SIMI°BioCell multichannel

A Tutorial

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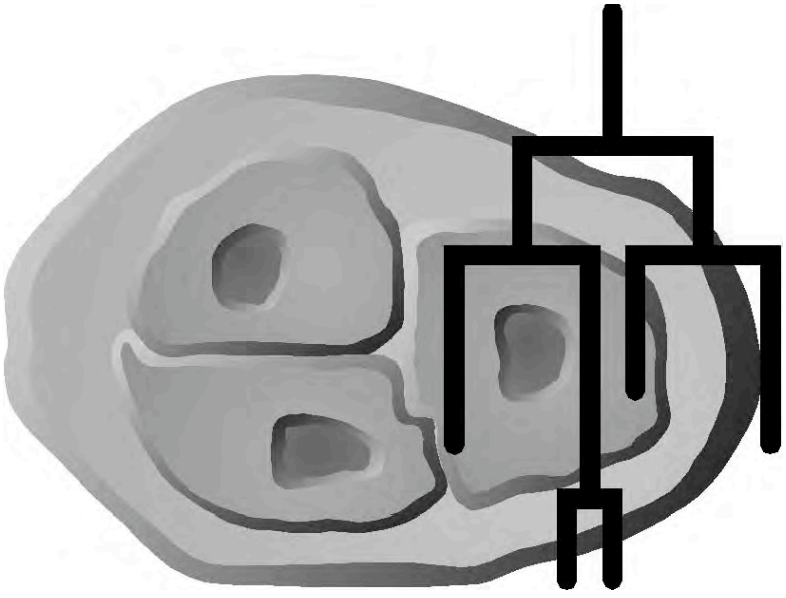
If you analyse an organism or cells with a 4D-microscope and SIMI°BioCell you will find that things are different of what was believed before.

Ralf Schnabel, 1997



SIMI°BioCell

Foreword

SIMI°BioCell was and still is developed in a co-operation of SIMI and myself. It is a tool to follow cells - in 3-dimensions in time (4D) - in organisms or in cell culture.

SIMI°BioCell is part of a modern philosophy of what is crucial to understand biology. To understand life we have to understand the organism or at least the cells, which build the organism. According to Sydney Brenner "not the molecules but the cell are the units at least of development". 4D-microscopy and SIMI°BioCell are tools to analyse the behaviour of cells in normal and experimental situations.

If you analyse an organism or cells with a 4D-microscope and SIMI°BioCell you will find that things are different of what was believed before.

Examples are the development of the nematode *C. elegans* or the Tardigrade *Thulinia stephaniae*, the development of the brain in *Drosophila* (see reference list page 9), or the differentiation of stem cells in the mouse brain (Hejnol & Schnabel, Development 132, 1349; 2005). I think if you want to get deep knowledge about an organism you have to look carefully at it yourself. My lab now tries since many years to develop an automatic lineaging software which does not make any use of fluorescent markers. This should make it suitable also for organisms which are not model organisms. However, I always force newcomers to undertake a manual analysis to get what Barbara McLintock called "The feeling for the organism".

Initially in 1994 SIMI°BioCell was developed for 4D-records acquired with DIC optics but today it has all features required to analyse multi-channel records. It is possible to either follow fluorescence labelled cells all the way through or a combinations of for example DIC optics and fluorescent optics using different colours to determine for example expression patterns. It does not really matter which kind of microscope you use. Microscopes and software for example from Zeiss also confocal ones, will do. There are special set ups based on Zeiss microscopes developed by me, which have highly sophisticated 4D-features. SIMI can read all kind of file formats and if you use a format, which is not yet implemented SIMI will do so.

Börßum, April 2008 Ralf Schnabel

Copyright Statement

Publisher

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Introduction

Who wants to read a manual? Especially from the beginning to the end? Who can remember what is written in a manual? I think one can only remember things on needs and uses on a regular base. Therefore, we list do not list here all functions in manual but offer tutorials which train you how to use **SIMI°BioCell.** Then we extend the tutorials by describing different tasks you may have. We recommend that you start, independently of what specimen you will analyse in your lab, with a DVD from Ralf Schnabel which supplies a recording of a *C. elegans* embryo together with **SIMI°BioCell** data files.

The program evolved now for more than 10 years with significant technical advances. Now digital picture handling is of sufficient high quality for 4D-microscopy, especially when high quality compression algorithms are used. **SIMI°BioCell** is able to read Lurawave (Luratec, Berlin) files which have a superb quality when pictures are compressed 10 fold. Please note that a license from Luratec is required to use this file format. Please contact Ralf Schnabel if you want to use this format. However recently we implemented also other compression modes which you can use as shareware. Initially the program was designed to read LVR-Discs from SONY laser video recorders. The program still contains all feature to work with Laser video discs since there are many records which must be still accessible today. However, since new users will only use digital records this parts of the program are ignored in this tutorial. In case you still need this functions please contact Ralf Schnabel.

If you once worked through the tutorial you an also use it as a reference for the supplied functions. The Contents list will guide you to the windows.

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. Good luck and a lot of patience with your analyses using 4D-microscopy and **SIMI°BioCell.**

Contents

Some publications using 4D-microscopy

Ralf Schnabel & Heinke Schnabel (1997) *Hox*-genes misled by local environments
Nature, 385, 588-589

Ralf Schnabel, Harald Hutter, Don Moerman & Heinke Schnabel (1997) Assessing normal embryogenesis in *C. elegans* embryo using a 4D-microscope:
variability of development and regional specification.
Developmental Biology, 184, 234-256

Titus Kaletta, Heinke Schnabel & Ralf Schnabel (1997) Binary specification of the embryonic lineage in *Caenorhabditis elegans*
Nature, 390, 294-298

Claudia Dolinski, Gaetan Borgonie, Ralf Schnabel, & James G. Baldwin (1998) Phylogenetic implications of buccal capsule development in some bacterial-feeding nematodes (Rhabditida: Nematoda)
Development Genes and Evolution, 208, 495-503

Karin Brunschwig, Claudia Wittmann, Ralf Schnabel, Thomas Bürglin, Heinz Tobler and Fritz Müller (1999) Anterior organization of the *C. elegans* embryo by the *labial*-like *Hox* gene *ceh-13*
Development, 126, 1537-1546

Ralf Schnabel, Biology in pictures: a question of fate (1999)
Curr. Biol. 12, R543**.**

Hoeppner DJ, Hengartner MO, Schnabel R (2001) Engulfment genes cooperate with ced-3 to promote cell death in *Caenorhabditis elegans*.
Nature 412, 202-206

Clucas C, Cabello J, Büssing I, Schnabel R and Johnstone I (2001) Oncogenic potential of a C. elegans cdc25 gene is demonstrated by a gain-of-function allele.
EMBO Journal 21, 665-674

Urbach R, Schnabel R and Technau GM (2003) The pattern of neuroblast formation, mitotic domains and proneural gene expression during early brain development in Drosophila.
Development, 130, 3589-3606

Karabinos A, Schulz E, Wan, J, Weber K and Schnabel, R. (2003) Functional analysis of the single calmodulin gene in the nematode *C. elegans* by RNA interference and 4-D microscopy
European. J. Cell Biology, 82, 557-563

Jason M. Kinchen Juan Cabello, Doris Klingele, Richard Feichtinger, Heinke Schnabel, Ralf Schnabel and Michael O. Hengartner (2005) Two pathways converge at CED-10 to mediate actin rearrangement and corpse removal in *C. elegans*
Nature, 434, 93-99

Andreas Hejnol & Ralf Schnabel (2005) The embryo of the eutardigrade *Thulinia stephaniae* has an indeterminate development and the potential to regulate early blastomere ablations.
Development, 132, 1349-1361

Andreas Hejnol & Ralf Schnabel (2006) From the Symposium on "The New Microscopy: Toward a Phylogenetic Synthesis" "What a couple of dimensions can do for you: Comparative developmental studies using 4D-microscopy – examples from tardigrade development"
International Journal of Comparitive Biology (in press)

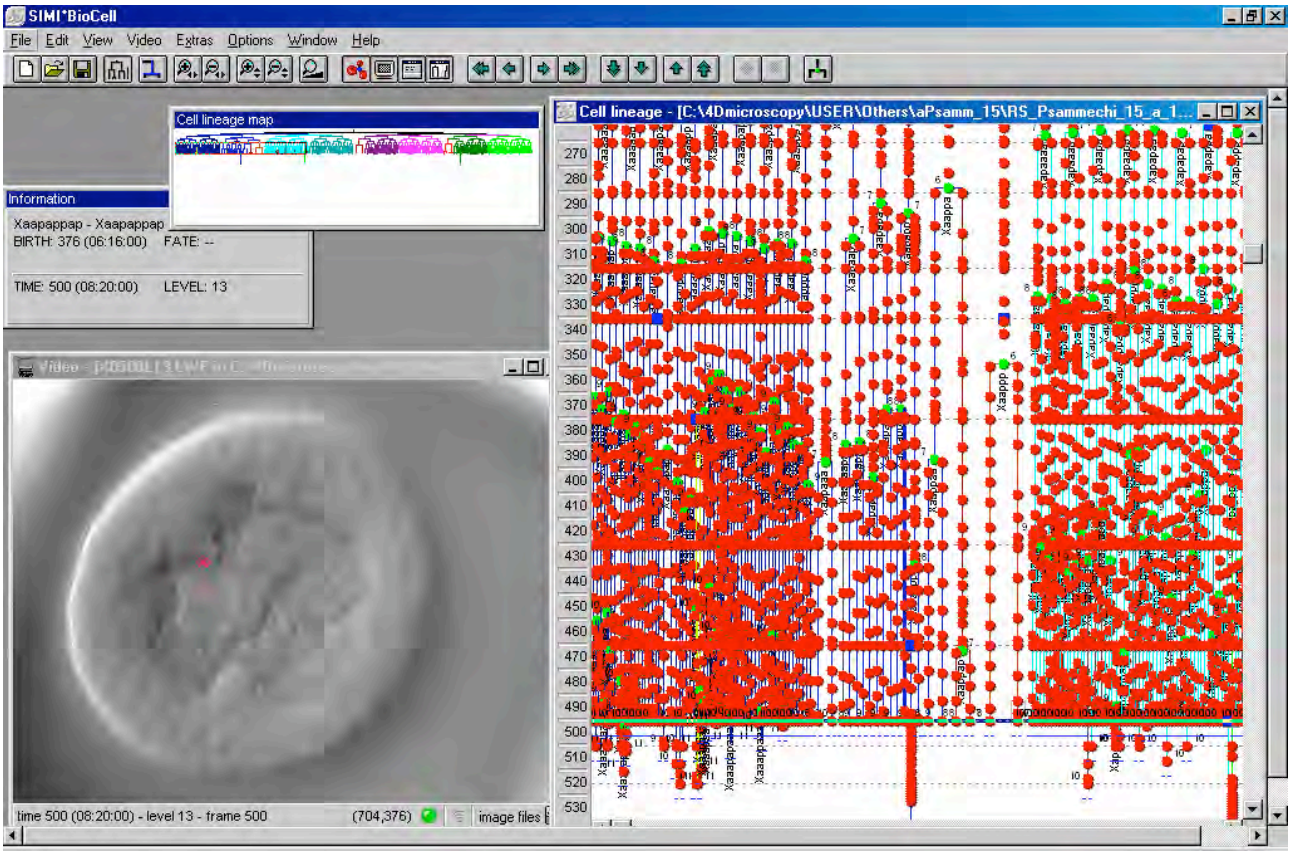
Ralf Schnabel, Marcus Bischoff, Arend Hintze, Anja-Kristina Schulz, Hans Meinhardt and Harald Hutter (2006) Fate dependent global cell sorting in the *C. elegans* embryo defines a new mechanism for pattern formation.
Developmental Biology, , 294, 418-431

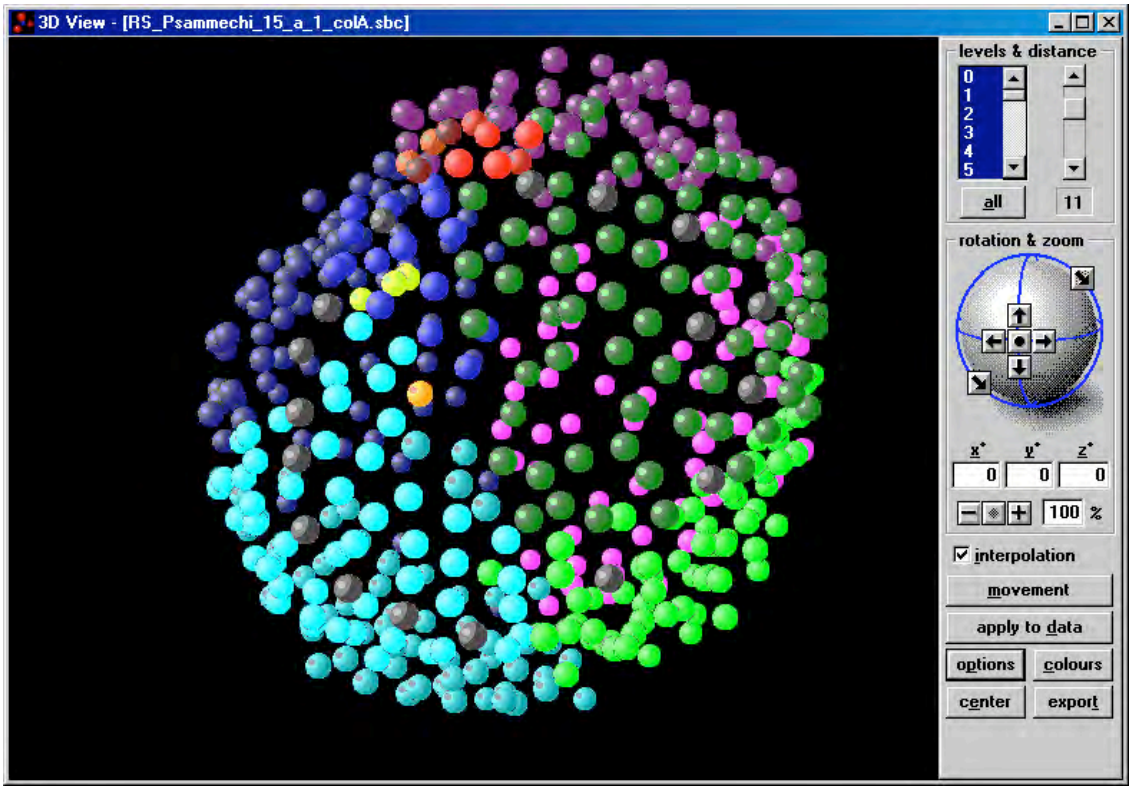
Marcus Bischoff and Ralf Schnabel (2006)
Global cell sorting is mediated by local cell-cell interactions in the *C. elegans* embryo.
Developmental Biology, 294, 432-444

Marcus Bischoff and Ralf Schnabel (2006) A posterior centre polarises the early *C. elegans* embryo by a Wnt-dependent relay mechanism.
PLOS Biology, 4, e237

Andreas Hejnol, Ralf Schnabel and Gerhard Scholtz (2006) A 4D-microscopic analysis of the germ band in the isopod crustacean *Porcellio scaber* (Peracarida, Malacostraca) – developmental and phylogenetic implications.
Development Genes and Evolution, 216, 755-267

Not only the worm.....





but a Sea Urchin and some others, like Gastrotriches and Tardigrades.

Installing software for Windows

Installing **SIMI°BioCell** you need...

• An IBM compatible PC with at least 250 MB memory and 100 MB free space on your harddisk running Microsoft Windows 2000 or XP.

• It is highly recommended to use two for example 19" monitors for **SIMI°BioCell,** since there are many large windows one should be able to view in parallel during an analysis. A modern wide field monitor is also suitable.

• Just execute the "install.exe" on the installation CD or if you downloaded the file from internet from your desctop.

• 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 install the driver for the dongle and connect the dongle to an USB or serial port!

• If you use compressed (Lurawave) picture files (highly recommended) please do not forget to implement the drivers and to activate your License. Please contact Ralf Schnabel

Getting Started with SIMI°BioCell

First Tutorial "Basic Lineaging"

In this session you will learn how to follow cells with SIMI°BioCell and how to correct mistakes you may have made.

Starting SIMI°BioCell

Assuming you have already installed the SIMI°BioCell software you are ready to start your first lineage session.

- Insert the DVD from Ralf Schnabel into your DVD drive.
- Double-click the SIMI°BioCell icon.

You will see the main window of SIMI°BioCell on-screen (Fig. 1). In case you use two screens pull the window to extent over both screens.

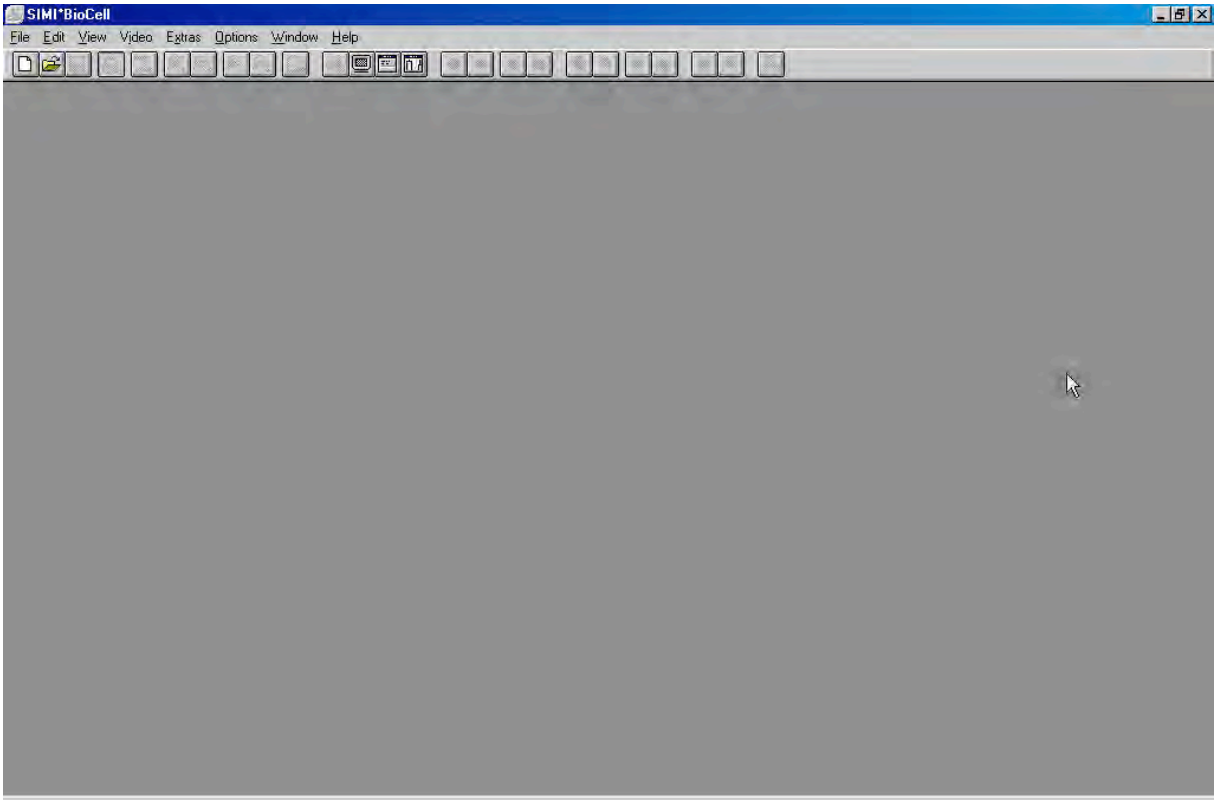


Fig. 1 The **Main window**

Click with the mouse either on the  icon, or go to **Open Project** in the **File** scroll down menu (Fig. 2). Navigate to the Folder **User_Tutorial_Biocell** on the DVD and open the project **RS_Start_IB+RS_N2_BMBs_lineage**.

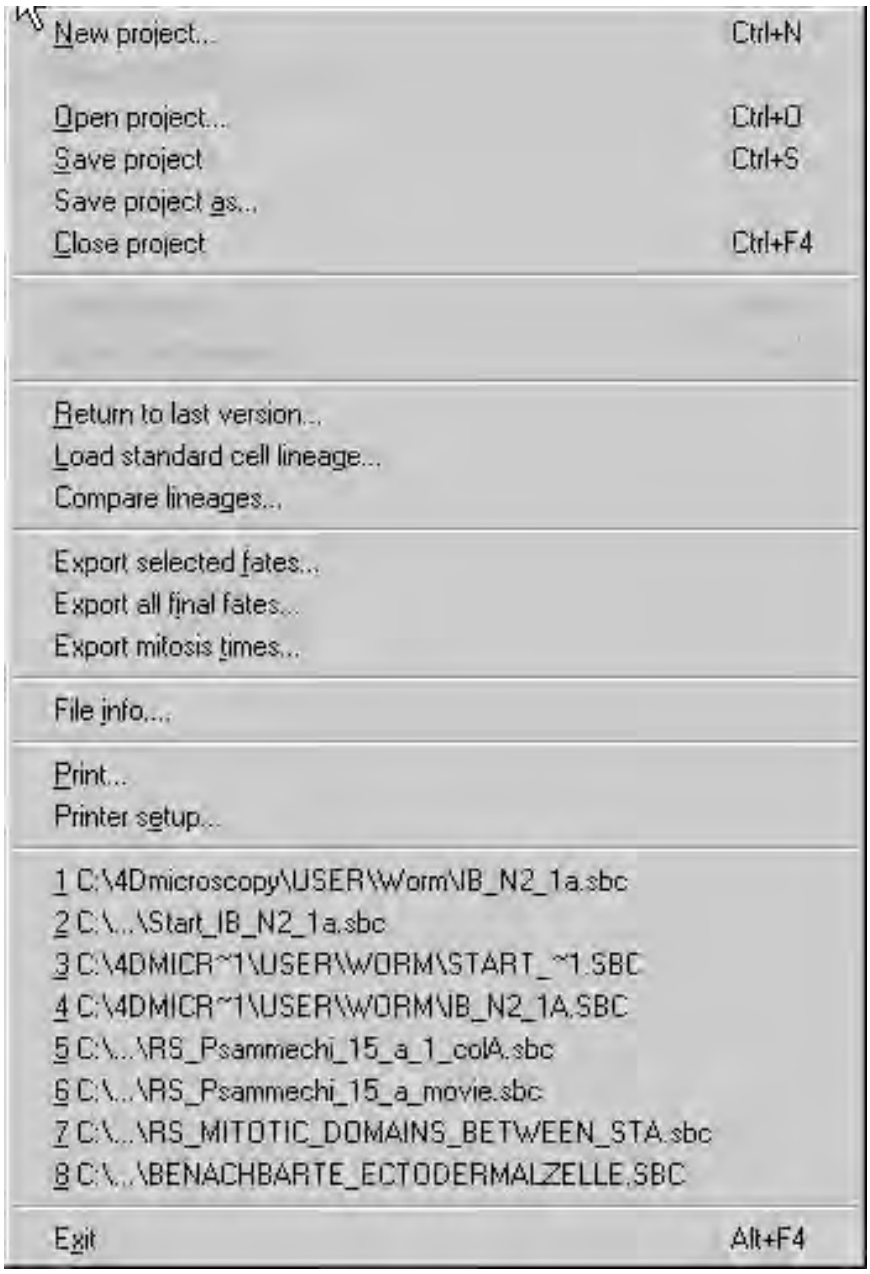


Fig. 2 The **File** menu. The window contains the normal file options to open and save files and to exit the program. The more advanced functions like **New project...** will be explained later. Functions which are only used for for Laser video recorders are shaded.
Please note that the program remembers the last 8 projects used in the window above **Exit**. Thus it is easy to recall these projects. When opening windows like this please use the chance to make yourself familiar with the listed functions.

You see the almost empty **Cell lineage window** with a grey *C. elegans* lineage in the background (if you see instead the **Image selection** window pleas go to the chapter "Addressing the video pictures" pp. 56 to 59 – Fig. 37 and assign the pictures). This lineage will guide you through the embryo if you identified and marked the cells in the first z-series properly. On the top you see a black lineage with only two cells (Fig. 3).

Open now the **Video window** by clicking in the  **icon.** You see now a 2-cell embryo at a medial level (Fig. 4).

