

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.

🚳 MRI Grid	_ 🗆 ×
File	
base 23x8.grid	
board 23x8.grid	
grid: 8-	23 ÷
g	
new	sort by:
	coordinates
set	Iabel
remove	grid position
	_
edit names	🖌 add empty
edit names	🖌 add empty
edit names	✓ add empty color

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.

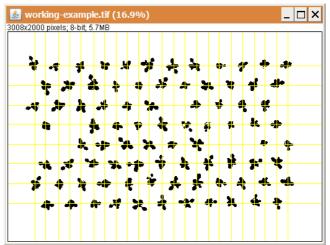


Illustration 2: A grid on a binary image.

£	grid m	easure	ements	_ 🗆	×							
File	File Edit Font											
	Cell	Area	Х	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	В3	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

Abstract	1
Quick start	3
Opening the grid tool	3
Creating a new grid	
Adjusting the grid	
Defining Labels	
Saving the grid	
Loading an existing grid	
Transferring the grid to another image	5
Removing a grid from an image	5
Deleting a grid	5
Adding labels to a results table	5
Options	6
Sort by coordinates	6
Sort by label	6
Sort by grid position	6
Add empty	6
Color	6
Example application	6
Introduction	6
Alignment of images	6
Rotating the image	8
Setting the scale	8
Segmentation of the plants	8
Using the grid	9

the positions of measurements
Illustration 15: Selecting the Montpellier RIO Imaging
toolset
application
Illustration 17: Images of the board taken at different time
points7
Illustration 18: The list editor allows to run applications on
a set of images
Illustration 19: The user selects multiple folders containing
images7
Illustration 20: Images from different folder have added to
the list7
Illustration 21: A known distance has been selected in the
image
Illustration 22: Setting the scale from a known distance8
Illustration 23: The Threshold Color tool9
Illustration 24: Objects that are more or less green have
been segmented
Illustration 25: Manual correction have been made to the
segmentation
Illustration 26: A z-projection of a series of masks can be
used to adjust the grid
Illustration 27: Pressing apply on the MRI Grid tool adds
the labels to the results table10
Illustration 28: Labels indicating the position of the object
have been added to the results table10

Illustration Index

Illustration 1: The interface that allows to define grids1
Illustration 2: A grid on a binary image1
Illustration 3: A results table with positions on the grid
(A1, A2,) and measurements1
Illustration 4: The grid tool is started from the toolset
MRI Tools
Illustration 5: The MRI grid tool
Illustration 6: A grid on a binary image
Illustration 7: The grid has been adjusted
Illustration 8: The default labels
Illustration 9: Customized labels
Illustration 10: Saving a grid4
Illustration 11: The user enters a name
Illustration 12: A grid can be loaded from the list5
Illustration 13: Deleting the selected grid
Illustration 14: A result table containing labels indicating



Quick start

Opening the grid tool

🧯 Image)	×
File Edit Image Process Analyze Plugins Window Help	
Switch to alternate macro tool sets	Startup Macros
Switch to alternate macro tool sets	Stack Tools*
	Counting
	Montpellier Rio Imaging
	MRI Tools
	Region Growing
	Tracing
	Help

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 \ddagger to open the *MRI Grid* tool.

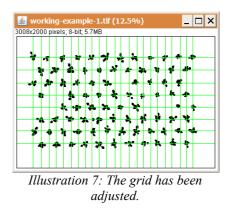


Creating a new grid

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.



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

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working-example-1.tif (12.5%)



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.

