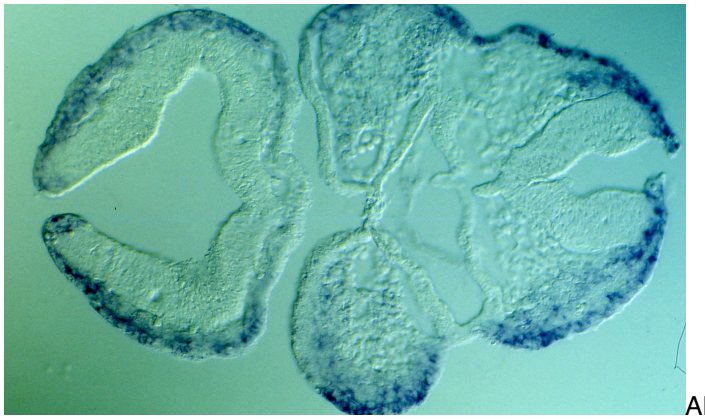
Mapping Section data with MAPaint

The data associated with this example is as follows:

This is a section from Ruth Arkell, MRC Mammalian Genetics Unit, Harwell. An antisense digoxigenin labelled mRNA probe for *AP2* (Mouse gene nomenclature committee approved symbol:*Tcfap2a*) was used on a wholemount and subsequently sectioned. The probe was transcribed from the entire insert of the RIKEN clone 2810443O20 which is an EST tagged clone (Genbank Accession number for the 5' EST sequence read is BY712305). Clone 2810443O20 was originally isolated from a C57BL/6J pooled 10+11 day embryo whole body cDNA library. The specimen used in the current study was a 9.0dpc CD1 mouse embryo. The visualisation method used in this experiment was Alkaline Phosphatase with NBT and BCIP and expression has been described in this case as being in the neural crest derived mesenchymal component of the head and specifically, the 1st branchial arch.

Original image:


AP2.jpg

This AP2.jpg file was saved into your **home directory** for the purposes of this course. To view this image, use the normal image viewing program available on your operating system (eg. preview, image magick, photoshop etc).

What Theiler stage is this sample?_____

Look at the EMAP mouse embryo staging criteria pages for help
(http://genex.mrc.ac.uk/Databases/Anatomy/MAStaging.shtml)

In an Xterm window, go to your home directory

```
% cd
```

Then make a directory for the source image and give it the name "section".

```
% mkdir section
```

Move the original image file (in jpg or tiff format) from your home directory (where it has been copied before the course) into this new directory.

```
% mv AP2.jpg section
```

Change to the new directory that you have made (ie. "section").

```
% cd section
```

To check the AP2.jpg file is in this directory, you can list its contents by typing

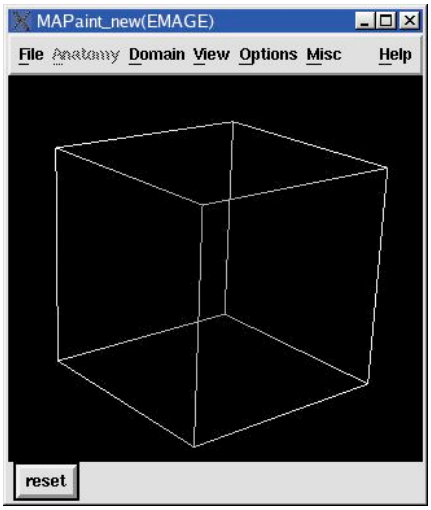
```
% ls
```

Open the version of MAPaint required for spatially mapping data for EMAGE by typing on the command line…

```
% MAPaint_EMAGE &
```

Since you started MAPaint in this directory ("section"), whenever you now save files during this session of MAPaint, they will all be saved into this "section" directory.

A window called "MAPaint_EMAGE" will open:



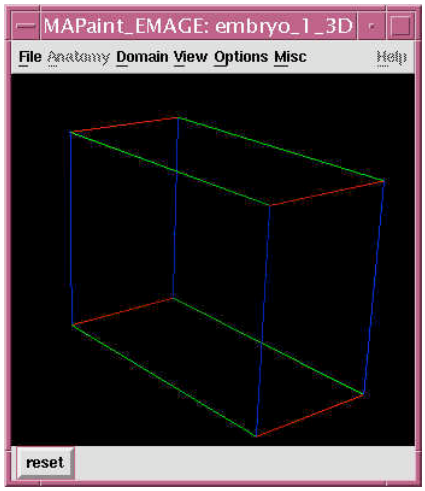
Select from the "File" menu, "Open EMAP Model". The 2D and 3D 'EMAP Model' image files of the standard embryos are on the CD. In your home laboratory you can copy these to your hard drive. If reading from the CD, make sure the CD-ROM is inserted, and navigate to it from within the MAPaint program (on MacOSX the CD is found in the Volumes folder; on Linux it depends where the CD has been mounted - it should usually be found at /mnt/cdrom; on Solaris is will be found at cdrom/dcrom0). To go up a directory at any stage, double click on "`..`"

On the CD there is a directory called "Models". Navigate to this directory. It contains a sub-directory for each of the EMAP models (these are denoted by Theiler stage) and within each Theiler stage

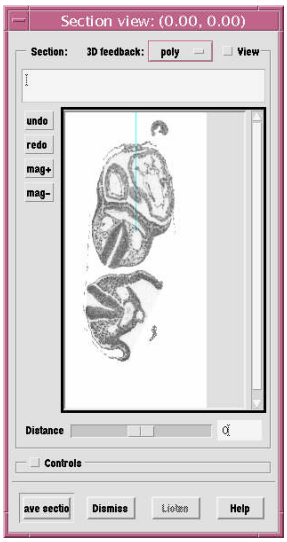
directory is a 3D file of that particular embryo model (`embryo_1_3D`) as well as left and right 2D whole mount views of the same model (`embryo_1_WM_left` and `embryo_1_WM_right`).

