SIMI°BioCell's menus, from which you can choose

You will find a detailed description of all menus in **Chapter 4**.

- **File** This menu enables access to various file management, printing and information options.
- **Edit** The Edit pulldown menu contains, in contrast to other Windows applications, specific cell lineage editing options like **Cell...** description and colouring menus (**Mark...**).
- **View** Toggles the visibility of fates, names and other information in the **Cell lineage** window. Furthermore, it offers access to SIMI°BioCell tools like the **3d-view**.
- **Video** This menu contains the play back functions like **Frame step forward** and **Level increase**.
- **Extras** This menu contains the **Navigator** and the **Collision Manager**.
- **Options** Presents a pop-up menu for selecting preference items like **autosave** and **auto center cell**. It is important to know that you could set up the **Cross wire**.
- **Window** Here, you could arrange the windows.
- **Help** This menu offers access to SIMI°BioCell's on-line help directory - but hey, you have got this book.

2.3 Toolbar

Tools, like the **3D-**model, the lineage navigator, are described in Chapter 3.

The toolbar contains buttons, that enable easy access to frequently used SIMI°BioCell commands and tools.


New project


Open project


Save project


Show standard lineage


Center active cell

 Zoom in horizontally

 Zoom out horizontally

 Zoom in vertically

 Zoom out vertically

 Free zoom

 Open the 3d model

 Video image

 Information window

 Map window

 Frame fast backward

 Frame step backward

 Frame fast forward

 Frame step forward

 Level fast decrease

 Level step decrease

 Level fast increase

 Level step increase

 Mitosis

For the Time-Challenged

Do not spend too much time trying to learn all the options on the menu bar. You will not use all of them frequently. Many options are available on the tool bar, a lot of them are accessible by double-clicks or keyboard commands and for the most important functions SIMI°BioCell provides short cuts.

2.4 Application Workspace

Here, SIMI°BioCell displays the file you are currently working on, the video image and all tools you have additionally activated. If the windows are bigger than the available application workspace, the scroll bars are activated. You can click the scroll arrows, or drag the scroll bars, to bring hidden portions of the document into view.

3 Basic windows callisthenics

3.1 Moving the Window

To place the various windows of SIMI°BioCell:

- Move the mouse pointer to the title bar, hold down the left mouse button, and drag.

- When you have moved the window to the right place, release the mouse button.

3.2 Sizing the Windows:

To size the SIMI°BioCell windows :

- Move the mouse pointer to one of the window's borders until the pointer change's shape, and drag the border.

- When you have sized the window the way you want, release the mouse button

Now that you have gone to all the trouble to position the window the way you want, SIMI°BioCell automatically saves your choices.

To size two borders at once, click on one of the corners and drag.

4 Creating a new project

In this section the integration of a recording into a SIMI°BioCell project is described. For a new project you need to link a standard lineage (that is normally the Sulston lineage) serving as a template with a time lapsed video recording of a C. elegans embryo.

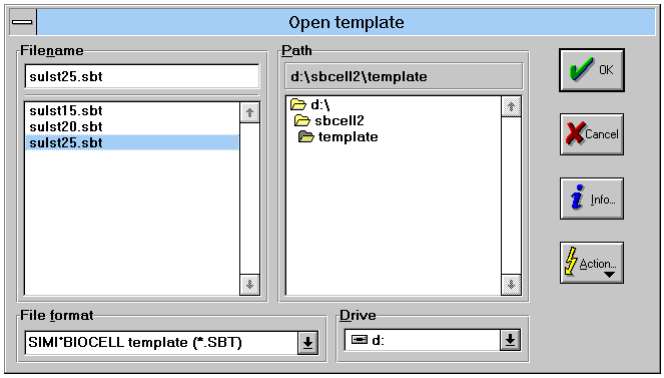


Figure 2.2 **Open template** dialogue box.

Lineaging with SIMI°BioCell means to build up your own lineage of a recorded embryo based on a template lineage.

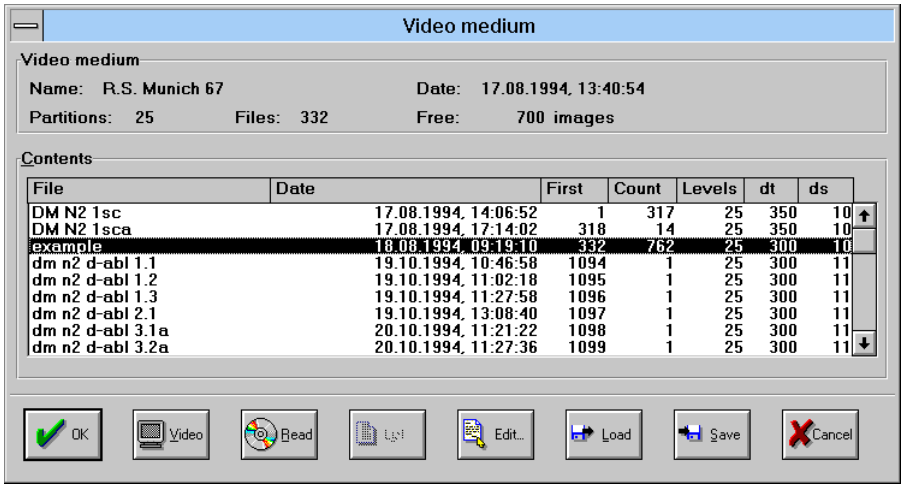
4.1 To access to one of your recordings

You have inserted a disk with a time lapsed video recording that starts (in our example) at around the 4 cell stage and lasts some division rounds.

- Click on the **File** menu and double-click on **New Project...**. The **Open template** dialogue box provides files with standard lineage trees at various recording conditions (e.g. temperature), so called templates, as shown in Figure 2.2.

- Select `sulst25.sbt`. Now, SIMI°BioCell starts to read the directory file of the disk, that may take some time. Afterwards you will see the **Video medium** dialogue box, as shown in Figure 2.3.

- This box displays a list of all recordings on the disk including information about the scan start, the length, level, time etc. Choose your desired recording by double-clicking on its name or by clicking and pressing the **OK** button.



Use the scroll bar to find your recording!

Figure 2.3 **Video medium** dialogue box.

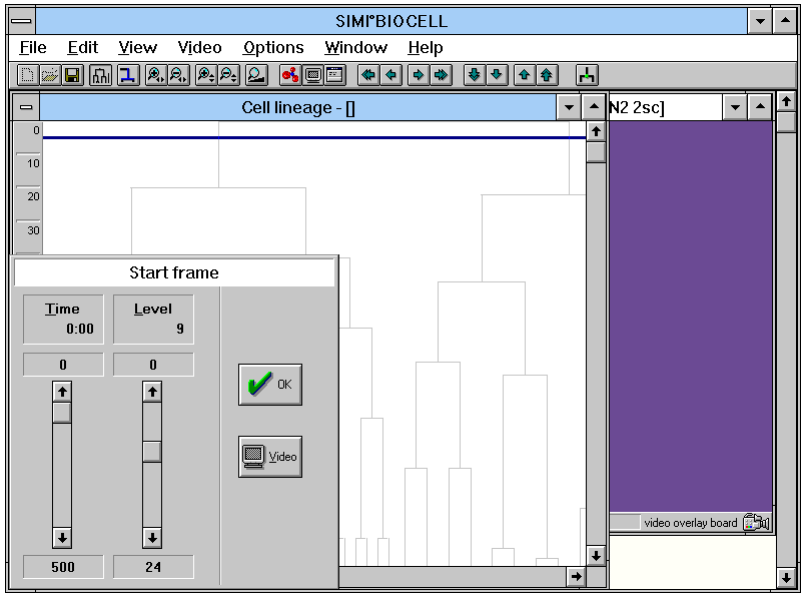


Figure 2.4 **SIMI°BioCell** main window, **Cell lineage** and **Video Image** windows, as well as the **Start frame** dialogue box. Here, the **Cell lineage** window is active!

4.2 To adjust a SIMI°BioCell template to your recording

On the workspace you will see the **Start frame** dialogue box that helps you to adjust the beginning of a recording to a certain template (which normally starts at P0, of course), as shown in Figure 2.4.

- Double-click on the **Video** button to open the first scan in the **Start frame** dialogue box.

- Focus on the nucleus of an AB daughter by using the scroll bar **Level**

- Click on the window **Video Image** to activate it and play the recording until the two AB daughters are dividing by using the cursor control → or the **Frame step forward** button on the tool bar.

- You either estimate the beginning of your recording or you count the number of scans (steps) between the first scan and the first division.

- Now, click on the window **Cell lineage** that contains the template lineage tree as a grey setting, as shown in Figure 2.4.

If you have counted x scans, place the time line on the ABx mitosis of the template setting, and go x steps back.

- Use the scroll bar **Time** to place the *blue time line* of the window **Cell lineage** between the AB and ABx division.

- Press the **OK** button or **return**. The template is now adjusted to your recording, as shown in Figure 2.5.