4	MRI	Grid	Nan	nes																							-		X
1, 1	2, 1	3, 1	4, 1	5, 1	6, 1	7, 1	8, 1	9,1	10, 1	11,1	1 12,	1 1	13, 1	14,	1	15, 1	16	1	17,	1 18	i, 1	19,	1	20,	1 2	1, 1	22	, 1	23, 1
1, 2	2, 2	3, 2	4, 2	5, 2	6, 2	7, 2	8, 2	9, 2	10, 2	11, 3	2 12,	2 1	13, 2	14,	2	15, 2	16	2	17,	2 18	1, 2	19,	2	20,	2 2	1, 3	22	, 2	23, 2
1, 3	2, 3	3, 3	4, 3	5, 3	6, 3	7, 3	8, 3	9, 3	10, 3	11,3	3 12,	3 1	13, 3	14,	3	15, 3	16	3	17,	3 18	1, 3	19,	3	20,	3 2	1, 3	3 22	, 3	23, 3
1, 4	2, 4	3, 4	4,4	5,4	6, 4	7,4	8, 4	9,4	10, 4	11,	4 12,	4 1	3, 4	14,	4	15, 4	16	4	17,	4 18	, 4	19,	4	20,	4 2	1,4	22	, 4	23, 4
1, 5	2, 5	3, 5	4,5	5, 5	6, 5	7,5	8, 5	9, 5	10, 5	11, 1	5 12,	5 1	13, 5	14,	5	15, 5	16	5	17,	5 18	1, 5	19,	5	20,	5 2	1, 5	5 22	, 5	23, 5
1,6	2,6	3, 6	4,6	5,6	6,6	7,6	8,6	9,6	10,6	11, 1	6 12,	6 1	3, 6	14,	6	15,6	16	6	17,	6 18	I, 6	19,	6	20,	6 2	1, (6 22	, 6	23, 6
1,7	2,7	3, 7	4,7	5,7	6,7	7,7	8,7	9,7	10,7	11,	7 12,																		23, 7
1, 8	2,8	3, 8	4,8	5,8	6, 8	7,8	8,8	9,8	10, 8	11,1	B 12,	8 1	3, 8	14,	8	15, 8	16	8	17,	8 18	, 8	19,	8	20,	8 2	1,8	3 22	, 8	23, 8

Illustration 8: The default labels.

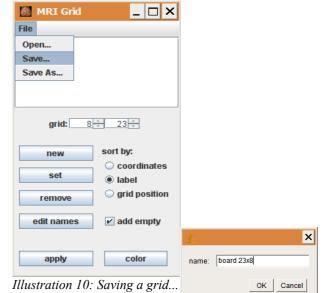
Edit the names according to the schema you want. Cells with empty labels will be ignored.

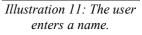
\$	MR	I Gri	d Na	mes																	_ 0];
A1		A2		A3		A4		B1		B2		B3		B4		C1		C2		C3		C4
	D1		D2		J1		K1		L1		M1		N1		01		P1		E1		E2	
D3		D4		J2		K2		L2		M2		N2		02		P2		E3		E4		
	Q1		Q2		J3		K3		L3		M3		N3		03		P3		R1		S1	
		Q3		T1		U1		V1		W1		X1		Y1		R2		R3		S2		S3
	F1		F2		T2		U2		V2		W2		X2		Y2		Z1		G1		G2	
F3		F4		T3		U3		V3		W3		X3		Y3		Z2		Z3		G3		G4
	H1		H2		H3		H4		AA1		AA2		AA3		11		12		13		14	

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.





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.



🚳 MRI Grid	_ 🗆 ×
File	
board 23x8.grid	
grid: 8	÷ 23 ÷
new	sort by:
set	 coordinates label
	 grid position
remove	o gra position
edit names	✓ add empty
apply	color
appiy	COIOI

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*.

🎊 MRI Grid	_ 🗆 ×
File	
board 23x8.grid	delete selected
grid: 8	÷ 23 ÷
new	sort by:
set	 coordinates label
remove	grid position
edit names	🖌 add empty
apply	color

Illustration 13: Deleting the selected grid.

Adding labels to a results table

4	grid m	easure	ments	_ 🗆	×						
File Edit Font											
	Cell	Area	Х	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	В2	8261	1343.466	313.371							
10	В3	7420	1579.073	328.644							
11	B4	6739	1787.026	319.691	•						
4											

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.

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.

🛓 ImageJ 📃 📃	<
File Edit Image Process Analyze Plugins Window Help	
Switch to alternate macro tool sets	Startup Macros Stack Tools* Counting ✓ Montpellier Rio Imaging MRI Tools Region Growing Tracing Help

Illustration 15: Selecting the Montpellier RIO Imaging toolset.

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

			X		
?	register series	0	P		
Illustration 16: The "register series"					
1	visual scripting appli	ication			



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.

🍝 list editor	×
add remove selected	close
	select

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

🍝 Ouvrir			×
Rechercher dans	test	- 6 6	
🗖 bas1 🗂 bas3			
Nom de fichier :	"bas1" "bas3"		
Fichiers du type :	all images		•
		Ouvrir	Annuler

Illustration 19: The user selects multiple folders containing images.