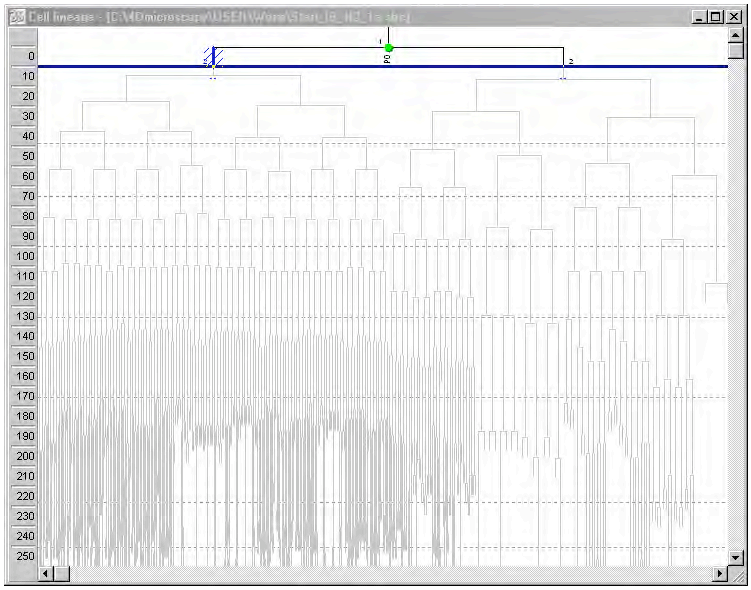


Fig. 3. The **Cell lineage** window ready to mark the first cell. In the background one sees the lineage of John Sulston et al.. The black horizontal line marks the time where the video of the embryo stands currently (first z-series).

May be it is a good idea now to look at the development of the embryo. To be able to do so pulls down the Extras menu and disable the **Fixed point enable function** (see p. 20). Now go down to level 8 using the cursor ↓ and then press the cursor → until the embryo starts moving. Using the four cursors you can navigate through the record as you like. It is so beautiful. When you are done go back to the first z-level. Enable the **Fixed points** again and proceed with the tutorial.

The embryo is in the standard orientation you should always use for your recordings. Anterior is to the left. Thus the cell which is placed to the left (it may be only a tiny little bit in respect to the orthogonal axis of the embryo) during a division is the anterior daughter. This cell is always assigned to the left (anterior) branch in the lineage scheme. The posterior daughter is assigned to the right branch. The 2-AB descendants cleave in the left-right direction. In this case the left daughters are assigned to the left daughter branch and the right daughters to the right daughter branch.

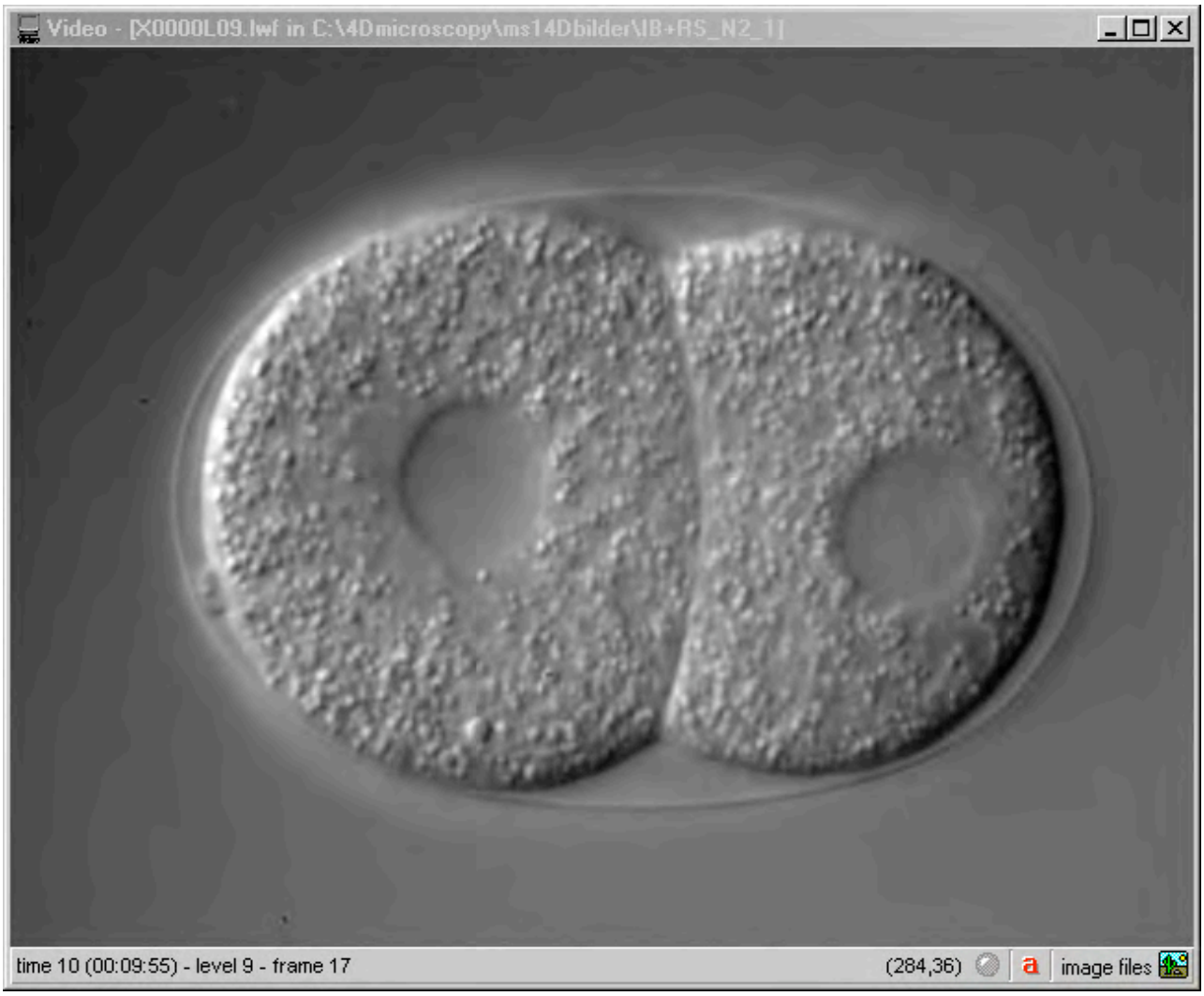


Fig. 4 The **Video window**. All relevant information about the picture is shown at the bars of this window. If a position is marked with the RIGHT mouse button a red cross will appear. The large cell on the left (anterior) is AB. The smaller cell on the right (posterior) is P1. **Due to the space restriction on the DVD level 24 is missing in the whole record.**

Saving your project

Pull down the **File menu** and choose the **Save project as ...** function (Fig. 2). A familiar window will open which allows you to save your project. During saving the **File Info window** opens which allows you to record worthwhile information about your project. The **Comment** section of this window (accessible through **File, File Info)** can also be used to save observations you make while you are analysing the embryo (there you find them for sure again). It is recommended to start the file name always with your initials (like "RS_my first lineage") save your file in a folder which participates in a schedule for making back ups on a regular base (every night). Projects can be very precious since many days or even weeks of work may have been spent to analyse a specimen. SIMI°BioCell automatically saves your project every 15 min. You can assign the interval to your convenience in the menu **Option, File Options.** Please save your project after you made

significant progress or before you undertake a challenging operation. Although we take great efforts to eliminate crashes you may be still able to find a combination of operations which will freeze the program.

Please note that upon saving two files are created by SIMI°BioCell. A **.sbc** file which stores the specific settings of your project and a .**sbd** file which contains the actual data.

Data analyses

The **.sbd** file can be also used to access the data for bio-informatical analyses (Schnabel et al., 2006) The File menu contains three basic functions to export some of the data (Fig. 2). Please contact Ralf Schnabel for software for basic analyses, like the movement of cells, the direction of cleavages and so on. It is actually a great advantage of SIMI°BioCell that you are able to get quantitative data about the behaviour of cells.

Program crashes

If the program froze or crashed you have to use the **Task manager** of the **System** to also quit the **wowexec.exe** process before you can start the program again. The program may offer you to go on with the last **Security back up** it created automatically. Please inform Toni Zeitler if you encounter a notorious freezing situation. It is very helpful if you transmit the exact circumstances which cause the freezing of the program.

Start lineaging

• In the **Lineage Window** the AB lineage branch is already activated. This can be seen by the fact that the branch is blue and marked by a hatched line. To confirm the identity of the branch you may click at the **Information icon**  and the **Information window** will open (Fig. 5) which shows you the name of the activated branch (cell). You can drag it, as any other window in any position of the screen you want.

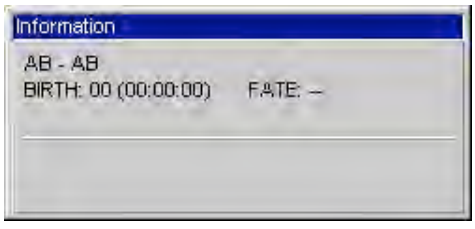


Fig. 5 The **Information window** displaying the identity of the activated cell.

Marking the first cell

• Activate now the Video Image window by clicking at it or by using the "**.**" on the numerical pad (the **Num Lock** must be on), which switches between the **Cell lineage** and the **Video** window (Fig. 8). The window is active when the top bar shows the "acitve" color. Here this is blue but this may depend on the configuration of your desk top**.**

• Focus on the centre of the AB nucleus (either using the tool bar or the cursor keys ↑ and ↓). AB is the larger cell in the anterior (left part of the picture) of the embryo. The smaller posterior cell is P1 (Fig. 4).

• 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 are stored. That a cell was marked is indicated by a **A Red point** at the corresponding time and branch in the lineage tree.

Retrieving the position of a cell

• If you click on a **Red point** in the lineage tree the corresponding cell marked with a **Red cross** will be displayed in the **Video Image** window.

Scaling the Lineage window

If the view of the lineage in the **Cell lineage window** is to small the computer may have difficulties to recognise the **Red point** or any other structure if you for example click on a lineage branch**.**

Please scale the view up by using the corresponding  buttons. It is more convenient to use the +/- keys or **ctr** and +/- keys for the time scale on the numerical pad for this purpose.

Navigating within the Cell lineage window

The active lineage branch may get outside of the **Cell lineage window.**

• Hit the **Space bar** and it will be centred. Alternatively you can use the scroll bars on the right and lower side of the window to search for your cell. By clicking at the  you get the **Cell lineage map window** (Fig. 6). By pulling the red **Box** with the mouse pointer you can navigate through the lineage.

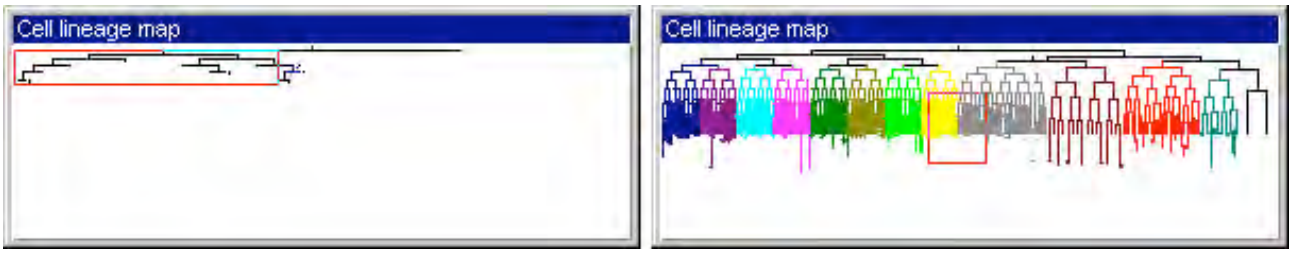


Fig. 6 The Cell lineage map window. On the left one sees a rather empty window with only a few lineages analysed. On the right the map of an embryo which was completely lineaged until the premorphogenetic stage (400 cells).

Navigating through the recording to follow a cell

Move onward in time using the cursor control → . One action will move the picture by one step forward. Always refocus the nucleus using the cursor keys ↑ and ↓. Click once and a while with the RIGHT mouse button to mark the cell. If you want to move back use the ← cursor. If you want to jump back to the last mark (red sphere) press the **0** on the numerical pad of the key board (the **Num Lock** has to be activated). Do not forget to save the data every few minutes by either clicking at the  icon or by pressing **ctr s** (<ctr> <s>).

The first mitosis

Go on with lineaging until the cell divides. When you see a pronounced cleavage furrow you press the **Return** button. A division is indicated in the lineage branch (Fig. 7).

• Now you have to decide which daughter of the AB blastomere you want to follow by either clicking at the corresponding branch with the mouse or by using the short cuts programmed on the numerical pad of the key board (Fig. 8). The short cut to activate the anterior daughter is to press the "**1**" on the numerical pad. This leaves the **Video window** active. Do not forget to activate the **Video window** again if you use the mouse to activate the lineage, otherwise the video picture will not move anymore when you press the cursors**.** The short cut to jump back and forward between the

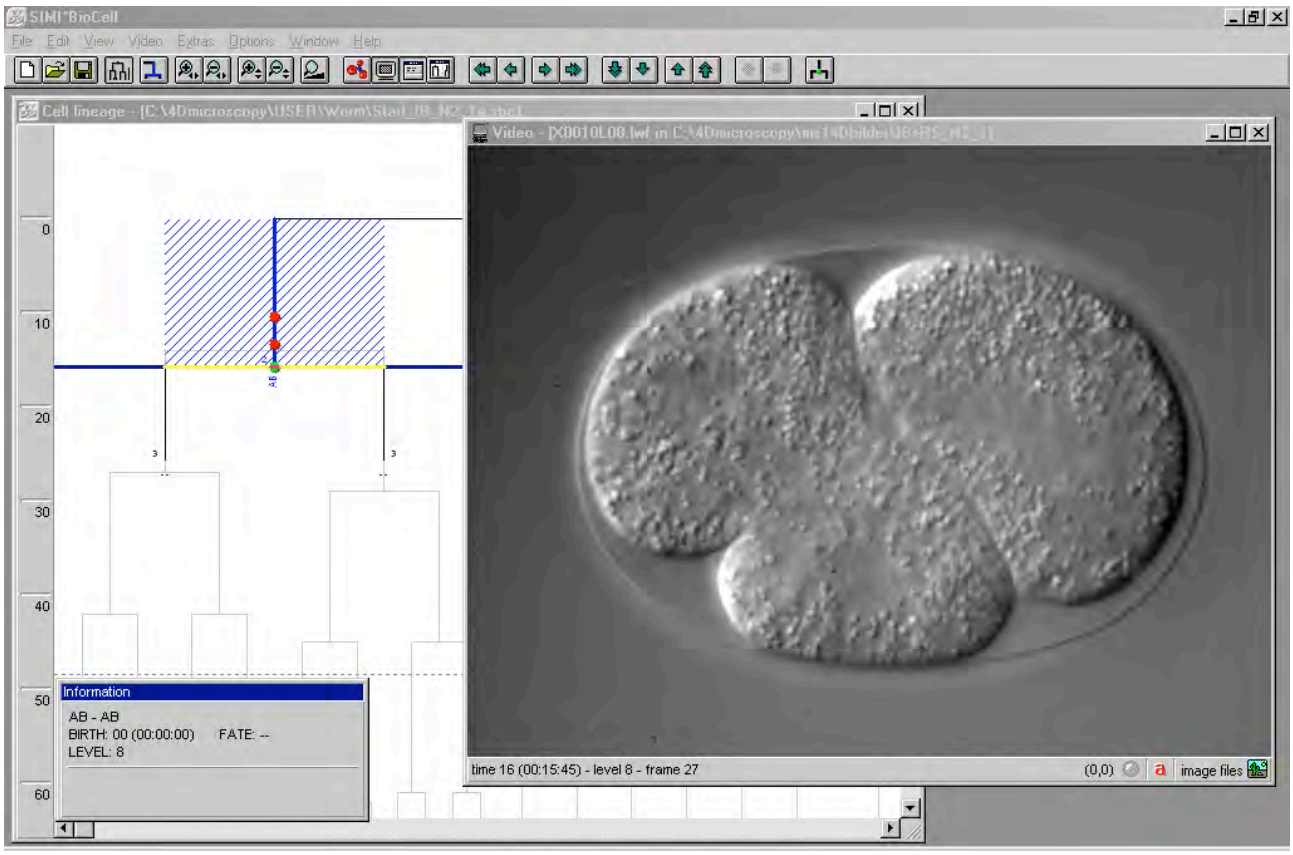


Fig. 7 The dividing AB blastomere. It is time to press **return** to introduce a cleavage in the lineage branch of AB.

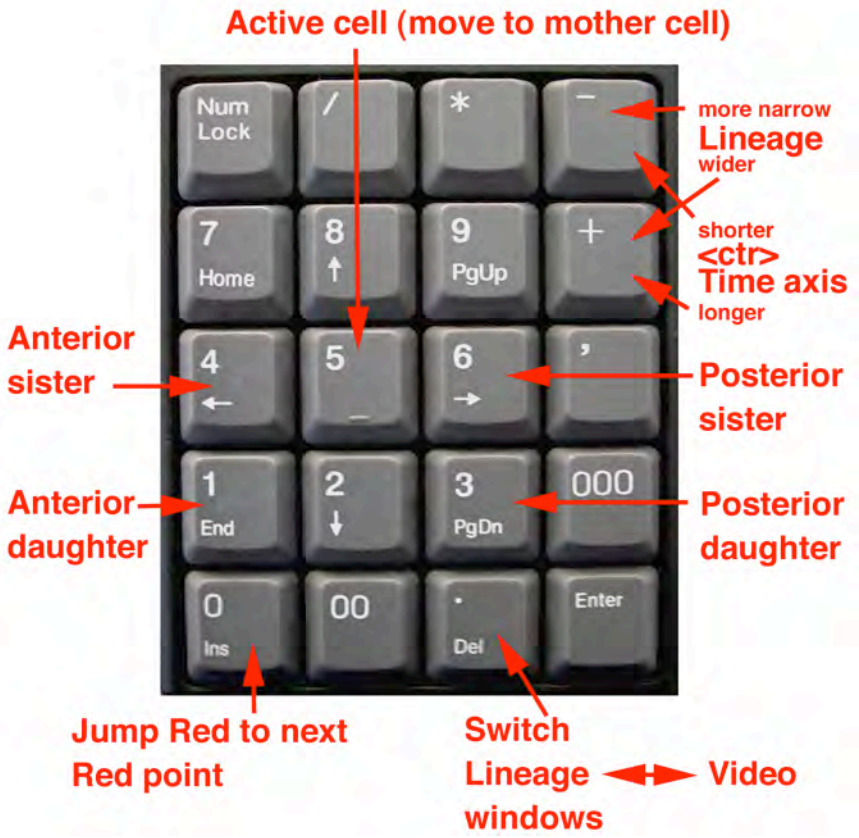


Fig. 8 The switch lineage functions on the numerical pad. The +/- key spread or shrink the lineage on the horizontal axis. If **ctr** is activated at the time axis will expand or shrink.

Cell lineage and the Video window is the "**.** " on the numerical pad. Now the anterior branch is activated, the **Information window** displays ABa as the active cell.

• Refocus on the centre of the cell (cursor keys ↑ and ↓). Move one step further in time and mark the cell with the RIGHT mouse click. Now go on lineaging the cell. As you proceed you approach a stippled horizontal line in the **Cell lineage window** (Fig. 9). This line indicates a so called **Fix point.**

Fixed points

When the time line the blue horizontal line spanning the whole window) hits this line you will not be able to proceed forward in time the video picture always jumps back. This line is a so called "**Fixed point"** it forces you to mark the cell (the fixed points are administrated in the Menu **Extras, Fixed points** or by pressing **F5).** Please also note that the **Fixed points** must be **enabled** in the Menu **Extras** to function**.** After you marked the cell you can move on. The purpose of this lines is to force the observer to mark all cells at the same time. Thus all cell co-ordinates are later available at the **Fixed points**. This is necessary to be able to reconstruct **3D-representations** of the cell positions and also to give the **Collision manager** a chance to surveil your analysis of cells. The collision manager checks if all cells were assigned to different lineage branches. If it finds cells whose co-ordinates are so close to each other that they may be only one cell. It warns the observer that there may be a problem in the assignment of cells. This feature is the best friend of the observer, it greatly reduces errors in the analysis.

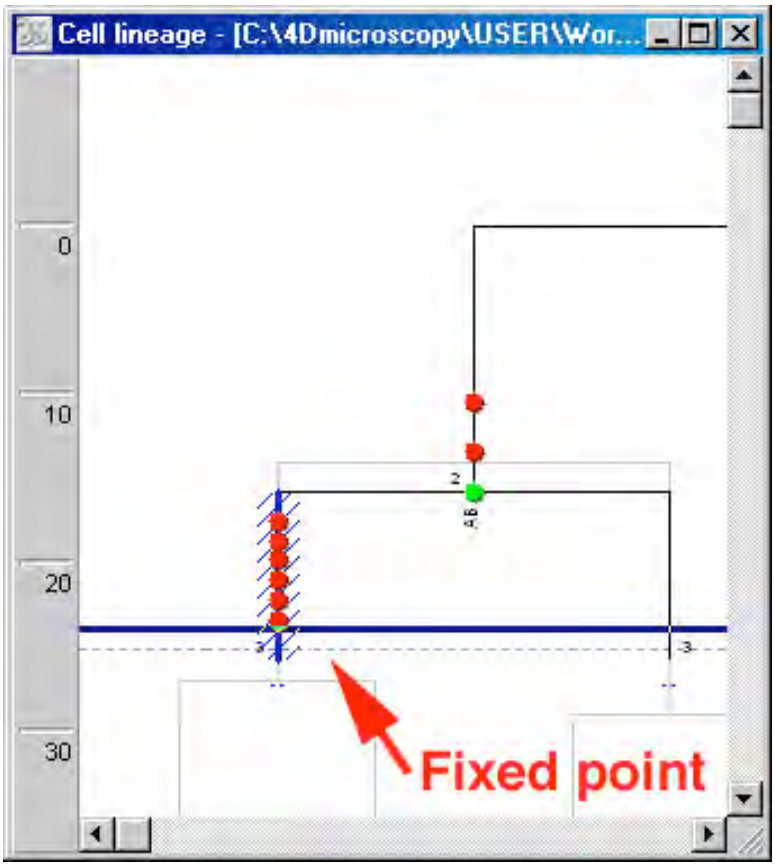


Fig. 9 The **Fixed point** is a horizontal line which enforces the observer to mark cells.

Lineage strategy and an important “Schnabel” rule

When you follow a cell its nucleus will sooner or later move out of the focal level you are just looking at. If this happens do not “jump after it”! One does not have any sense for the direction in which it escaped. I assure you will find also a nucleus in the wrong direction. use the “**0**” on the numerical pad to jump back to the last marker. Go again forward and take great care to keep the nucleus always in focus. In my experience it is also better to move rather fast through the video, since the human eye is trained to follow moving patterns. Mitoses in which the daughters move out of the focal level can also be quite difficult in the beginning. Move up and down in the focal levels and observe how a daughter is “jumping” in the picture. Do not get desperate too fast, it takes some weeks to learn the in and outs of lineaging. Use either the **My first lineage_finished.sbc** or the **RS_IB+RS_N2_lineage.sbc** project - supplied with this tutorial - to find the solution for a specific lineage (if you find a mistake in the lineages please tell us). Or, even better, come to my lab and learn lineaging.

The grey lineage tree in the background

The grey tree in the background reflects the database which is used for lineaging. It assigns for example the cell names if you introduce a cleavage. Do not use it for example to search for a mitosis. The timing of mitoses is variable. Development also depends strongly on the temperature. Please do not worry if the timing of your embryo deviates from the grey lineage. The overlay also depends on a good mapping of the first stage in your recording on the lineage tree (see Chapter 4)A good rule of thumb is that the next cell cycle will be approximately 20 t0 30% longer than the last one. If the lineage branch you work on is double as long as the one before you missed for sure a cleavage. An exception from this rule is the early E lineage.

Correction of errors

Correction of a wrong mark

- Go on with lineaging. ABa will divide in the left-right direction so up or down on the focal levels. The cell which divides towards EMS (the top of the screen) will be ABal. Lineage towards the ABalaap blastomere (Fig. 10). When you arrive at the **Fix point** 70 min (ABalaa) purposely do not mark the centre but the margin of the nucleus. To correct the “error” just click again at the

centre of the nucleus. A window will pop up and ask you if you want to correct the position of the mark, please answer "**Yes**". The marker will move to the centre of the nucleus.

