

## 1 Background

The GE In Cell analyser produces separate tiff files for every field and channel captured by the instrument. In High Content Analysis users often what to pick example fields from a well which reflects a certain treatment condition of the experiment. Furthermore, a user may want to produce examples images from control and treated conditions to show a visual example of the dynamic range of a particular assay. Therefore, it is important that any pixel mapping used by image analysis software is consistently applied across all the example images.

The software produced by GE to deal with these challenges back in 2011 was limited. However, there is an open source software available called Image J which has very powerful image handling functions. It has been possible for us to write some extensions to Image J which allow users to load well images from an InCell Experiment and create a 3 dimensional hyperstack that allows one to quickly apply a consistent pixel map to all the images within the stack. Image J can be downloaded from <http://rsbweb.nih.gov/ij/>.

This document is a brief instruction manual that will help users understand the extensions we have created in Image J to allow it to work with In Cell images.

## 2 Installation

For installing Image J, please follow the instructions on their website <http://rsbweb.nih.gov/ij/>.

Once you have installed image J, you can the install our macro extension as follows:

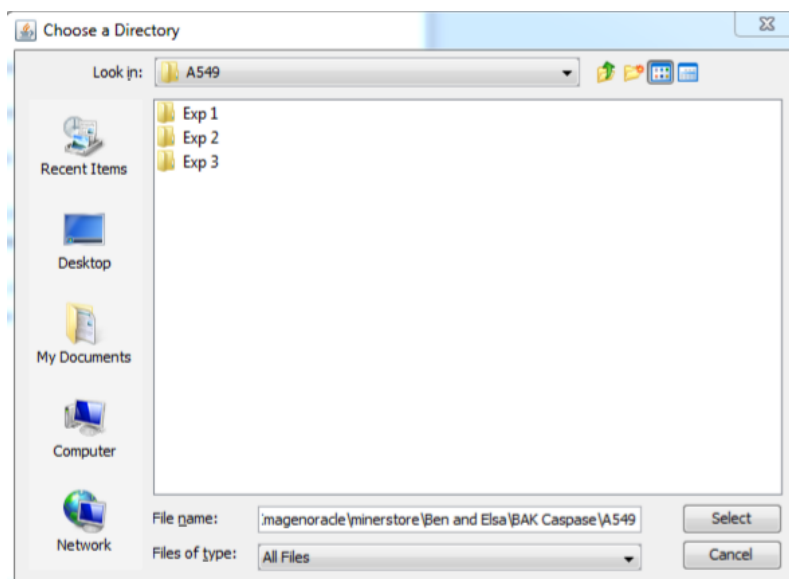
1. Go to C:\program files\ImageJ\macros or if you have a 64 bit operating system it will be C:\program files (x86)\ImageJ\macros.
2. Rename the file StartupMacros.txt to StartupMacros Old.txt
3. Copy the StartupMacros.txt downloaded from our website into the folder path above.

Now restart Image J. You will not notice any difference to Image J but our text file provides several useful keyboard shortcuts for working with In Cell Files and these shall now be described.

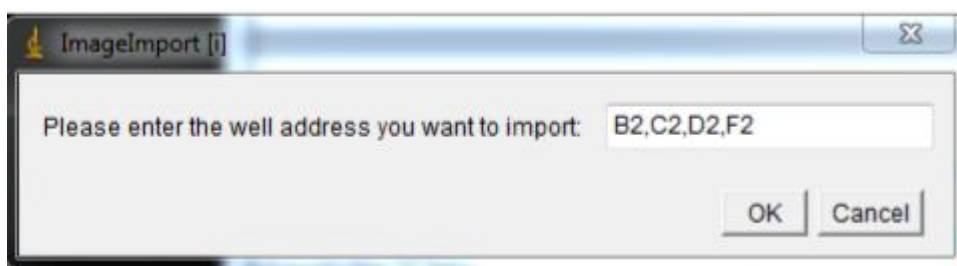
## 3 Operation

### 3.1 The I(i) Key

With the Image J menu selected push the “I” key.



A Windows “choose a directory” prompt will appear which allows you to navigate to the directory that contains In Cell images. Once you have selected the directory push select. A small dialogue box will appear that allows you to enter the wells you want to import



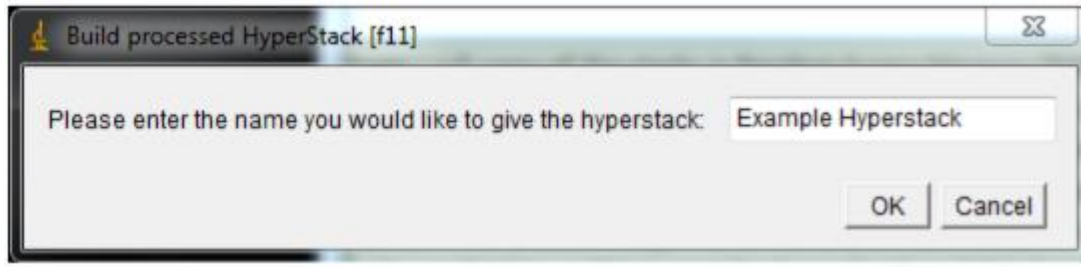
You can enter a multiple set of wells by separating each one with a comma (,) as in the above example. Another example: if I have my positive controls in wells F2 to F4 and my negative controls in wells A2 to A4 and I also want to open 2 compound treatment examples (say wells B2 to B4 and D2 to D4) I could enter in the dialogue box “A2,F2,B2,D2”. This will create 4 separate initial stacked images containing all the images for all the channels of wells A2, F2, B2 and D2.