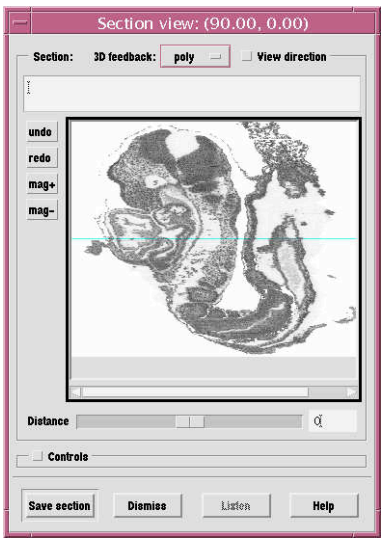
The AP2 image is a section through the head of a TS13 embryo and as opposed to simply choosing the left or right side views of the whole embryo in the wholemount case, this time a matching section must first be selected from within the 3D embryo model before the data can be warped and thresholded.

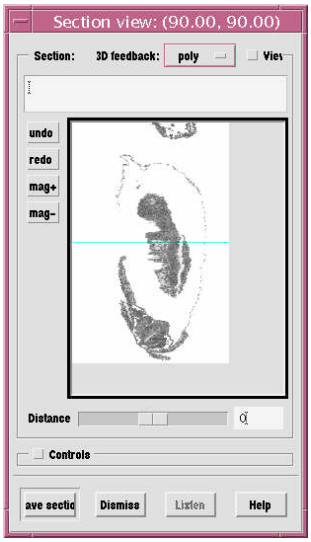
Navigate to the **`ts13`** folder and open it by double clicking on it. Inside is a 3D embryo model file called **`embryo_1_3D`**. Open this file by double clicking on it. The white box in the MAPaint window will change to one with red, green and blue sides. This box represents the space that the embryo model resides in.



Select all of the following from the "View" menu: "X-Y view ...", "Y-Z view ..." and "Z-X view ...". Three windows with sectioned views of the embryo model in the three standard axes will open.

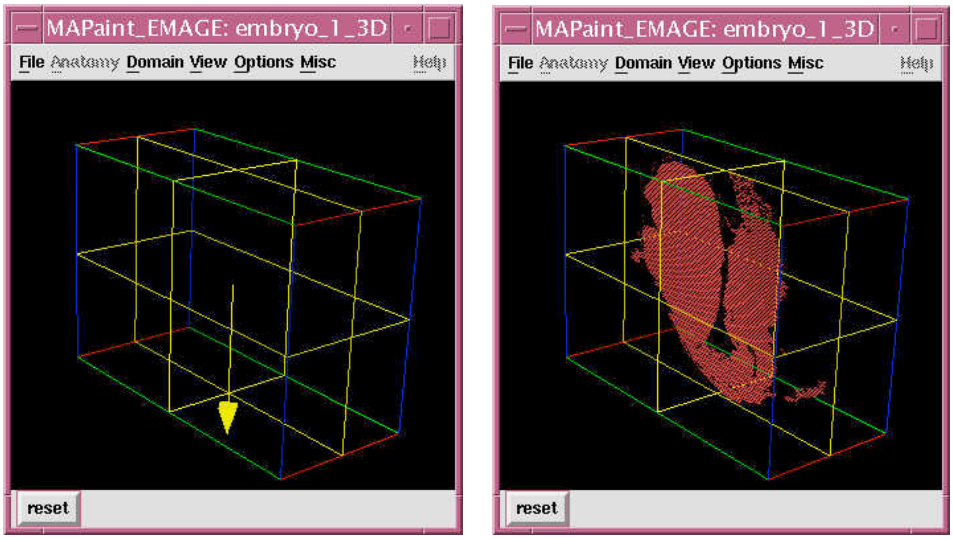




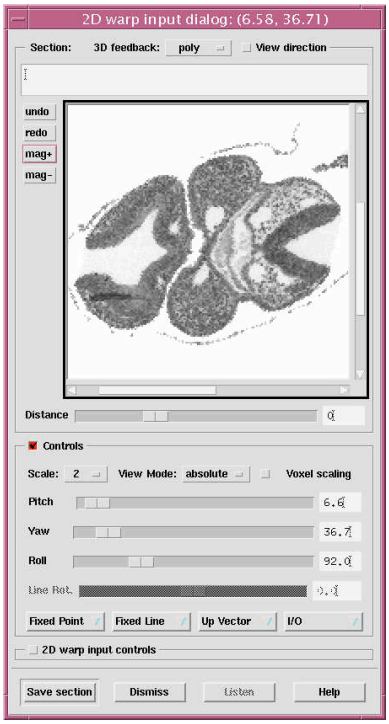


If needed, resize the individual images using the "mag+" and "mag-" buttons. When the cursor is positioned within one of these windows, the plane of the section shown in that window is also depicted in the other two windows by the light blue lines. The position of the section plane along each axis is controlled using the 'distance' slider. The default distance is set to 0. Move the distance slider and the plane will move accordingly in the box in the MAPaint window.