- Alternatively you can delete the wrong mark (and the **Red point** in the **Cell lineage window**) by double clicking the **Red point** in the **Cell lineage window.** The **Point** window will pop up (Fig. 16) and ask you if you want to delete the point. Please answer "**Yes**". Now you can set the correct mark with the RIGHT mouse button.

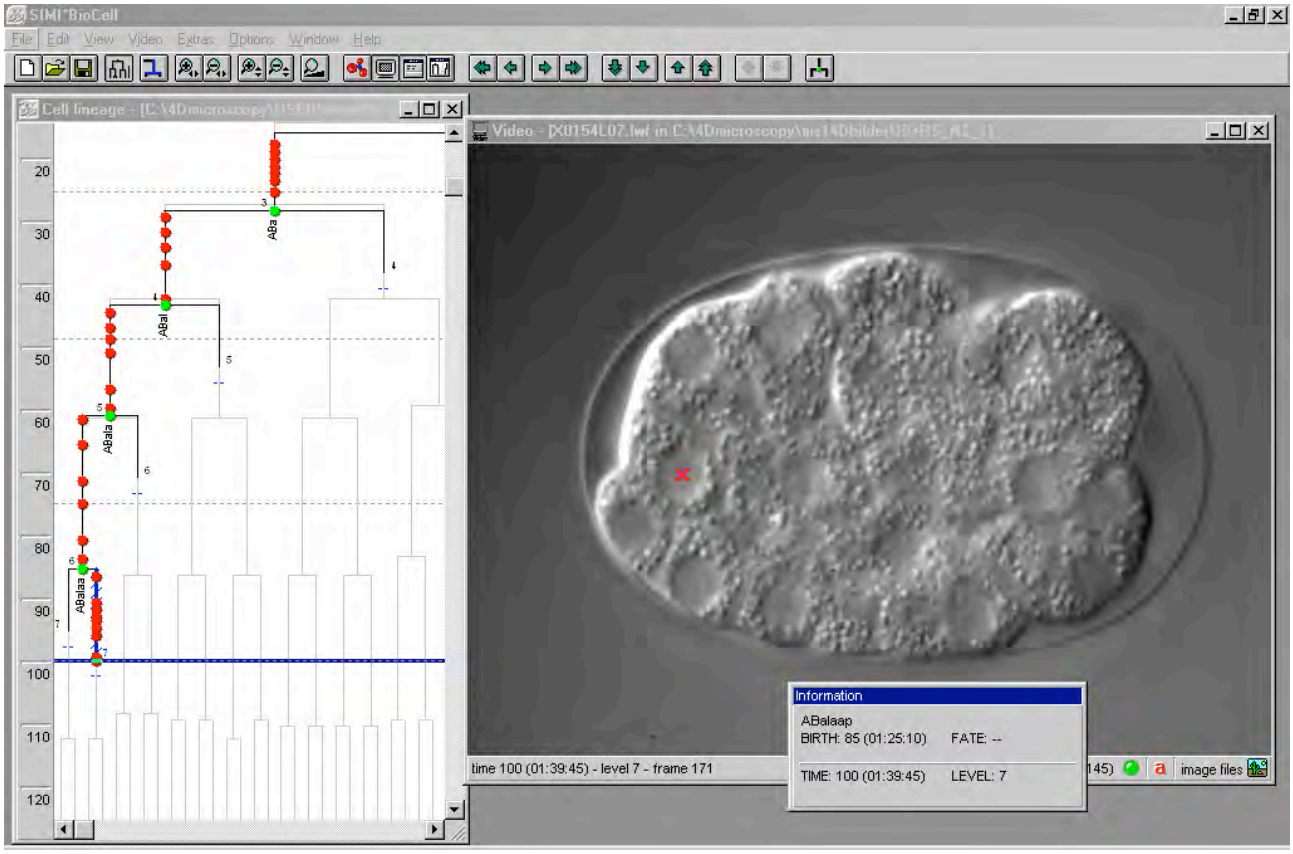


Fig. 10 The ABalaap lineage. If you do not see this cell marked at 100 min in the **Video window** of your project please go back and search for the mistake.

Correction of lineage errors

- Move on and mark the cell once and a while. Ignore the next division of ABalaa (do not push return) and follow and mark the anterior daughter till 100 min. Now you obviously made a bad mistake (which is quite common when the mitosis occurs on the z-axis), which you need to correct. To do so you have to go back in the lineage (to 83 min) before the mitosis happened. There are several possibilities to achieve this.

• You move back in time by using the ← cursor. The **Traffic light** feature will help you to follow the cell backwards.

The "Traffic light" feature

If you go again through a lineage you will pass through marks you set before. The **Red cross** will appear. At the same time the **Traffic light**  on the right side of the lower bar of the **Video window** will indicate if you are now at the same focal level in which you marked the cell before. If it is green you are at the same level. Yellow indicates that you are close, you are one or two levels above or below the level of the mark. Red means that you are further off than two levels. This is very useful to spot mistakes. The **Information window** indicates the level on which the mark was set. At the bottom bar of the **Video window** the level of the picture you see is indicated.

• Go back to 100 min by clicking at the corresponding **Red point**.

• You click through the **Red points** using the mouse. This is quite cumbersome.

• Go back to 100 min by clicking at the corresponding **Red point**.

• You hit the "**0**" on the numerical pad (the **Num Lock** must be activated). The program will jump each time back to the next **Red point.** If you press <**ctrl**> at the same time the program will move forward in time.

• You are now positioned at the time point (83 min) just before the cell divides and you are thus ready to correct your error. There are now two possibilities to do so.

The Delete all points downward function

• Point with the mouse at the **Red point** and hold the RIGHT mouse button down. The **Edit Point... window** displaying several functions will appear (Fig. 16). Sometimes - if you do not aim properly and you hit the line (branch) in between the **Red points** - a different window, **Edit cell... window** which contains another set of functions appears (Fig. 11). These functions will be addressed later. Just try again until you get the right window (you may have to zoom the window up a little bit) and then choose "**Delete all points downwards"** and release the mouse button. You will be asked if you want to delete indeed the **Red point**. Please answer "**Yes**", the **Red points** below will be deleted.

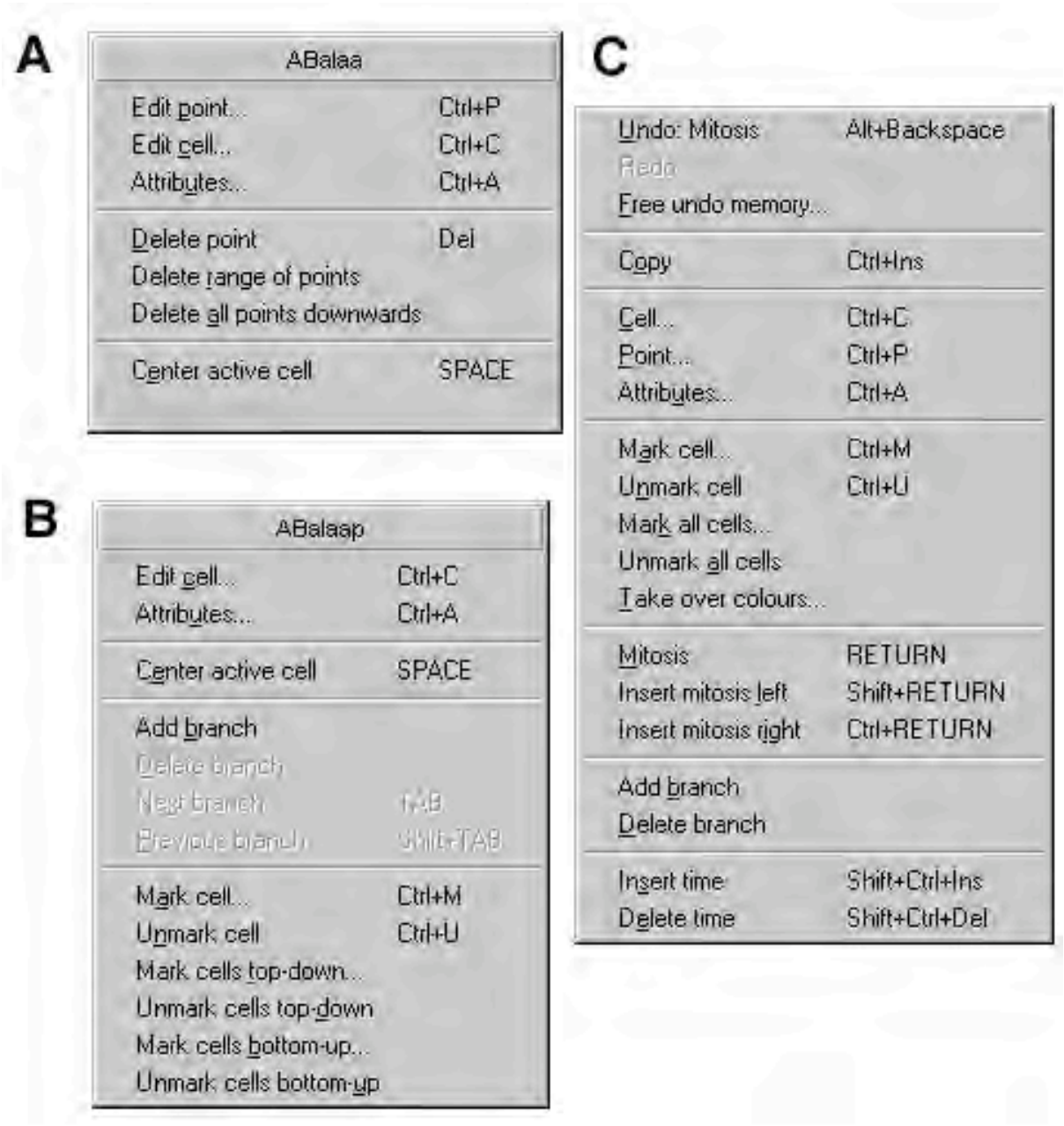


Fig. 11 (A) The **"Edit point"** window activated when a **Red point** is activated with the RIGHT mouse button. In the centre are the **Delete** functions. The function **Set cross wires** is shaded since it is only used for analog video devices. (B) The **Edit cell window** which may pop up accidentally instead of the window with the **Delete** functions. It will be discussed later. (C) The **Edit window** from the main menu. It contains among others the **Insert Mitosis** functions. **Delete all points downwards** will also delete mitoses downstream of the position you start from.

With this procedure the **Red point,** you started with, will persist. If you press <**ctrl**> when you activate the deletion this point will disappear also. This function is sometimes very useful, for example, if you use the first wrong point to delete everything downwards. With the same window you can also **Delete a range of points**. If you accidentally marked a wrong cell in between but you

came back to the correct cell. You will see later that this will happen to you too. The window also allows to **Delete** a single **point**. This is an alternative to the procedure described above.

• Sorry, lineage again what you deleted and miss again the cell cleavage. Follow the anterior daughter of ABalaa (or use the **Undo function** in the **Edit pull down menu,** p.28).

The "Insert mitosis" function

Since you just missed the mitosis but followed the anterior daughter of ABalaa correctly there is no need to delete anything. You move to the time point 86 min where the cell divides (if the position is marked with a **Red point** remove it or move one step further in time).

• Then you activate in the **Main menu Edit** (Fig. 11). At the bottom you see the functions **Insert mitosis left** and **Insert mitosis right.** Choose the **Insert mitosis left.** A mitosis will be inserted and the following marks will be transferred to the anterior branch which corresponds to the anterior daughter of ABalaap. If required use the **Insert mitosis right** function to assign the lineage to the posterior daughter. The lineage is now correct again and you can proceed with the lineaging. To get to ABalaap you have to follow the posterior daughter in the cleavage you just corrected.

- **Follow a second lineage**

Go back to the AB blastomere and lineage towards the ABprpaa cell until you hit the **Fix point** at 100 min. Please consider that the cleavage of ABp occurs on the left-right axis of the embryo. Thus the daughter moving towards the top of the recording (levels with the lower numbers) is ABpr. If you do not end up with the cell marked in Fig. 12, please search for mistakes.

- **Follow a second lineage**

• Go back to the AB blastomere and lineage towards the ABprpaa cell until you hit the **Fix point** at 100 min. Please consider that the cleavage of ABp occurs on the left-right axis of the embryo. Thus the daughter moving towards the top of the recording (levels with the lower numbers) is ABpr. If you do not end up with the cell marked in Fig. 12, please search for mistakes.

The Collision manager finds mistakes automatically

• Go back to ABprpa and follow the posterior daughter ABarpap up to the **Fix point** at 100 min. Now you have to introduce purposely a mistake to activate the **Collision manager** to learn about its function. Hit the **4** on the numerical pad, which will activate the anterior sister lineage and the **Red**

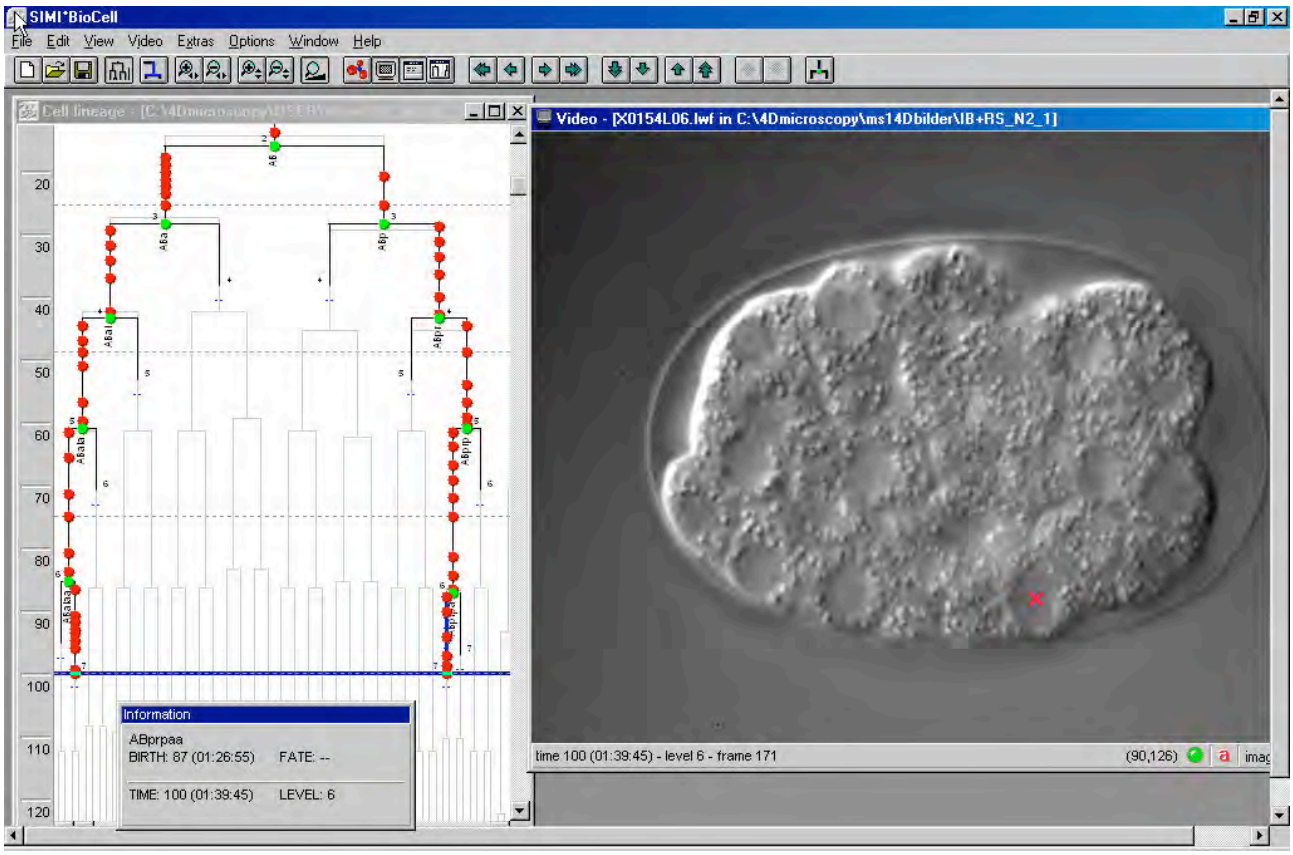


Fig. 12 The ABprpaa cell. Please check if you labelled this cell at 100 min when lineaging the ABprpaa cell.

cross will show you the cell. Point with the mouse pointer on the **Red cross** and activate now the posterior lineage again by pressing the **6** on the numerical pad**.** Click with the RIGHT mouse button to mark the cell now**.** Answer "**Yes**" to the question if you want to change the position of the mark. Now you assigned the ABarpap cell to two lineage branches which is obviously a mistake.

The **Collision manager** will take only a few ms to spot this mistake and alarm you by informing you that it spotted a collision. You will develop a "love and hate" relationship to this window. On one hand its nice to know you made a mistake on the other hand it may be a pain to find the error. Answer "**Yes**" that you want to know the details.

A new window the **Collision manager** (Fig. 13) will pop up which helps you to fix the problem. The details of this window are explained in the legend to the figure. By clicking at the cells in conflict, shown in the lower window, you can jump back and forward between the involved cells. Click at the name of the cell listed under **Cell 1**. The program now jumps to the cell which was there before and which produces the conflict. In case you cannot see the cell in the **Cell lineage window** activate the window by clicking at its top bar and hit the **Space bar** of your key board.

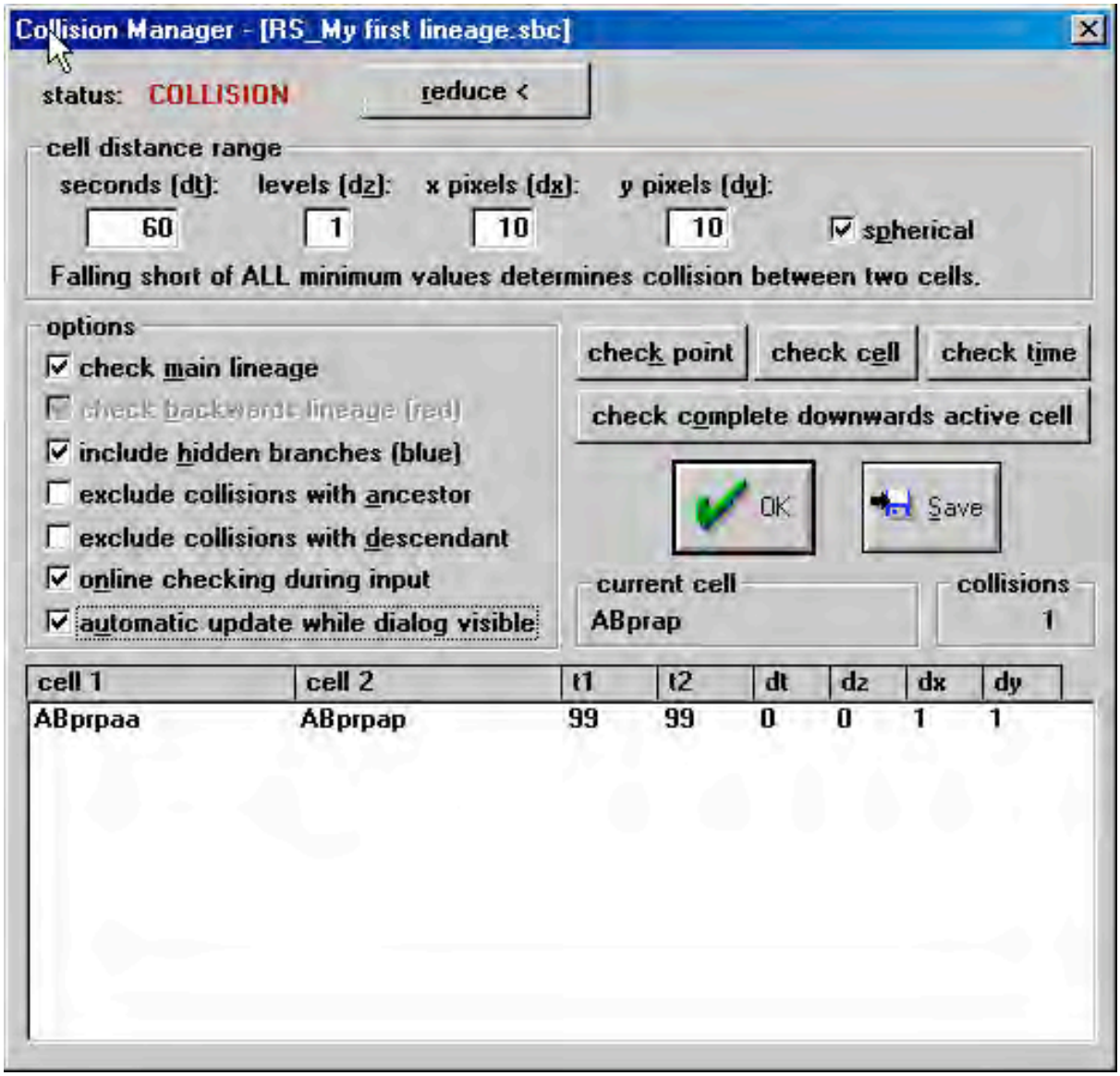


Fig. 13 The collision manager. In **Cell distance range** you can assign the sensitivity of the collision manager by setting the range in pixels, the number of focal levels and the time interval in which a second mark must fall to activate the manager. The pre-setting is optimised by our experience. If you lineage in late embryos you may want to restrict the range to not provoke too many false alarms. In the **Options** section you can choose more options to prevent false alarms. It is important to have the **online checking** and the **automatic update** activated. The last function notifies you when you resolved the conflict, you may, however, have to move one time point forward and then again backward to get the information on the screen. The buttons in the right part allow you to check lineages for conflicts. Please note that checking a lineage of hundreds of cells may require some time. The lower part of the window shows you the details of the conflict.

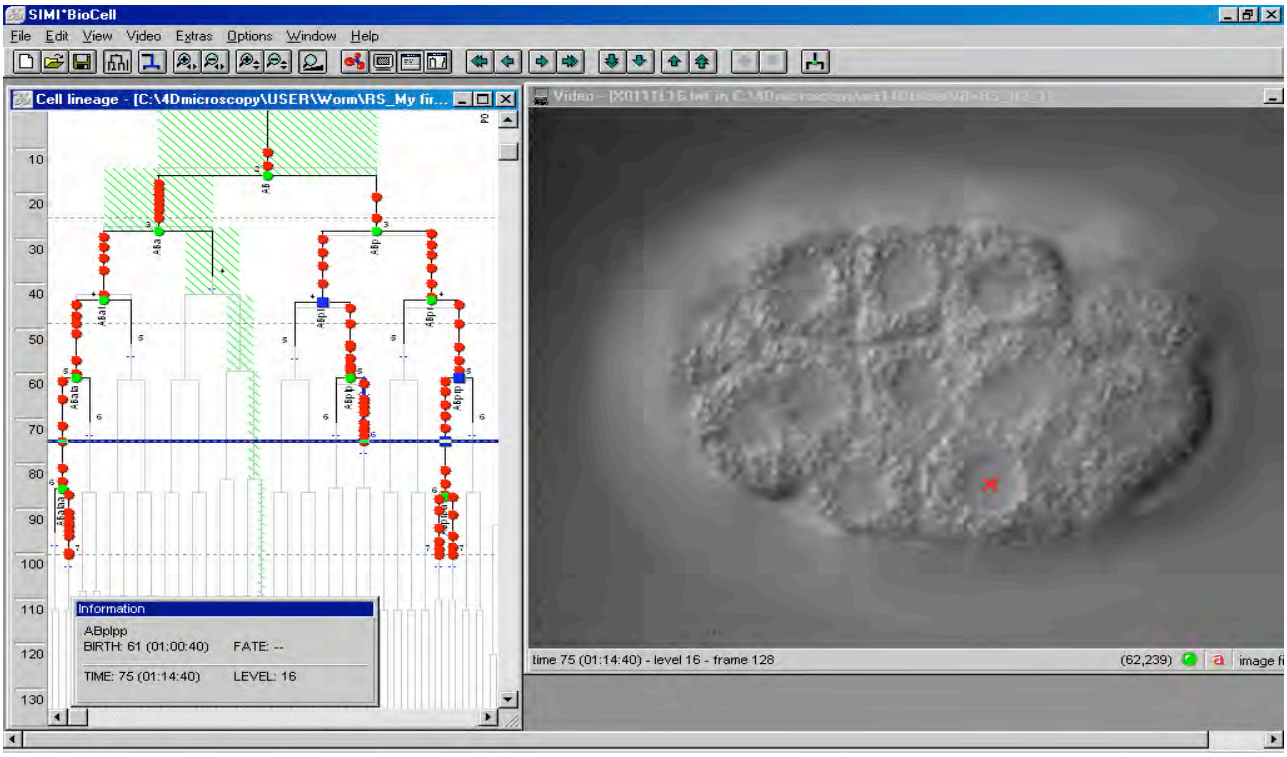


Fig. 14 The position of the wrong"ABplapp" cell.