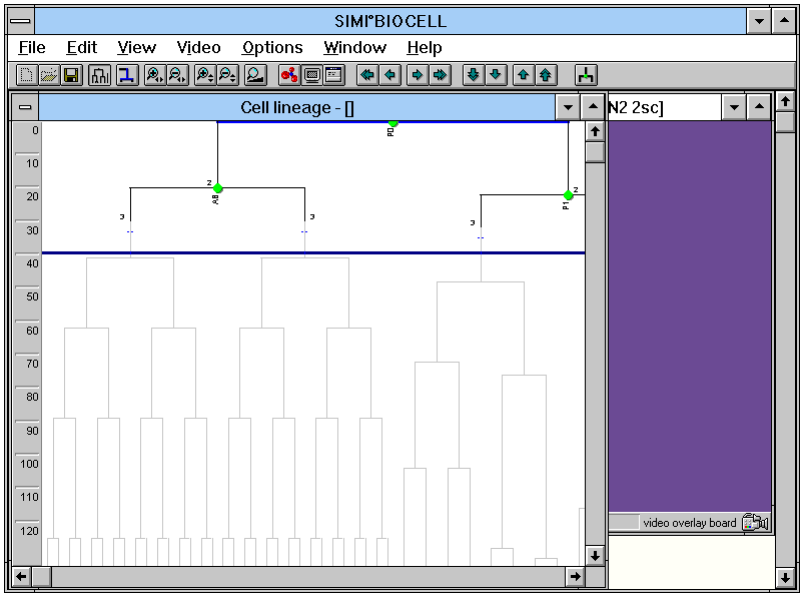


Figure 2.5 **SIMI°BioCell** main window, **Cell lineage** and **Video Image**. Here, the **Cell lineage** window is active. Note, that the new part of cell lineage adjusted to your recording is *black* and the mitosis are marked as *green* points.

4.3 The Cell lineage window

The **Start frame box** is disappeared. You see the **Cell lineage** window with the Sulston tree as *grey* setting and the new *black* cell lineage of your project, as shown in Fig. 3.5. The blue time line indicates the beginning of the recording referring to the template. In our example the recording starts during

the 4 cell stage, therefor the branches of the previous stages are automatically generated and are already displayed.

- Click on the *black* **branch** of the ABa cell. It switches to *blue*, which means it is active now, as shown in Figure 2.6.

- Click on the **Video Image** window and focus the ABa nucleus (either using the tool bar or the cursor keys ↑ and ↓)

- Move the tip of the mouse pointer so that it is positioned at the centre of the nucleus on the video image and click on it with the RIGHT mouse button. Forget all the double-clicking stuff; you just click once.

A *red* cross appears indicating that the position of the cell on the video image and the scan number as well are stored, as shown in Figure 2.7. This information is shown off with a *red* point at the corresponding time in the lineage tree. If you click on a *red* point in this lineage tree the corresponding cell marked with the *red* cross will be displayed in the **Video Image** window.

- Activate the right branch (that of ABp) and mark ABp, continue with EMS and P2.

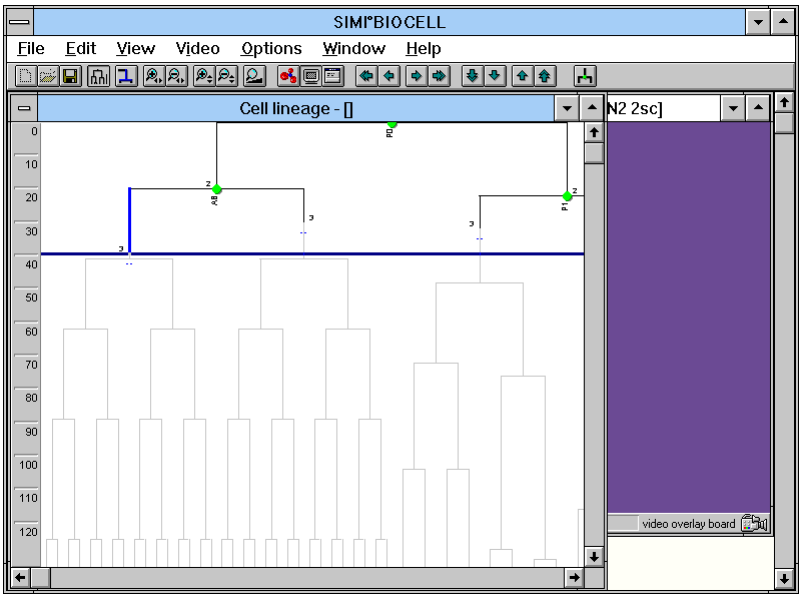


Figure 2.6 **SIMI°BioCell** main window, **Cell lineage** and **Video Image**. Here, the **Cell lineage** window is active. Note, the branch of the AB daughter ABa is blue (bold type)now, that means it is activated.

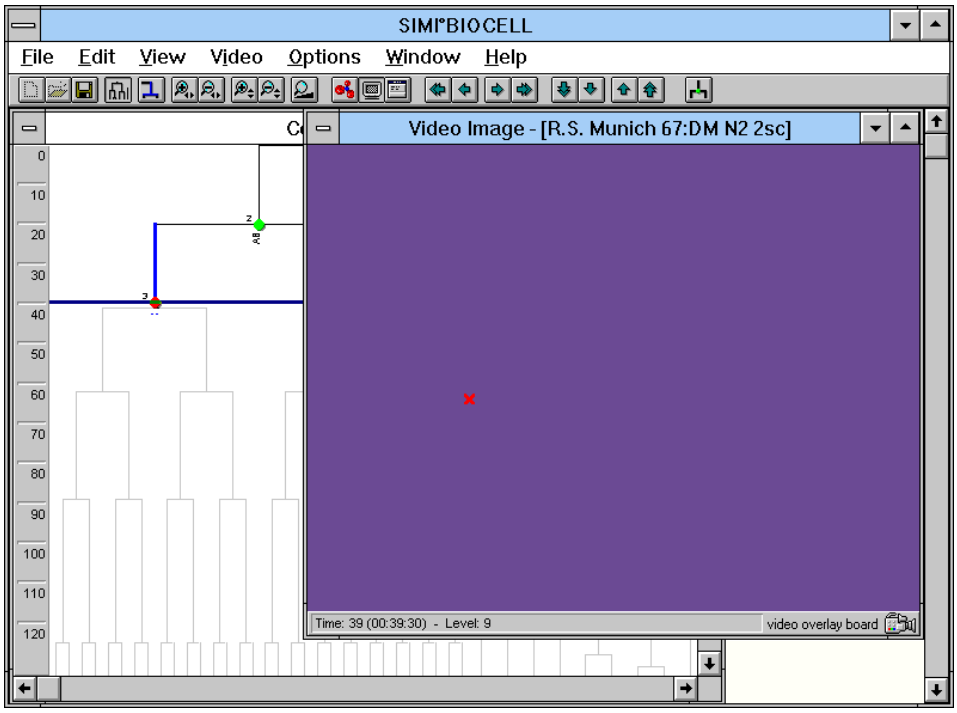


Figure 2.7 **SIMI°BioCell** main window, **Cell lineage** and **Video Image**. Here, the **Video Image** window is active. Note, the red cross on the **Video Image** is indicating the position of ABa and the red point in the **Cell lineage** window is indicating the corresponding position in the cell lineage.

4.4 Save your project!

To save a project:

- Choose from the toolbar. A **File Info** dialogue box appears. Fill in your name and press the **OK** button or simply return.

The **File Info** dialogue box allows optionally to fill in the author's name, the project and comments like the recording conditions, see Figure 2.8.

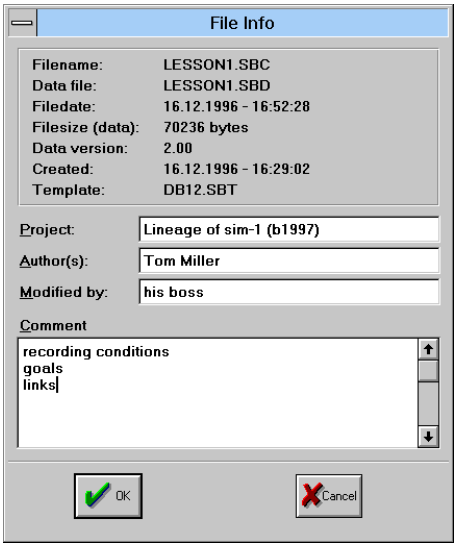


Figure 2.8 **File Info** dialogue box.

5 The first lineage session

The real work starts now. You have to follow cells, mark them with the RIGHT mouse button on the video image, to enter mitosis and finally to determine the cell fate at the end of a recording. Follow the next section to learn how to use the SIMI°BioCell lineage software.

5.1 Entering a mitosis

Lineaging means to follow a cell until it is dividing and then to continue with one of its daughters.

