MRI Grid

18/02/08

Abstract

The *MRI Grid* is a tool in *MRI Cell Image Analyzer*, that can be used to associate measurements with labeled positions on a board.

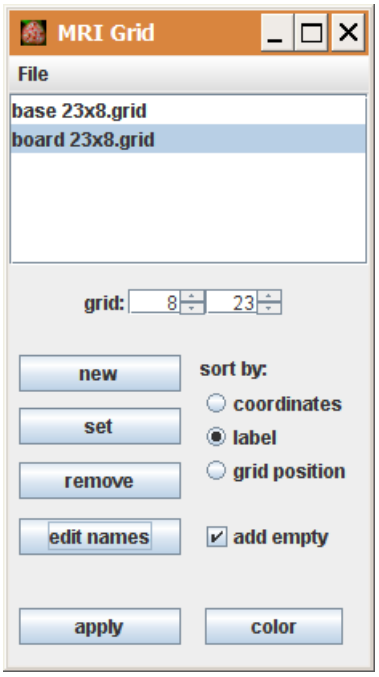


Illustration 1: The interface that allows to define grids.

The user can define a grid with an arbitrary number of rows and columns. The positions on the grid can be labeled. A grid can be overlayed to an image and the positions of the rows and columns can be changed. A results table of measurements can be modified to include the label of the nearest grid point for each measurement.

The measurements can be sorted by image coordinates, by labels or by grid positions. For each empty positions a row containing only the corresponding label can be added.

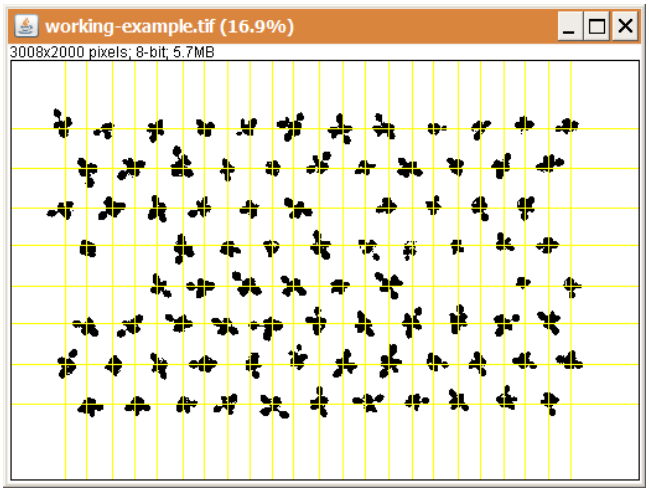
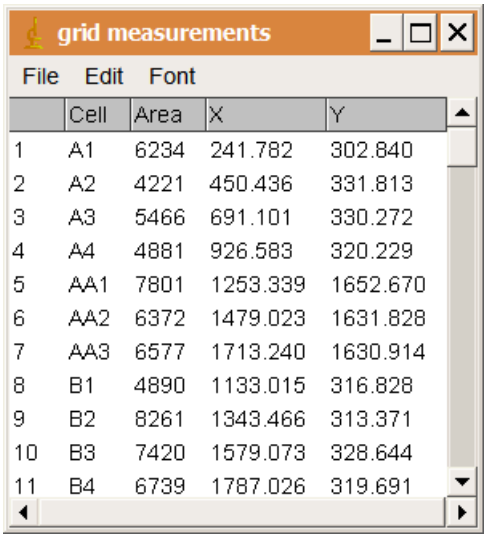


Illustration 2: A grid on a binary image.



	Cell	Area	X	Y
1	A1	6234	241.782	302.840
2	A2	4221	450.436	331.813
3	A3	5466	691.101	330.272
4	A4	4881	926.583	320.229
5	AA1	7801	1253.339	1652.670
6	AA2	6372	1479.023	1631.828
7	AA3	6577	1713.240	1630.914
8	B1	4890	1133.015	316.828
9	B2	8261	1343.466	313.371
10	B3	7420	1579.073	328.644
11	B4	6739	1787.026	319.691

Illustration 3: A results table with positions on the grid (A1, A2, ...) and measurements.