**Note:** In Cell images are large and Image J will open all the stacks in Random Access Memory (RAM) so if you do not change the default RAM assigned to Image J you will probably get an out of memory error. You can fix this by assigning more RAM to Image J (see installation section above). However, if you are running a 32 bit operating system then sadly the largest amount of memory you can assign Image J is approximately 1700MB. If you are running a 64 bit system then you can assign more memory but be careful not assign more than about 75% of real RAM to the application as this may make it run slowly and also it might become unstable. For more details see <http://imagej.nih.gov/ij/docs/menus/edit.html#memory>

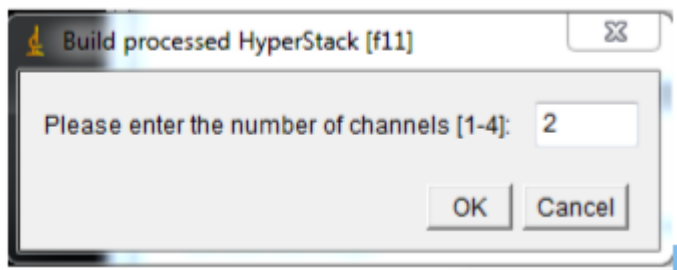
If you have captured more than one field per well then it is not normally necessary to open up multiple wells of the same treatment as you already have multiple image examples from the single well and the more wells you open the larger the final hyperstack.

### 3.2 The F11 Key

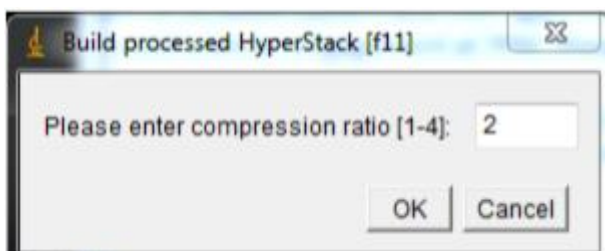
Once you have your stacks open, push the F11 key. You will then be shown a few dialogue boxes which allow you to complete your hyperstack. The first dialogue box allows you to give the hyperstack a new name:



The next dialogue asks you to enter the number of channels used in your experiment (normally between 1 and 4).



The final dialogue box asks you for the number of channels in the experiment and the third asks you for a resizing ratio:

