GoldenEye How-to:

Ver 1p6

A. Database .txt file
You will need a text file describing your collection. The column are organized as follows: Plate number, row (number or capital letter), column, Blank, ORF name.

	A	B	C	D	E
1	Plate	Row	Col		ORF
2	1	1	1		YAL001C
3	1	1	2		YAL012W
4	1	1	3		YAL020C
5	1	1	4		YAL030W
6	1	1	5		YAL037W
7	1	1	6		YAL044C
8	1	1	7		YAL055W
9	1	1	8		YBR060C
10	1	1	9		YAL069W
11	1	1	10		YBR102C
12	1	1	11		YBR154C
13	1	1	12		YAR035W
14	1	2	1		YAL003W
15	1	2	2		YAL013W
16	1	2	3		YAL022C
17	1	2	4		YAL031C
18	1	2	5		YAL038W
19	1	2	6		YBR029C
20	1	2	7		YAL056W
21	1	2	8		YAL064C-A
22	1	2	9		YAR007C
23	1	2	10		YAR015W

	A	B	C	D	E	F
	Position finale					
	Plate	row	col	record #	ORF	strain
	1	A	1	338	YAL068C	BY4741
	1	A	2	339	YAL067C	BY4741
	1	A	3	340	YAL066W	BY4741
	1	A	4	341	YAL065C	BY4741
	1	A	5	345	YAL062W	BY4741
	1	A	6	346	YAL061W	BY4741
	1	A	7	347	YAL060W	BY4741
	1	A	8	348	YAL059W	BY4741
	1	A	9	349	YAL058W	BY4741
	1	A	10	351	YAL056W	BY4741
	1	A	11	352	YAL055W	BY4741
	1	A	12	354	YAL053W	BY4741
	1	B	1	355	YAL051W	BY4741
	1	B	2	356	YAL049C	BY4741

You can generate this with excel but it needs to be saved as a .txt file

There is a script ConvertDatabase.m that allows you to change the order of ORF entries from an original excel sheet if you for instanced started from a 384 spotted library to a 96 strains per plates with 4 dilutions.

B. Plate Pictures.
The images of the plates have to be saved as XXX.tif files where XXX is the plate number (i.e. plate 13 will be 013.tif). Plates from different conditions (glucose / Galactose or Drug X / Drug Y / Ctrl) have to be saved in separate folders.

When you measure the pictures, make sure you position the plates always in the same position. The software can compensate for some shifts in the image but it will run faster and more reliably if all the images are similar.
All the screen I have analyzed were of same types: 96 strains per plate with 4 dilutions each arranged in a square. These number could be modified but I would have to check some of the code for consistency.

C. Connection to AmiGO database to get protein names

Optionally, you can allow the program to look up directly ORF names in the AmiGO database (http://amigo.geneontology.org). To enable this feature the database toolbox from Matlab is required. You also need to copy the JDBC drivers to enable the connection to the database.

On Mac OS

1. Copy the file GoldenEye_V1a6/Code/mysql-connector-java-5.1.19-bin.jar to /System/Library/Java/Extensions
2. Re-start Matlab