Table of Contents

Illustration Index

Quick start

Opening the grid tool

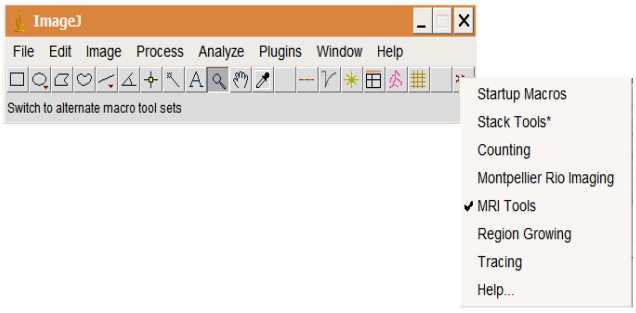


Illustration 4: The grid tool is started from the toolset MRI Tools.

To use the grid tool you need *ImageJ*[1] together with the *MRI plugins*[2]. Select the toolset *MRI Tools*. Click on the grid button to open the *MRI Grid* tool.

Creating a new grid

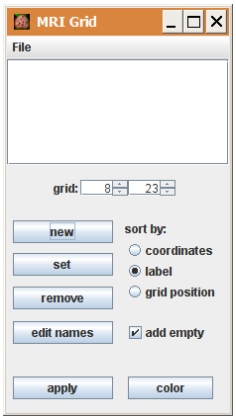


Illustration 5: The MRI grid tool.

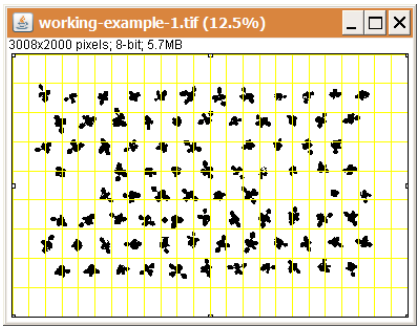


Illustration 6: A grid on a binary image.

Open an image using *ImageJ*. Enter the number of rows and columns on the grid tool and press *new*. The grid will appear on the active image.

Adjusting the grid

When the mouse-pointer is over a grid-line, the cursor changes. Left-click and move the mouse, to move a vertical line to the left or right or a horizontal line up or down.

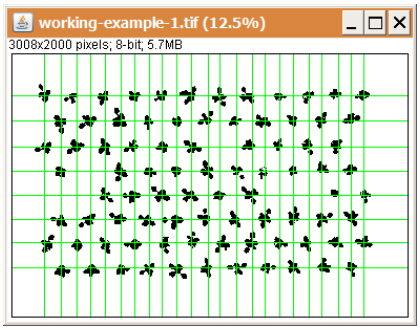


Illustration 7: The grid has been adjusted.

By grabbing a vertical or horizontal line and pressing the *ctrl-key* you can move the whole grid. (Remark that this way you can move lines out off the image. If this should happen move the whole grid back, move the line at the border closer to the inner lines and move the whole grid again.)

1 http://rsb.info.nih.gov/ij/
2 http://www.mri.cnrs.fr/mriwiki/index.php?pagename=ImageJ%20plugins%20from%20Montpellier%20RIO%20Imaging

A line can be moved until it touches its neighbor. The order of lines cannot be changed.

Defining Labels

Press the *edit names* button. A table representing the grid positions and the corresponding labels is opened. By default the labels correspond to row and column indices.

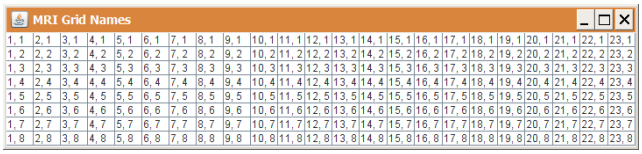


Illustration 8: The default labels.

Edit the names according to the schema you want. Cells with empty labels will be ignored.

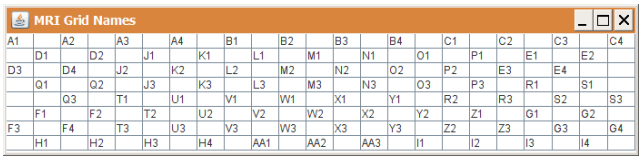


Illustration 9: Customized labels.

Saving the grid

Select the *Save...* command from the *File* menu of the *MRI Grid*. In the dialog enter the name of the grid. The grid will be saved to the *_grids* folder in the *ImageJ* installation directory. It will appear in the list of the *MRI Grid* interface.

