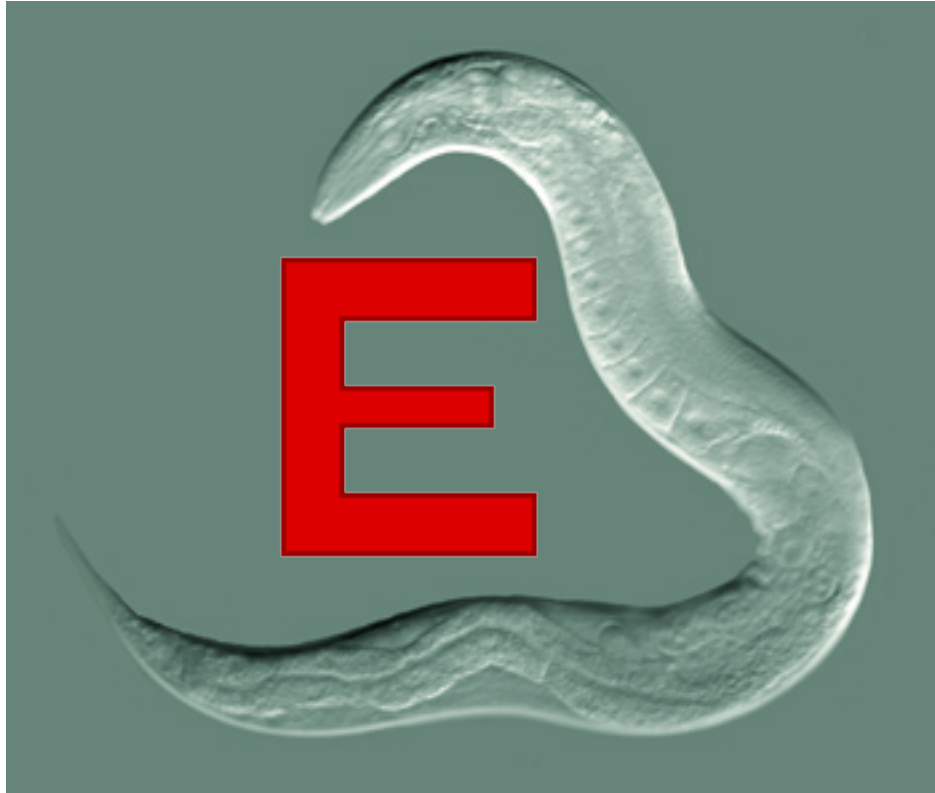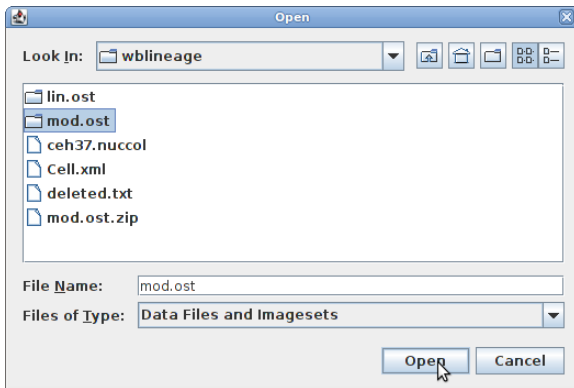# Using Endrov to explore nematodes

Johan Henriksson
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Opening files

Our native file format is OST. To open an OST-file with data, you have to select the folder and then press *Open*. Double-clicking the folder will *not* have the desired effect!

It is important to note that OST-files can contain a bit anything – image, lineages, expression patterns. The summary file does *not* contain the raw image data but it can be obtained from us separately.

Endrov can also open and annotate other files. In fact, it is a general image processing framework and microscopy controlling program. Go to www.endrov.net if you want to use the advanced features.