After having inserted a mitosis, instead of clicking on a branch press **1** (**3**) on the numeric pad for the anterior (posterior) daughter. Switching to the sister goes with **4** and **6**.

- Activate the branch of the ABa cell (it is now highlighted in blue). Follow this cell by playing the recording.
- Mark the ABa cell time by time.
- If it starts to divide press either return or the mitosis button on the tool bar. A fork is created (a mitosis is indicated by a green point).
- Choose a daughter and follow it up to the 12 cell stage, see Figure 2.9 and 2.10.

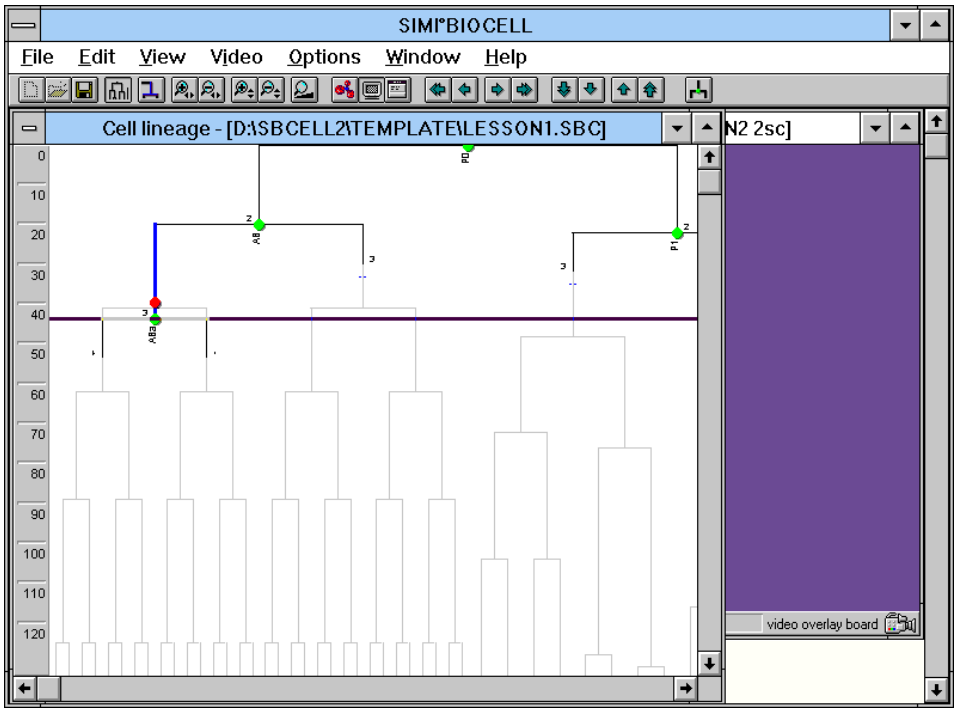


Figure 2.9 **Cell lineage** window. A fork is created for the two daughters ABal and ABar. The point for the mitosis is coloured green and named with the mothers name, in this case ABa, of course.

5.2 Making use of the software

Up to now you spent more time on this program than you got out! This will change right now. While lineaging you "lose" a cell you are following for many reasons (and it happens all the time):

Use the **0** key of the numeric pad to jump to the last point or **control** + **0** to jump to next red point!

- Click on the last red point of the current cell.

The recording immediately jumps back to this position displaying a red cross on the corresponding position.

- Try again!

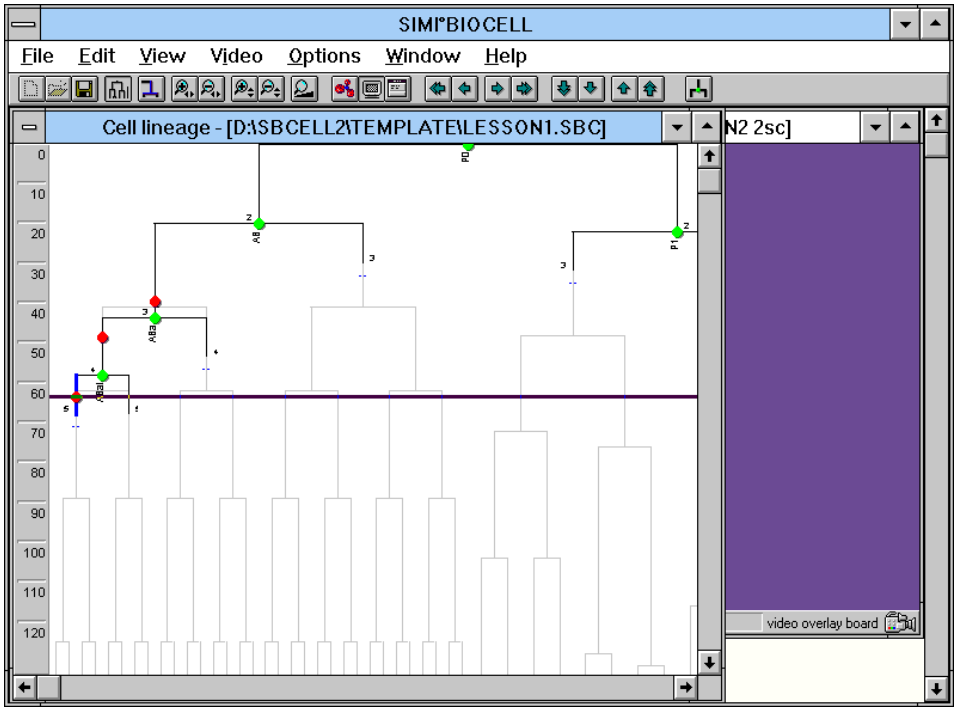


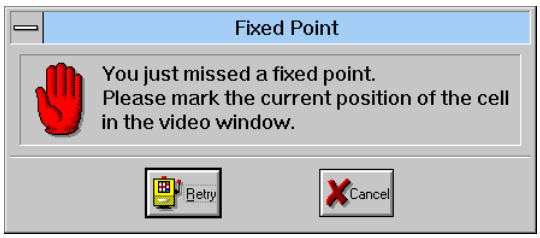
Figure 2.10 **Cell lineage** window. Lineage of ABala.

5.3 Fixed points are indispensable for the SIMI°BioCell tools!

It is useful to have every 20 minutes a fixed point line!

Most of the SIMI°BioCell tools require that all cells at a certain time are marked. This function helps and forces you to do so. You have completed the lineage up to the 12 cell stage.

- Click on one of the points.
- Choose **Edit** from the menu bar and click again on **Fixed points**. A **Fixed Points** dialogue box will be shown.
- Press the **add** button and fill in a name (optional). Press twice **return** to return to the **Cell lineage** window.



A stippled time line is visible. You can only pass this line if you mark a cell at exactly this time!

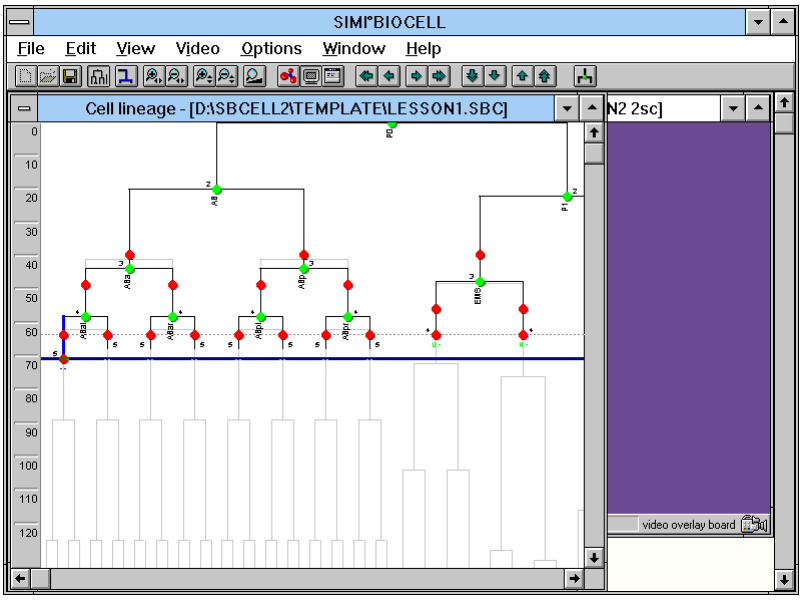


Figure 2.11 The complete lineage of an embryo to the 12cell stage. A stippled line at 60min (note the time strip at the left side of the **Cell lineage** window), the fixed point, is visible.

The interactive windows of SIMI°BioCell

If you have marked a cell on the video image, a data set for this cell will be created (its name, its position in the embryo, its birth time etc.) and stored in a database, a SIMI°BioCell project. These data sets are organised as members of the lineage and displayed on the **Cell lineage** window as points. To access to certain cell information and the corresponding video image you have simply to click on a point *or* a branch. Furthermore you could edit and analyse these data sets, of course. **See Chapter 5.1!**

5.4 Determine a cell fate

Watch out **chapter 5.1** for advanced actions on points and branches!