To see which direction the section plane is being viewed from (it can be viewed from either side depending on the settings), click on the "view direction" option at the top of the relevant window. An arrow appears in the MAPaint window showing the direction of view. Click on "view direction" again to remove the arrow. Select "Mask" from the "3D feedback" drop down menu to display a representation of the currently shown section in the 3D space.



In any of the 'Section view' windows, click on the box beside "controls" and the frame will expand to show extra controls including "pitch" and "yaw". Using distance in conjunction with pitch and yaw in the three views, find a target section that corresponds to the input *AP2* data section:



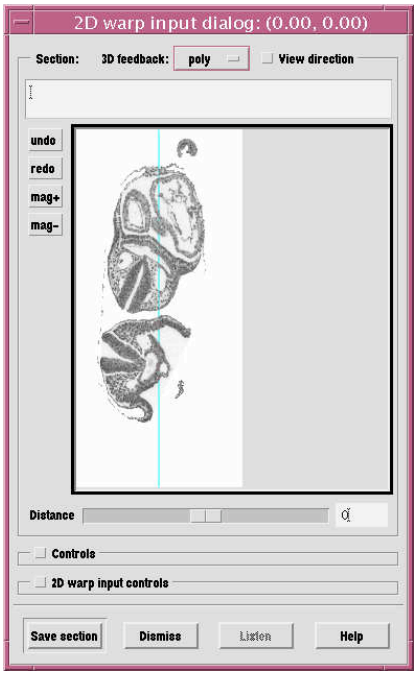
Instead of simply using pitch and yaw, you may find it easiest to rotate the 3D embryo model around a line within the 3D space to find the desired section. Do this by selecting "point define" from the "fixed line" menu using the right hand mouse button. A cursor (as a very small black dot) will appear. Click once on the section image using the left hand mouse button to define the start of the line and then position the cursor at the end of the line and click again using the left hand mouse button. The "pitch" and "yaw" options beneath the image will be replaced by "line rotation". Use the slider to rotate the 3D object around the selected line. The section view will change in the section window and the plane will move accordingly in the box within the MAPaint window. To deselect the line rotation option, move the distance control.

When happy with your choice of section, write a ".bib" (bibliography) file to record the section you have chosen by clicking on the "I/O" button using the right hand mouse button and selecting "write" from the list that appears. In the "Get Filename Dialog" window that appears, change the default file name `MAPaintSectParams.bib` to **`AP2.ts13.1.bib`**, click on "OK" and then close the "Section View" window. This can be used to read in this section again later.

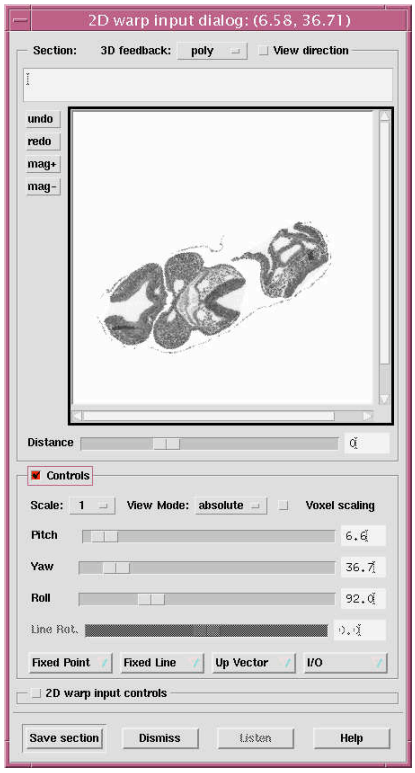
The standardised way for writing bib files for section data in MAPaint for later inclusion in the EMAGE database is **`filename.ts##.X.bib`** where **ts** and the two following numbers **##** refer to the Theiler stage of the model you are mapping onto, **X** refers to the number of the embryo model (e.g. for TS15 and TS16 there is more than one embryo model)**.**
bib files must be saved using this standardised nomenclature in MAPaint otherwise it will not be possible to enter this data into the EMAGE database later on.

Now close all "section view" windows you have open.

Having chosen the target section, now commence the warping procedure to place the data from the input section onto the standard section you have chosen by going back to the MAPaint window and selecting the "Options" menu and then "2D warp input". A 2D warp input dialog window will open:

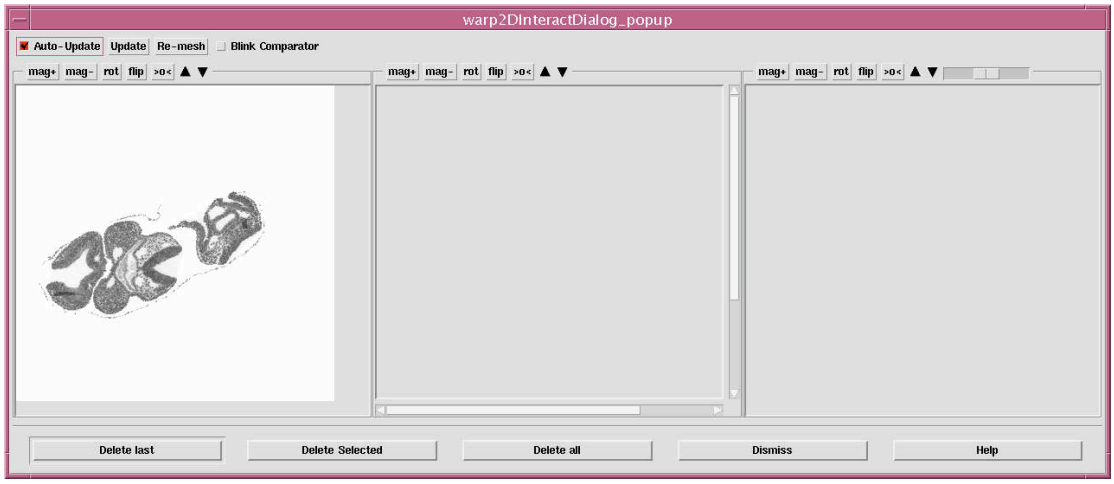


Click on the square beside "Controls" to expand the window and with the right hand mouse button, click on "I/O" and select "Read" from the list. A "Get Filename Dialog" window opens. Click on "Browse" to show a new window displaying your previously saved **`AP2.ts13.1.bib`** file. Select this file by double clicking on it and then click on "OK" in the next "Get Filename Dialog" box that appears. The file is read and the corresponding section displayed in the window.