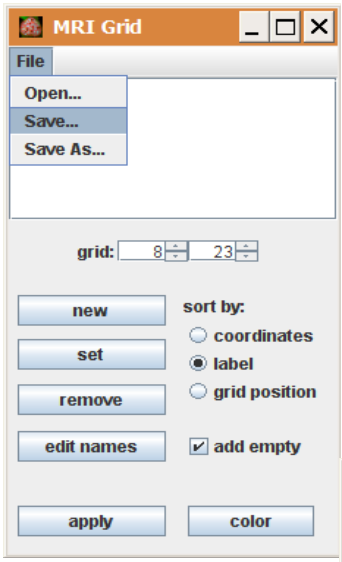


Illustration 10: Saving a grid...

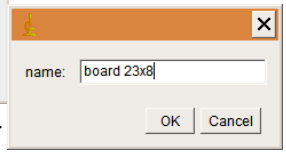


Illustration 11: The user enters a name.

Use the *Save As...* command to save the grid in a folder different from the *_grids* folder

Loading an existing grid

You can open a grid by double-clicking on the name in the list. The name of the opened grid will become green until changes to the grid are made.

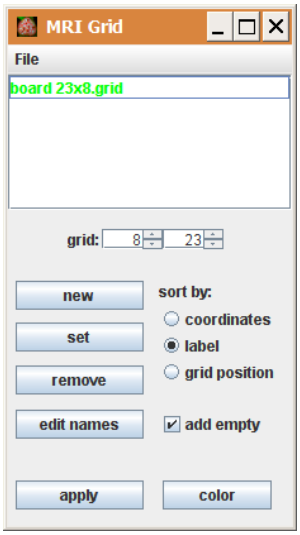


Illustration 12: A grid can be loaded from the list.

Use the *Open...* command to load a grid that has been saved in a folder different from the *_grids* folder.

Transferring the grid to another image

The active grid is either the last loaded grid (shown in green in the list) or the grid that is set on the last active image. To set a modified grid to a new image remove the grid from the target image if there is one, select the image with the source grid, select the target image and press the *set* button.

Removing a grid from an image

Select the image and press the *remove button* on the *MRI Grid* tool.

Deleting a grid

To delete a grid from the list and from the _grids folder, select it in the list, right-click on the list to bring up the context menu and select the command *delete selected*.

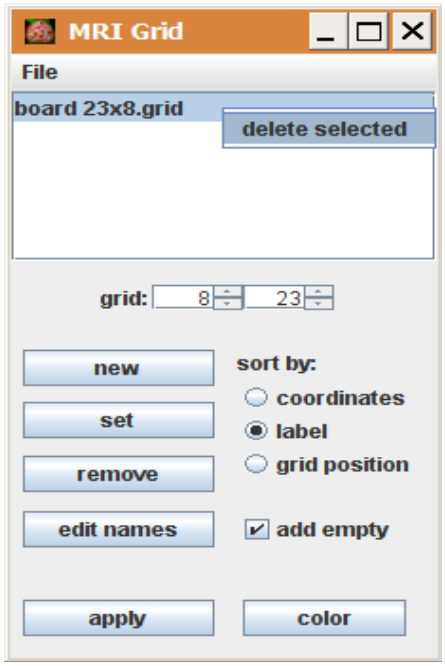


Illustration 13: Deleting the selected grid.

Adding labels to a results table

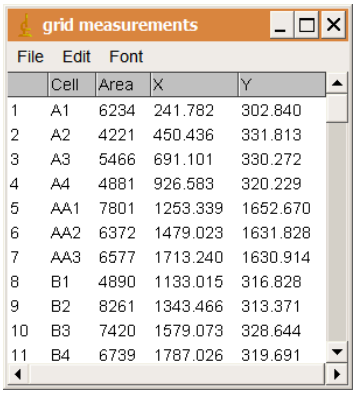


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Illustration 14: A result table containing labels indicating the positions of measurements.

Use the *Particle Analyzer* to measure objects in a binary image as usual. Make sure that the *centroids* are measured. Click the *apply* button on the *MRI Grid* tool. A second *results table*, containing the results together with the labels from the grid will be opened.

Options

Sort by coordinates

The order of the original *results table* is kept. The results are sorted by the y-coordinate and the x-coordinate of the upper left corner of the objects' bounding boxes.

Sort by label

The results are sorted by labels in alphanumerical order (A1, A2, A3, A4, AA1, ...).