The window will centre to show you the appropriate lineage branch. You have to lineage both cells to resolve the conflict. In this case it is easy but ....., anyway good luck in the future. If you have more experience you may find the **Drag & Drop function** introduced next also useful to move, swap or copy whole branches through the lineage tree.

The Drag & Drop function

Sometimes one realises after extensive lineaging that for example very early a mistake has been made. It would be a pity to delete everything and to start all over again. There is a possibility to rescue the work, with the Drag & Drop function.

- To simulate such a mistake go back to the ABa blastomere and move just after its division. The more posterior daughter is ABar, you lineaged ABal before. Do not activate the ABar but the ABpl branch by clicking at it with the mouse pointer and then set the first mark. Lineage until you labelled the "ABplpp" cell at the **Fixed point** 75 min. Looking carefully at the position of the cell (Fig.14) you may start to wonder that it is so close to ABprpp cell which you lineaged before - click at the **Red point** marking this cell to see again its position and then move back to "ABprpp".

Are left and a right homologs at that time of development almost neighbours? To be sure you start the lineage again with the ABa blastomere and you realise that you have made a bad mistake. You assigned the ABar blastomere to ABpl lineage. It is now dead easy to correct this mistake.

• Make sure you saved the project (moving large branches may occasionally crash the computer) and that the lineage window is activated and direct the mouse pointer on the lineage branch of the ABpl blastomere. Press the LEFT mouse button and pull the pointer to the left. If you get it right you should move a little lineage scheme, which is attached to a little fist, with this action. Move the structure to the ABar branch until it is highlighted by a little grey square. Trop the lineage by releasing the mouse button. Now the **Drag & Drop** menu pops up which offers you now several possibilities how to proceed (Fig. 15). The default function is to **Swap** to branches**.** Choose **Move** and click **Ok** in the next window popping up to agree with the operation. Now the lineage branch moves to ABar and the error in assigning the ABar to ABpl is fixed.

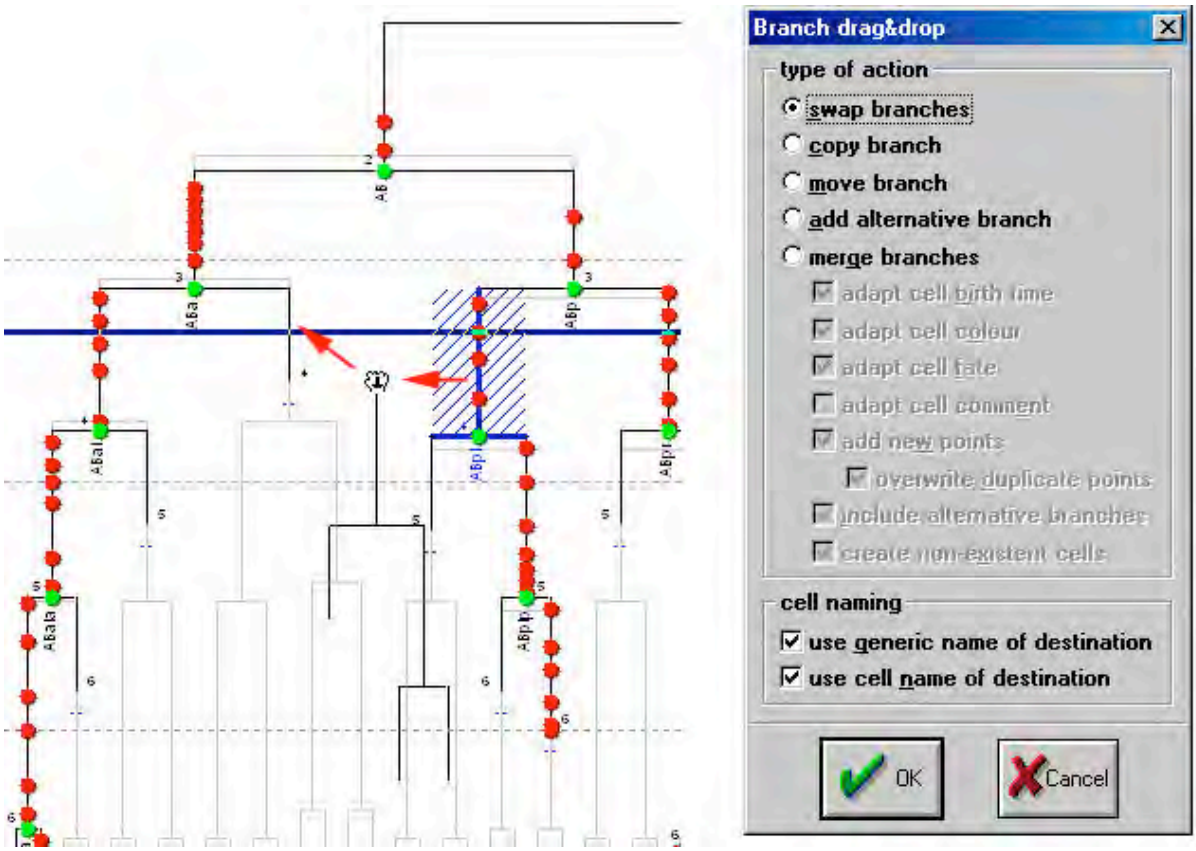


Fig. 15 The **Drag and Drop window.** The left part shows the moving of the lineage branch. After dropping the lineage at the target branch the menu shown on the right pops up. Please study the options, they are more or less self explanatory.

After such “extensive” alterations I always save the project under a new name usually by extending the name with for example _a. The I enter in the **File info** the information how the new version arose from the previous one. Thus I am able to reconstruct how a project evolved. The last version is always the one I continue to work on.

More “error” functions

The Undo function

You carry an operation out and you realise that this was a mistake. I always save the project before I do something which causes a major alteration of the project for example when I plan to use the **Drag & Drop function** or before I insert a mitosis I am not really sure about. Thus I always can return to the old version by closing the project without saving and loading the previous version. If you just saved a project you can also use the

Return to last version function to do so.

An alternative to undo an operation is the **Undo function**, which is displayed on the top of the **Edit** pull down menu. This function undoes all the operations listed in the **Edit** pull down menu (also if they are accessed by other means) and the **Load standard cell lineage** function. You are also able to **Redo** the last operation. The function memorises many operations you did in the past until the assigned memory is full. You can clear this memory with the **Free undo memory...** function if required (I never used this feature so far).

Alternative branches

You may have had problems to follow a cell and you get in conflict with another cell (the Collision manager gets active). Thus the chances are high that the lineage (cell) you just followed, and not the lineage which is in conflict, is wrong. You hesitate to delete all the work you did in the last 20 min or so but you want to follow the cell again independently. The marks you set before may bias you in the same error in case there is one. You get a new empty branch by choosing the **Add branch function** in the **Edit** menu. Alternative branches are indicated by a little yellow triangle marking the mitosis the branch is originating from. A small yellow window indicates which of the branches is just active. You can add several branches, if you wish one for everybody in the lab who wants to try to solve that lineage. Now you can follow the cell again. One can switch back and forward

between the branches using the (**Shift**) **Tab** keys on the keyboard or by using the corresponding function in the **View** pull down menu. If you made up your mind which one is the correct branch you can delete the wrong branch - when it is active - with the **Delete branch** function in the **Edit** menu.

Functions for annotations

You already learned how to note an observation in the **File Info**. However you may want to annotate or comment specific events within the lineage. You can annotate the **Red points** and thus a specific mark of a cell**.** If you want to comment a mitoses you can annotate the **Green point** marking the mitosis. And you can annotate a cell, i.e a whole lineage branch, which is usually done to assign a fate (tissue) to a cell.

• Double click a **Red point.** The **Point information window** will appear (Fig 16). Type a comment in the corresponding field as shown in the figure.

Please note that this window offers also seven other functions, you may find useful. One can also delete a point using this window.

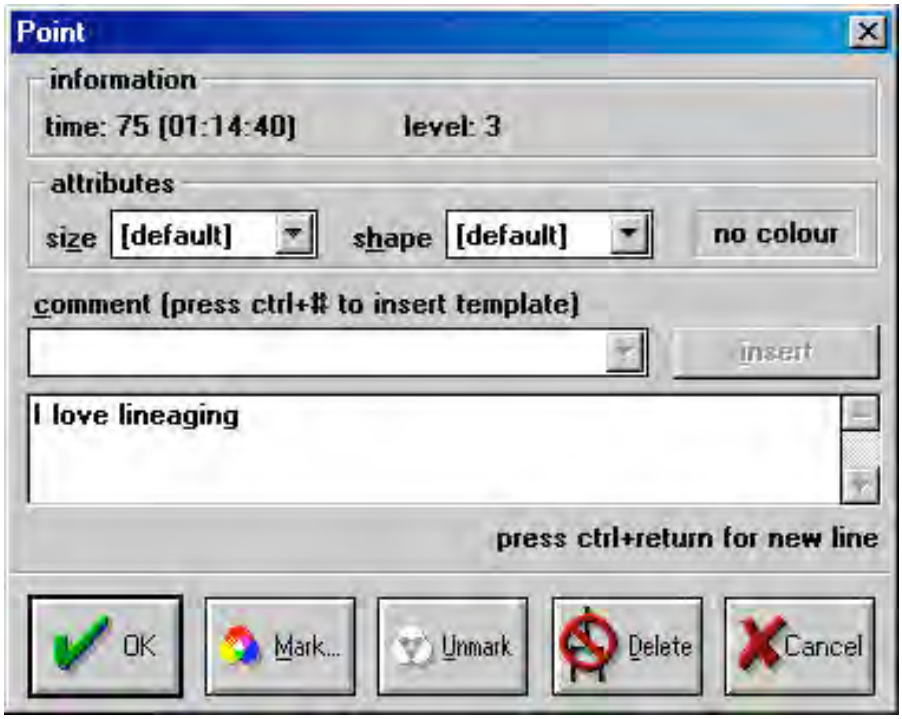


Fig. 16 The **point information** window

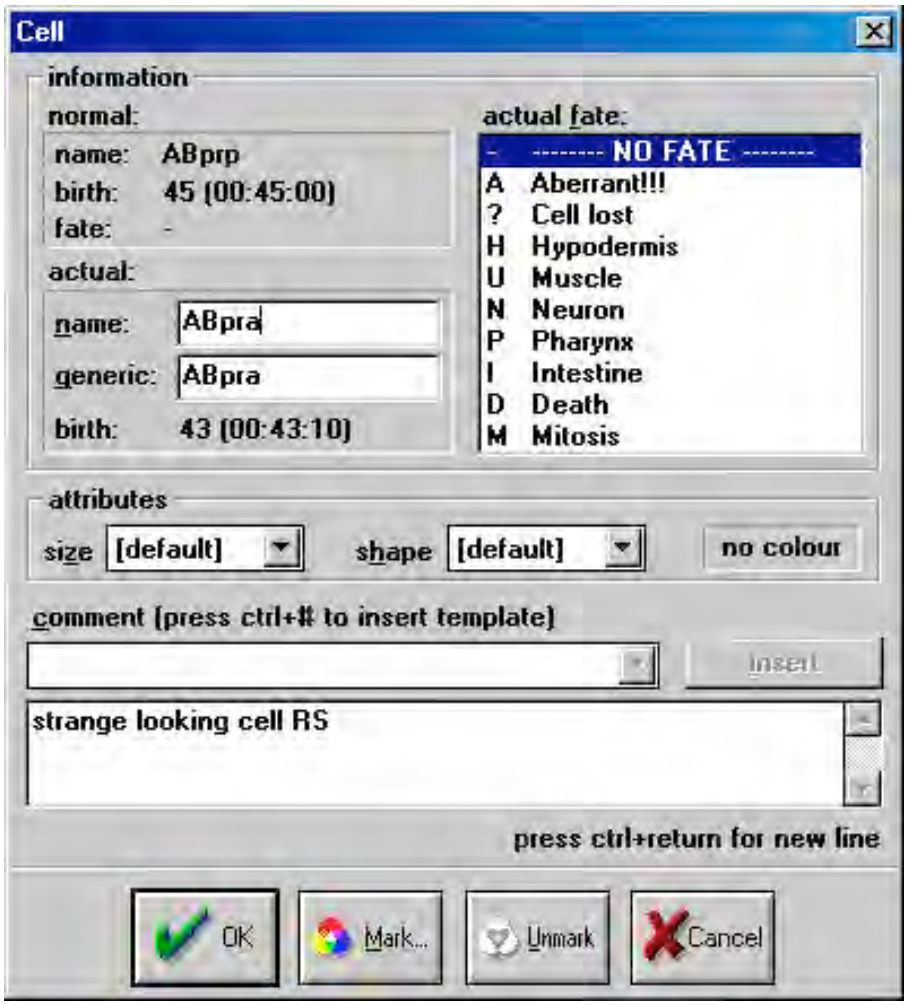


Fig. 17 The **Cell information** window

• Please activate **OK** and the window will close. The **Red point** now differentiated into a **Blue square** which indicates that this point was commented. The comment is displayed in the lower part of the **Information window.** So you can read the comment when you pass through the point or by just clicking once at it**.** I sometimes just annotate a cell with the comment "xxxx" to label the branch for example when it is involved in a collision and I have to check it. Use this feature for example when you are not sure if your lineage is really correct. For example it helps to guide your PI to problematic cells and he can solve the lineage for you. My students love this use of the **Blue square.** The same procedure can be used to annotate the **Green points** marking the mitoses. The corresponding window is called the **Mitosis information** window. It is worthwhile to note for example clearly asymmetric divisions or cross deviation from the normally expected a-p direction of a cleavage.

• If you double click at an activated lineage branch the **Cell information** window will pop up (Fig. 17). This window is more sophisticated then the two others before since it is also used to assign cell fates when differentiation has started. If you insert a comment it will be displayed in the upper part of the **Information window.** The information is displayed along the whole lineage branch a cell exists. Put a comment in the lower window "strange looking cell" and close the window by clicking **OK**. Your comment is displayed in the **Information window.**

The Navigator

• Navigate through the **Cell lineage window** and click at a **Red Point** marking the **ABarpp** cell**.** When you see the **Red cross** in the nucleus press **F7** (or activate **Extras, Navigator).** Now the **Cell lineage window** switches in the **Navigator mode** and all the lineages you followed disappear (Fig. 18). The lineage branch which was active when you started the navigator remains activated. Now orientate yourself in the window and zoom up and move to the ABarpppppp cell down at 227 min. Click at the branch of this cell. Now the **Cell lineage window** comes back and a hatched green line shows you the way from ABarpp to your aim the V6R cell. Please follow the cell till 227 min. This cell is a hypodermal cell which never divides again. You may follow the cell until the embryo starts to move at 337 min of development.If you get lost in between just press the **0** on the numerical pad and SIMI°BioCell will jump back to the last mark you set. Just try again.....

The "Small cell markers"

Lineaging towards the V6R cell cells get smaller and smaller and you may find that the large **Red cross** marking the cells obscures your view of the cell. Choose the **Video pull down menu** and activate the **Small cell markers**. Now the **Red cross** converts into a small **Red dot** and you can see the cells much better. I use only this dot to lineage.

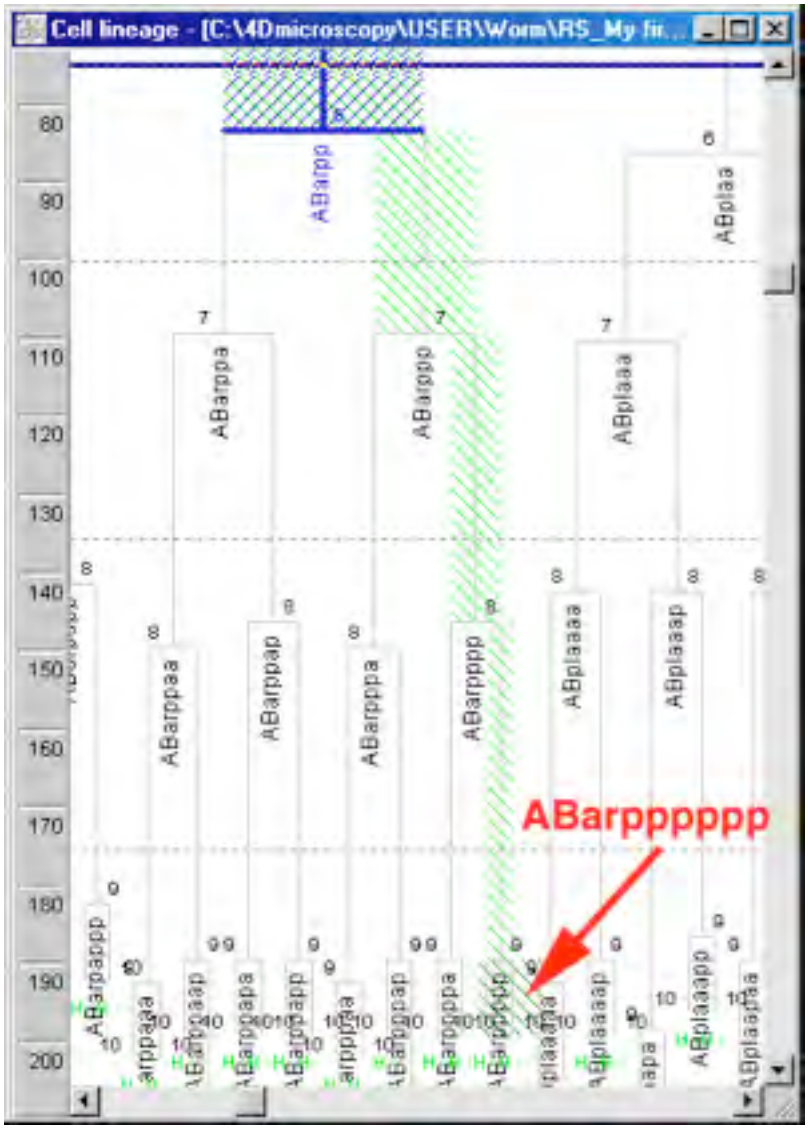


Fig. 18 The **Navigator window**

Lineage strategy and an important rule (once again)

When you follow a cell its nucleus will soon or later move out of the focal level you are just looking at. If this happens do not "jump after it"! One does not have any sense for the direction in which it escaped. I assure you will find also a nucleus in the wrong direction. Use the "**0**" on the numerical pad to jump back to the last marker. Go again forward and take great care to keep the nucleus always in focus. In my experience it is also better to move rather fast through the video, since the human eye is trained to follow moving patterns. Mitoses in which the daughters move out of the focal level can be also quite difficult in the beginning. Move up and down in the focal levels and observe how a daughter is "jumping" in the picture. Do not get desperate too fast. Use either

the **My first lineage_finished.sbc** or the **RS_IB+RS_N2_lineage.sbc** project - supplied with this tutorial - to find the solution for a specific lineage (if you find a mistake in the lineages please tell us). Or even better, come to my lab and learn lineaging.

At the **Fixed point** 227 min you should end up at the cell shown in Fig. 19. If not search for the error(s). It is no problem to have two projects open at the same time and to jump back and forward between them to compare the lineages. Three are ok too, however, if you open too many large projects you may crash the memory. I would not be surprised if you had problems with the division of the ABarpppppp cell. If you get desperate please open the project **My first lineage_finished.sbc.**

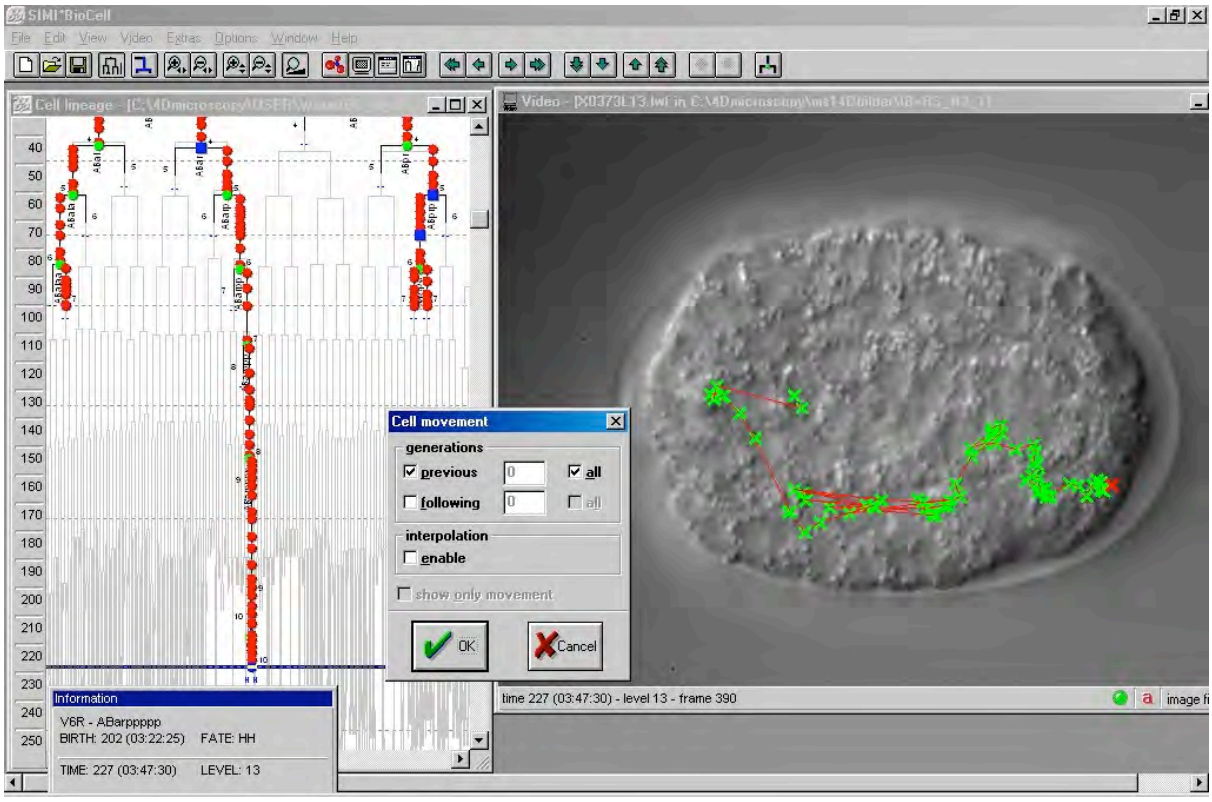


Fig. 19 The Position of the ABarpppppp (V6R) hypodermis cell and the **Cell movement** function.

The cell movement function

• If you identified the V6R go to **Extras** and choose **Cell movement.** The **Cell movement window** will open (Fig. 19). Activate **previous** and **all.** Now all marks you have set are shown and connected by red lines. With this function you can see the cell movements and the mitoses of the precursors and the cell itself you activated. Play by using only 1, 2, ... generations instead of **all.** Is it not astonishing how far ABarppppp and its precursors migrated? Jump back to the AB blastomere and try the **following** function.

Backwards lineaging

There are situations where you may be curious what the identity of a certain cell is, for example in a mutant embryo or a cell expressing a GFP marker as will be explained in the tutorial for the multichannel version of SIMI°BioCell. For this purpose we created a special window, but before you activate this window please make sure that you use your own first project again in case you looked at **My first lineage_finished.sbc** before (just close it). Now activate ABarpppppp at 227 min again.

• Press **F8**, a new window called **Backwards lineaging** will open. Now mark the cell which looks like the bilateral homologue of ABarpppppp (V6R) in the **Video** window, (Fig. 20). Now you see a **Red point** and the blue line goes back to P0 in the lineage tree (see **Information window).**

• Follow the cell backwards in development (← cursor) and mark it once and a while. If a cell division occurs use the **Insert mitosis** function to introduce the mitosis in the branch. Probably you have to use the **Insert mitosis right** function several times and then it will be rather obvious. When you arrive back at 80 min or so mark the cell every frame or at least very often. If you are lucky suddenly the **Collision detected window** opens and warns you that a collision occurred (Fig. 21). Yes, of course you want to see the details. The **Collision manager** now informs you that you marked in the **Backwards lineaging window** the same cell which was identified as ABarpp in the normal **Lineage window**. Congratulations you identified the cell at 227 min as V6L (ABarppappp, the bilateral homolog of V6R.