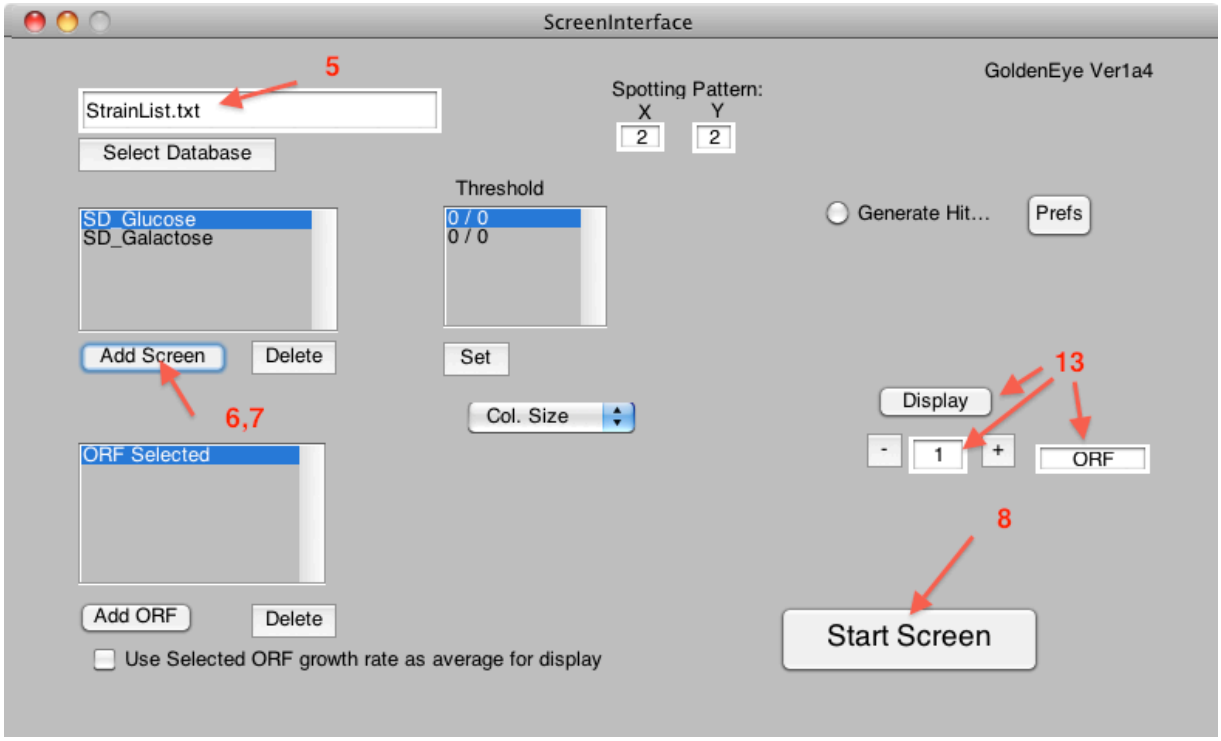
On Windows

1. Edit the classpath.txt file in *matlabroot*\toolbox\local to add the path of the mysql-connector-java-5.1.19-bin.jar file on your disk.

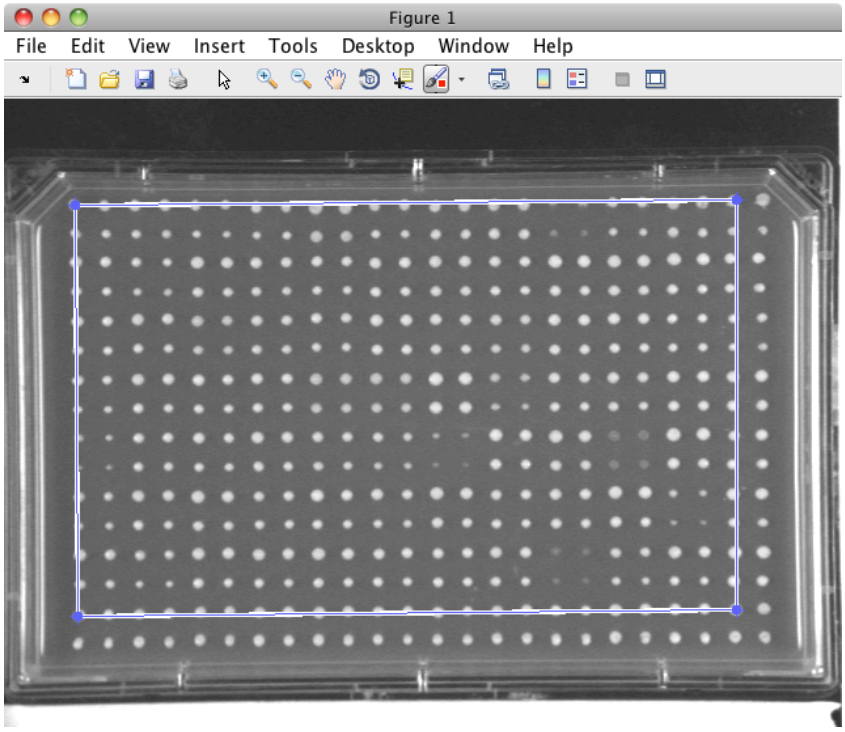
D. Running the program

1. Start Matlab
2. Navigate to the directory (.../GoldenEye_V1a6/Code) where the program is located
3. At the Matlab prompt type: `ScreenInterface`
4. The main window of the program will appear
5. Select Database to get the .txt file containing the database information
6. Add Screen to select the different images you want to analyze. You only need to select one .tif file from a given folder and all the other ones will be selected automatically
7. Repeat 6. if you want to compare different results