😹 list editor	×
Z:\baecker\muller\2007-11-26\test\bas1\01-bas1.JPG	
Z:\baecker\muller\2007-11-26\test\bas1\02-bas1.JPG	
Z:\baecker\muller\2007-11-26\test\bas1\03-bas1.JPG	
Z:\baecker\muller\2007-11-26\test\bas1\04-bas1.JPG	
Z:\baecker\muller\2007-11-26\test\bas3\01_bas3.JPG	
Z:\baecker\muller\2007-11-26\test\bas3\02_bas3.JPG	
Z:\baecker\muller\2007-11-26\test\bas3\03_bas3.JPG	
Z:\baecker\muller\2007-11-26\test\bas3\04_bas3.JPG	
add remove selected	close
select	

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 Open the Set Scale dialog from the Analyze image. Use the angle tool \checkmark from the ImageJ menu. In the field known distance enter the launcher window to measure the angle. Open the length of the board in cm. Change the unit to cm rotate dialog from the Image>Rotate>Arbitrarily. Enter the measured scale will be set for the active image. Check the 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 Open the Threshold Colour dialog from the will be made in a given unit like for example cm. menu Plugins>Segmentation. Switch to the Cie

Make a line selection of a known distance, for disappears. Move the maximum just before that example the long side of the board, using the line point. Close the Threshold Color dialog. selection tool from ImageJ.



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

🛓 Set Scale	×			
Distance in Pixels:	2838.000			
Known Distance:	40			
Pixel Aspect Ratio:	1.0			
Unit of Length:	cm			
Scale: 70.950 pixels/cm				
Global				
	Reset			
C	K Cancel			

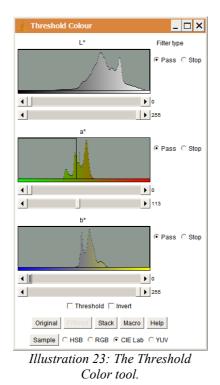
Illustration 22: Setting the scale from a known distance.

menu in the unit of length field. If you click ok the Global checkbox if you want to use this scale with all images.

Segmentation of the plants

Lab color space. In the green/red component Open an image from the *aligned* sub folder. adjust the maximum until the first part of a plant 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.





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.

plants touching each other, by drawing a thin

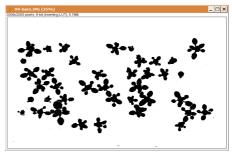


Illustration 25: Manual correction have been made to the segmentation.

Using the grid

the color picker to select white or black as Import foreground color.

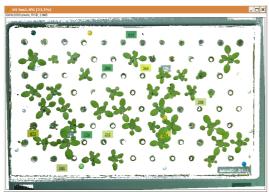
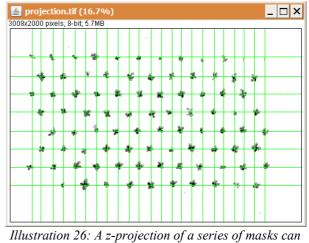


Illustration 24: Objects that are more or less green have been segmented.

Remove all objects that are not plants (Alternatively you can remove the objects before changing the image type to grey-scale). Separate

You can either use the whole stack of input Select the *startup macros* toolset. Use the *Pencil* images or the stack of masks or a *z-projection* of Tool and the Paintbrush Tool to cleanup the one of these, to define the grid for one series. In image. To draw or paint in white or black, use this example a z-projection of the masks is used. the mask images using File>Import>Image Sequence and create the zprojection from Image>Stacks>Z Project...



be used to adjust the grid.

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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 doubleclicking 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.

📓 MRI Grid 📃 🗆 🗙						
File	£	grid n	neasurer	nents	_ □	×
base 23x8.grid	File	e Edit	Font			
board 23x8.grid		Cell	Area	X	Y	
	16	D1	32046	309.078	498.857	
	17	D2	8043	558.377	487.284	
	18	D3	5328	220.279	672.913	
	19	D4	0	0	0	
grid: 8 - 23 -	20	E1	0	0	0	
new sort by:	21	E2	31680	2592.274	481.221	
new sort by:	22	E3	8933	2268.647	669.699	
set label	23	E4	0	0	0	
grid position	24	F1	6071	319.707	1260.152	
remove	25	F2	6142	555.036	1256.248	
edit names v add empty	26	F3	4555	200.882	1457.124	
	27	F4	20952	450.644	1446.610	
	28	G1	30831	2393.882	1264.407	
apply color	29	G2	0	0	0	
	30	G3	26963	2499.528	1464.646	_
Illustration 27:	Q1	C1	9110/	9717.070	1/50 0/9	-

Pressing apply on the MRI Grid tool results table.

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

10/10