Opening new windows

New windows to explore the data can be created by using the Windows menu. For this particular tutorial, we use 3 windows:

- Image – For displaying 2D annotation over the raw images

- Model – To show the 3D model

- Lineage – To view the linage tree

Endrov allows multiple instances of each type of window, to allow you to make full use of multiple screens, and to have several views of the same data.
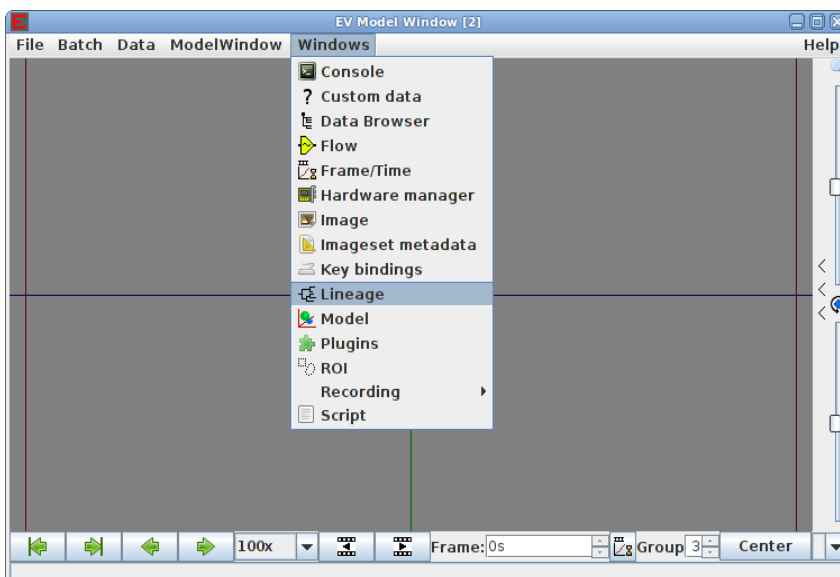
The Lineage Window

This window allows you to explore and interact with the lineage. Select cells by *left-click*. Select additional cells by holding *Shift*.
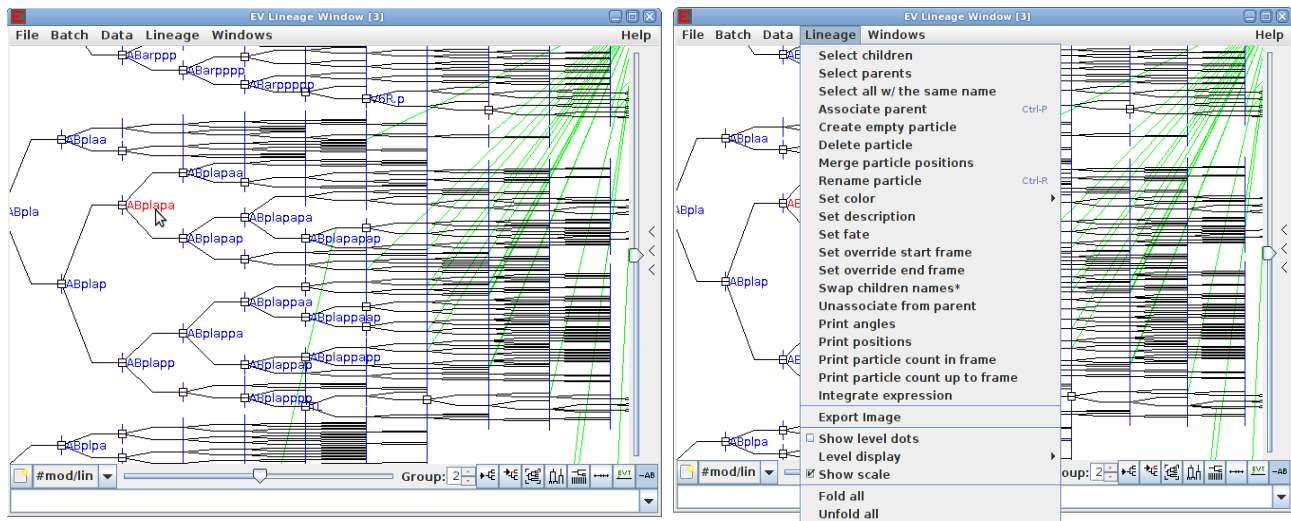

The Lineage menu contains many options, of which the most interesting for a starter are

- Select children – If you e.g. have selected AB, it will select ABa, ABp, ABal, ABar... and hence you can highlight in the model all the cells which are descendants of your selection