Sort by grid position

The results are sorted by the position on the grid from left to right and top to bottom.

Add empty

Normally empty positions, i.e. positions on the grid without an object, do not appear in the *results table*. This can make comparisons more complicated. If you select *add empty,* a row containing the label and a zero for each measurement is added for each empty position.

Color

Click the *color* button to change the color of the grid. Changing the color might be helpful in case the current grid color has not enough contrast with the colors in your image.

Example application

Introduction

Arabidopsis plants are grown on fixed positions on a board. Plants on the board are grouped according to different experimental conditions. At different time points photos are taken. With help of image analysis the growth of the leaf surface of the plants shall be measured for the different conditions.

Alignment of images

Each series of images, representing the same board at different time points, should be stored in one folder and the images should be numbered according to their age.

Select the Montpellier RIO Imaging toolset.

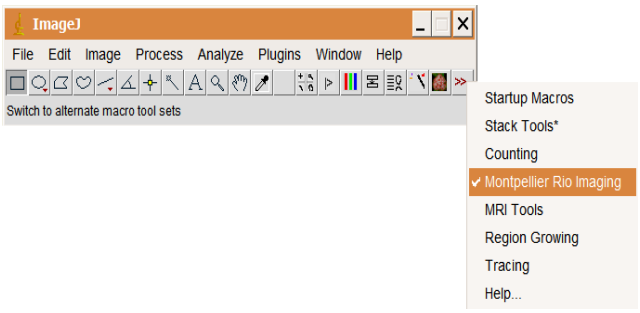

Illustration 15: Selecting the Montpellier RIO Imaging toolset.

Click on the  button to open the *visual scripting launcher*. From the *visual scripting launcher* open the application *register series* from the menu *Applications>applications>stacks*.

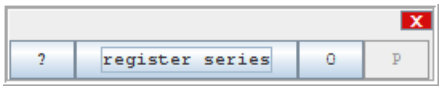

Illustration 16: The "register series" visual scripting application.

Click on the *register series* button. This will open the *list editor*. Click on the *add* button. A file dialog will be opened. Set the field *file types* to *all images*. Select all folders containing your images and click the open button. The names of the images should now appear in the *list editor*.

Illustration 17: Images of the board taken at different time points.

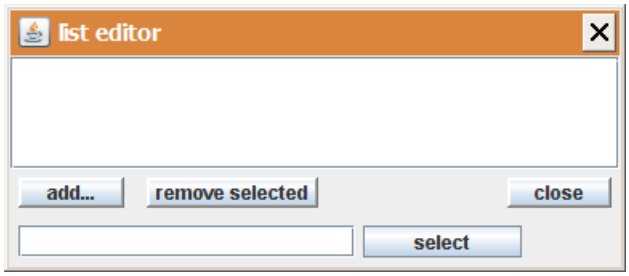

Illustration 18: The list editor allows to run applications on a set of images.

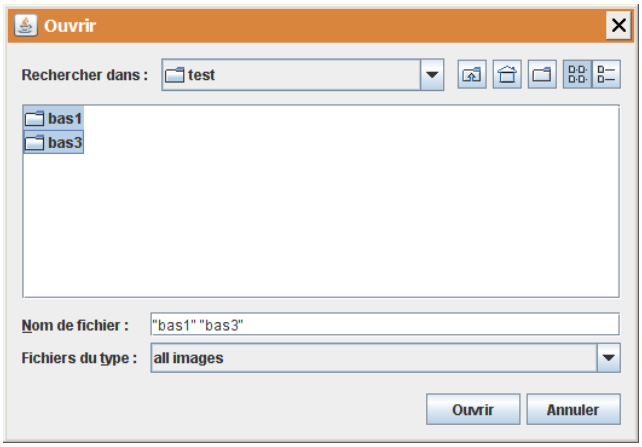

Illustration 19: The user selects multiple folders containing images.