Information about a cell are accessible in the **Cell** information and dialogue box. Here, you could enter deviations to the Sulston lineage, too.

- Follow one cell to the end of a recording and determine its cell fate.

- Double-click on the branch or click on **Edit** and again on **Cell...** .

The **Cell** dialogue box will appear, as shown in Figure 2.12. It gives information about the actual cell. Choose a fate! The last red point of this cell in the lineage has now a legend.

Choose the **Information** box from the tool bar; it is very useful to show notes written in the **Point** dialogue box.

5.5 Deleting a point

Information considering a red point, that is like a snapshot during a cell life, are accessible in the **Point** information and dialogue box. This box is also very useful, if you want to delete a point, for example if you have mixed up two cells.

- Double-click on a red point. You will see the **Point** dialogue box, see Figure 2.13.
- Press the **Delete** button to remove the current point.

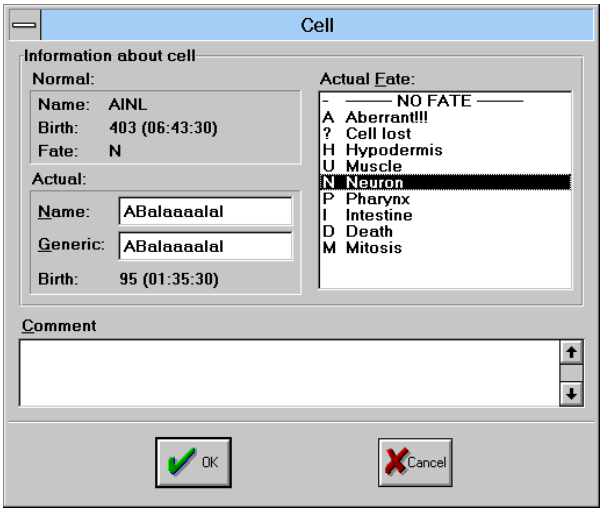


Figure 2.12 The **Cell** dialogue box. Abalaaaalal is a neurone!

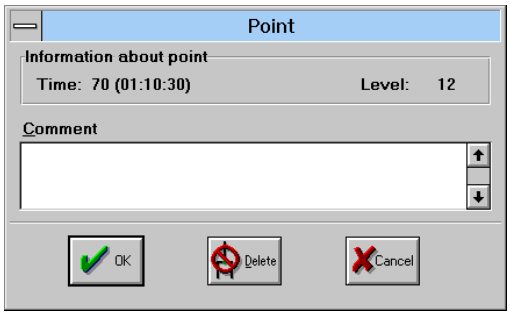


Figure 2.13 The **Point** dialogue box. It tells you the time and level. Fill in a comment and press the **OK** button, the comment will be shown in the **Information** box. Press Delete to delete the point.

5.6 Deleting a sub-lineage

In chapter 3 a complete section describes how to copy or to exchange complete sub-lineages.

Of, course, it is possible to act on a complete sub-lineage!

- Click with the RIGHT mouse button on a point. You will see a cell edit menu.
- Choose **Delete all points downwards**.

6 Quitting SIMI°BioCell

So, you are done. It is about 5 AM, isn't it? Hope you had fun. To quit SIMI°BioCell :

- From the **File** menu, choose **Exit** or press Alt + F4.

6.1 From Here

Now, you have learned to lineage with SIMI°BioCell. ...

- Read Chapter 3 to see the SIMI°BioCell tools.
- Use Chapter 4 as a complete reference of all functions.
- Running into problems - Check out Appendix B.

Chapter 3

SIMI°BioCell tools

In this chapter all SIMI°BioCell tools are introduced.

The SIMI°BioCell tools require a database with lineage information. These tools produce based on this once generated database secondary information. Some otheer tools may be still used as lineage support like the collision manager. Others help to analyse the cells within a three dimensional embryonic context.

1 The 3D-model

The 3D-model is the projection of the lineage data into the third dimension. Every point or cross is represented by a sphere that is used to build up a 3 dimensional embryo.

1.1 Requirements

In **Chapter 2** „Creating a new project“ you have learned to create a database or lineage project. These information are necessary to build a three-dimensional embryo. You need a project that contains at least a complete lineaged cell stage or a complete lineaged blastomere. Furthermore, it has to be lineaged with the **Fixed points** option.

1.2 The 3D model window

- Click on a point of the fixed point line, e.g., at the 12cell stage.
- Open the **3D model** window by pressing  on the tool bar.

The **3D model** window has an application workspace that shows the 3 dimensional model of the lineage and several option buttons, combo boxes and scroll bars to edit the presentation and orientation of the model, as shown in Figure 3.1.

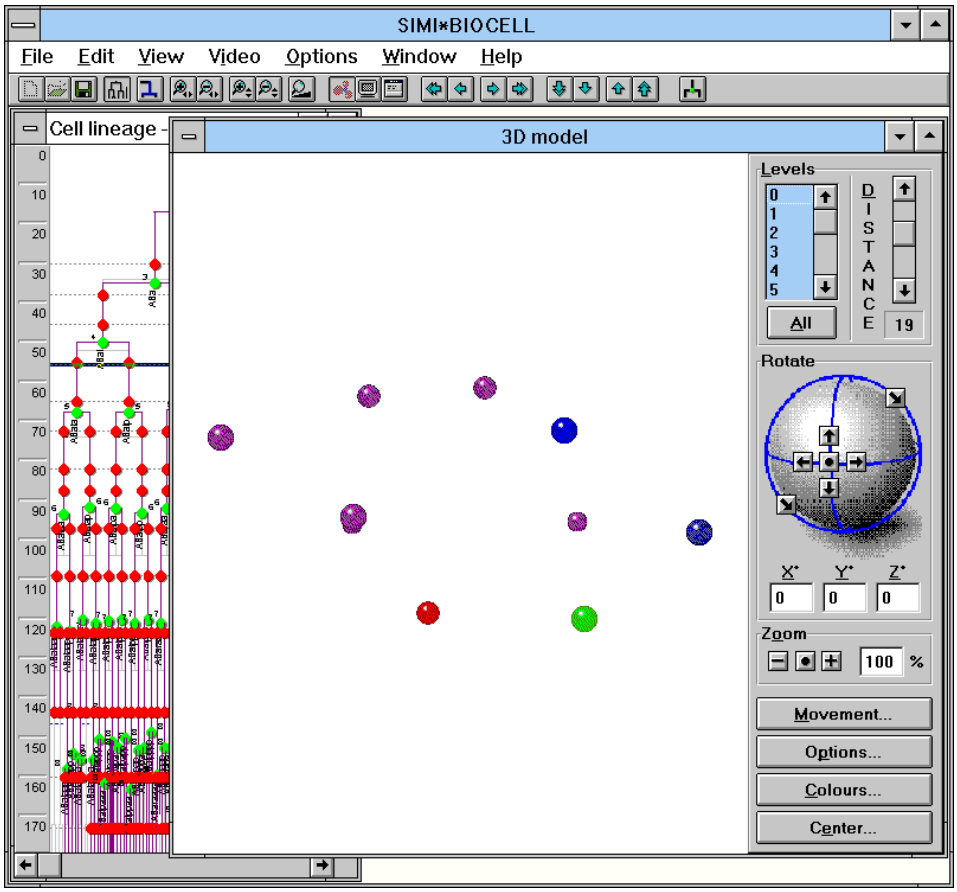


Figure 3.1 The SIMI°BioCell main window, the **Cell lineage** window and the active **3D model** window. A 3d model of an 12cell stage is shown.

Like the **Cell lineage** window the **3D model** window is also interactive.

LEVEL: tells you the real level of the scan whereas CURLEV: is the fictive level after the embryo was rotated.

- Place the mouse pointer on a sphere. A yellow flag the **Fly over information** tells you the cell's identity, its fate, the time of birth and the level, as shown in Figure 3.2.

- Double-click on a sphere and the corresponding point in the **Cell lineage** window is shown.

Double-click on a sphere and the corresponding point in the **Cell lineage** windows is shown, double-click on a point and the corresponding sphere is highlighted in the **3D model** window and have a look at the **Video Image** to see the cross at the corresponding position.