- Set color – Give the cell a particular color

The Model Window

The model window allows you to see annotation in 3D. The first thing to do is to set up the view – If you have several files open, select the object of interest (lower-right corner). Make sure the right frame is selected; you can go to the first and last frame using *|<=* and *=>|*. Then press *center* to display everything.
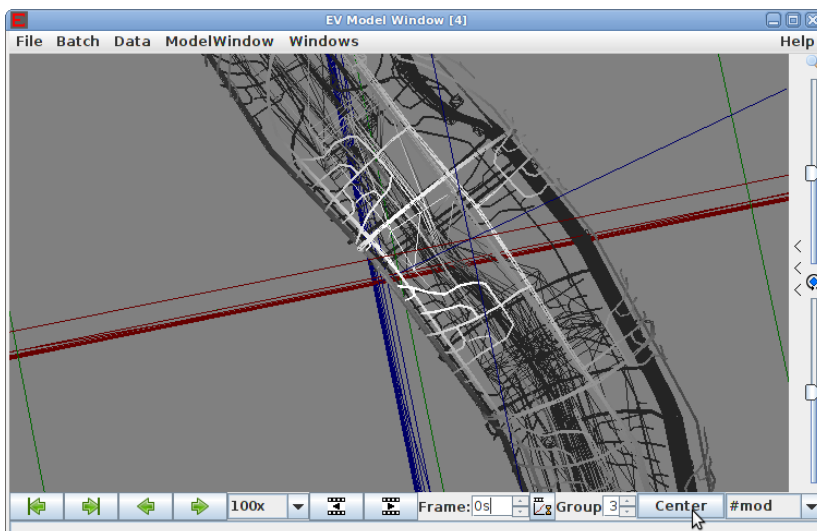
Pan around by *drag+mid button pressed*, rotate by holding the *left/right mouse button*. Zoom using the *scroll wheel*.

Cells and meshs (cell outlines) can be selected by *left-click*.
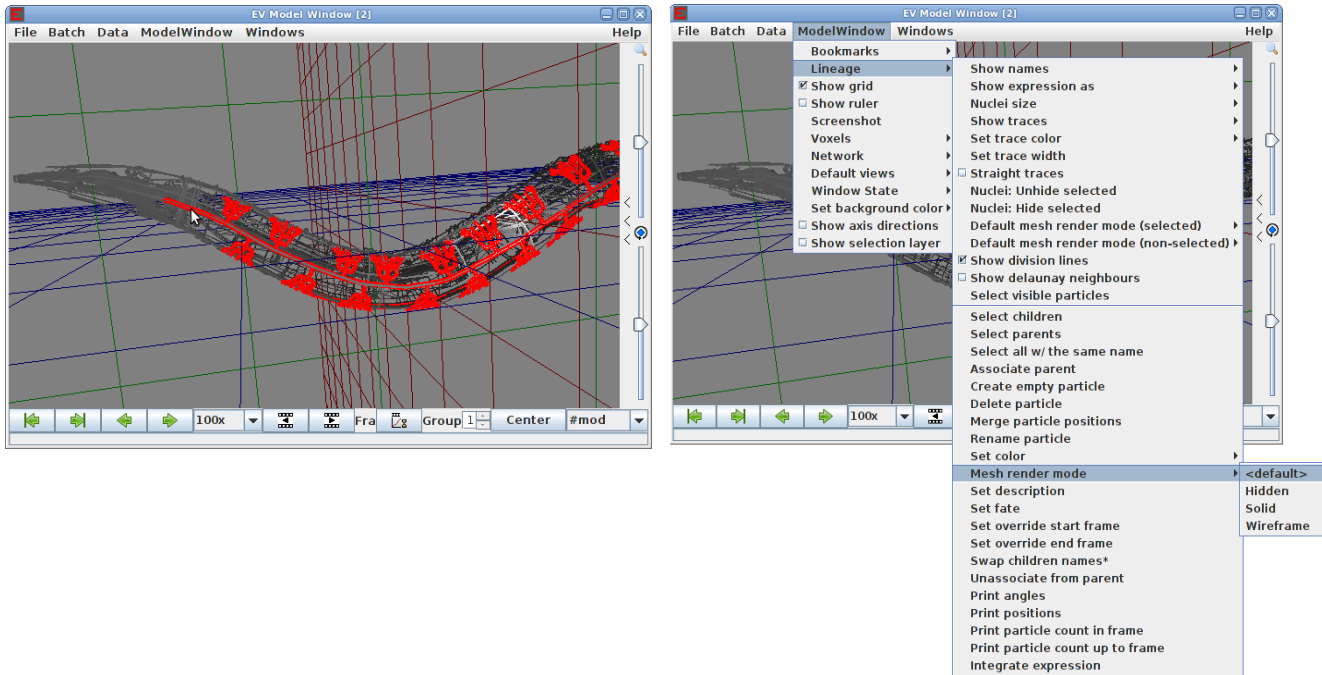
There are many options in the menu – Some of the most interesting ones for viewing are