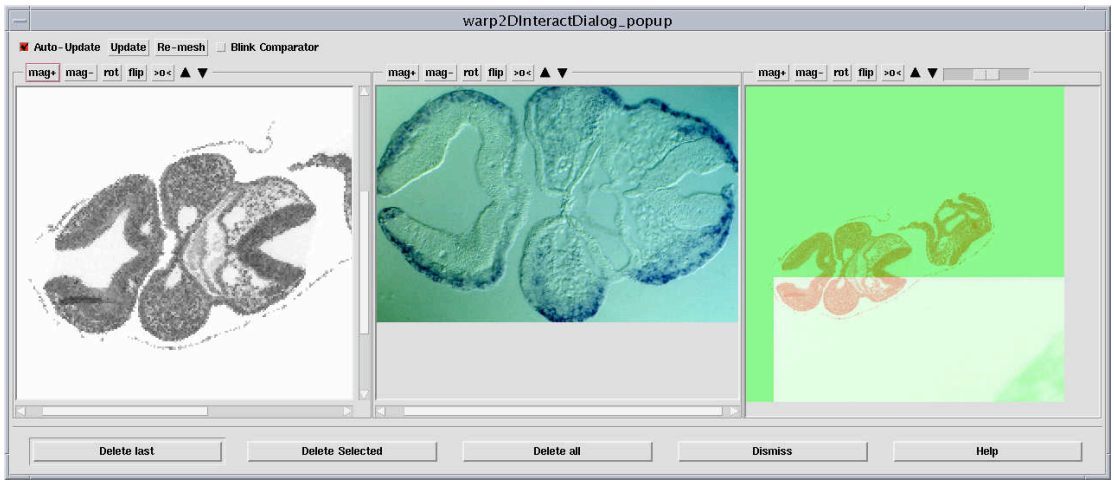


Click on the "controls" box again to minimise the window.

Now click on the "2D warp input controls" box to open a new "warp2DinteractDialog_popup" window which contains three panels, the left hand one of which will contain the target section you have chosen.



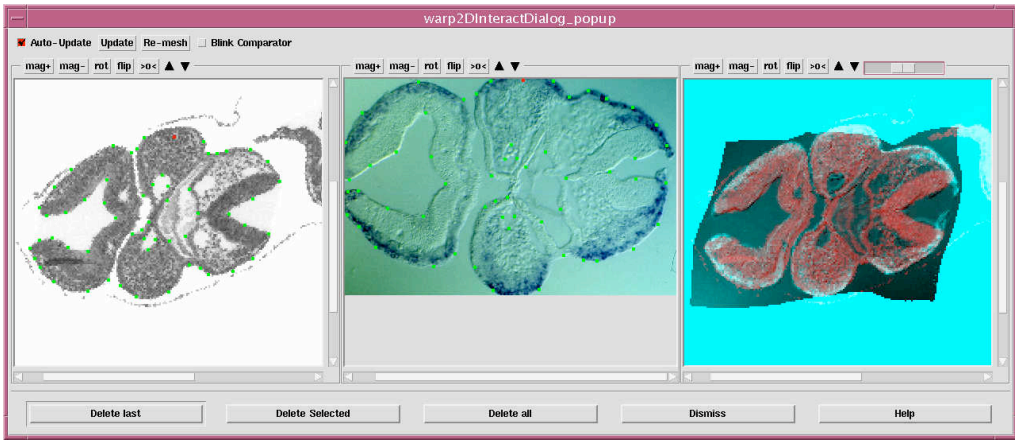
Back in the "2D warp input dialog" window click on the "ReadSource" button to open a "Read Warp Input Source Object" window. Select **`AP2.jpg`** from the list by double clicking on it. (For source images other than jpg's, select the appropriate file type from the drop down "image type" menu). This image is loaded into the middle panel of the "warp2DinteractDialog" window.



The left hand panel contains the target section within the model. The middle panel contains the input image file and the right hand panel contains an overlay of both of these files. To get the images into the fields of view, either magnify (click on "mag+" button) or reduce (click on "mag-" button) or rotate (click on "rot") each separately. To adjust the contrast of the images in each panel, click on the up and down arrows. To change the two colours in the overlay panel, click on this panel using the right hand mouse button, select "overlay method" and then select a colour combination that you like (red-cyan gives good contrast).

Start entering tie-points between the selected section within the target section (left hand panel) and the input data (middle panel). To add a tie point, move the cursor to an appropriate place on the selected section on the left and click with the left hand mouse button (a red dot appears), then move the cursor to the equivalent point on the input image file and click with the left hand mouse button (both dots will change to green). Moving the cursor back over the dot will change the colour of the two linked dots to red and can be used to determine which dots are linked to each other in the two panels and can be used to adjust their position independently of each other by clicking on and dragging one of the points. To remove two linked points, move the cursor over either and use the middle mouse button to remove both.