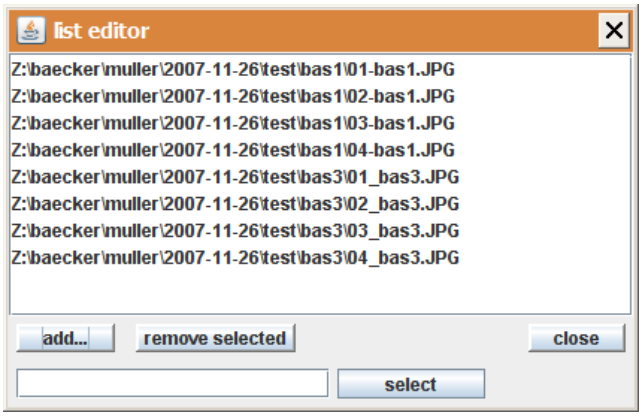

Illustration 20: Images from different folder have added to the list.

If the order of the images is not correct, select all images and remove them using the button remove selected. Then use the add button multiple times to add the images folder by folder,

by selecting all images in a folder in the file dialog.

When you press the close button the application will start to work. A sub folder *aligned*, containing the aligned images, will be created in each image folder. The calculation of the alignments can take a long time.

Rotating the image

Open an image from the *aligned* sub folder. If the edges of the board are not parallel to the edges of the window you should rotate the image. Use the *angle tool* from the *ImageJ* launcher window to measure the angle. Open the *rotate dialog* from the menu *Image>Rotate>Arbitrarily*. Enter the measured angle multiplied by -1 and click ok.

Setting the scale

Per default, measurements are made in pixels. You can set a scale so that your measurements will be made in a given unit like for example *cm*.

Open an image from the *aligned* sub folder. Make a *line selection* of a known distance, for example the long side of the board, using the line selection tool from *ImageJ*.

Illustration 21: A known distance has been selected in the image.

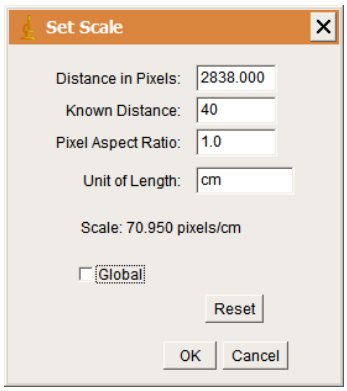

Illustration 22: Setting the scale from a known distance.

Open the *Set Scale* dialog from the *Analyze* menu. In the field *known distance* enter the length of the board in *cm*. Change the unit to cm in the *unit of length* field. If you click *ok* the scale will be set for the active image. Check the *Global* checkbox if you want to use this scale with all images.

Segmentation of the plants

Open the *Threshold Colour* dialog from the menu *Plugins>Segmentation*. Switch to the *Cie Lab* color space. In the green/red component adjust the maximum until the first part of a plant disappears. Move the maximum just before that point. Close the *Threshold Color* dialog. Transform the image into a grey-scale image with the *Image>Type>8-bit* command. Open the *Threshold* dialog (*shift-t*) and set the minimum to 0 and the maximum to 254. Press the *apply* button. You now have a binary image.

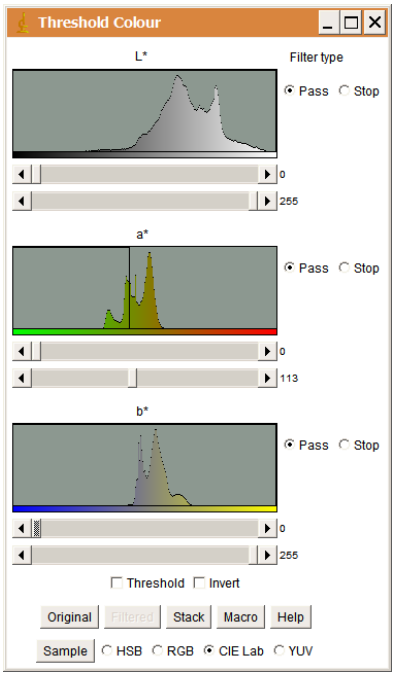

Illustration 23: The Threshold Color tool.

Select the *startup macros* toolset. Use the *Pencil Tool* and the *Paintbrush Tool* to cleanup the image. To draw or paint in white or black, use the *color picker* to select white or black as foreground color.

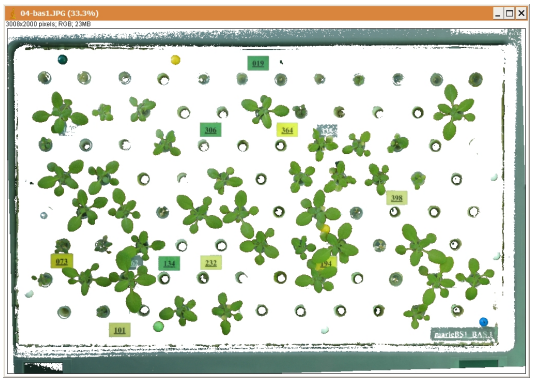

Illustration 24: Objects that are more or less green have been segmented.