- Default mesh render mode selected/non-selected – How to show the outlines of cells
- Mesh render mode – For the select cells, override their display method as set above
- Show names – Show the name of cells (currently only seen in embryos)
- Set color – For selected cells, use a different color

There are 3 rendering modes for outlines:

- Hidden – Do not show
- Solid – It is not possible to see through these cell outlines
- Wireframe – Only the edges of the cell outlines will be shown so one can partially see through

Viewing expression patterns

Expression patterns can be shown in the lineage window and in the model window. You find it in the menu to the right, use *Add expression* to show one more expression pattern. Several can be shown at once but it is difficult to understand more than two genes at a time.

In the lineage window it helps to set *Lineage → Level display → Filled* but it is really only useful for showing a single gene. There is a slider for setting the magnification of the expression pattern.
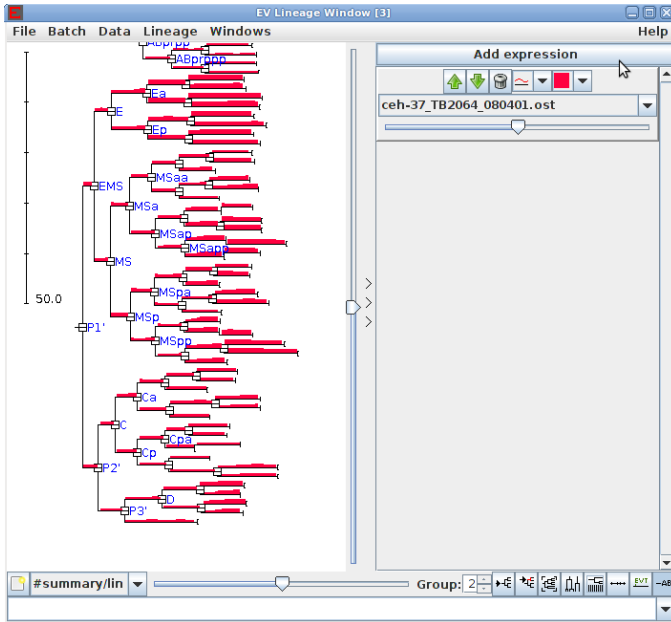

The model window has two sliders: One for contrast and one for brightness. Alternative rendering modes are available under *Lineage → Show expression as*.

Browsing the raw images

The image window allow you to see the annotation as it was made on the original images. Hovering the mouse cursor over a cell will show it's name. Pan around by *drag+right mouse button*, zoom using the *scroll wheel*. To go up and down use the *Z scroller*, and control frame by the *Frame scroller*. A much faster way is to use the keys *A* and *D* for time, and *W* and *S* for Z (make sure the image view is in focus).

For each channel you can set the contrast (left slider) and brightness (right slider).