8. Press Start Screen

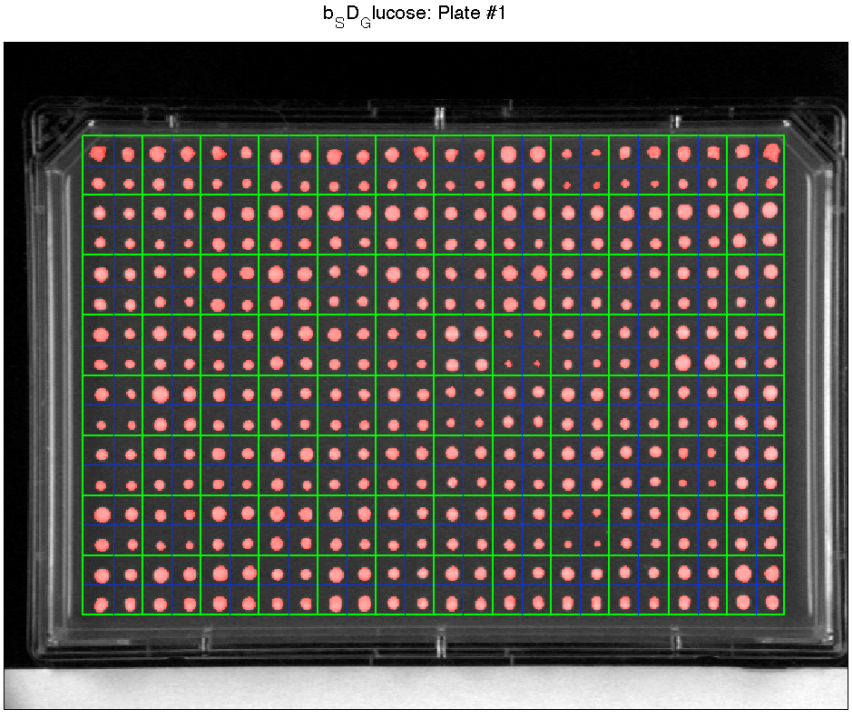


9. Select the upper left colonies from the four corner strain. This will allow to place a grid

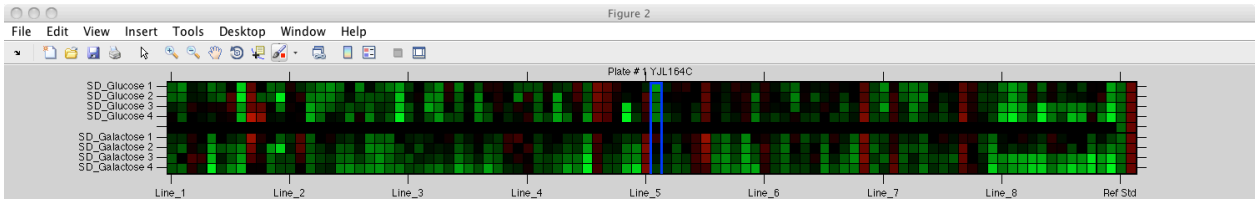


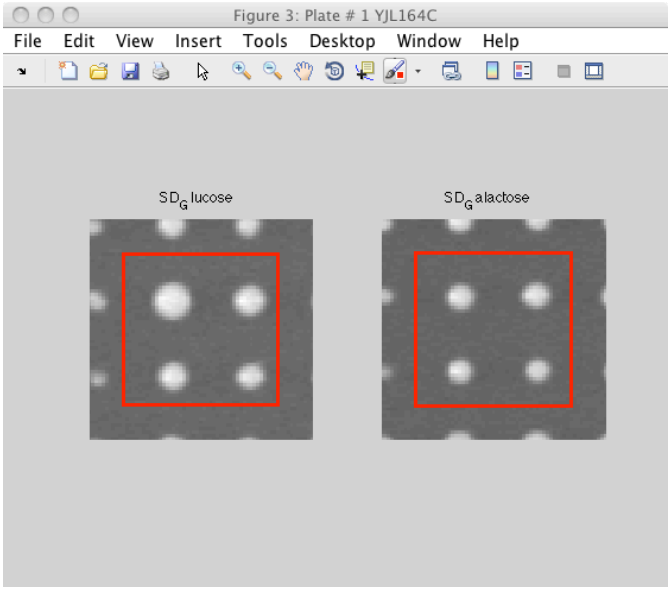
on the picture of the plate to recognize individual colonies.

10. The program will then attempt to place a similar grid automatically on all images of the screen. If it fails, it will ask the user to provide the grid position.



11. Once the grid is placed the program segments individual colonies and measures their sizes. In the image above: the separation between strains is in green, the separation between dilutions in blue and the recognized colonies in red. You can use that image to judge wether the program performed correctly the grid positioning and the spot's segmentation.
12. The size measurements are save in a text file and the grid position in a mat file
13. You can display the results for a given plate by selecting a plate number or ORF name and pressing Display. The programs displays in green the colonies that grow better than the average and in red less than the average. Sometimes you have to be careful with the first and last lines of a plate because there are some edge effect.
14. Selecting a row on this figure will display the ORF name (and the protein name if the connection to AMIGO is enabled) and the dilution spots for that strains in the different conditions chosen.





15. Once a dataset has been quantified, the measurement can be easily reloaded. When pressing the StartScreen button (8) it will ask the user if he wants to use previously saved measurements. This allows to speed up the comparison of different plates since the slow segmentation part doesn't have to be performed again.