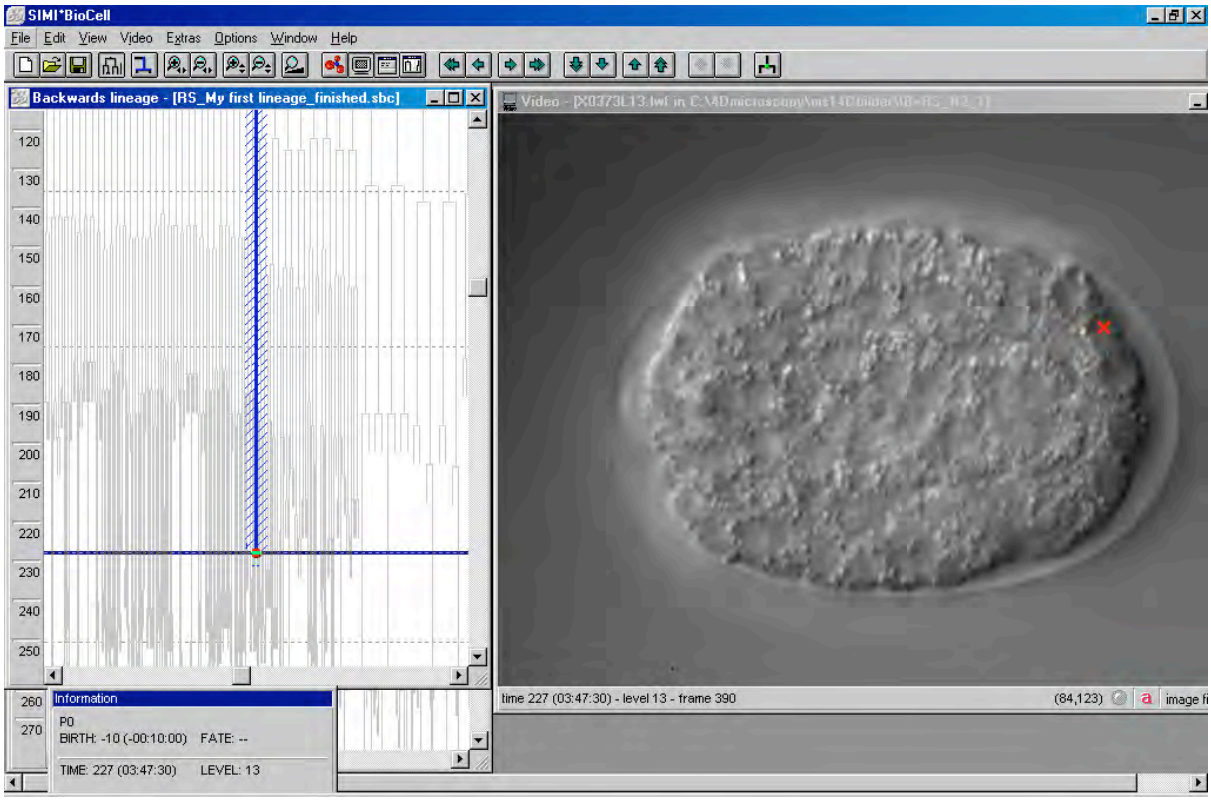


Fig. 20 The **Backwards lineage window**. The **Video window** shows the cell which should be identified by backwards lineaging.

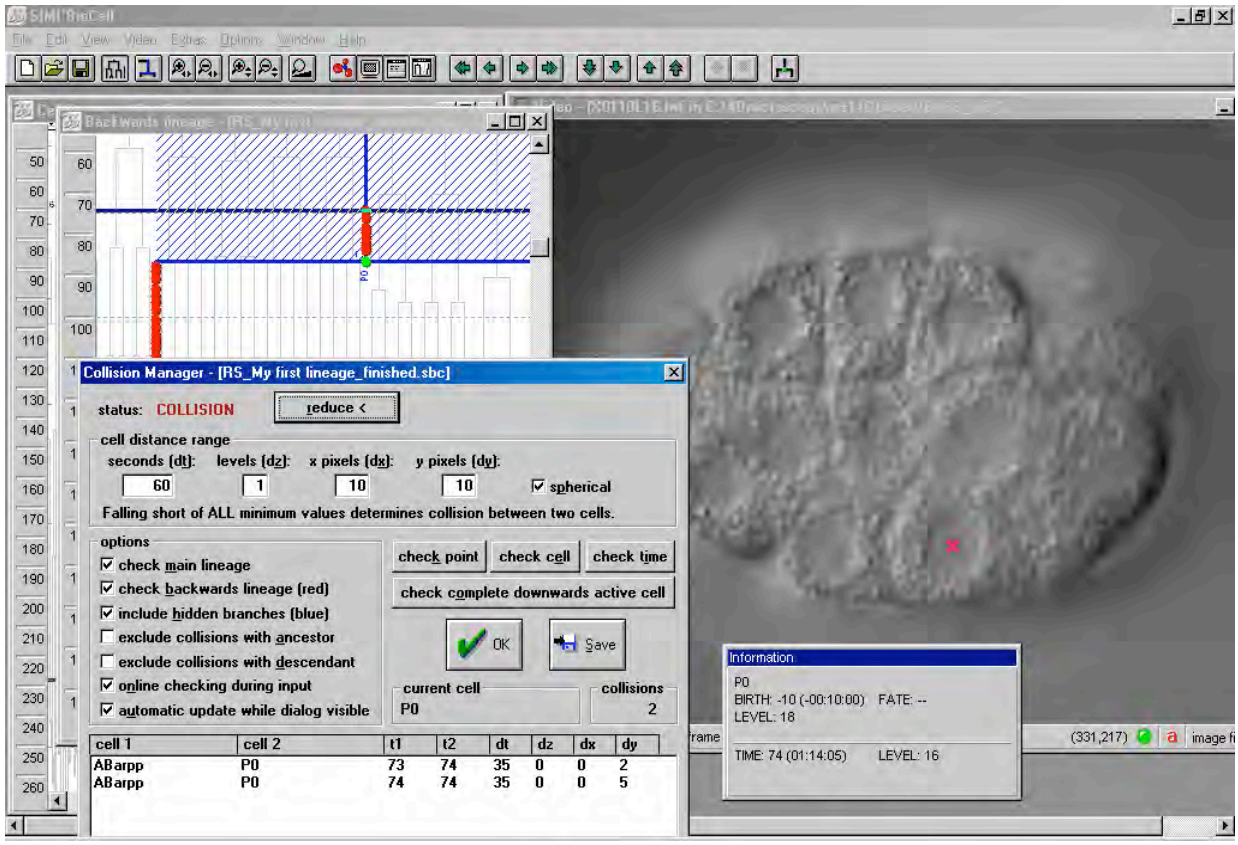


Fig. 21 The **collision manager** identifies the mother cell of the backwards lineage as ABarpp.

• Use the **Drag & drop function** to transfer your backwards lineage into the normal **Lineage window**. Do not forget to save the project. If you could not succeed please do not get desperate. Just lineage a couple of days and try again. After all everybody who tried seriously also learned it.

The cell movement function again

• Click at some **Red point** marking the ABarp blastomere and activate the **following** function and **all**. You now see how the V6L and V6R cells were placed by cell movements through the embryo. I find these movements really fascinating (Schnabel et al. 1997 and 2006).

Some more interesting lineages

• Next lineage two cell death. ABalaapapa (CD#1) is an easy one due to the specific orientation of this embryo. Observe the asymmetric cleavage of the mother cell producing the cell death. Annotate when the cell dies and when it is engulfed (Hoeppner et al. 2001). ABplpappa (CD#9) occurs also on the ventral side of the embryo. It is the posterior sister of the precursor of the excretory cell. This is the largest cell on the ventral side which is not too surprising if you consider that this cell will be the kidney of the worm. Identify the engulfing cells by lineaging backwards. Next lineage two cells producing nervous system. ABaraaappp first its 10th cleavage characteristic for this tissue is easy to score. Then try the anterior sister, its cleavage occurs in the dorsal-ventral direction - tough. If you have difficulties you may look at these lineages in the **RS_IB+RS_N2_lineage.sbc** project which shows an wild-type embryo lineaged by my former graduate student Ingo Büssing. This project will be also used for the next tutorial which introduces more functions of SIMI°BioCell.

Yet another “Schnabel” rule for lineaging

Please consider that a lineage analysis is much more effort than making a new recording. So do not lineage embryos which were recorded with bad optics or were strange in one or the other way, like having a big bump in the eggshell. Only lineage really nice recordings!

Automatic assignment of “anterior” and “posterior” after a division.

So far you always had to activate the appropriate branch to lineage a daughter cell after a division. Only the **Navigator** activated the appropriate branch after a division to guide you to a cell. However, you can teach SIMI°BioCell where anterior and posterior are in your embryo. After a division it will then choose the right lineage branch for you when you click at one of the daughters after you entered the mitosis. To do so the program compares the relative position of the new mark to the last one you inserted before the division. Thus to make the automatic assignment reliable you always have to set a mark right before the cleavage. I usually do so just before the first indications of the cleavage furrow are seen. This also gives reliable data when you want to calculate division angles by bio-informatics. If you plan to do so it is also a good idea to mark the centre of the cell and not the small reforming nucleus after the division and only to mark the nucleus again when it returned to the centre of the embryo. Alternatively one can just use the mark set 2 min after the division for the calculation of angles and transport of cells during a cleavage.

• Pull down the menu which hides behind the little computer screen icon on the left of the top bar of the **Video** window (Fig. 22). Choose **Calibration** and release the mouse button**.** The **Calibration “First Step”** window will pop up (Fig. 23).

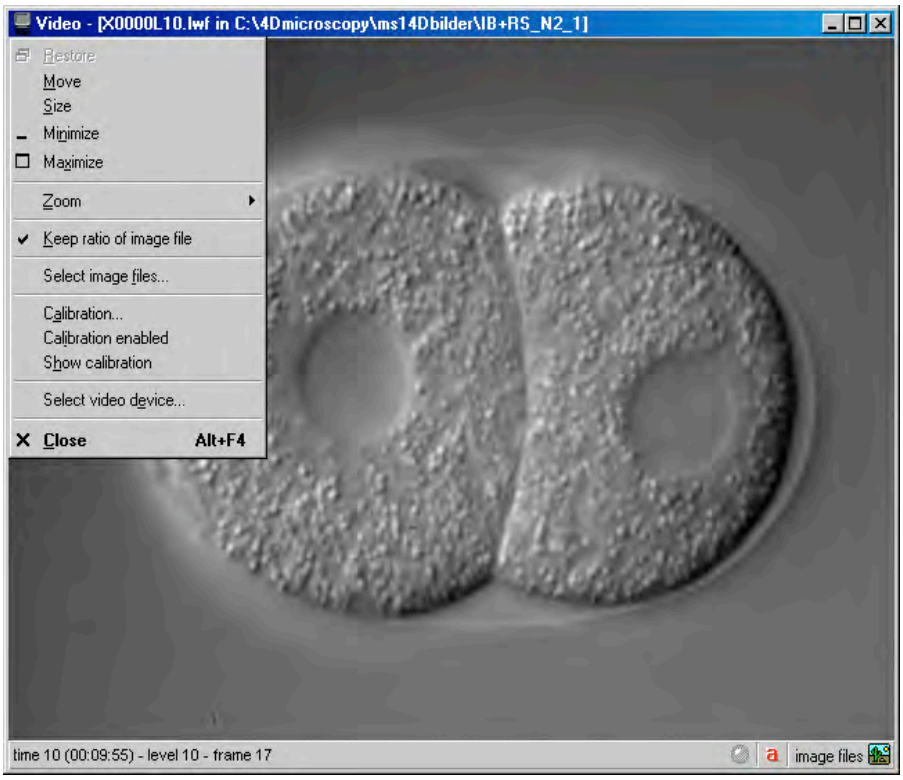


Fig. 22 The **Video** window pull down menu. Here you find also the **Zoom** function to scale the video window and again some useful functions you saw before in other menus. The **calibration functions** which allow to define the a-p axis of the embryo and to enable the automatic assignment of the daughters of a cleavage are accessed here conveniently.

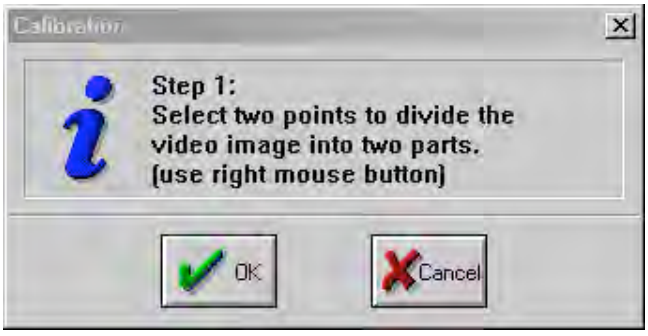


Fig. 23 The **Calibration "First Step"** window

• Pull down the menu which hides behind the little computer screen icon on the left of the top bar of the **Video** window (Fig. 22). Choose **Calibration** and release the mouse button**.** The **Calibration "First Step"** window will pop up (Fig. 23).

• Now try to determine the a-p axis of the embryo by just looking at the eggshell at a medial level.

Any orientation is possible for the automatic function. Anterior can be at the top, to the right or at the bottom of the picture. To avoid mistakes during lineaging I, however, always record embryos with the anterior to the left, or if I record two embryos sometimes with the anterior to the top of the picture. One gets so used to these orientation that one makes all the time mistakes in embryos with other orientation. One then finds all kind of "interesting a-p lineage transformations after the lineage is finished. Its hell to fix that again afterwards using the **Drag & Drop feature.** New students sometimes record embryos with the anterior to the right. They are usually a head shorter after that but especially in these cases I use then the automatic assignment function to avoid mistakes.

• Click "**OK**" and draw a line orthogonal to the a-p axis using the RIGHT mouse button as shown in Fig. 24 and release the mouse button at the end of the line. The **Step 2** window will appear. Click "**OK**" and click once again with using the RIGHT mouse button in the anterior part of the embryo. A new window will acknowledge that the calibration is finished. Now check in the pull down menu of the **Video window** that the **Calibration** is enabled and the **Show** calibration is disabled. Otherwise the grey line will go through each mark you set. Now the automatic a-p assignment will work after you introduced a lineage branch during a mitosis. Lineage a cell and introduce a mark just before the cleavage furrow ingresses. Push return when the furrow is appearing to introduce a lineage branch. Go one step further in time and click at one of the daughters to mark it. SIMI°BioCell will now assign the **Red cross** to the correct anterior or posterior lineage branch.

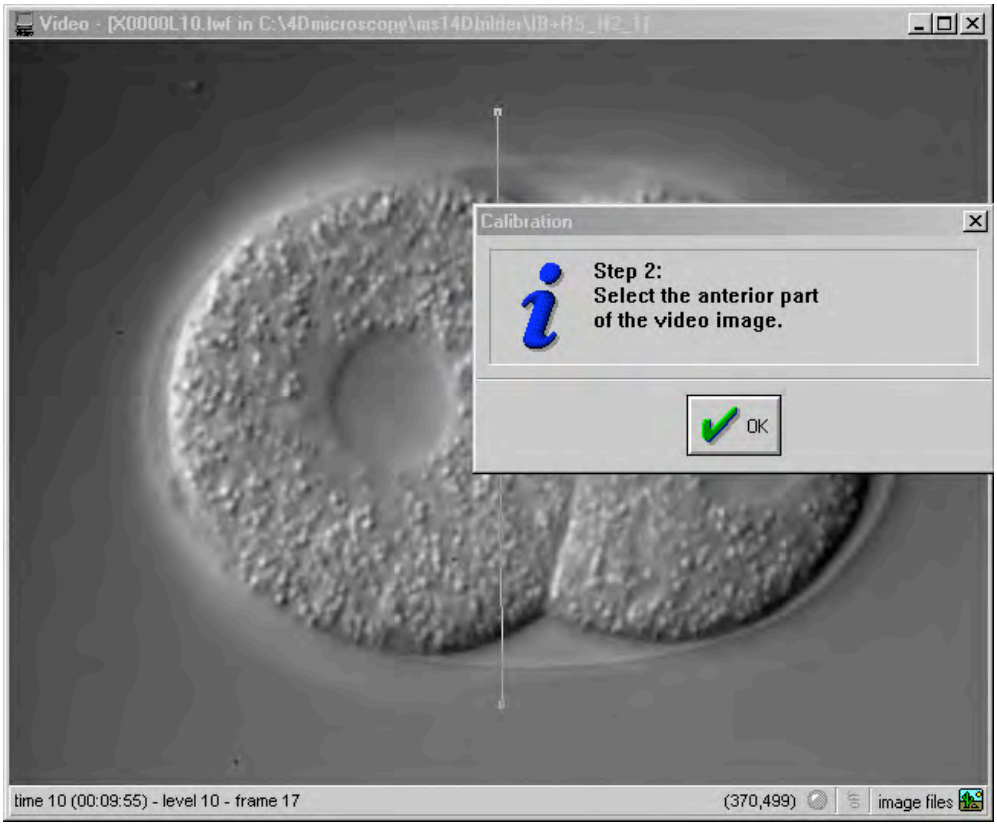


Fig. 24 Drawing a line orthogonal to the a-p axis of the embryo.

•Please note that you always have to check the correct assignment. The program just calculates if the new coordinate after the cleavage is more anterior or posterior than the last mark before the cleavage. So if the cell moved far posteriorly before it divides and the anterior cell is then in a more posterior position than the last mark before the division, the program will assume that it is the posterior daughter. This happens once and a while if one is not careful. I then use the **Drag & Drop function** to exchange the assignment of the cell to the anterior branch.

Optimising the SIMI°BioCell outlay using the options functions

• Activate the **Options pull down menu** (Fig. 25). Choose **Display Options...** A large window will open which allows you to change some basic settings of the program (Fig. 26).

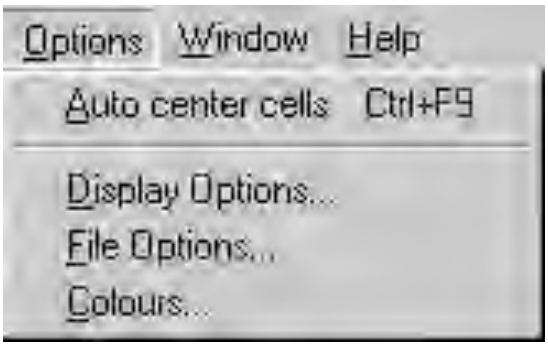


Fig. 25 The **Options pull down menu**

Please look also at the **File Options...** window (Fig. 27). The functions displayed in these windows are explained in the legends of these figures. The **Colours** window (Fig. 29) permits you to create your own colour outlay for the program if you wish to do so. The only function I really use here is to change sometimes the colour of the background in the **3D View window** from white to black or vice versa. If you are once used to the outlay we pre-set for SIMI°BioCell you may not really want to change it anymore. It is also optimised for printing and slide shows.

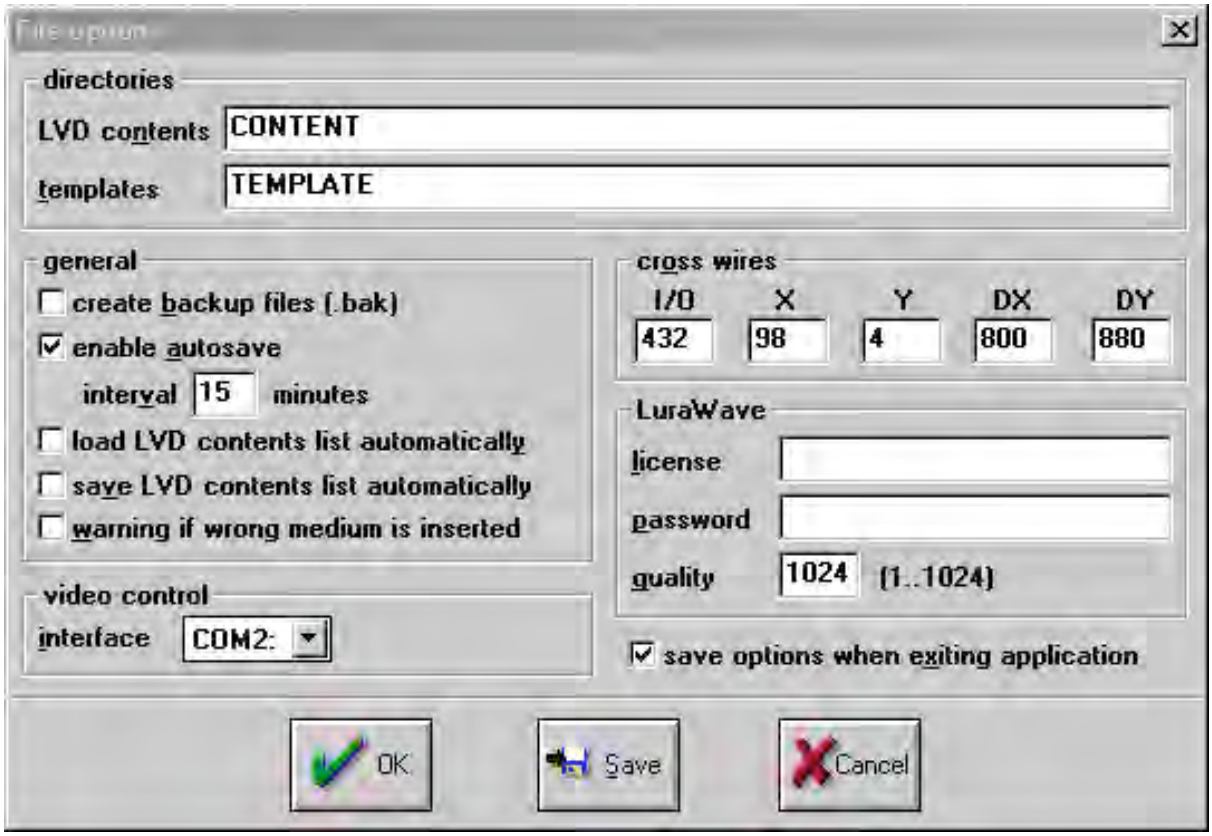


Fig. 27 The **File options...** window. In directories only the name of the folder **TEMPLATE** is relevant for you. This folder locates in the SIMI°BioCell folder and contains the templates for new projects. How new projects are created is explained in Chapter 4. If you make your own templates they either have to be stored there or you have to create a new folder and you tell the program in this window the name of the new folder. Well..... In the general part of the window you should enabled the **autosave** function! Choose a time interval you feel save with. You can also tell the program to create back up files upon saving. I feel that this does not make much sense anymore considering the safety of modern hard disks. But make sure that the original files are backed up on a regular base. The **LuraWave** section is important for you if you want to use pictures with LuraWave compression. I strongly recommend to do so! See the Introduction. Please contact me for a licence number to enter in this window. All LVD functions are not relevant for you including the **cross wires.**