Channels can be overlaid; just create a new channel using *[+]*.
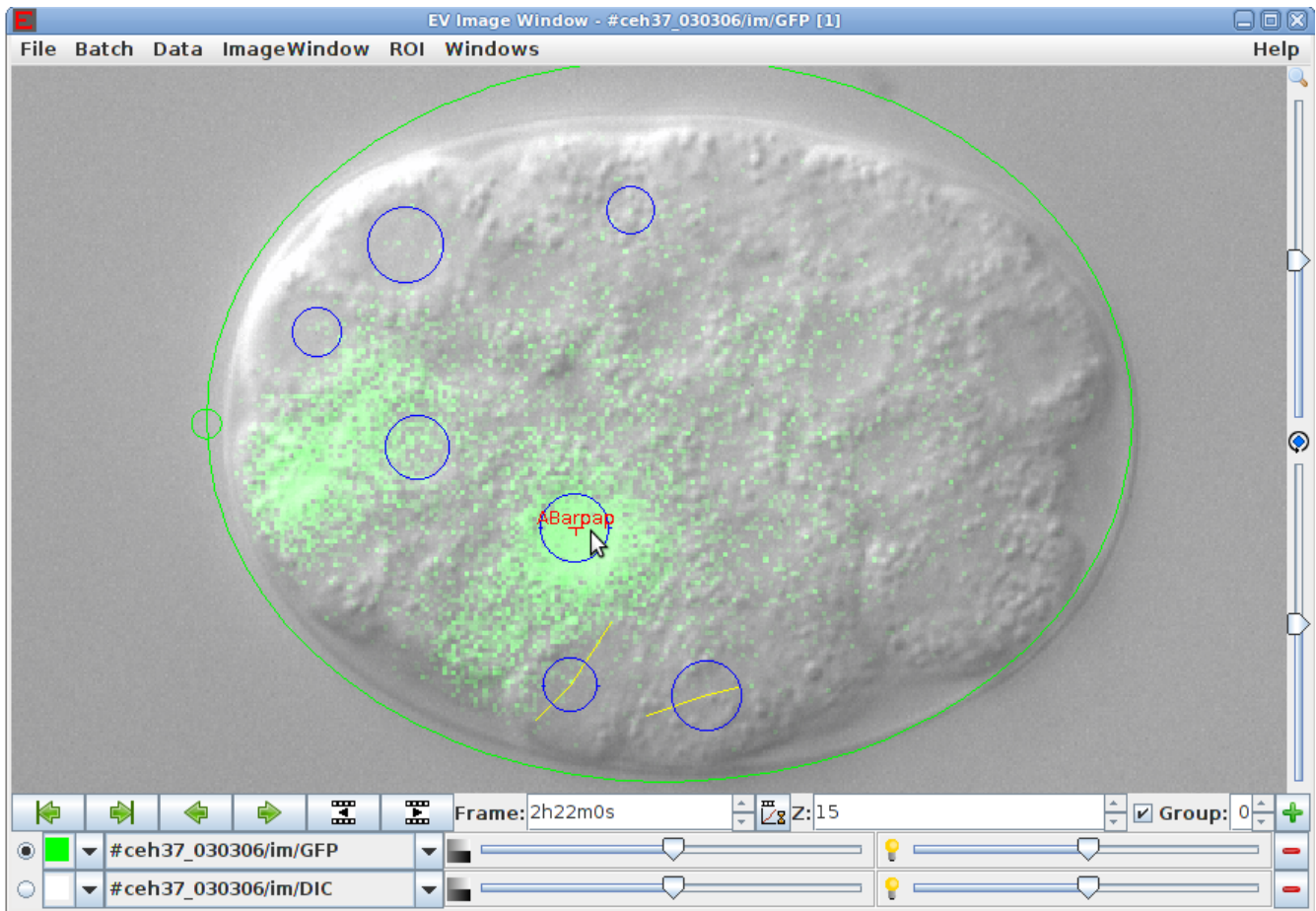
Extracting expression patterns

Before extraction, you need a lineage. It can be made in the following ways

- Manually annotate the cells you are interested in. See website for instructions.

- Superimpose the standard model (as in the paper). See below.

- Use another program to do automatic lineaging, and load it in Endrov

Superimposing the model

Create a new lineage and annotate:

- The first 4 cells. Call them ABa, ABp, EMS, P2'.

- Optional: The E cell

- Optional: **: gast – at the gastrulation

- Optional: **: venc – at the ventral enclosure

- Optional: **: 2ftail – at the tail of the 2-fold embryo

** it need not be in any particular position. Only the time of the marker will be used

Not all markers are needed but increases time precision. At the very least, one of the three last optional markers should be made. Then

1. Go to Data → your file → … the lineage → Map ce model

2. Open a standard model for *C. elegans* (e.g., Ce2008).

Integrating the fluorescence

Go to Data → your file → … the lineage → integrate expression

There are two modes; if you choose "integrate only within cell radius", only the fluorescence within the nucleus is integrated. This can be good if have annotated the minimum number of cells.

The other mode is to assign each pixel to the closest cell. In this case as many cells as possible should be annotated, and you should also add a Shell annotation.

Superimposing patterns

It is possible to put two patterns onto the same model, e.g., for mixing colors. To do this

1. Open both recordings

2. Go to Data → your file → … the lineage to have both expressions → Map expression to here

3. Give it a name, make sure the patterns have different names

Exporting patterns

It is possible to create a file with only the pattern, and no images. This is one way it can be done:

1. Open your recording

2. File → New
3. Windows → Data browser
4. Move/Copy the annotation to the new file
5. Data → the new file → save as, e.g., mypattern.ost