Initially enter 4-8 tie-points around the area you are mapping at easily recognisable positions. This ensures both images are of similar size and helps in subsequent tie-point mapping. Enter more tie-points at easily recognisable landmarks paying particular attention to regions of gene expression. Typically around 30-50 tie points may be required to adequately warp the expression pattern.



This process works by warping an underlying triangular mesh across the two images. For every triangle in the left hand image there is an equivalent triangle in the central image. *You can see the underlying mesh by right clicking on the left hand and middle panels and selecting the "show mesh" option.*

If you try to enter a point that generates an invalid mesh (eg. when the mesh has to 'fold' back over itself - in these cases the problem part of the mesh will be seen in a different colour to the rest of the mesh), an error message will appear in a window entitled "confirm dialog" giving the options of 'select yes to attempt to correct the mesh automatically or no if you want to move some of the tie points or reset the mesh parameters and try again".

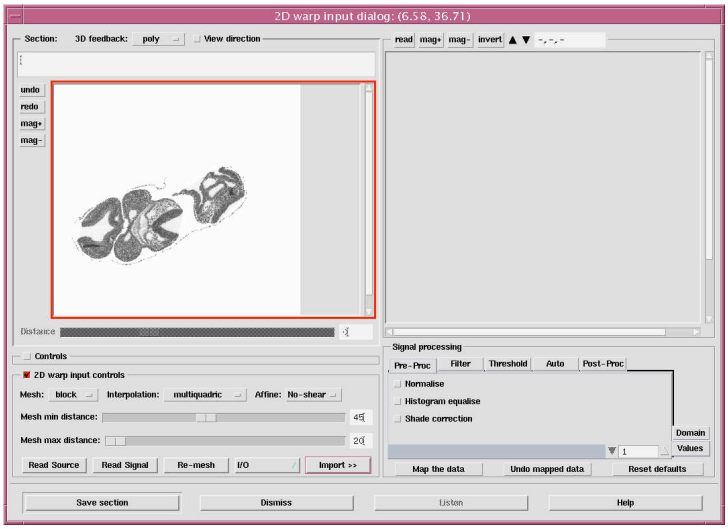
Initially it is a good idea to select "no", remove the inappropriate tie-point using the middle mouse button and try again. If you still have a problem, zoom in to help when adding tie points. Depending on the original data, another way to help in getting the mesh to tolerate the warping required may be to adjust the "Mesh min distance" and "Mesh max distance" parameters in the "2D Warp Input" window. These are the minimum and maximum distances that the sides of the triangles will stretch/contract to during the warping.

Write a ".bib" file to record the tie-points you have entered by clicking on the "I/O" button under the left hand panel while using the right hand mouse button and then selecting "write warp data" from the list that appears. A window will appear entitled "Get Filename Dialog" with the default file name of "`MAPaintWarpParams.bib`"

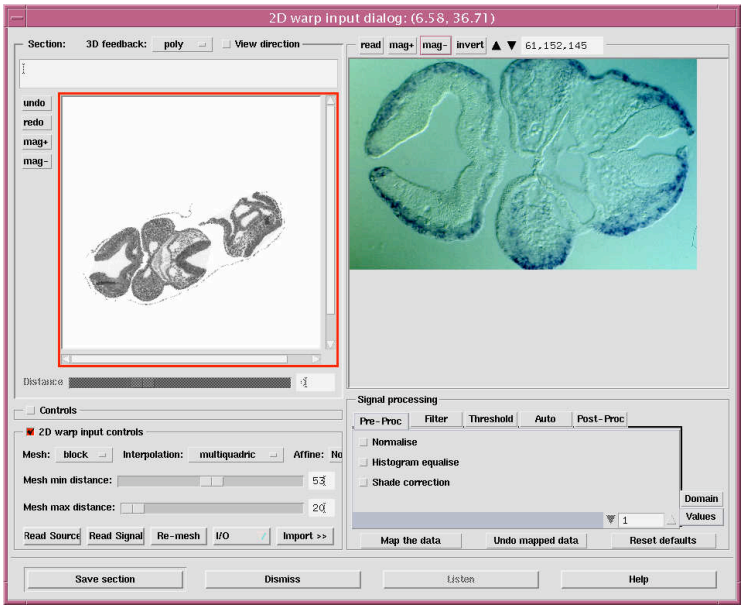
Change the name to **`AP2.bib`** and click on the "OK" button.

Now regions will be denoted on the section representing different areas of AP2 expression (or non-expression) using a thresholding method. The program allows for regions of strong, moderate, weak and possible expression to be denoted as well as regions that have no detectable expression and areas that have not been examined. Not all of these may be required. Bear in mind not to over-interpret the data (ie. adding strong, weak and moderate levels of gene expression may not be any more meaningful than adding two regions). In the case of this example, denoting regions of strong and moderate expression is enough (along with regions that have no expression detected and regions that have not been examined).