There is an interactive link between these windows, o.k., that's fine -nice software. No! Hey, haven't you realised that - this is the first time in your life *that you can identify a cell in an organism by simply clicking on it!*

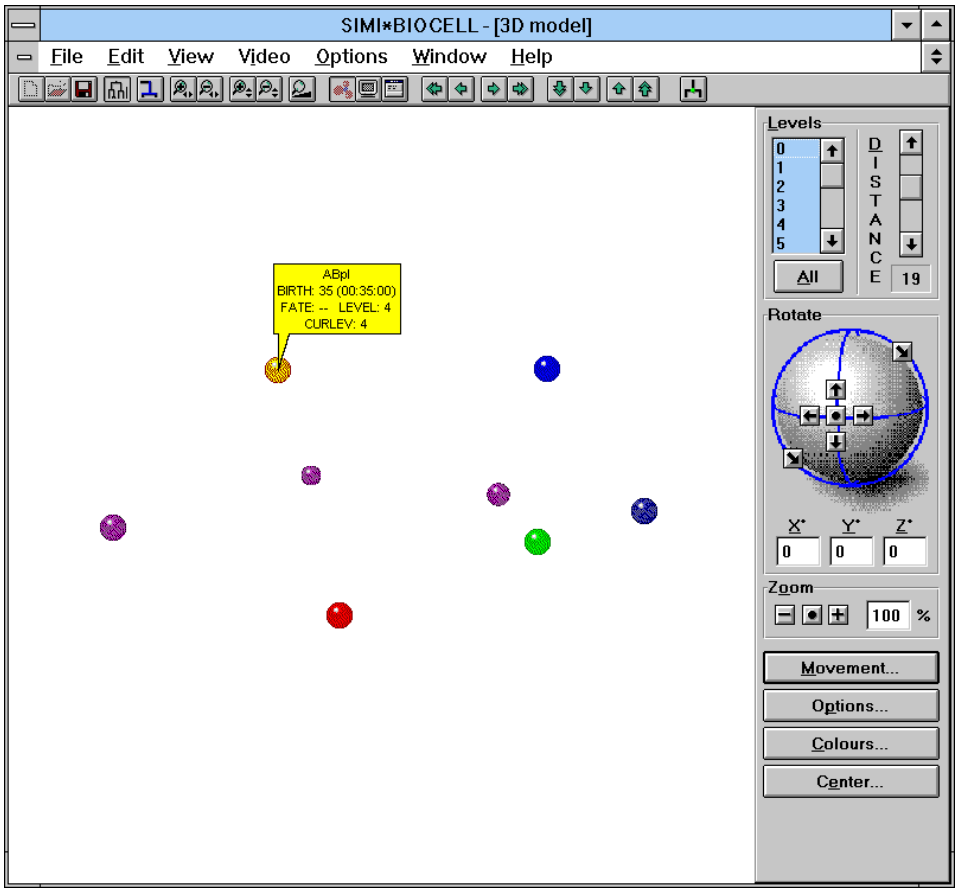


Figure 3.2 Fly over information of the ABp blastomere.

This chapter focuses on the **Rotate** control sphere and the **Options...** button. You find a complete description of all functions in Chapter 4.

1.3 The Rotate control

To rotate the 3D model simply use the cursor buttons on the sphere. The degree of a horizontal, vertical or circular rotation is displayed below. Since most of the embryos do not behave like an ideal body one has to set up the geometry of an embryo for a certain purpose (see Chapter 4).

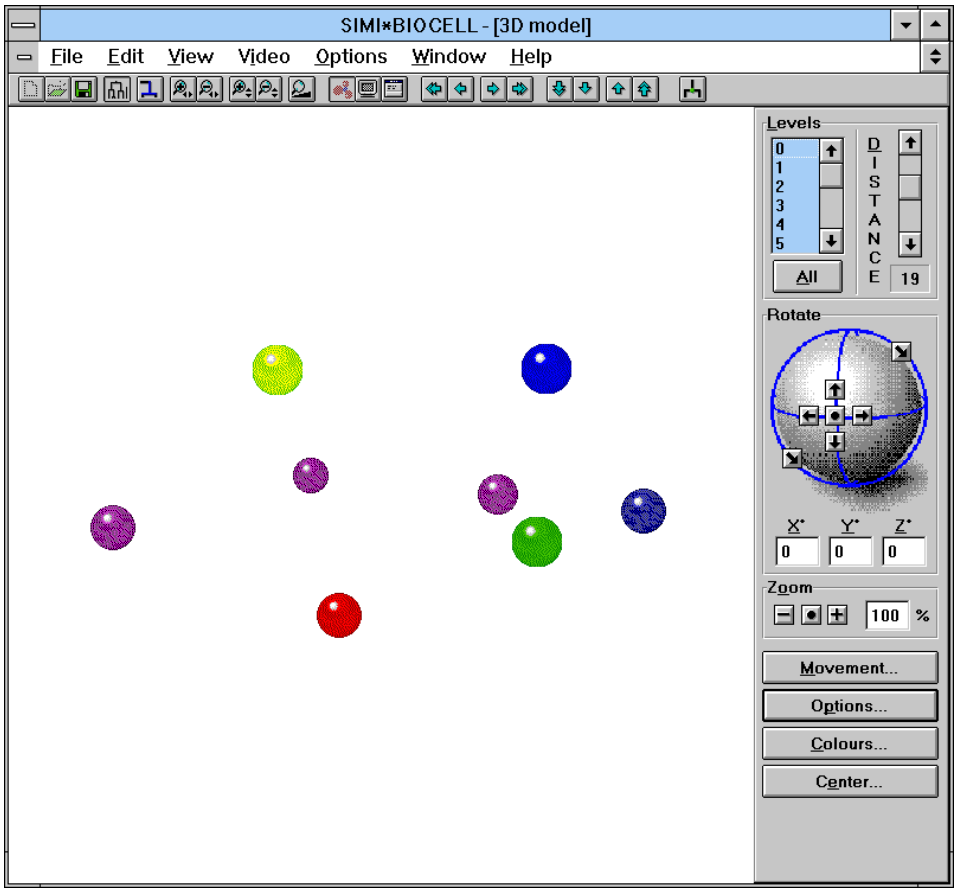


Figure 3.4 3D model window with big spheres.

1.4 Options

- Open the **3D options** dialogue box by pressing the **Options...** button, as shown in Figure 3.3.

The **3D view** group box is to size the spheres the way you want, e.g. Figure 3.4.

- Fill in a number between 1 and 13 to settle the number of different sizes.

- Check off small, medium or big to limit the maximum sphere size.

The **Fly over information** group box provides information of a cell point to select.

- Check off the information you want to see in the yellow **Fly over** flag

2 Cell movement

The cell movement function displays either the ancestors or the descendants of a certain blastomere.

2.1 Cell movement displayed on the Video Image

- Click on the desired point.
- Choose in the **View** menu **Cell movement…** .The **Cell movement** box appears. Check off **Previous** for the ancestors or **Following** for the descendants.
- This box lets select you 0 for only the active cell or 1, 2, …up to all, for a certain number of generations of ancestors or descendants.

All chosen ancestors will be displayed on the **Video Image** as crosses that are linked to each other. If you have to many points or generations it might look a little bit overcrowded. If you would like to study cell movement in detail it is recommended to clean out a project having only points on the Fixed points line. This tool is also valuable to check your lineage. Assuming that before morphogenisis most descendants stay together. A blastomere that suddenly moves out of that region might be a lineage error.

Note, that all points between two mitosis are displayed. As consequence, 0 produces also some crosses.

2.2 Cell movement displayed on the 3D model

- Click on the desired point.
- Open the **3D model**.
- Press the **Movement…** button. The **Cell movement** box appears. Check off **Previous** for the ancestors or **Following** for the descendants.
- This box lets select you 0 for only the active cell or 1, 2, …up to all, for a certain number of generations of ancestors or descendants.

All chosen ancestors will be displayed on the **Video Image** as spheres that are linked to each other. Note, that every point will be displayed. If you have to many points or generations it might look a little bit overcrowded. If you would like to study the cell movement in detail it is recommended to clean out a project having only points on the Fixed points line. To facilitate the 3D view you have to options.

- Check off **Show only movement** in the **Cell movement** box.

- Choose different colours for the first and the last blastomere of a cell movement. Press the **Colours…** button!

3 The Collision Manager

The collision manager compares only cells represented by points in the **Cell Lineage** window. It does not interpolate putative positions of a cell between two points!

The collision manager has an eye on your work. The collision manager checks the cellular environment of a given active cell. It calculates the distance to neighbours and warns you if you think lineaging different cells but actually do tracking the same cell.

- Choose **Collision Manager…** from the **Extras** menu. The **Collision Manager** dialogues box will appear.

- Fill in the **Range** values to set the tolerance of the collision manger. Every pair of cells with values within the specified range will be considered as a collision and therefore displayed in the window.

- Press **Ok**!

- Enable the collision manager in the **Options** menu.

To keep the application workspace with the various windows manageable make use of the **Reduce <** button

While you are lineaging (assigning crosses to cells) the collision manger checks automatically whether you are following a new cell or whether you are lineaging a previously lineaged cell that is derived from another ancestor. In the latter case the collision manager displays an info box and next the **Collision Manager** dialogue box again. The current cell and the collisioning cell(s) are listed. Differences in timing and distance are shown beside. By clicking on the cell name the program jumps to the corresponding position in the **Cell lineage** window helping you to find quickly the other cell.

To correct the lineage SIMI°BioCell offers several possibilities that are described in the following chapter “Copy and Paste”.