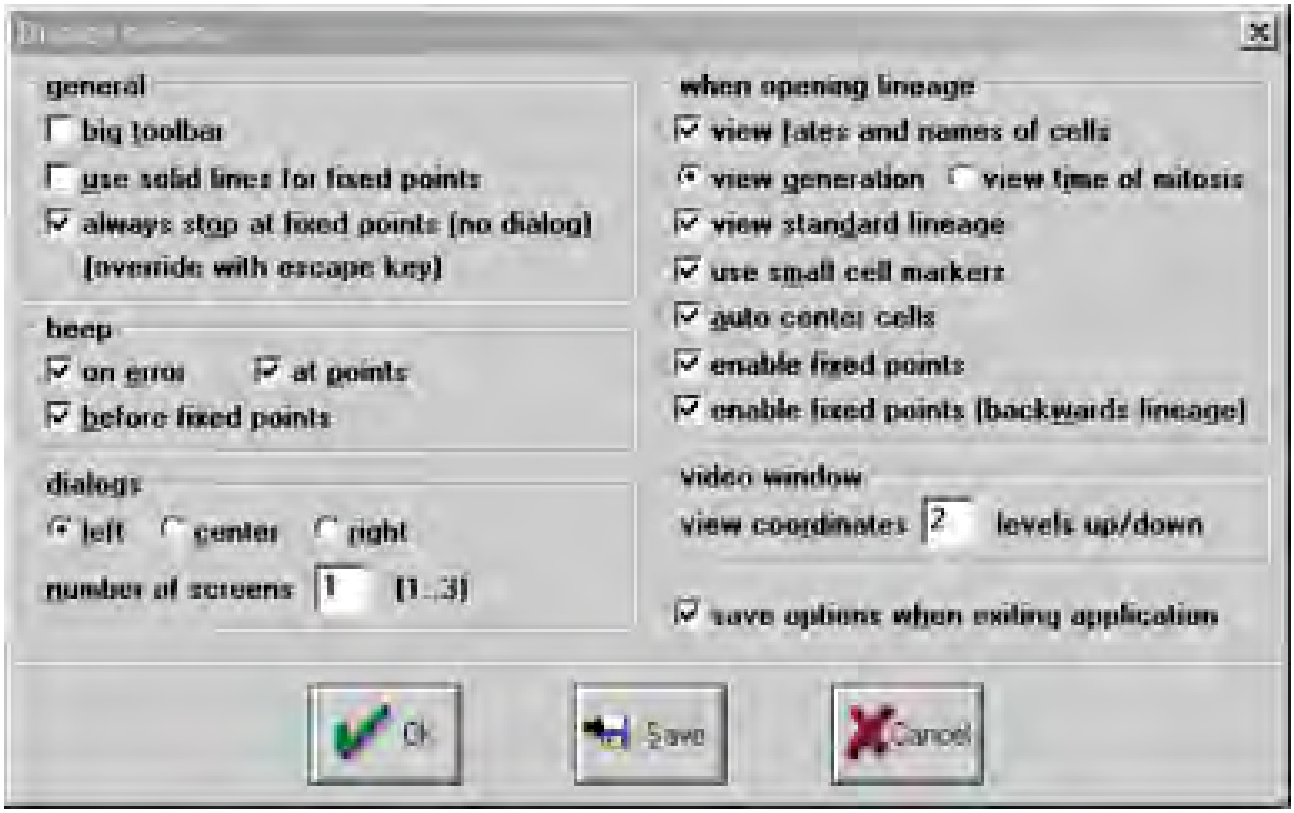


Fig. 28 The **Display options window.** The functions in this window are rather self explanatory. The function **always stop at fixed points** causes the program to stop at a fixed point and you can only proceed when you marked the cell or by pressing <**esc**> while you move the video. If this function is not enabled SIMI°BioCell always opens a window at the fixed point which asks you to set a mark. This can be really cumbersome. Therefore I asked Toni for this function. The function **view coordinates x levels up and down** in the **video window** section determines how far off from the level the **Red cross** was originally set it still can be seen. You may want to alter this number according to your personal requirements if you lineage for example tissue culture cells or other animals than *C. elegans.*

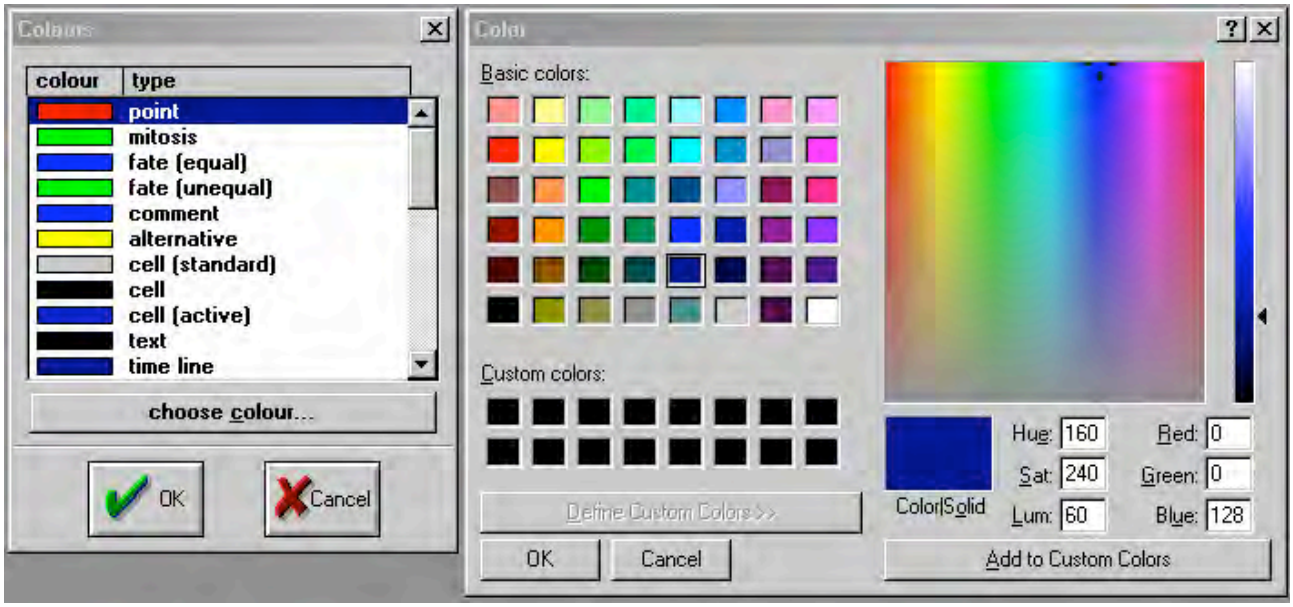


Fig. 29 The **Colours windows**. With the left window you can choose your own colour layout in the windows of SIMI°BioCell. If you select **choose colours...** the right window will pop up. There you can choose from the **Basic colours:** or mix your own ones and save them in the **Custom colours:**. As will be explained in Chapter 3 I recommend to use the Schnabel set especially to mark the founder cells present in the 12-cell stage embryo.

Exporting windows or pictures for presentations or publications

You can either export windows using programs like SnagIt to choose and save the windows. Or you copy the whole desktop with the print function on your keyboard in the system memory. Afterwards you paste the picture in a program like Photoshop and you process it there. I use this a lot to get nice pictures from for example 3D views.

Second Tutorial “Embryo Analysis”

Chapter 3

In this session you will learn how to

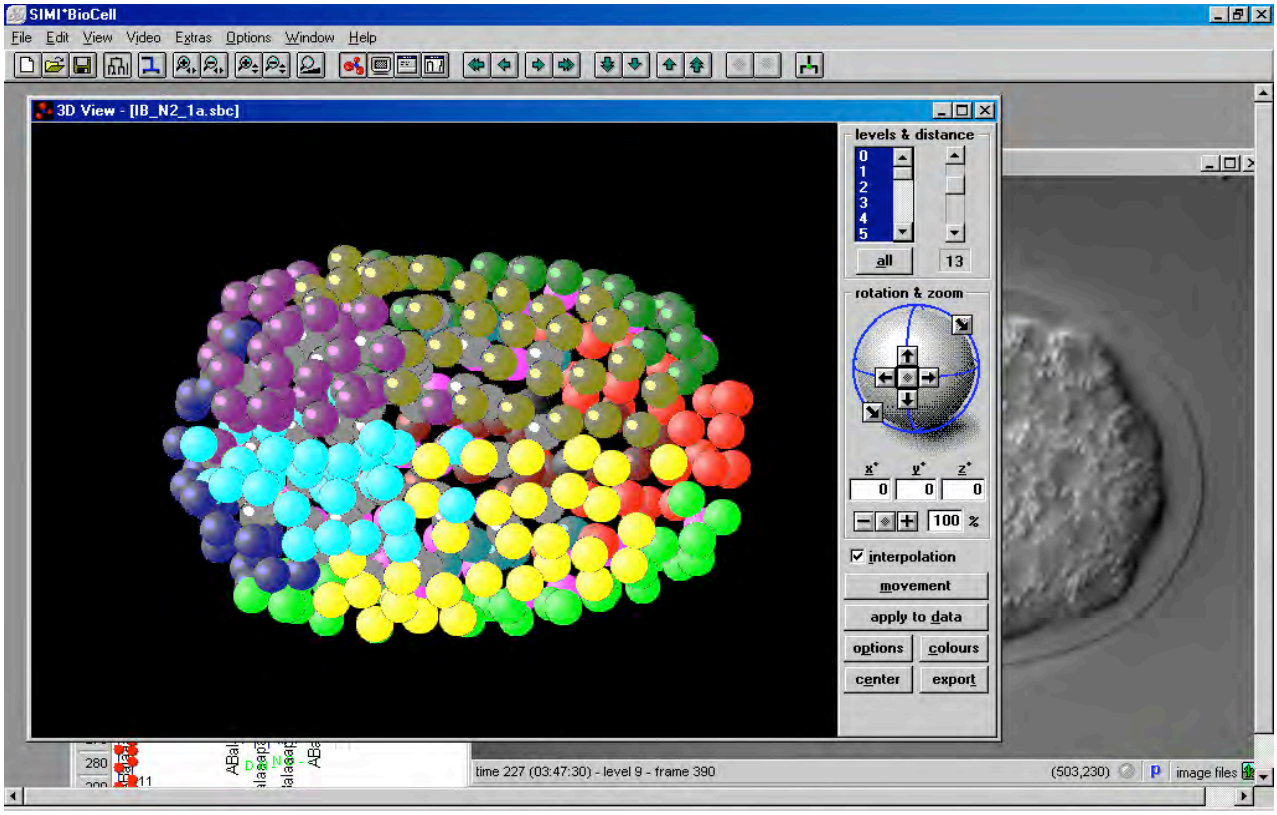
analyse embryos with SIMI°BioCell.

The 3D-representation of cell positions

• Open the project **RS_Start_IB+RS_N2_BMBs_lineage** which is supplied on the SIMI°BioCell Tutorial DVD. The **Cell lineage window** is now crowded since it contains the lineage up to the premorphogenetic stage (227 min) and some lineages which go further. However, the pictures end at 250 min, since they are not compressed and thus take a lot of space. The time line should be positioned at 227 min, if this is not the case move it there. Save the project (add your initials in front of RS).

• Open the 3D-representation function either through **Extras, 3D-representation** or by clicking at the  icon. A new window **3D View** will open and you see a 3D-representation of the positions of all nuclei (Fig. 30). When you click again at the icon the window will close again. You see the

Fig. 30 The **3D View window**



ventral view of the embryo as it was recorded under the microscope. Please note that embryos “rotate” under the microscope during development (Schnabel et al., 1997). At the 4-cell stage (25

min) the EMS blastomere - which by convention defines the ventral side of the early embryo - is located on the lateral left side (level 8) of the embryo - as it is then positioned under the microscope. At 227 min the ventral side of is located on the top (level 0) of the embryo. Thus by definition the dorsal-ventral axis rotated by 90°. Whether this is a true rotation or due to internal reorganisation of the embryo during development is a subject of fierce discussions in my lab (Schnabel et al., 1997). The problem is that we do not have a fixed reference point which would help to solve this question.

• Use the "globe" in the **3D View window** (Fig. 31) to rotate the 3D-representation of the embryo. The other functions of this window are explained in the figure. You can also turn the 3D-representation with the mouse pointer while pressing the RIGHT button. Now press **Pos1** on your keyboard. The program will jump back to the first time point. You will see two grey spheres which represent the AB and P1 blastomeres.

3D-Videos

• Use the → cursor to move forward in time (the **Video** window has to be active). Alternatively you can use  to move in time. In this case the **Video** window can be closed or no pictures are required at all to play the 3D-movie. You will see in a video how the embryo develops. When **interpolation (**Fig. 30) is activated the video interpolates the positions of spheres between the marks.

• Stop at some point and move the mouse pointer over a sphere.

The Show fly-over information

A yellow flag will pop up and show you the **Show fly-over information**, which identifies the cell and shows you some details. When it was born, the level it occupies in the original recording and in case you turned the representation the new level it was projected in. Also annotations will be displayed. Click at the cell its colour will turn orange which tells you that its lineage branch is now activated. The video window jumps to the appropriate level and shows you the cell marked with the **Red cross**. If you wish you can now explore the neighbourhood of this cell by clicking through the spheres. Who touches whom? If you are done activate the **3D View window** again and proceed in time until you reach 227 min. You can play now around for example turn the 3D-representation and observe development from a different perspective. If you look from the anterior you can watch

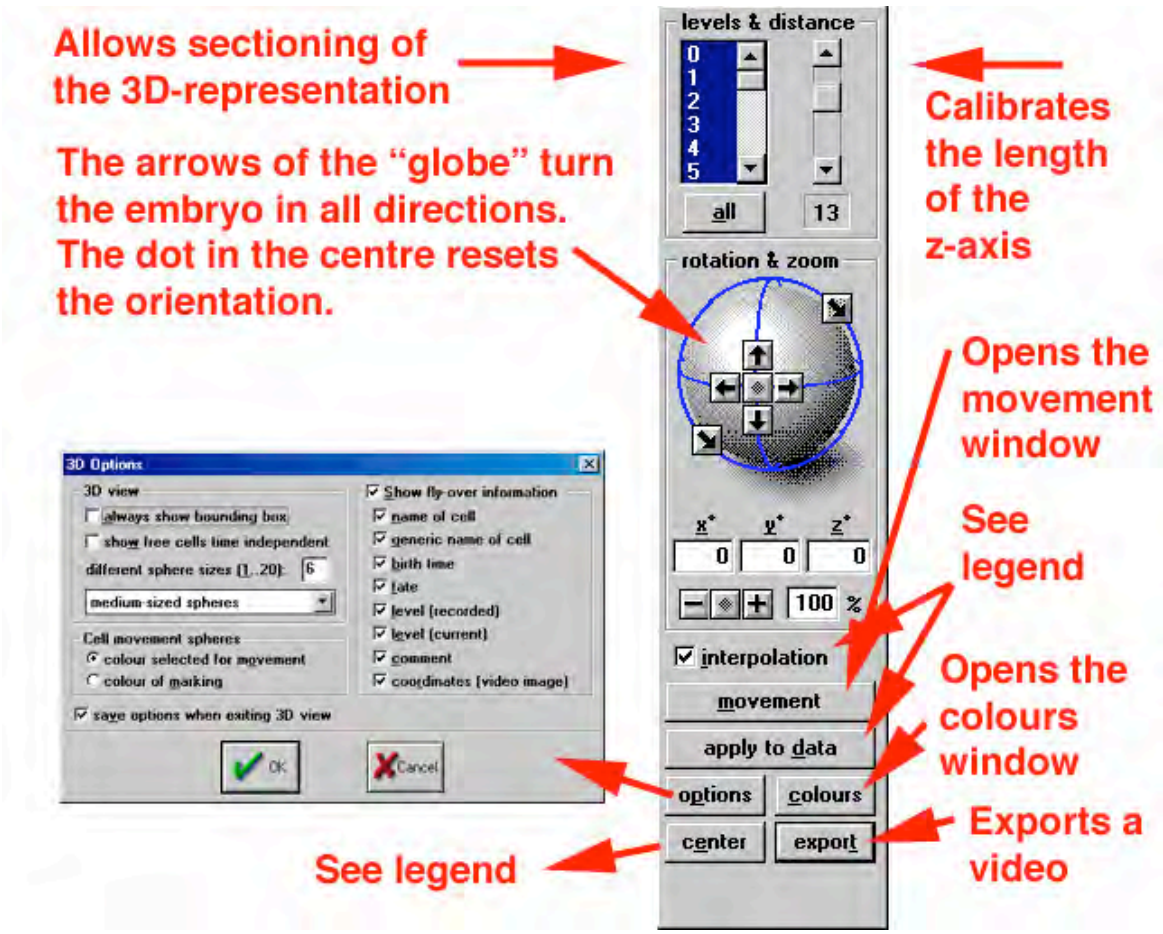


Fig. 31 The functions panel of the **3D View window**. Important is the **different sphere sizes** option in the **3D Options window**. Here you can determine the size of the spheres and the factor by which a cell will become smaller at the lower levels of the embryo. Please play around. You can activate a bounding box if you wish which helps to judge the orientation. Since SIMI°BioCell has no idea of the dimensions of the embryo you have to calibrate the relative length of the z-axis. The representation should be "round" when you rotate it around the long axis. If you want to play the development as a video in principle all cells have to be marked at all time points, which is a lot of work. If the **interpolation** function is **on** the program interpolates the co-ordinates between the marks of the cells. Thus if you label cells every few minutes a realistic movie will show you the details of development. The **apply to data** function will calculate a new set of co-ordinates corresponding to a new view of the embryo. Thus you are able to create a video representation which shows a dorsal view of development of this embryo which in reality developed with the ventral side up. This function is also important for bioinformatical analyses which may depend on standardised axes. **Export** will create and save a movie which can be shown for example in Power Point shows**.** The **center function** allows to calibrate the 3D-representation onto the embryo**.** Please try if you feel like it. It fixes the 3D-representation. Normally the position is determined by the centre of gravity of all spheres.

the gastrulation movements of the MS-derived cells. AB derived cells do not internalise up to the premorphogenetic stage. The yellow cells (ABplp and ABprp) just close the gap the MS derived cells create by moving in. As already mentioned in the legend to Fig. 30 one can export videos using the **export** function of the **3D View window**. However, I get better results by recording the video using programs like Hypercam or Snagit.

Editing colours and shapes of spheres

You may already have wondered about the different colours of the spheres in the 3D-representations. This "Schnabel" colour code corresponds to the cell fates of the 12-cell stage embryo. It was worked out with great care to print well and to work on the web and different computerscreens. It would be very kind if you would also use these colours in papers. It should make it easier for the audience to follow our work.

• Go back to the 12-cell stage (**Fixed point** at 48 min) and explore the code with the **Show fly-over information**. The D lineage is coded a little bit later when it was born with turquoise spheres. The germ line is coded in a darker grey than MS.

• Move back to the start of the recording and point with the mouse at the P0 branch of the lineage and press the right mouse button. The **Edit cell menu** will pop up. Activate the function **Unmark cell top down**. Move down to 227 min again - just click at a cell down there -and activate the **3D view window**. Most spheres are now grey, you have deleted most colours. One exception is the cell you activated which is orange. Just click at another sphere now it will turn orange. Some other cells are still blue, these are those cell which have been annotated.

There are three methods to activate the colour annotation functions. The first you just used. One can access the same functions through the **Edit** pull down menu. The third very convenient way is to click with the right mouse button at a sphere in the **3D View window.**

• Move back to the 12 cell stage (**Fixed point** at 48 min**)** and click with right mouse button at the most anterior (ABala) sphere, the **Edit cell window** will pop up. Choose **Mark cells down.** Now the **Colour window** opens. Activate the darkest blue square in the **Basic colours:** panel **(**4th row 4th colour from top) and click **OK**. The sphere remains orange, now move with the mouse pointer

to the grey sphere slightly posterior and down (ABpla cell) and click at this cell again with the right mouse button. Now the **Edit cell window** will open again and you choose again **Mark cells down** . Activate the green square **(**3rd row 3rd colour from top). Meanwhile the ABala sphere turned blue as will ABalp if you click at another cell. Please play around the marking functions are self explanatory. If you activate **Cell function** only the cell (the corresponding lineage branch) will be coloured. The function referring to cells will colour all cells up or down from the activated cell.

• Play now the 3D-video down to the premorphogenetic stage. You will see how the regions of the embryo develop (Schnabel et al., 1997).

• Please play around and test the different functions. Check the results by looking at the **3D Videos**.

Advanced editing using the Attributes Window

To analyse embryos or to display data in publications and/or supplementary videos you may soon wish to have more sophisticated features. These can be accessed through the **Attribute window** (Fig. 32)**.** It can be opened with the same methods just introduced for the colour procedures in the **Cell edit** window.

• Please play around.

Now you finally "ruined" the project. But it can be restored easily. First go to P0 and set the **Attributes (**access them through **Edit** or with the RIGHT mouse button**)** back to spheres. Activate the **Load standard cell lineage** function in the **File** pull down menu. With this function you can also take over the Schnabel lab colour code in any new project you created.

• Alternatively close the project without saving and open it again. Or just load the file from the DVD again to proceed with the next exercises. Just go back to P0 and unmark again all cells downwards. At 227 min all spheres should be grey again before you proceed.

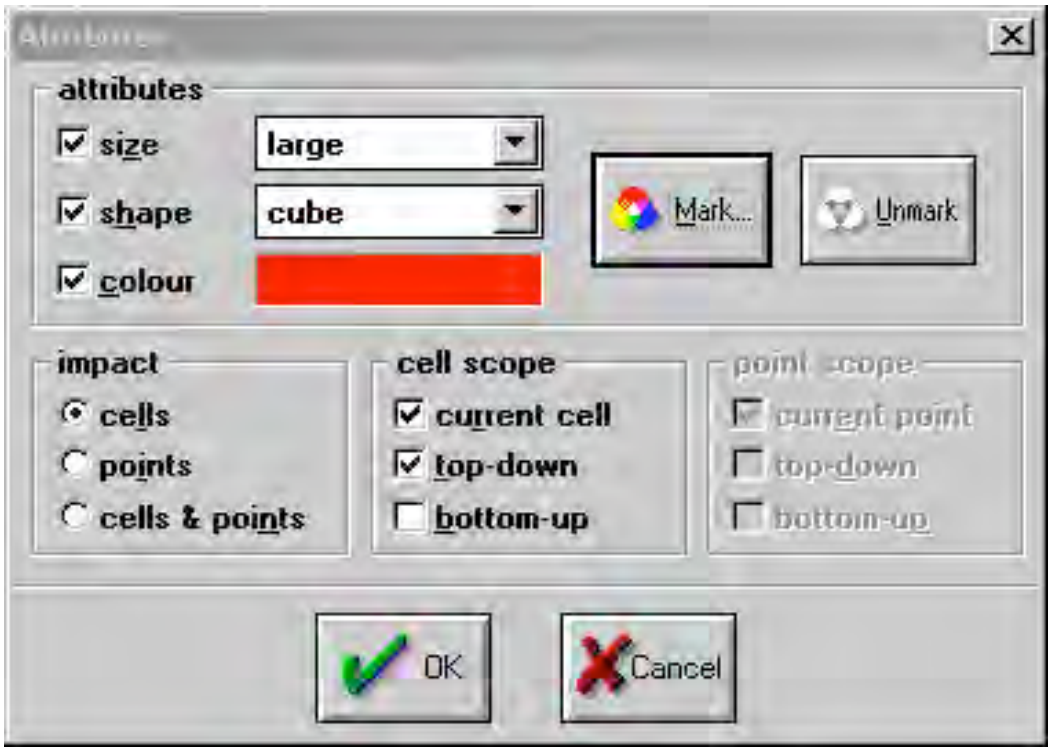


Fig. 32 The **Attributes window.** In the top panel one can choose the attributes which will be manipulated including the colour. Below the **impact** of the attribute can be selected. **Cell scope** defines the direction in which the **attributes** will be assigned.

The Load standard cell lineage function

Importing the standard colours in a project

• After you chose this function the standard Windows **File open** window will pop up. Navigate to the User_Tutorial_Biocell folder and choose the RS-standard_colours file and open it. First one sees no obvious changes, however, now all cell names and cell fates are set to those corresponding to the lineage of Sir John Sulston (Sulston et al., 1983). To also change the colour code choose **Take over colours** in the **Edit** pull down menu. Immediately you see that spheres in the 3D View window will be stained in the colour code which was displayed when you opened the original project.

With the same function you can take over the “Schnabel lab colour code” in any new project you created. It would be very kind if you would use this code also in publications. I found that it is sometimes difficult for the audience to appreciate the new features introduced here to display

embryonic development. Thus it may make things easier if a kind of standard would be used to present data (thank you).