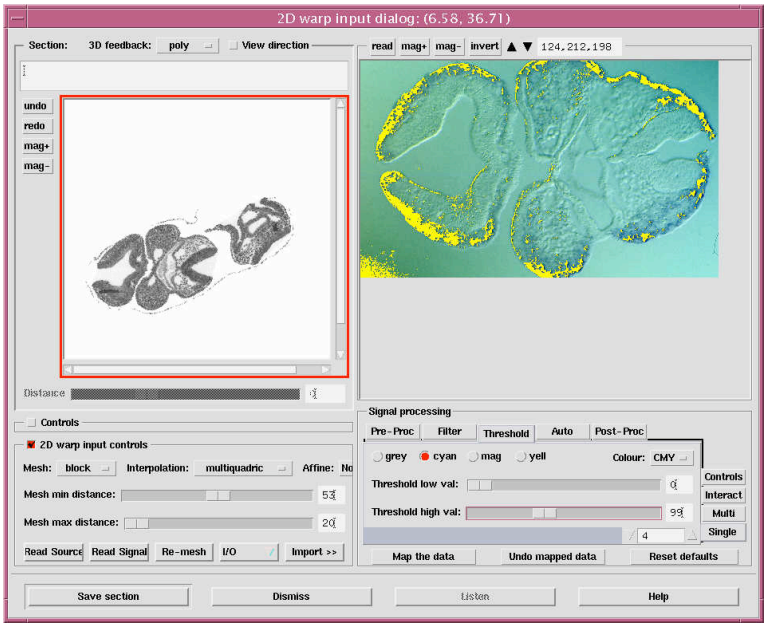
Get ready to read in the colour image for thresholding by going back to the "2D warp input dialog" window and clicking on "Import>>". A new panel will appear on the right hand side of the window.



Click on the "read" button above the new panel and a window entitled "read signal object" will open. Select the **`AP2.jpg`** source image and click on "OK". (For source images other than jpg's, select the appropriate file type from the drop down "image type" menu). The image file will then appear in the right hand panel of the 2D warp input dialog window. To fit the image in the panel, either magnify (using "mag+" button) or reduce the image (using the 'mag-' button) as required.

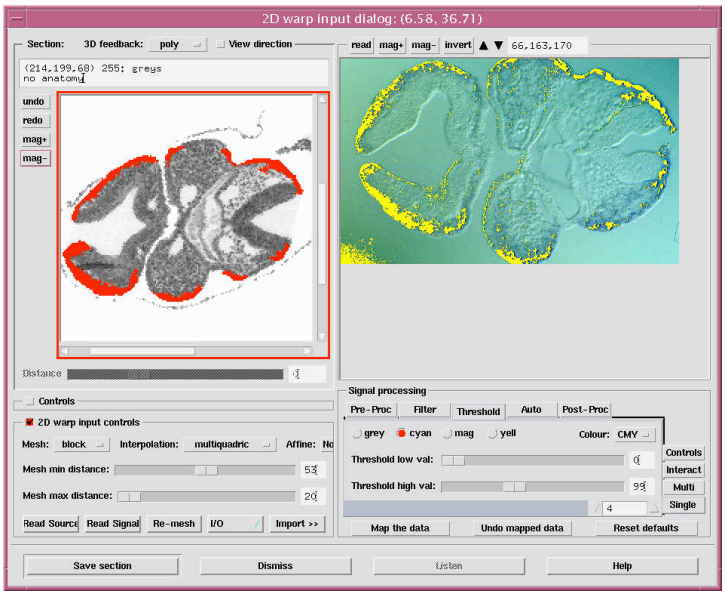


Look at the colour image of AP2 to discern the region of highest signal intensity and then go to the "2D warp input dialog" window. Select the “Threshold” tab in the “Signal processing” panel, and then select the “Interact” tab on the right. Click once with the left hand mouse button on the region of the data image with the strongest signal. Continue to hold the button down while dragging the cursor to an appropriate region on the image that denotes the lower limit of strong gene expression. Then release the mouse button to define the lower limit of strong gene expression. Don't worry at this stage if you also incorporate some yellow areas corresponding to other areas such as debris as these will be removed later. If the region selected is not appropriate, click on "reset defaults" and try again.



Note: it may sometimes be easier to extract signal by using other strategies apart from the "interact" method. For example the "single" option sometimes works better to extract either the overall image density (ie. in grey mode) or just the blue, red or green colour channel (while in RGB mode) or the cyan, magenta or yellow colour channel (while in CMY mode). Try some different methods in this AP2 example to see how these signal extraction methods differ.

Click on the "map the data" button under the right hand panel and the data will be transferred onto the target section in the left hand panel in red:



If you are satisfied with the positioning of the transferred data, proceed to the next step. If it is not satisfactory, click on the "undo mapped data" button and re-adjust the tie points in the "warp2DinteractDialog" window and then re-save the bib file `Ap2.bib` to record the re-adjusted position of the points.

Write another "bib" file that contains information on the thresholding levels for the regions of strong gene expression by clicking with the right hand mouse button on the "I/O" button under the left hand panel and selecting "write warp data" from the list that appears. As before, a window will appear entitled "Get Filename Dialog" with the default file name `MAPaintWarpParams.bib`. Change to **`AP2.strong.bib`** and then click on the "OK" button.

Remove any red areas of debris that have been carried across with the thresholding by going to the MAPaint window. The program is set by default to 'paintball' mode with the cursor appearing as a black dot. Use the middle mouse button to remove any areas of debris. This may not be required. If you make a mistake, click on "undo".