4 Copy and Paste

Once the collision manager has ringed the alarm bell the trouble starts because you have mixed up two cells. This sections describes how to deal with that problem. First, you should think about alternative routes to come to another cell. Second, you need to repair the lineage either by deleting points or by rearranging sub-lineages.

4.1 Alternative lineages for one cell

- Select a cell that is indicated by the collision manager.

- Choose **Add branch** from the **Edit** menu. A blank lineage is provided. The original lineage still exists as shown by small line beside the line of the next cell.

- Try to find a new route for the lineage to create an alternative lineage.

- You can switch between alternatives by using the "TAB" key.

You may check the alternative lineage with collision manager. There are lots of cases where a situation remains unclear. In this case keep the alternatives and make use of the **Information** box.

4.2 Copy and Paste lineages

You have found the right route for the cell. If the original version of the sub-lineage was totally screwed up you may delete it as well as any other alternative lineage by choosing **Delete branch** from the **Edit** menu. Most of the time it turns out that you have mixed up two cells but the subsequent lineage is correct. Deleting this alternative and lineage the same stuff from the right cell again will be a enormous amount of work- you want to keep the points but put them to another place. SIMI°BioCell offers to rearrange sublineages - copy and paste sub-lineages.

- Save the current project!

- Choose a destination cell.

- Move the mouse pointer to the desired source lineage that should moved, hold down the left mouse button and drag.

Please, pay attention to the generations. It should be the same or an earlier one!

- When you have moved the lineage to the destination place, release the mouse button. The **Move branch** box appears.

- Check off for **Swap** to exchange the source and destination lineages, **Copy** to overwrite the destination lineage with the source while keeping the source, **Move** to overwrite the destination lineage with the source without keeping it and **Add** to automatically create an alternative lineage including the source one.

Done! The **Add** option is the safest one, of course. Anyway, if larger parts are rearranged review them.

5 Tips and Tricks

The goal of the previous sections was to work out and then analyse the complete lineage of an embryo. This section focuses on lineaging a specific cell or subset of cells. For example one would like to lineage in different

embryos always the hypodermal cell *H0R* that is ABa r p a p p p a . The **Navigator** automatically provides the cleavage directions and prevents you from figuring out the a/p code. Or vice versa, you might have a fluorescence image that corresponds to the last scan of a recording and you would like to identify all stained cells. SIMI°BioCell provides "backward lineaging" features for this purpose.

5.1 The Navigator

- Choose a free branch of any precursor of *H0R*.

5.2 Backwards lineaging

- Choose a free branch.
- Disable the fixed points in the **Options** menu.
- Play the recording to the end (or to any other scan)
- Mark the desired cell
- Play the recording back; always mark it!
- Shortly before a mitosis occurs, this time the fusion of your cell with its daughter, mark the cell.
- Step a few scans back to the event of the mitosis. If you have followed the anterior (posterior) daughter choose **Insert Mitosis left** (**right**) from the **Edit** menu.
- Go ahead!

Often it turns out that one has chosen the wrong starting branch. For this problem see section 4.2.

Chapter 4

User's Reference

In this chapter are all functions and commands listed and described.

User's Reference gives a complete list of all functions and commands. It is organised the way, that you start, e.g., with the **File menu** and get information about all submenus, buttons etc.. Cross-references to **Chapter 2** and **3** facilitate an efficient access.

1 File

1.1 New Project

Creates a new project; see **Chapter 2** „Creating a new project“ for an introduction; here, you find the details.

1.1.1 Open template window

`Name.sbt` are templates for various recording conditions, for example `sulst25.sbt` should be used for recordings at 25°C (see Figure 2.2). It is possible to create a new template files (see **Chapter 5** (3.2)).

Buttons in the **Open template** window work as follows

OK Click on **OK** to accept selection; press **return** for short..

Cancel Back to the main window without any changes.

Info... Displays the **File info** dialogue box.

Action... Produces a file manger pull-down menu.

1.1.2 Video medium

The **Video medium** dialogue box is basically thought to simply select a certain recording. Buttons like **Load** are only useful, if you want to organise all your recordings on several disks as a small database.

Buttons in the **Open template** window work as follows

OK Or return opens the selected recording.

Video Opens the image of the selected recording to preview.

Read Reads the current video disk. SIMI°BioCell stores all recordings of a disk to files named `file0000.sbl` in the **content** directory. Read creates an update.

List If available this displays a list of changes of record intervals and focus changes done during the recording of a file.

Edit Produces the **Record attributes** read-only dialogue box. All information of a file on a certain disk are displayed. If the disk is a ROM data storage, the attributes cannot be changed!

Load Lets you select a content file other than displayed. Useful when checking several disks since the size of such a file is limited.

Save Only necessary if you want to save the current file but under a different name in a different directory, e.g., instead of `file0001.sbl` `disk_01.sbl` in the directory `usergroup_1`. The dialogue box lets you select the file's name and place.

1.2 Open project

Lets you select a file to open from a dialogue box.

1.3 Save project

Saves the current Cell lineage file.

1.4 Save project as...

Creates a new file of the current cell lineage file. A dialogue box lets you select the file's name and place. It opens also the **File Info** box that displays file information and offers also the opportunity to fill in additional ones.

1.5 Close project

Closes the current cell lineage file. A dialogue box lets you choose between saving the file (press **Yes**) or keeping the last version (press **No**).

1.6 Load standard cell lineage...

Produces a **Load standard cell lineage** box that lets you select either the standard Sulston lineage or a yourself created file.

1.7 Compare with lineage...

Compares the points of two lineages. First you can chose a lineage to which you can compare the current lineage, the SIMI°BioCell asks for the output file. This produces a ASCII text file with the following format:

<file name 1>
<file name 2>
...
<cell name file 1> <time of point> <x position of point><y><z>
<cell name file 2> <time of point> <x position of point><y><z>
<cell name file 1> <time of point> <x position of point><y><z>
<cell name file 2> <time of point> <x position of point><y><z>
...

1.8 Export fates...

Exports the fates from selected cells into an ASCII text file. See at SBIOCELL.INI, section TEXTFATE for more details.

The resulting text file has also a "tree structure", the cells are separated by tabulators. If a cell has a fate, this is marked with the corresponding letter.

1.9 Export mitosis times...

Creates an **Export to text file** dialogue box that allows you to save the time of all mitosis in a text file.

1.10 File Info...

Displays the **File Info** dialogue box.

1.11 Print...

It is a simple output option to print directly. It prints the lineage tree from an activated point without adjustment to the full page. The **Copy** dialogue box in the **Edit** menu is much more convenient!

1.12 Printer setup...

To set up a printer.

1.13 Exit

Closes the current Cell lineage file and quits the SIMI°BioCell application. A dialogue box lets you choose between saving the file (press **Yes**) or keeping the last version (press **No**).

2 Edit

The most options of the **Edit** menu are accessible by clicking on a point or a branch!

Only the sublineage downstream of the active cell point will be copied!

2.1 Copy...

Copies the current cell lineage file to the clipboard. The content of this clipboard can be pasted into a graphic application's file like of Paintbrush or Corral Draw. The **Copy** box provides several options to either use the complete content of the **Cell lineage** window or only part of it. Note, that some graphic applications require a bitmap format!

2.2 Mitosis

To generate a new mitosis. Better use the short cuts!

Double-click on the branch of the desired cell to access this box faster!

2.3 Insert Mitosis left

An option required for backwards lineaging. If one traces a cell at late stages backwards to earlier ones, one can insert mitosis by mitosis to build a sub-lineage. The copy and paste function can place it to the right ancestor.

2.4 Insert Mitosis right

See 2.3

2.5 Add branch

In some cases several routes seem to apply for one cell. They can be stored as alternatives by **Add branch** and are indicated by a small line beside the line

of the next cell. The comparison of these alternatives should facilitate a final decision for one rout (See chapter 3, 5.1).

2.6 Delete branch

To remove alternatives.

2.7 Cell...

The **Cell** dialogue box provides information of the current cell. Fate assignments can be chosen and comments can be written. The fate is shown in the **Cell lineage** window as shortcut, e.g., *H* for hypodermis, and the comments are displayed in the upper part of the **Information** box. Since those comments are always shown write only general comments that consider the complete lineage of the current cell. For notes referring to a certain point use the **Point** dialogue box (see below)!

The information of the group box „Normal:“ is derived from the current used standard cell lineage. If you plan to design your own standard cell lineage, it is necessary to fill in the group box „Actual:“. This information will be displayed in „Normal:“, if you use the current project as standard cell lineage in a new project.

2.8 Point...

Double-click on the red point of the desired cell to access this box faster!

The **Point** box contents information of a certain point in a certain lineage of a cell. You can fill in a comment that is displayed in the lower part of the **Information** box. Furthermore you can delete this point by pressing **Delete**.

2.9 Mark...

It is useful to highlight a few cells with a special colour for some purpose, e.g., checking two cells in a close neighbourhood that derive from different blastomeres.