Remove all objects that are not plants (Alternatively you can remove the objects before changing the image type to grey-scale). Separate plants touching each other, by drawing a thin white line. By double-clicking on the *Pencil*- or *Paintbrush*-tool you can open a dialog, in which you can set the width of the tool. If parts of a plant are not connected with the main part, draw a small connection in black.

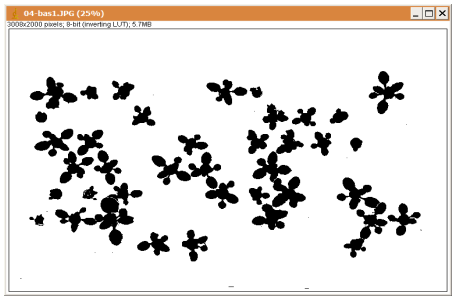

Illustration 25: Manual correction have been made to the segmentation.

Using the grid

You can either use the whole stack of input images or the stack of masks or a *z-projection* of one of these, to define the grid for one series. In this example a *z-projection* of the masks is used. Import the mask images using *File>Import>Image Sequence* and create the *z-projection* from *Image>Stacks>Z Project...*

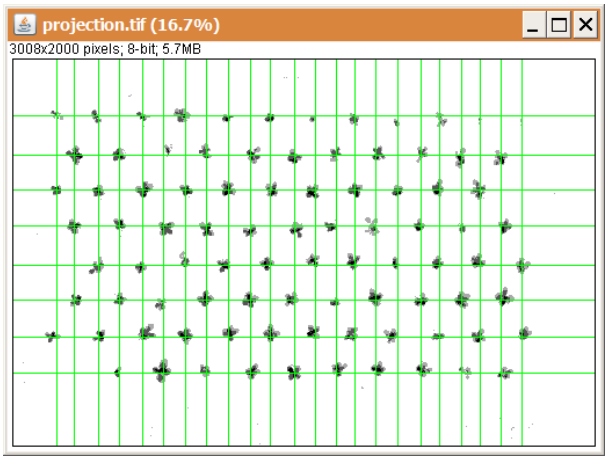

Illustration 26: A z-projection of a series of masks can be used to adjust the grid.

Open the *MRI Grid* tool. To define a new grid, enter the number of rows and columns and press the *new* button. Click the *edit names* button to change the labels. Positions with empty labels will be ignored.

Alternatively open an existing grid by double-clicking on its name in the list and set it to the image, by clicking on the *set* button.

Adjust the rows and columns in a way that they best fit the rows and columns of plants.

Now open one mask image after the other and execute the following steps.

Make sure that, in the *Analyze>Set Measurements* dialog, at least area and centroid are selected. Run the *Particle Analyzer* from *Analyze>Analyze Particles*. Enter a minimum size, that is just above the area of the smallest plant in the image, to remove remaining noise. Click the *apply* button on the *MRI Grid* dialog. Copy the results from the new results table and paste them into a spreadsheet file.

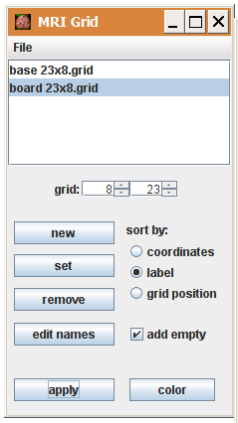

Illustration 27: Pressing apply on the MRI Grid tool adds the labels to the results table.

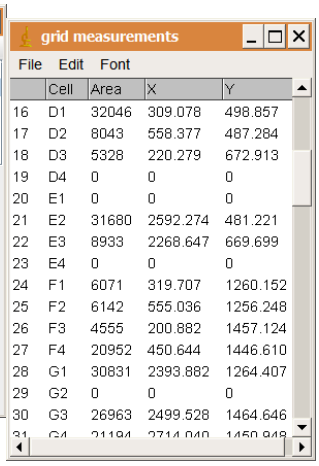

Illustration 28: Labels indicating the position of the object have been added to the results table.