The size of the paintball can be adjusted by selecting "tool controls" from the "options" menu and then changing the Paint size with the slider. The painting mode can also be changed to other formats (draw, fill, threshold, affine etc) by selecting these from the

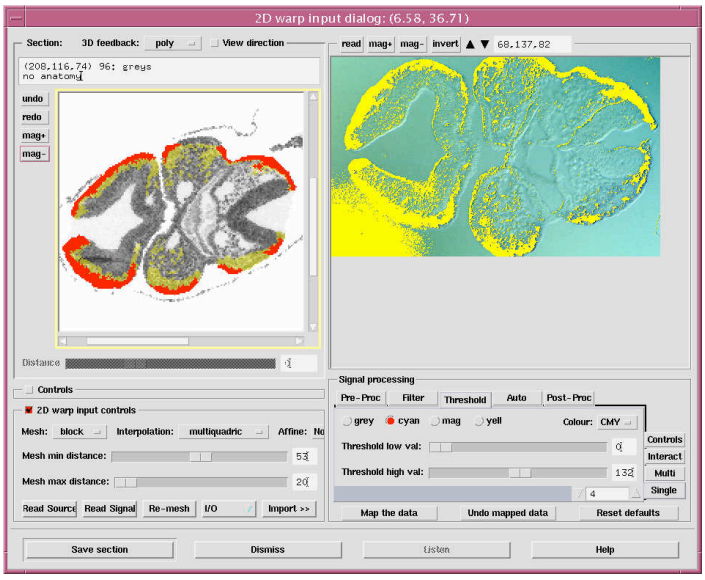
"Paint tools (2D)" menu within the "options" menu. The behaviour of all of these tools is such that using the left hand mouse button generally is used to add colour whilst using the middle hand mouse button is used to remove colour.

Now add any red areas that you feel represent true expression and have been missed by the thresholding technique by using the paintball with the left hand mouse button. It may be necessary to adjust the "Paint size". Once again, If you make a mistake, click on 'undo' using the left hand mouse button.

When happy with the areas representing strong expression, the process is now repeated for areas of moderate (yellow), weak (blue), possible (green) expression as well as areas with no detectable expression. The process used is exactly the same for each however the domain has to be selected separately for each. Remember that not all of these levels may be required.

Select the moderate domain by choosing the "Domain" menu and then the "Select" menu and finally "Moderate Expression". The border surrounding the embryo model on the left will change from red to yellow. Repeat the thresholding procedure so that the yellow areas in the right hand panel now adequately cover sites of moderate gene expression. This can be done either by starting the thresholding procedure again, and extending to include moderate areas of expression, but is most easily achieved simply by moving the threshold distance slider up which automatically extends the range of the selected expression intensity. Again, don't worry at this stage if you also include some yellow areas corresponding to other areas such as debris as these will be removed later.

Click on the "map the data" button under the right hand panel and the data will be transferred onto the standard model in the left hand panel in yellow:



Write a new "bib" file that contains information on the thresholding levels for the regions of moderate gene expression by clicking with the right hand mouse button on the "I/O" button under the left hand

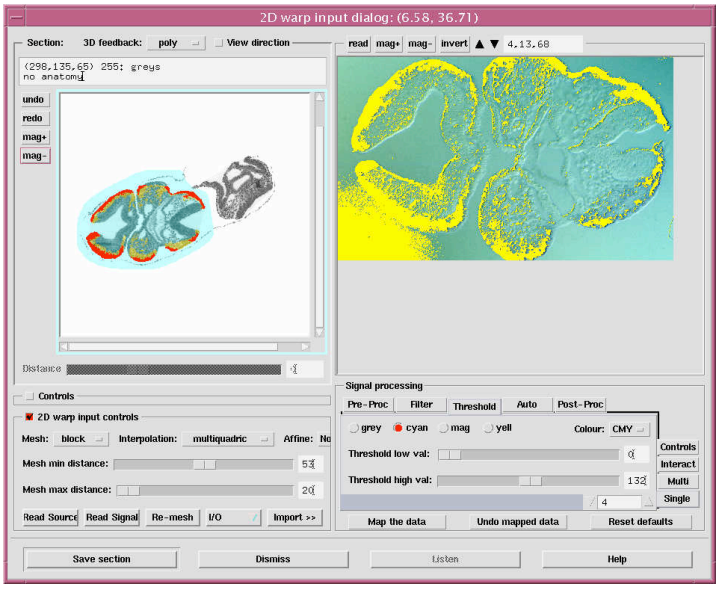
panel and selecting "write warp data" from the list that appears. As before, a window will appear entitled "Get Filename Dialog" with the default file name `MAPaintWarpParams.bib`. Change to **`AP2.moderate.bib`** and then click on the "OK" button.

Remove any yellow areas of debris that have been carried across with the thresholding by going to the MAPaint window and selecting the "Options" menu and then the "Paint Tools (2D)" and "Draw". Back in the "2D warp input dialog" window use the middle mouse button to circle all yellow areas that represent debris and then click the right hand mouse button to remove the areas of yellow within the line. Again, this may not be required. If you make a mistake, click on the "undo" button using the left hand mouse button.

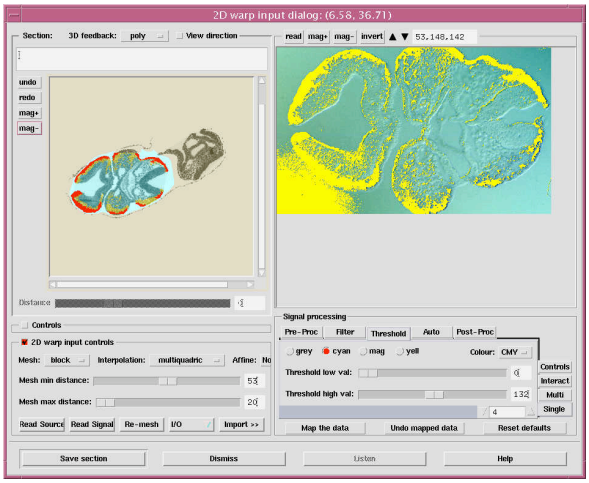
Add any yellow areas of moderate expression that you feel have been missed by the thresholding by going to the MAPaint window and selecting the "Options" menu and then the "Paint Tools (2D)" and "Paint Ball". In the "2D warp input dialog" window use the left hand mouse button to paint in any yellow areas that represent true expression that have been missed by the thresholding technique. If you make a mistake, click on "undo" using the left hand mouse button.