The Cell manager

• Move to the **Fix point** at 227 min and then choose **Cell manager...** in the **Extras pull down menu**. The window shown in Fig. 33 will pop up. The cell manager allows to list, sort and activate either all or a selection of cells. Explore the options you can select in the pull down menus **view** and **sort by.** The **view "cells with point at current time"** is very useful to count the cells at a **Fixed point** to define a developmental stage. With the button **select all/select none** one can activate or deactivate all cells at a certain time point. Try these options while the **Video** window is open. If all

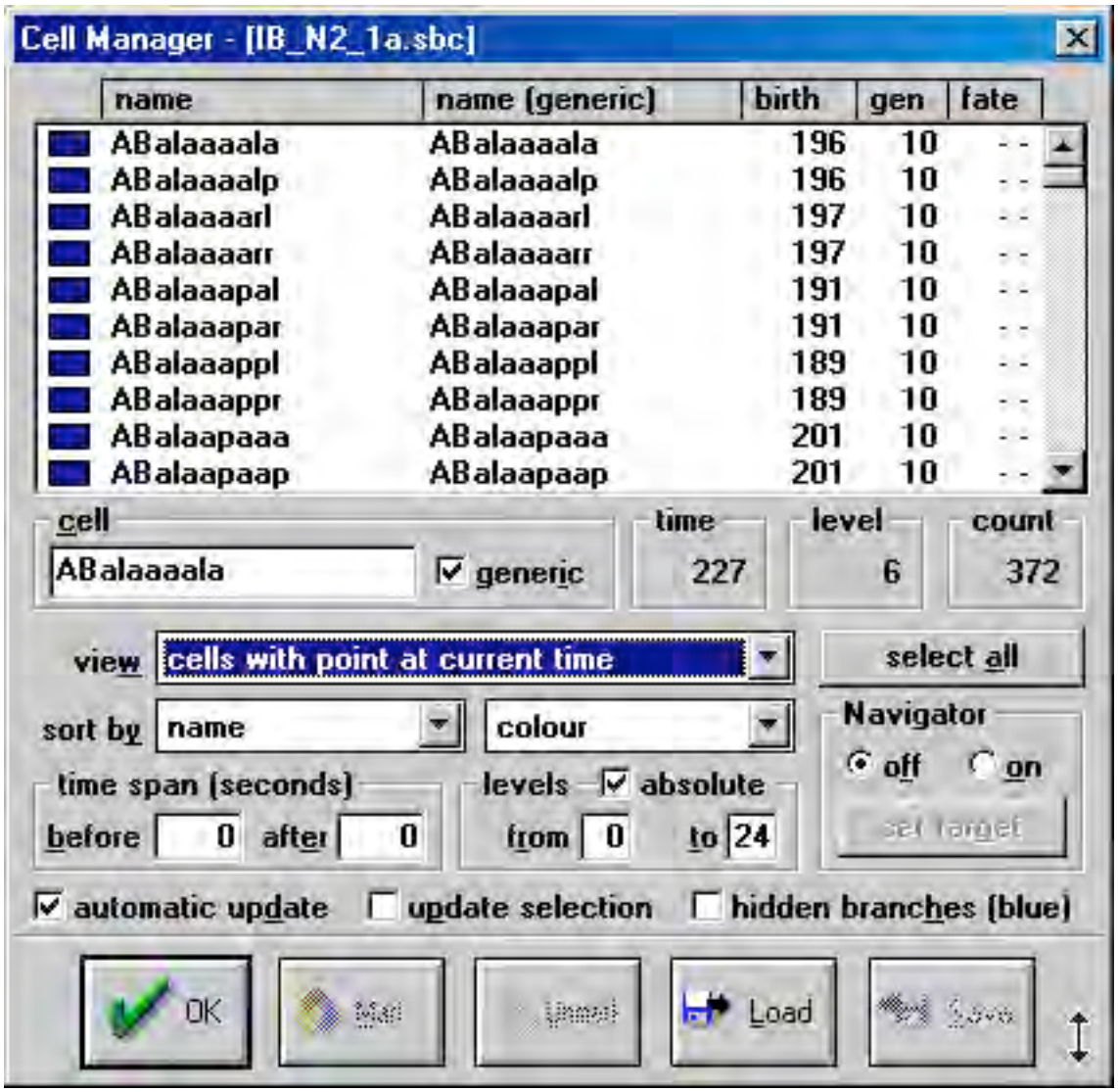


Fig. 33 The **Cell manager**

cells are selected **green dots** will show all the cell positions marked at that developmental stage. This is not really useful but a variation of the procedure is a function I use quite often to find "lost" cells in the embryo.

Resolving very difficult lineages using the Cell manager

The strategy I propose now to resolve very difficult lineages is a kind of cheating but I assure you it works very efficiently. You have resolved more than 90% of all lineages up to the premorphogenetic stage. However, there are these damned difficult lineages from ABala where you do not find one or the other daughter cell, or even both, after a cleavage. Or the **Collision manager** gets all the time on your nerves. Please try the following strategy. Move to the next **Fixed point** and activate the

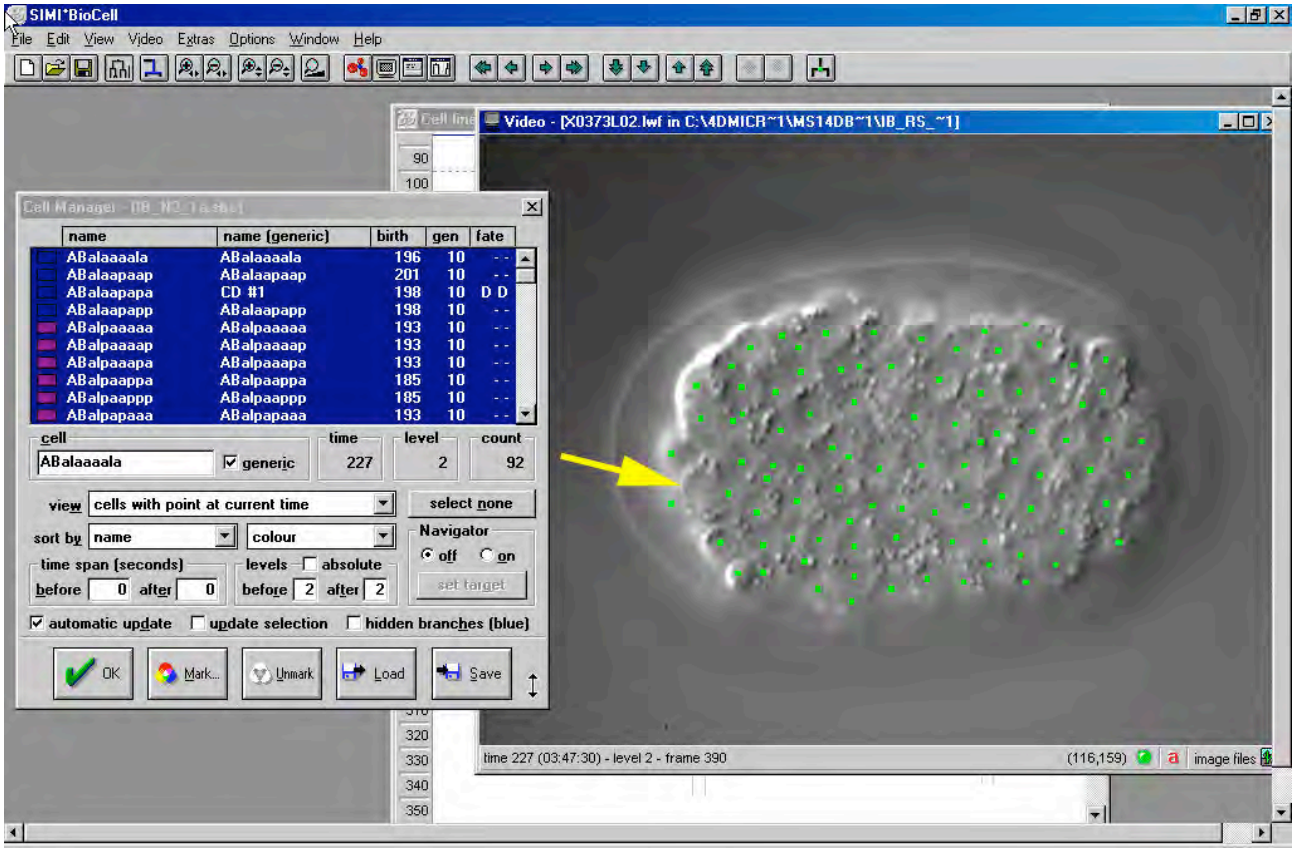


Fig. 34 Searching for unmarked cells using the **Cell manager.**

settings in the Cell manager as shown in Fig. 34. These settings cause the **Green crosses** - please use the **small cell markers** - to behave like the **Red cross** and the **Traffic light** if you follow a

lineage again after the marks were set. The **green dot** will only mark a cell on the level it was set and ±2 levels. Thus you can scan through the focal levels and find out if there are cells which were not marked before. To be able to train this procedure go on like described below.

• You are positioned at the **Fixed point** 227 min. Open the **3D View window** and search for the ABaraaaaap cell (the third light blue cell from the anterior) by sliding over the spheres with the mouse pointer and by looking at the cell names in the yellow flags popping up. When you found the cell activate it by pushing the LEFT mouse button. Activate the **Cell lineage** window and hit the space bar. The activated **Red point** the corresponding lineage **branch** will be in the centre of the window. Delete now with the **Delete range of points** function (RIGHT mouse button) this points and all points back to the mitoses by which the cell was born. Go back to 227 min and look at the Video window. All cell nuclei will be marked but the cell you just deleted. Thus you introduced a missing cell in the lineage tree. The cell is labelled with a yellow arrow in Fig. 33. If not **undo** your operation and try again.

• Open the **Backwards lineaging window** and mark the cell using the RIGHT mouse button. Now lineage backwards and you should identify with the help of the **Collision manager** ABaraaaaa as the mother of this cell.

Explore the embryo

Please click through the lineage and look at cells. May be you want to open an **Alternative branch** at some point and lineage again some cells. If the **Collision manager** gets on your nerves (it is only active in this operation if in the **Collision manager (Extras**) window the box **Include hidden branches (Blue)** is activated) switch it off using **Extras, Collision manager enabled.** Actually it should get on your nerves as long as you follow the right cell. Do not forget to activate it later again.

Now you are familiar with the functions you need to lineage and you should be able to lineage your own recording. But to do so you have to learn how to start your own project.

In case you have no descent 4D-microscope or you have problems to make brilliant recordings with your microscope please contact me (RS) for advice

Third Tutorial

"Create a New Project"

In this session you will learn how to open your own project and how to address your video record with SIMI°BioCell. Create your own lineage template

Opening a new project (using the IB+RS_N2_1_bmps video files)

• Chose the function **New project** in the **File** pull down menu (Fig. 2). The standard window **Open** will pop up. The path should already be directed into the T**emplate** folder. Select the **John_Sulston_25C.sbt** and open it. This is the template for recordings at 25°C. The other files can be used for 20°C or 15°C. The only difference between these templates is the time scale. At lower temperature causes of course a slower development. Now the **New SIMI°BioCell project window** will open. Fill in the appropriate parameters for your recording as explained in the legend to Fig. 35. You should take great care to write down these parameters when you record a new embryo.

• Click "**OK**" and the **Cell lineage window** (Fig. 3) and the **Start frame window** (Fig. 35) will open**.** Ignore the **Start frame window** for now. You have to address your video pictures first to see the in which developmental stage of the embryo your started the recording (see legend to figure).

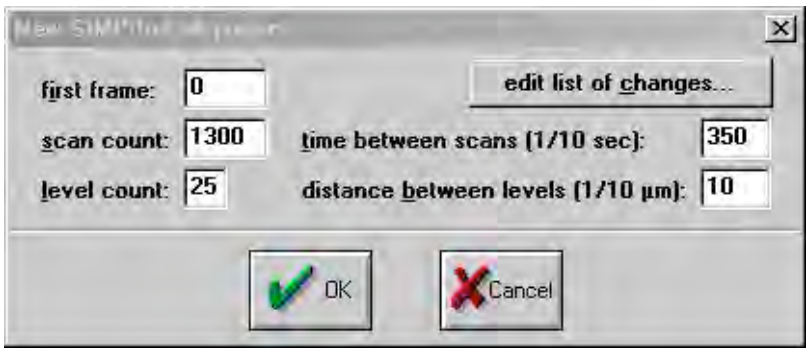


Fig. 35 The **New SIMI°BioCell project window**. **First frame:** Defines the number of the z-series (scan) the analysis should start with. There are sometimes occasions where one wants not to start with the first scan but usually it is the first frame, which due to some strange philosophy of informatics guys has the number 0. The scan count should be at least as long as your recording. In doubt take a high(er) number. Otherwise they are there but you cannot access them. **Level count:** the number of focal levels in your z-series. **Time between scans (1/10 sec):** Here you enter the time between the scans in 1/10th of seconds. For 35 sec the value is 350. These parameters are very important, if you get them wrong the video in the **Video window)** will end at this number and you may wonder where your pictures are. The same is true for the scan count either focal levels are missing or you see (black) pictures of levels you never recorded. If the **time between scans** is wrong the timing of events will be wrong, since the time is calculated on the basis of the scan number in the picture file coding system used here (see further down). However, if you realise after a lot of work that a parameter is wrong not everything is lost. You can open the **.sbc file** with a text editor and edit the numbers. **Distance between levels (1/10 µm)** helps you to remember this

Addressing the video pictures

• Activate the pull down menu **Video** and choose **Select video device...** The window pops up and it allows you to select the type of video you want to use (Fig. 36, see legend for further information about video types).

Fig. 36 Choosing **none,** you can work with a project without a video**.** This may be useful if you want to edit in a project for example the colour code for a 3D-representation video. Or you get a project from some friend without the videos to look at it. Just open the window and select **none**. **overlay board** addresses frame grabbers if you want to feed in an analog video. **digital video** has the same function but you can load video files for each level from the hard disc. single images will open the **Image selection window**.

• Select **single pictures** and click "**OK**", the **Image selection window** will appear. The details about this window which will allow you to address all kind of name codes of pictures of a recording are explained in Fig. 37.

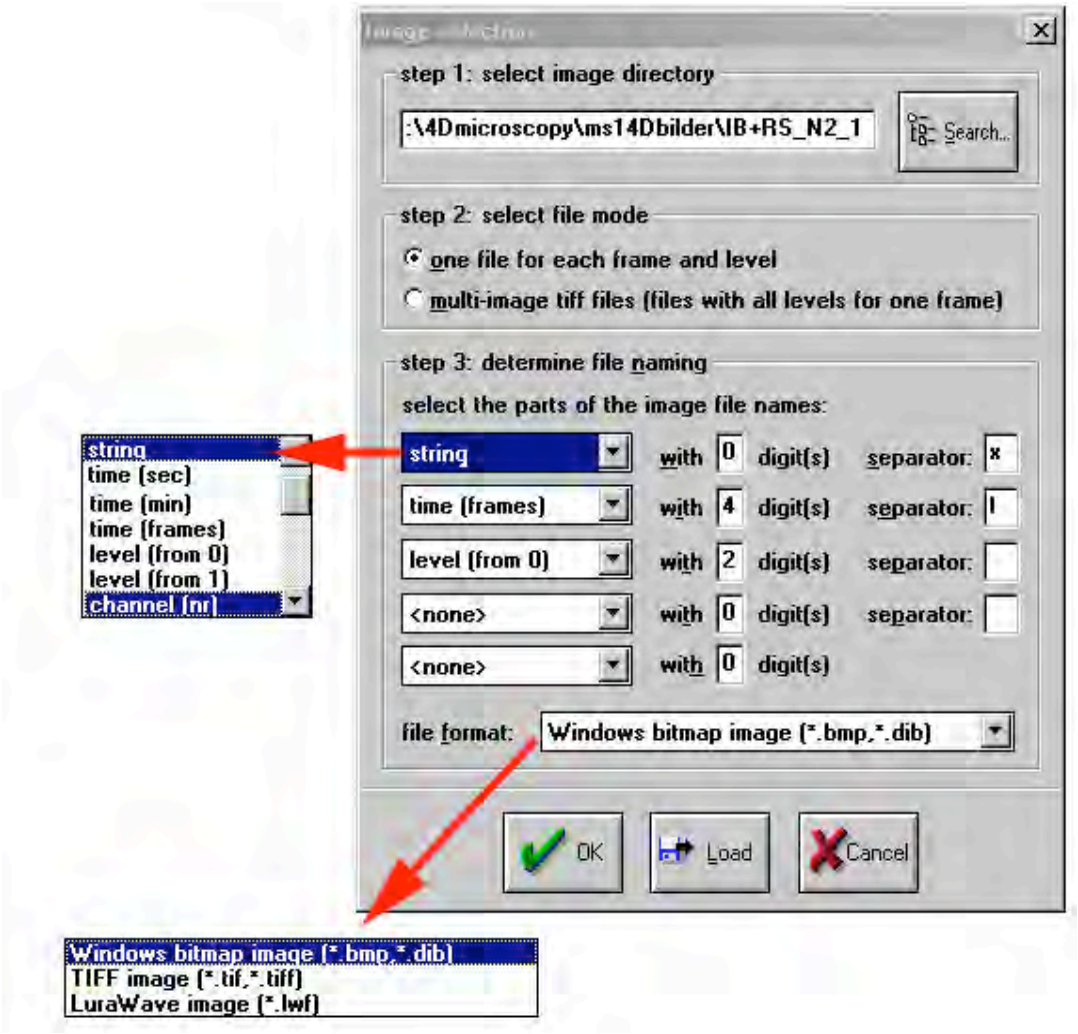


Fig. 37 The **Image selection window**. In **step 1** you select the folder containing your pictures or video files. Do not open files containing for example 20.000 pictures. Just activate the folder. **Step 2** selects the type of recording. **Step 3** decodes your **file naming system**. Files of the following structure can be decoded: *000000000a000000000b000000000c000000000d000000000.* So you have four times 9 digits each separated by a letter. I use, as you have seen before the code X0000L00. Behind the X is the number of the focal series with 4 digits so I can record 9999 z-series. Behind the L is the number of the focal level here 99 is my limit. Since there are no digits before the first letter X one uses **string**, which has the meaning "ignore". First separator is "X" followed by four digits. Since a recording was done ever 35 sec the number automatically contains the time code which corresponds to the setting **time (frames)** (you entered this in the New SIMI°BioCell project window Fig. 34). However, you can also code seconds or minutes after you time separator. After separator L for level there are 2 digits. In this case the first level has the number 0, setting **level (from 0)**. There is also the option to start from 1. The two next separators are not used thus they are digits which count the focal levels from 0, the first level has the ignored with "none". In case the name of the pictures contain a separator followed with digits you want to ignore just choose **string.** You can choose now from many file formats.

• Click at **search** and yet another window, the **Browse for folder window** will open. Navigate to the folder as exemplified in Fig. 38. It is important just to activate the folder containing the picture and **not** to double click it. Otherwise the system will load the 20.000 files or so and this may take a while, Click "**OK**".

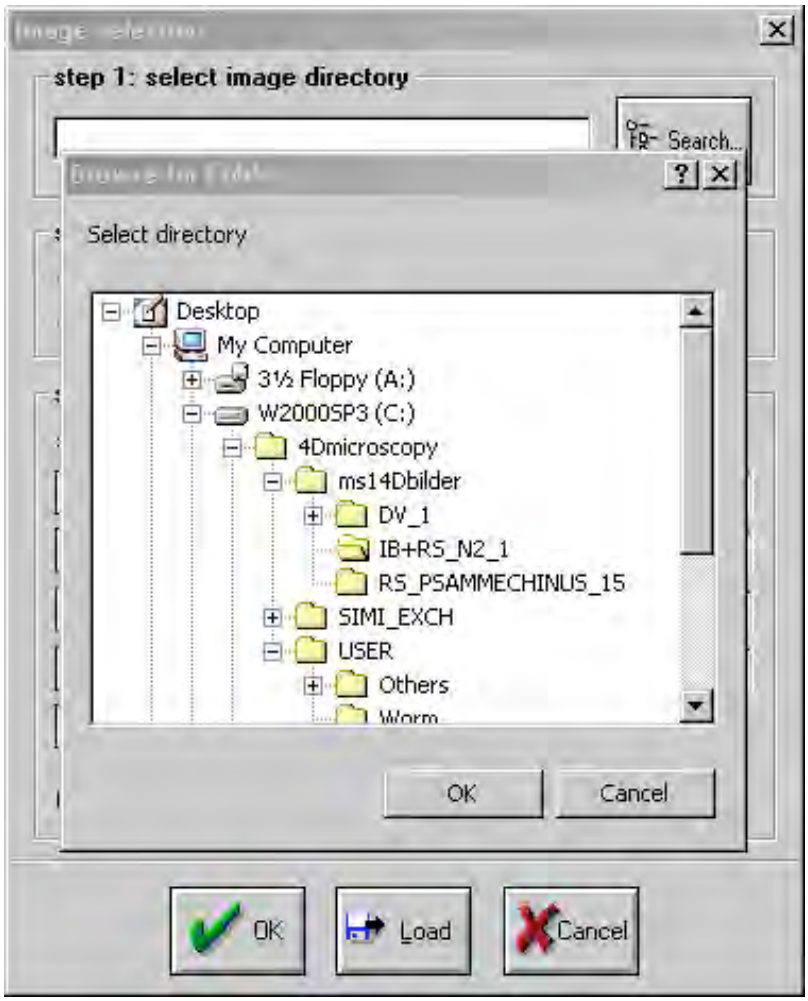


Fig. 38 The **Browse for Folder window** in the **Image selection window.**

• Now you have to enter the naming code used for your pictures. In this case just fill in the fields as shown in Fig. 38 (the multichannel procedure will be explained later). If you use your own pictures with another naming system consult the legend of this figure for an explanation of the name reader. Activate "**OK**" and the window disappears.

• Click at the  icon (or activate **View, Video window**) and the **Video** window will open. If you are lucky and you got everything right you will see the top level of your first scan. If you are unlucky you will be informed by a window that there are no video pictures. I am afraid you have to search for the problem. A good candidate for an error is that you chose the wrong file format. In case you used compressed .lwf files the top bar of the **Video** window may inform you about the

problem with the .lwf file system. Otherwise your code for the name system may be wrong. If nothing else helps ask Toni or me for advice (calling may be more efficient than email since it is much more interactive). Sometimes it happens that the warning window **no video device** is faster than the file system. If you just press the **move right** curser once or twice the picture will appear.

Finally you have to align the grey lineage tree with the start of your recording.

• You had all the time the **Start frame window** (Fig. 38) on your screen and I hope you did not close it prematurely. Now you use this window to align the grey lineage in the background of the

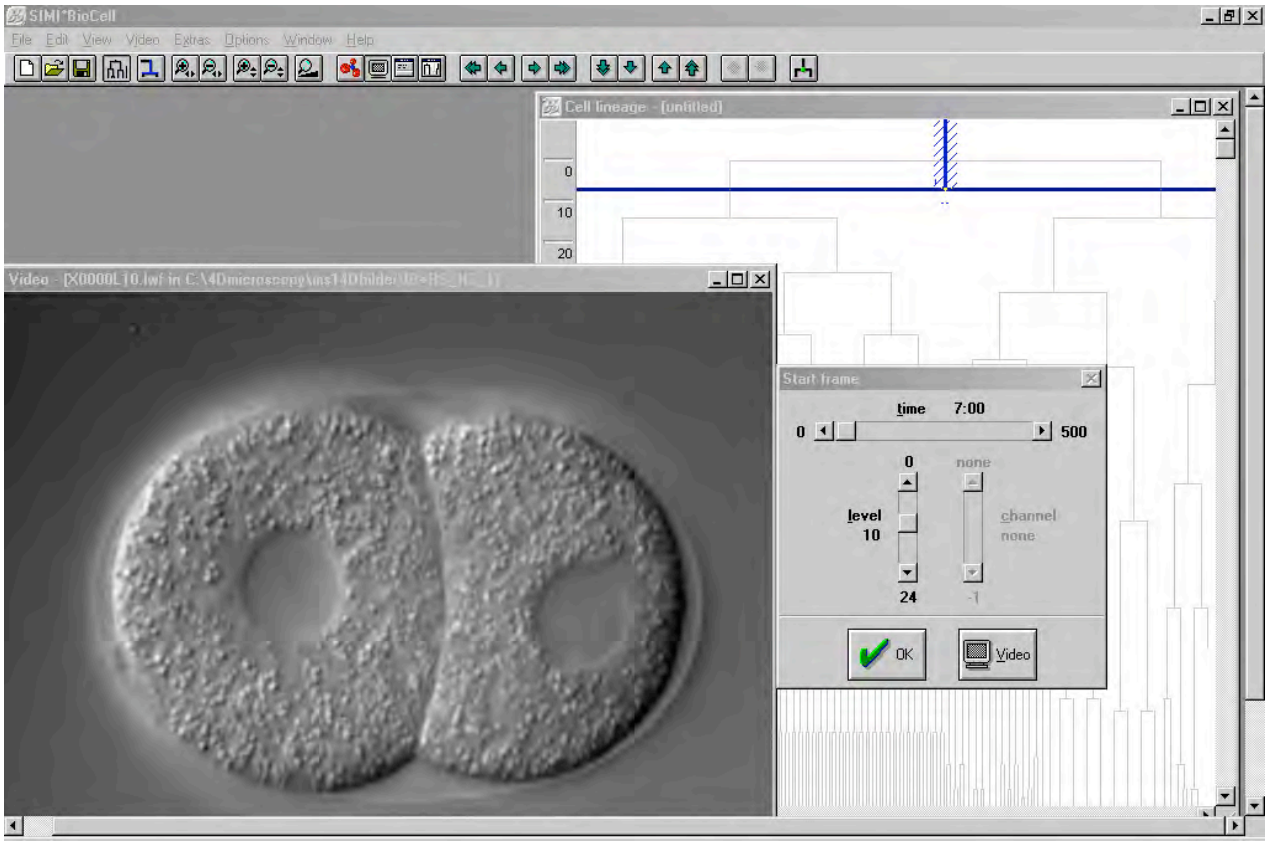


Fig. 39 Aligning the start of the record to the grey cell lineage in the background of the **Cell lineage window**. Use the **time** slider to set the time line in this case to the 2-cell stage. You can navigate through the first z-series by using the **level** slider. Click "**OK**" to finish.