With the **Colours** menu you can mark the current cell branch between two mitosis with a colour.

2.10 Unmark...

Returns the coloured cell branch to black.

2.11 Mark all...

To colour all cells of the Cell lineage tree.

2.12 Unmark all...

Returns the colour of all cells to black.

2.13 Fixed points...

This option generates a barrier visible by a stippled line in the **Cell lineage** window. To pass by you have to mark the current cell. Some tools of SIMI°BioCell requires the position of all cells at a certain time.

3 View

3.1 Fates and names, Generations and standard lineage

Toggles the visibility of Fates, names, of the division round and of the standard lineage.

3.2 Video window, Status window

Toggles the visibility of the **Video Image** windows and the **Information** box.

3.3 Cell movement...

This option is helpful, if you want to study the movement of one cell or a clone of cells during embryogenesis.

It produces a **Cell movement** dialogue box. Check off Previous (Following) to show all points of the previous (following) generations. On the **Video Image** window you will see all crosses joined with a line that resembles the movement of the current cell (bold type).

3.4 Cell list...

Only cells of the database, that means already lineaged cells will be coloured. Practically, you have sometimes to colour your lineage again or to load a pre-setting.

This option is required to colour the branches in the **Cell lineage** window as well as the spheres in the **3d-model** window. The SIMI°BioCell software provides two ways to use colours. Here, it is described, how to colour descendants of a certain blastomere or cells of a certain tissue. In the **Options** menu the **Colours** box could be used to colour the layout of the lineage like the colour of the active point, of the mitosis or of the background.

It produces the **Cell list** box that displays a list of a set of cells, that you could define on your own.

- Check off **Cells existing at current time** for cells that are existing at the current time or **All cells** for all existing cells etc. to generate the corresponding list.

Scroll down and press **shift** (⇑) + first and last E-descendant to mark <u>all</u> in-between. Or Scroll down and press **control** (**Strg**) + first and last E-descendant to mark <u>only</u> the first and the last one.

- Now select a subset of cells to either mark or unmark it, e.g., select all E derived cells.
- Press **Mark** to open the **Colour** box and choose a colour, e.g., *green* to highlight the intestine in the embryo.
- Repeat this procedure to colour every desired cell or clone of cells.
- The subsets with the corresponding colours can be stored as presettings by pressing **Save**. Of course, you can load this presetting by pressing **Load**.
- **Navigator**

3.5 3D-model...

Produces the **3D model** window, as shown in Figure 3.1. Chapter 3 describes the interactive application workspace. The following section details the option buttons of this window.

3.5.1 Levels

This combo box toggles the visibility of a single level, a group of levels or all.

- Click on level **0**.
- Scroll down and press **shift** (⇑) + level **8**. Only the upper third of the 3D model of the embryo is visible.
- Scroll down and press **control** (**Strg**) + level **8**. Only level **0** and **8** are visible in the 3D model.

This allows you to cut virtually the embryos into sections and facilitates massively the analysis of a 558- cell embryo.

3.5.2 Distance

If you want to rotate an embryo, try to optimise it with the **Distance** scroll bar. If that does not work properly use the **Center...** button in addition.

This scroll bar controls the distance between the levels, that means along the z-axis. If you want to rotate the embryo you adjust the distance until the embryo rotates like a cylinder. If the distance is to short, you will rotate a disk, if it is to far, the embryo will crawl over the application workspace.

3.5.3 Rotate

To rotate the embryo in three axis. The ⊡ button is to reset.

3.5.4 Zoom

This box controls the overall size of the 3D model, that means zooming along all axis and is therefore different from the **Distance** scroll bar. Use the buttons zoom in, reset and zoom out or just fill in.

3.5.5 Movement...

This button works exactly the same as the **Cell movement...** option in the **View** menu.

3.5.6 Options...

The **3D options** dialogue box controls the lay out of the 3D model, see also Figure 3.4. Fields in the **3D options** dialogue box work as follows:

Zoom Controls the Zoom box to zoom either linear or exponential.

3D view Select the number of different sphere sizes and limit the maximum size with small, medium and big size. Note, that you only get the full range of sphere sizes, if you have checked off big size!

Cell movement spheres You can define with the **Colour...** button (see below) a set of colours to mark certain spheres in the 3D model and a set to mark spheres specifically for the option **Cell movement**. If you want to use the same colours for both sets, you can switch between the two modes „colour selected for movement“ and „colour for marking“.

Fly over information Toggles the visibility of different information in the yellow fly over flag.

Check off „Save options when closing“, if you want to keep the options after closing the project or quitting SIMI°BioCell.

3.5.7 Colours...

This button works exactly the same as the **Colours...** option in the **Options** menu. Note, changes apply always to both options!

To adjust your embryo, be aware of the options **Distance**, **Zoom** and **Center...**!

3.5.8 Center...

This dialogue box helps to adjust the 3D model of an embryo to its shape and orientation of the **Video Image**. Default is always the automatic centring of the available spheres in the **3D model** window. For user's defined placing check off „check during input“ in the **Edges (x-axis, y-axis)** field.

- Place suitably the **Center...** dialogue box and the **Video Image** window.
- Check off „check during input“ in the **Edges (x-axis, y-axis)** field
- Click on *top* in the dialogue box, a red point in the left field is visible.
- Click on the top of the embryo on the **Video Image**. This position is now stored and the *right* position is highlighted. Click on the most right position of the embryo on the **Video Image**. Continue for bottom and left.
- If you are not satisfied, press the **New** button.

For example, you have three embryos on the **Video Image** window and you have adjusted the upper right, the 3D model of it should now appear at the upper right on the **3D model** window. If you want to centre this embryo, use the **Move** field in the **Center...** dialogue box.

If you are not using recordings with 25 levels, it might be useful to set up the levels in the **Level (z-axis)** field.

All options will be saved for a certain project.

3.6 Center active cell

Places the current branch in the centre of the **Cell lineage** window.

3.7 Center time line

Places the current time line in the centre of the **Cell lineage** window.

3.8 Zoom in etc.

Zooming gradually.

3.9 Free Zoom

Zooming infinitive variable.

3.10 Next (Previous) branch

Brings the next (previous) alternative (see 2.5).

4 Video

Scan control commands. Use the cursor control keys instead!

Frame step forward	Brings the next scan
Frame step backward	Brings the previous scan
Frame fast forward	Acceleration of **Frame step forward**
Frame fast backward	Acceleration of **Frame step backward**
Level increase	Brings the lower level of one scan
Level decrease	Brings the upper level of one scan
Level fast increase	Acceleration of **Level increase**
Level fast decrease	Acceleration of **Level decrease**

4.1 Select video device...

The Video device box appears. Check off the used video device.

4.2 Set default video device...

The **Set default video device** box appears. Check off the normally used video device.

5 Extras

5.1 Navigator

The **Navigator** info box appears, press **OK**! Only the standard cell lineage is displayed. Select a cell by clicking on its branch.

5.2 Collision Manager...

This tool is completely described in chapter 3, 3.

5.3 Take over colours...

Displays the **Take over colours** info box. If your standard lineage is already coloured (though it is displayed in grey when used as background) you may use the same colours for the corresponding lineages in the new cell lineage. Pressing **OK** will take over the colours.

These two functions need to be handled very carefully otherwise you will screw up your lineage!

5.4 Insert time

Allows to shift the time of a mitosis between mother and daughters without affecting the lineage. This option may be used to correct the starting time of a lineage if the new project was not properly set up or when mitosis are not precisely marked.

- Save your project!
- Click on the last point of the branch of the mother.
- Step one scan forward
- Insert as many time intervals as required to assign the right scan / time for the mitosis. Remember the amount of insertions!
- Click on one daughter and step backward.
- Delete as many time intervals as inserted previously.
- Repeat the last two steps for the other daughter.

Now, you have left unaffected all points above and below but changed the time of the mitosis.

5.5 Delete time

See 5.4 .

6 Options

6.1 Auto center cells

Places the active cell automatically in the middle of the **Cell lineage** window.

6.2 Enable fixed points

To disable the fixed points means to remove the lineage barrier. To delete fixed points access the **Fixed points...** option

6.3 Enable collision checking

Enables or disables the collision manger.

6.4 Options

Presents a pop-up menu for selecting preference items.

Fields and buttons in the dialogue box work as follows:

6.4.1 General

Beep...	Sound presettings
Big toolbar	Enlarges the toolbar

Create backup files Creates a `name.bak` backup file of the current project.

Enable autosave Creates automatically a `~asv0000.sbd` backup file that is used after SIMI°BioCell was unexpectedly closed and restarted again.

Load contents... Loads and saves the content list of a disk automatically.

Video co-ordinates The cross on the video image of the active cell can be also displayed at x level above or below. This option takes into account that the size of blastomeres of each generation changes and that therefore the range of levels showing parts of the blastomere differs. A traffic light is displayed on the bottom corner of the **Video Image**; green means current scan level is coincident with stored level of the point, yellow means x levels above or below and red when the current level is out of range.