Continue on as before thresholding, painting and saving the .bib file for each level of expression detected in the original image.

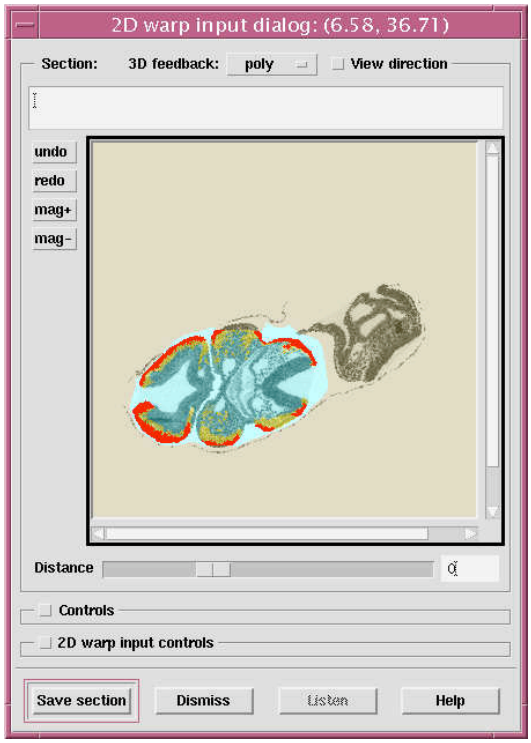
Denote areas in the target section with no detectable expression by firstly selecting the "Domain" menu and then the "Select" menu and "Not Detected" and then the "Options" menu, "Paint Tools 2D" and then "Draw". In the "2D warp input dialog" window use the left hand mouse button to circle regions that you are examining which have no detectable expression (in this case it is the rest of the head) and then click once on the right hand mouse button. The area within the line becomes cyan and represents areas of non-detectable gene expression within regions of the section that you are mapping.



Denote all of the areas of the section that have not been examined by firstly selecting from the "Domain" menu, "Select" and then "Not Examined". Then select from the "Options" menu, "Paint Tools (2D)" and then "Fill". In the "2D warp input dialog" window click once with the left hand mouse button on part of the section that you have not mapped data to. The area outside the region you have already mapped data to become brown. This represents areas of the section that have not been examined in your experiment. If required, make any fine adjustments using the "paint' tool. (eg in this case, the distal tips of the branchial arches, the tail and extra-embryonic membranes are regions that have not been examined - see below).



Save the files for the regions of different expression levels by firstly clicking on the "2D warp input controls" box. The border surrounding the image of the target section in the "2D warp input dialog" window changes to black signifying the files are ready to be written.



In the MAPaint Window select from the "Domain" menu, "Save All Domains". This automatically saves the files in "woolz" format (.wlz) to your working directory (i.e. the directory you were in when you started MAPaint) for whichever domains you have created as: **strong.wlz**; **moderate.wlz**; **weak.wlz**; **possible.wlz**; **notDetected.wlz** and **notExamined.wlz**

Quit the MAPaint program. A window appears entitled "confirm dialog" with the message "really quit? " Click on "yes". Another "confirm dialog" window appears with the message "really really quit? " Click on "yes" and the program quits.

Check that you have the relevant files that will be required to load this entry into the EMAGE interface later.

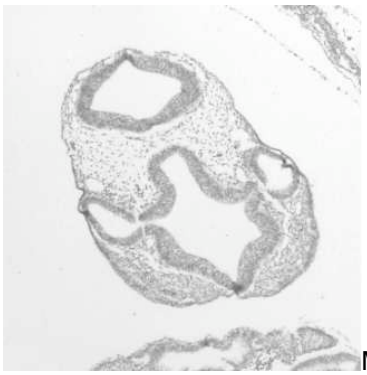
These are:

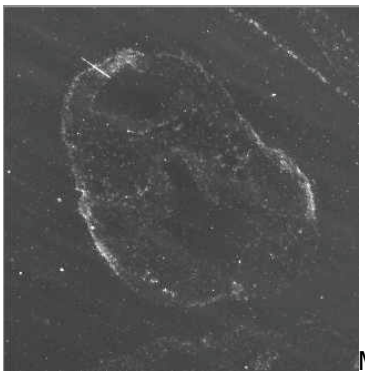
- the original image file **AP2.jpg**,
- the bib file to tell the EMAGE interface which section to use when visualising the mapped expression and will in this case be **AP2.ts13.1.bib**.
- the appropriate .wlz files that denote the different regions of expression you denoted: **strong.wlz**; **moderate.wlz**; **notDetected.wlz** and **notExamined.wlz**

All section data can be spatially mapped using the same process apart from radio-active in situ data that has been imaged using separate bright and dark-field images. In this case it is usually necessary to extract signal from the dark-field image which often cannot be used for the warping procedure due to a lack of morphological features. In these cases the bright-field image can be read in as the source object for

tie-point placement and warping whilst the dark-field image can be read in separately as the source image for signal thresholding. This process requires however that the two images are of exactly the same size and magnification.

For example these two images of Msx2 have been digitised without moving the slide and are suitable for use in this manner:


Msx2.bf.jpg


Msx.df.jpg