Cell lineage window with the number of cells your embryo has in the first z-series. Use the **level slider** to scan through your embryo and count the number of cell and estimate where in the cell stage you are. In the example shown in Fig. 39. It is the 2-cell stage about in the middle of the S-phase. Use now the **time slider** and set the **time line** half the way through the 2-cell stage. click **OK.** Now a black lineage with the AB and P1 blastomeres will appear. Save your project. You are ready to start lineaging.

Good luck with lineaging! I hope you make a lot of interesting observations.

Any problems? Start again with Chapter 2.

Create your own lineage template

In case you do not lineage *C. elegans* embryos you may want to create your own lineage template for SIMI°BioCell. There are two functions to help you to make your own template which are found at the bottom of the **Extras pull down menu.** I will come soon back to these functions**.**

As a service two templates are provided in the **RS_Template folder** on the DVD, which you may copy into the **TEMPLATE** folder in the program folder of SIMI°BioCell. Both templates use "X" as a cell name but in one cells are numbered in a binary 0/1 system, whereas in the other an a/p code is used. Both templates are prepared for 10 generations. You will see soon how to extend the generations if necessary.

You can use either of the files to start to lineage any embryo and think later of good names for the cells when you know more about the development.

You can use the **Cell information** window (Fig. 17) to edit the **name: (**for example V6R or EMS**)** and the **generic name:** (the name corresponding to the genealogy of the cell, for example ABaraaap) of a cell at any phase of your project.

You find also an empty template which you can use to create your own lineage tree by using for example the classical blastomere names for a specific animal.

If you would lineage *C. elegans* the first time like Sir John you would name the cells as the founder cells are born. But when AB divided you would call the anterior daughter ABa and so on. If you finally arrived at MS you would proceed the same way. Editing cell names through a whole lineage is very cumbersome (or hell on earth to be honest). Therefore, Toni programmed the **Generate lineage** and **Cell labelling...** functions in the **Extra menu** (Figs. 40 and 41). Both function lead to the same result but in different ways. The **Generate lineage** function creates immediately a template, while the **Cell labelling...** function labels cells as you lineage the embryo. The use of the functions are explained in the figure legends.

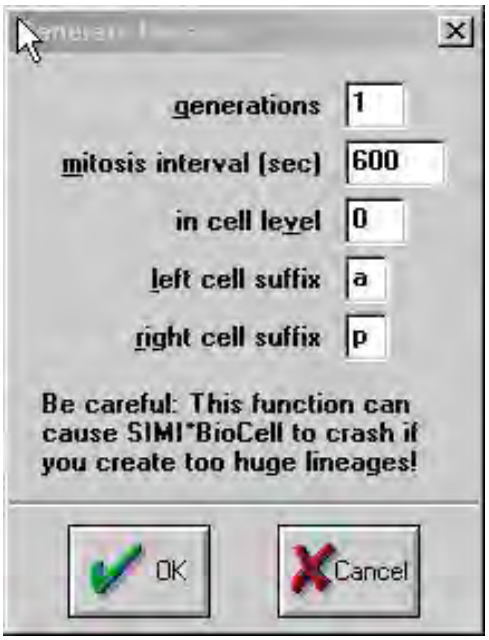


Fig. 40 The **Generate lineage function.** This function will create a lineage tree downwards of the lineage branch you activated in the **lineage window**. You can pre-set the naming system and the number of generations. You thus can create pre-named lineages downstream of the founder cells you named manually. To be able to use the lineage as the grey background tree you have to save the project. Start a new one with the empty template and then reload the lineage you just created using the **Load standard lineage...** function. The lineage will appear as a grey lineage in the new project. Save the project and start lineaging with the new Database you created.

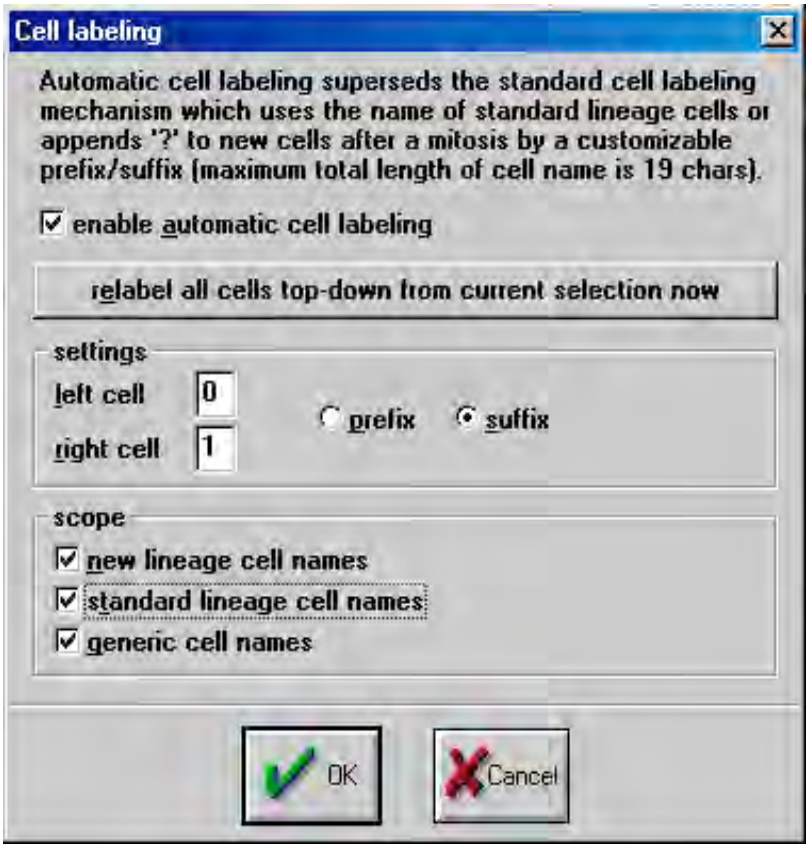


Fig. 41 The **Cell labelling... window**. If you activate the **relabel all cells top-down from the current selection now** button cells will be labelled according to the settings you pre-set. When you are finished with the first embryo you can use the lineage as a grey tree (new database) by loading it into an empty project using the **Load standard lineage...** function.Using the **Cell labelling...** function has the advantage that you get a grey tree with a realistic timing. With the **Generate lineage** function you have to estimate the generation time for crating the lineage. Thus the first real lineage will most probably deviate significantly from the grey tree. You may heal this in your second lineage by using the first as the standard lineage.

Create your own template

You can create your own template for a new project by opening the **empty.sbt** file with Notepad which is part of the system of your computer. Just rename the .**sbd** file with the name of the project you want to use as the template. Save the **.sbt** file then with a new name, for example the name of the animal you work on. Of course you must put a copy of the corresponding **.sbd** file in the **TEMPLATE** folder.

Good luck with making your own lineage trees!

Fourth Tutorial

"Analysis of multichannel records"

In this session you will learn how to work with multichannel recordings. For example analyses where DIC and fluorescent optics was used.

Expression patterns.

With the analyses introduced so far one can perfectly analyse the "behavior " of cells and with some training also recognize their fates, identify tissues. I believe one can learn more about development like that as by looking at the expression of molecules. However, since not many peers share my taste it is unavoidable to also include molecules in the analyses. The live recording of the expression of fluorescent marker genes not only offers an unique opportunity to identify the individual cells expressing a gene and but also the kinetics of the expression (see also Newbury et al. PlOS Biology 5: e237, 2007). There are always surprises if one undertakes careful analyses. I guess the "unexplainable" expression patterns found in this analysis reflect the binary specification also unraveled with the 4D-microscope (Kaletta et al., 1997).

Some commercial microscopes, especially confocal microscopes, now also allows to collect 4D-records using different channels. Since the programs never really do what you would like to see I also developed software for multi (three) channels recordings. Please consult the manual if you are interested.

Using the multichannel version of SIMI°BioCell is not very different of the single channel version you used the far. It just requires some more steps when setting up the project.

Opening a new project

Starting the project you use the very same procedure (**Opening a new project)** as described on page 55. Three new steps are required before you address the video with the **image selection window** (p. 56). First you pull down the video menu (Fig. 42) and you enable the channel

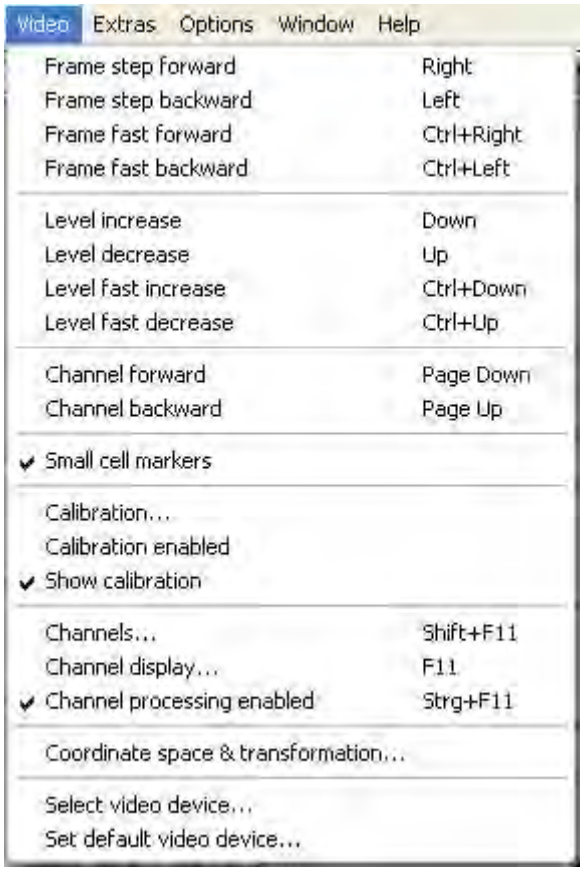


Fig. 42 Enabling channel processing.

Fig. 43 The **Channels window**. Insert the channel number and assign a name. Then press the button **add**. You may list as many channels as you wish. The **Mark button** can be used to colour the pictures in a specific channel. For example you may stain a GFP expression pattern green (see below).

processing. Next to have to instruct the program how many channels will be used. Click at channels in the same window. The **Channels window** will pop up (Fig. 43). Now add two new channels as shown and explained in Fig. 43. After that go back to the **video menu** and select the **video device window.** Address the video images as shown in Fig 44. If you use a different code for the pictures

please consult again the paragraph **Addressing the video pictures** (p. 56, figs 37 and 38). Please be not surprised that - although you filled in everything properly - the **no video pictures window**

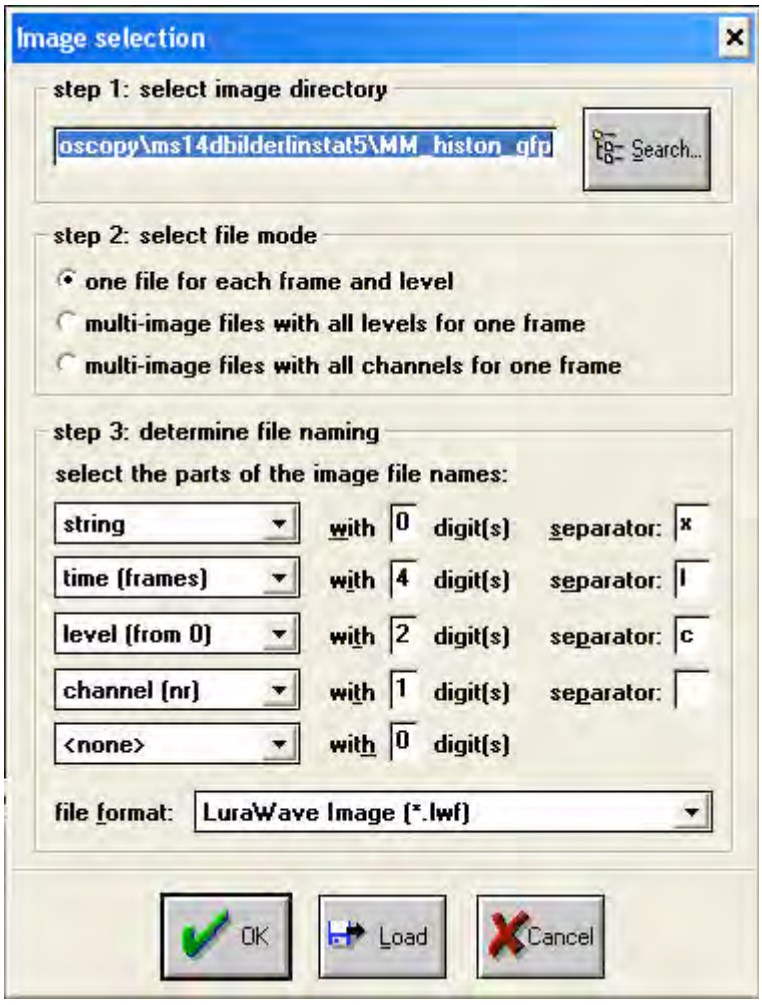


Fig. 44 The **Image selection** for a multichannel record. The channels are addressed by adding a C in the third separator and selecting channel (nr) with one digit. Do not forget to choose the appropriate file format.

will pop up. Just click it away. You have to still work on the **Channel display window** (Fig. 45) till you can see the pictures. Add the channels you defined before in the **Channel window** into the Channel stack. In the **display sequence (global)** you choose **all channels in stack (sequential)** and you tick the box **skip missing pictures automatically**. This causes the program not to display pictures which are not there - otherwise you would see only a black picture, because, since there are no pictures recorded in a channel at the specific time point. Now the first pictures should pop up and you can look at your record. If a pictures exists in an additional channel. for example channel number two, at a certain time point it will be displayed after the first channel (Fig. 46).

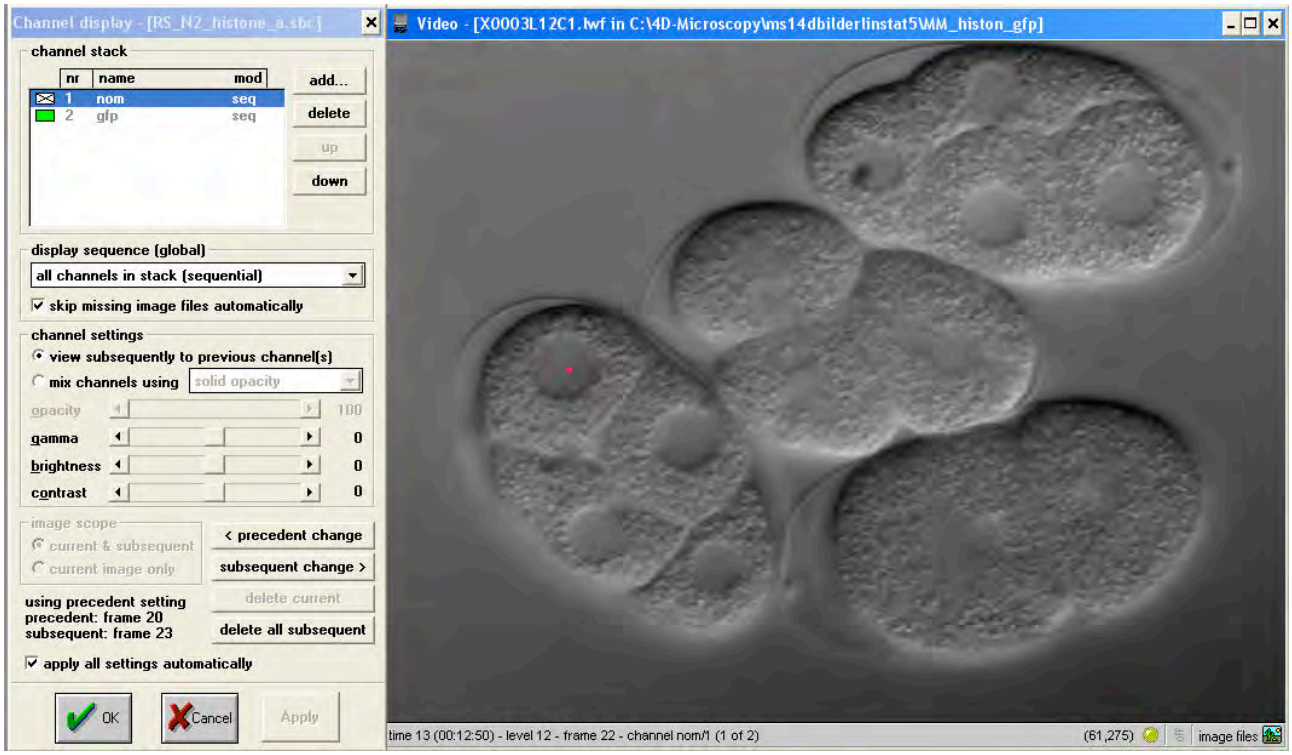


Fig 45 The **Channel diplay** and the **Video** window showing the DIC channel.

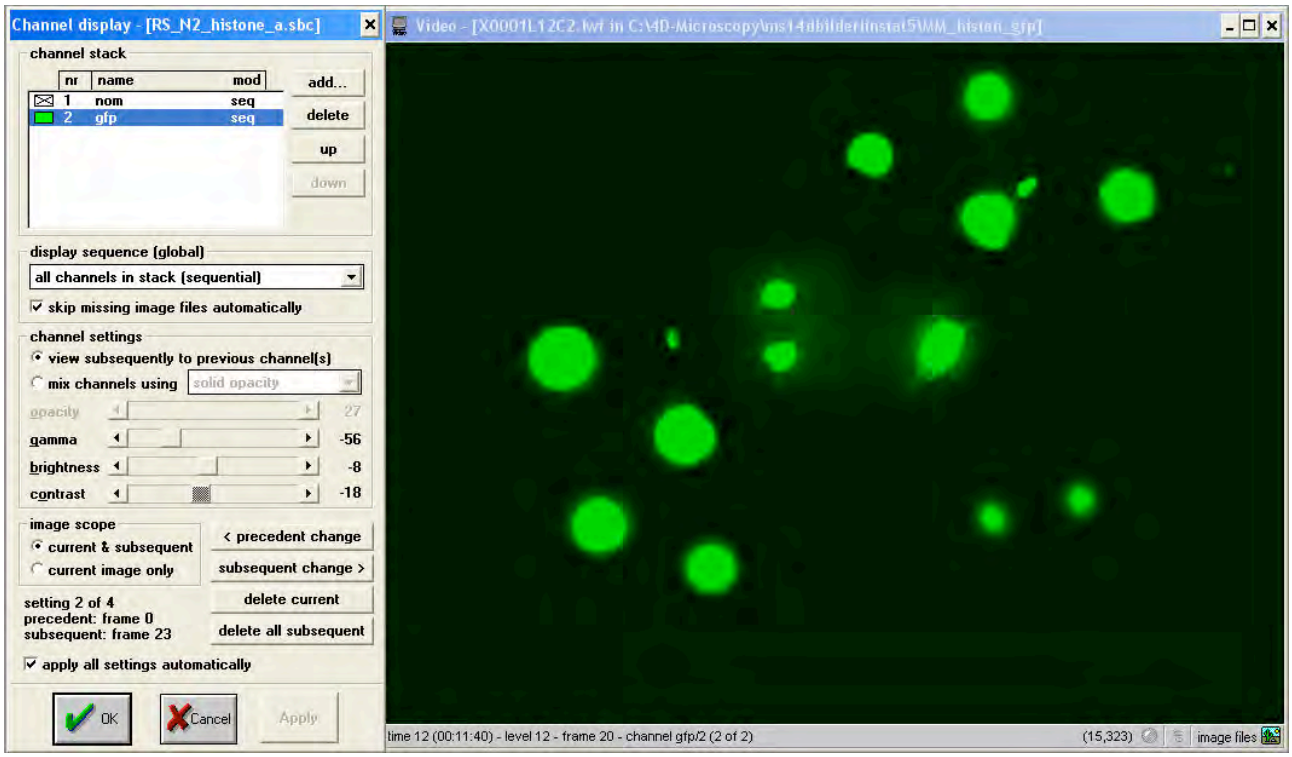


Fig 46 The **Channel diplay** and the **Video** window showing the GFP channel.

In the example shown the nuclei of early embryos are stained with a histon::GFP marker gene. Thus it is very easy to assign the fluorescence to the cells. However this may be not be as easy if neurons are stained in late development. Therefore, SIMI°BioCell will also crate a picture overlay in real time if instructed to do so. Activate the GFP-Channel in the **channel stack** and then choose **mix channels** in the **channel settings**. The sliders below allow to manipulate the overlay according to your taste. Please explore the functions. The last feature of the **Channel display window** is the **image scope.** This function allows you to specify which setting is applied to a specific image stack. Thus you can adapt the pictures to different expression intensities. The function **apply all settings automatically** is useful in most recordings.

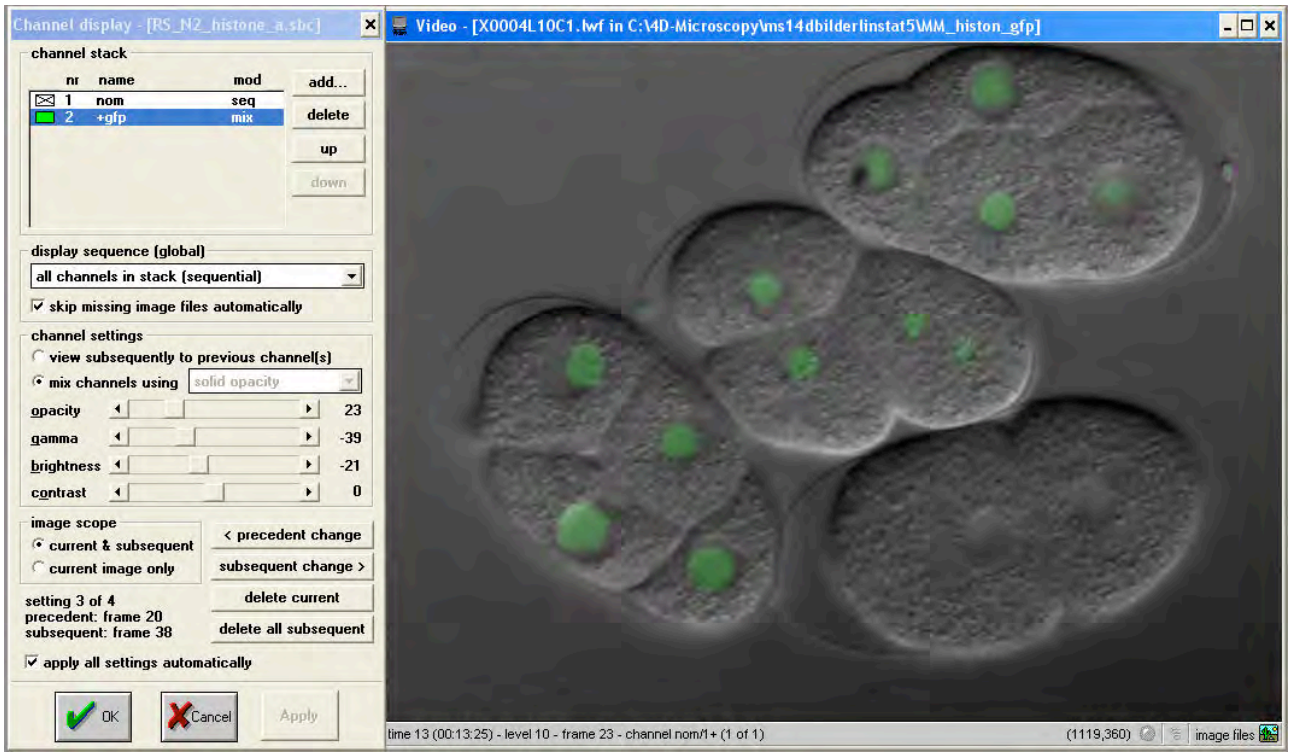


Fig. 47 The **Channel diplay** and the **Video** window showing an overlay between the DIC and the GFP channels.

Good luck lineaging your expression patterns!