6.4.2 Presettings

This field works exactly the same as the various options in the **View** and **Options** menu.

6.4.3 Video control

Defines the port of the graphic board.

6.4.4 Cross wires

The position of the cross of any cell on the **Video Image** is stored in a project file and independent of the hardware set up (if you are using an overlay board you have to calibrate the overlay). In contrary the adjustment of the cross wire on the high resolution screen to the cross on the **Video Image** is dependent of the interface and the currently loaded driver. X and Y settle the absolute position of the co-ordinates and DX, DY settle the spreading of the axis units.

6.4.5 Directories

Directories of the contents list and the template files.

6.5 Colours...

This dialogue box controls the colour of the lay out of cells, mitosis and the background. It has to be used for the **Cell lineage** and the **3D model** windows.

Point Colour of any point in the **Cell lineage** window

Mitosis Colour of any mitosis in the **Cell lineage** window

Fate (equal) Colour of fates as expected from the standard lineage

Fate (unequal) Colour of fates different from the standard lineage

Comment Colour of a point with a comment

Cell (standard) Colour of the standard lineage

Cell Colour of the new lineage of a certain project

Active cell	Colour of the active cell
Text	Colour of the fates and names in the **Cell lineage** window
Time line	Colour of the time line
Fixed points	Colour of the stippled line
Background	Colour of the window background
Video - Cross	Colour of the any cross displayed on the **Video Image**
Video - Active cross	Colour of the active cross of the corresponding level
Video - line	Colour of the cell joining line in the function cell movement
3D - Sphere	Colour of any sphere in the **3D model** window
3D - Active	Colour of the active sphere in the **3D model** window
3D - Fate (equal)	Colour of fates as expected from the standard lineage
3D - Fate (unequal)	Colour of fates that are different from the standard lineage
3D - Movement sphere	Colour of the spheres of a movement set
3D - Movement start	Colour of the first sphere of a movement set
3D - Movement end	Colour of the last sphere of a movement set
3D - Fly-over information	Colour of the information flag in the **3D model** window
3D - Background	Colour of the background of the **3D model** window

7 Window

7.1 Tile, Cascade...

To place windows on the SIMI°BioCell application workspace see Chapter 2.

7.2 Repaint

Brings a fresh copy of the current cell lineage to replace the original. The command affects only the window appearance.

7.3 Help

The on-line help is under construction!

Supplemental Material

Chapter 5

1 Pulldown menus of the Cell Lineage window

Action	Pull-down menu / Dialogue box
Double-click on point	Point information
Double-click on branch	Cell information
Right-button-click on point	Edit options
Right button-click on branch	Edit + Mark options

See **Chapter 1** (5.4-5.6) and **Chapter 4** (2 Edit) for descriptions of the options!

2 Keyboard Shortcuts

2.1 General

F1	Help
F5	Standard Lineage on/off
<Ctrl>F8	Online Collision Checking on/off
<Ctrl>F9	Fixed Points on/off
<Ctrl>F10	Local Cell Menu
+	Zoom in horizontally
-	Zoom out horizontally
<Ctrl>+	Zoom in vertically
<Ctrl>-	Zoom out vertically
<Ctrl>C	Cell Dialogue
<Ctrl>P	Point Dialogue
<Ctrl>S	Save current File
<Ctrl>W	Repaint Window
<Ctrl>M	Mark Cell
<Ctrl>U	Unmark Cell
<Ctrl><Shift><Insert>	Insert Time (one Frame)
<Ctrl><Shift><Delete>	Delete Time (one Frame)
<Ctrl><Insert>	Copy
<Space>	Center active Cell in Window

<Ctrl><Space>	Center Time Line in Window
<Ctrl><Shift><Space>	Center active Cell and Time Line in Window
<Return>	Mitosis
<Shift><Return>	Insert Mitosis and fix following lineage on the left
<Ctrl><Return>	Insert Mitosis and fix following lineage on the right
<NumPad ,>	Switch between Lineage Window and Video Window

2.2 Dragging a Branch

<Shift>	Cut current Cell at Time Line

2.3 Moving in the Lineage

<Tab>	Switch between alternative Branches forwards
<Shift><Tab>	Switch between alternative Branches backwards
<NumPad 0>	Jump to previous Point
<Ctrl><NumPad 0>	Jump to next Point
<NumPad 1>	Jump to left Successor
<NumPad 2>	Jump to last active Successor
<NumPad 3>	Jump to right Successor
<NumPad 4>	Jump to left Neighbour
<NumPad 5>	Jump to Predecessor
<NumPad 6>	Jump to right Neighbour
<Ctrl><Home>	Jump to Root Cell
<Ctrl><End>	Jump to Bottom Cell (last active)

2.4 Deleting all Points downwards

<Ctrl>	Delete also current point

2.5 Scrolling

<Left>	Scroll Lineage left
<Right>	Scroll Lineage right
<Up>	Scroll Lineage up
<Down>	Scroll Lineage down
<Ctrl>...	Page scrolling

2.6 Video Window

<Home>	Jump to first Video Frame
<End>	Jump to last Video Frame
<NumPad ,>	Switch between Lineage Window and Video Window

<Left>	Previous Frame
<Right>	Next Frame
<Up>	Level down
<Down>	Level up
<Ctrl>...	Bigger Steps

2.7 3D Window

<ScrollLock><NumPad 2>	Rotate X downwards
<ScrollLock><NumPad 8>	Rotate X upwards
<ScrollLock><NumPad 4>	Rotate Y left
<ScrollLock><NumPad 6>	Rotate Y right
<ScrollLock><NumPad 1>	Rotate Z anticlockwise
<ScrollLock><NumPad 9>	Rotate Z clockwise
<ScrollLock>+	Zoom in
<ScrollLock>-	Zoom out
<Ctrl>...	Speed x2
<Alt><Ctrl>...	Speed x4
<ScrollLock><NumPad 5>	Zero position
<Shift>	Keep Flyover Information

3 SIMI°BioCell Files

All files created by SIMI°BioCell can be edited with a text editor. There is no reason to edit or change any of the files created by SIMI°BioCell.

SIMI IS NOT RESPONSIBLE FOR ANY PROBLEMS OR DAMAGES CAUSED BY CHANGES OF ANY OF THE FILES LISTED BELOW!

3.1 .sbd and .sbc

A SIMI°BioCell project consists of two files. All created data of a project are stored in a text file **.sbd**. The **.sbc** file contains information of the corresponding disc and settings of the last lineage session as window size etc.

WHEN COPYING A PROJECT YOU NEED TO COPY BOTH!

3.2 .sbt

It is a template file. If you have created your own standard file that you would like to define as a template file, you have to open an existing template file. In the line DATAFILE=*.SBD, you insert the name of your project selected for being a template file. You may also want to change the time extension or compression. This can be changed in the line TIMEFACTOR=800. In the most cases it is recommended to use the **Load standard cell lineage** option (see **Chapter 4** (1.6)).

3.3 .sbs

This file contains your colour selection.

3.4 SBIOCELL.INI

3.4.1 Section [BIOCELL]

PAINTDELAY	delay time after painting a new video image for drawing something on it (milliseconds)
AUTOSAVE	filename of autosave file if it exists

LVDOUT	dump laserdisc directory to this file
LVDIN	read laserdisc directory from this file, not from the disc
SCREENS	number of screens connected to your computer
DIALOGS	alignment of dialogs: 1 = left, 2 = center, 3 = right

3.4.2 Section [COLOURS]

SIMI°BioCell saves its colours here. They can be set in the colours dialogue.

3.4.3 Section [COMMENTS]

COMMENT%i Here you can define as much standard comments as you want. Just give them successive numbers (instead of %i). The comments appear in the cell and point dialogues.

3.4.4 Section [FATE]

FATE%i Here you can define the possible fates for the cells. This setting is used for new files and then saved in the project file. Number each entry and start with 1 (FATE1=...)
Attention: The fates are saved by their numbers!
Format: FATE<No.>=<shortcut letter>,<description>

3.4.5 Section [HISTORY]

FILE%i the last used files (%i = number)

3.4.6 Section [OPTIONS]

SIMI°BioCell saves its options here. They can be set in the options dialogue.

3.4.7 Section [TEXTFATE]

CELL%i You can define here, which cells should be exported when you want to **Export fates**. Moreover, you can set how many generations following the specified cell should be exported. Number each entry and start with 1 (CELL1=...)
Format: CELL<No.>=<gen. cell name>,<generations>

3.4.8 Section [VIDEO]

COM	the COM port used for controlling the video device
DEFAULTDEVICE	the videodevice which is taken for a new file: NONE, OVERLAY or DIGITALVIDEO

3.4.9 Section [WINDOWS]

SIMI°BioCell saves its window positions here

3.4.10 Section [3D]

SIMI°BioCell saves its 3D options here. They can be set in the options dialogue in the 3D window.