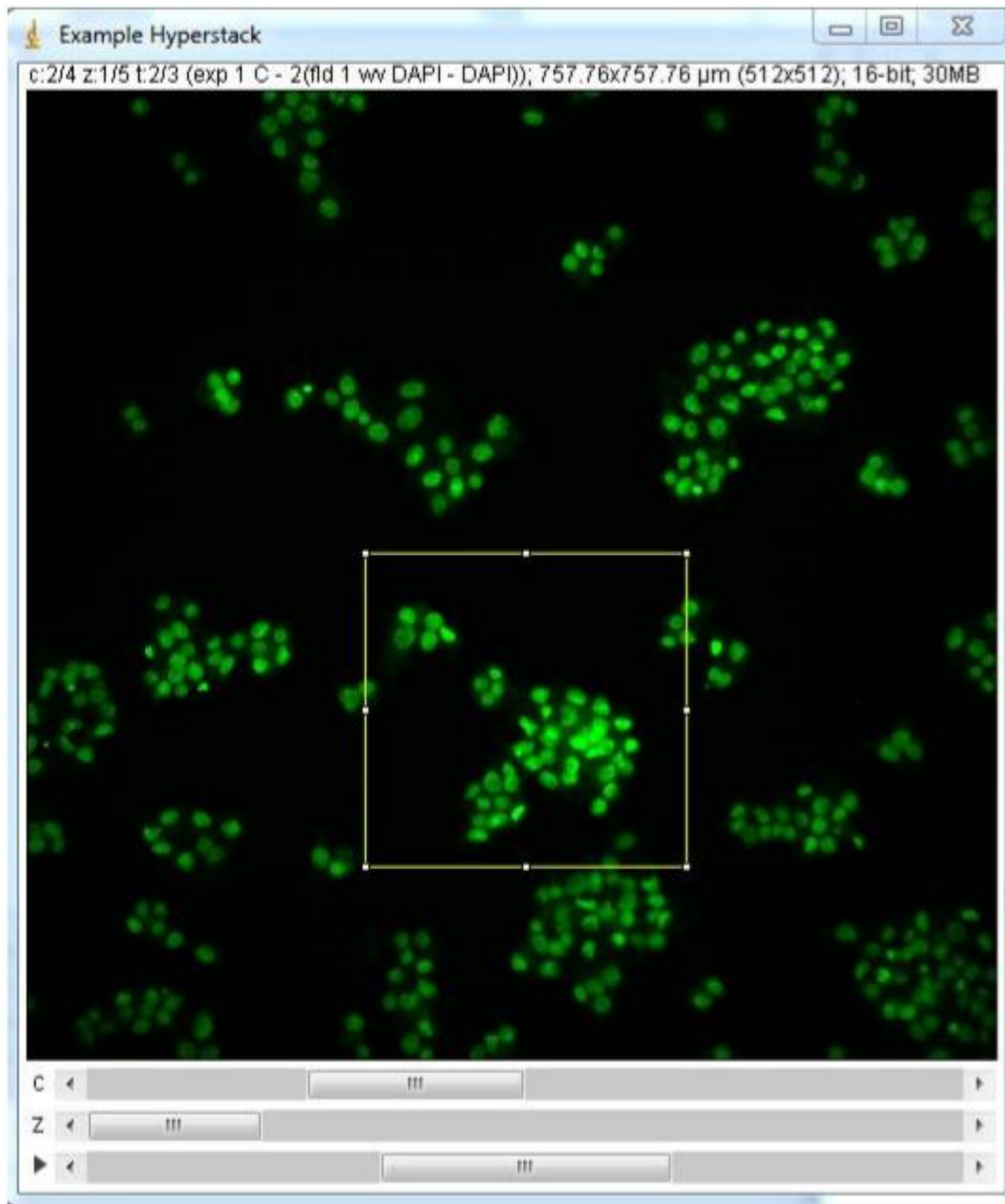


The ratio entered should be from 1 – 4 and the meaning is as follows:

- 1: No resizing applied images saved in original size. It should be noted that the In Cell uses a large field camera so the images are quite large.
- 2: The images are resized to half the original size.
- 3: The images are resized to a third the original size.

If you enter a number less than 1 then the program, if possible, takes the reciprocal of the number and converts it to a number between 1 to 4. So a ratio of 0.25 is the same as 4. If you enter a number outside this range it is ignored and the image is not compressed.

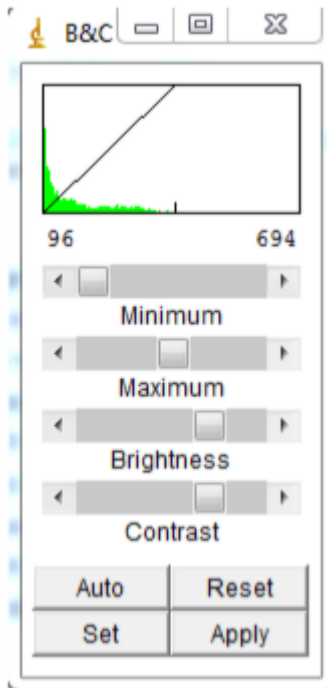
Once these dialogues have been entered, the 3D hyperstack will then be created. An example of a typical stack created by our addin is shown below.



Each of the dimensions can be accessed using one of the three sliders. The top slider will allow you to view each channel (in the example above we have 4 channels. If you have a two-channel experiment then this slider will only have 2 positions – in general the number of positions of the slider is determined by the number of channels. The middle slider allows you to step through the number of fields that make up the well. The number of positions in this slider is determined by the number of fields you originally selected for capture in your In Cell Experiment. Finally, the bottom slider steps through the wells you imported with Import function above. You will see that the well identity has been placed in the image information block so that you can easily track which wells you are currently examining.

The main aim of creating the hyperstack is to allow you to prepare example images of an HCA experiment. Therefore, once the hyperstack is created, the next step is to use the inbuilt Image J functions to map the pixel information of the image so that the image details are maximised for human vision. Image J provides a simple Contrast brightness tool which is switched on and selected

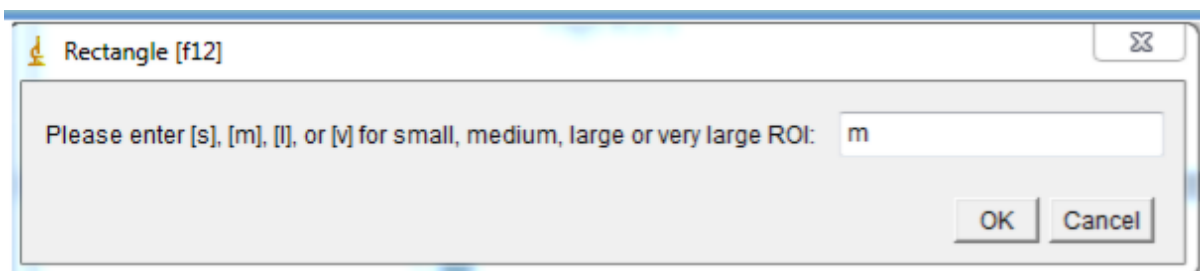
by our function F11. One can use this on each channel to alter the image so that it best displays the pertinent information of the HCA experiment. The real power of the hyperstack is that whatever changes you make to one image is applied to all images simultaneously so it is possible to switch from the positive and negative control images and adjust the brightness and contrast (background threshold and gradient map from black to white) until both images reflect the underlying HCA data derived from them.



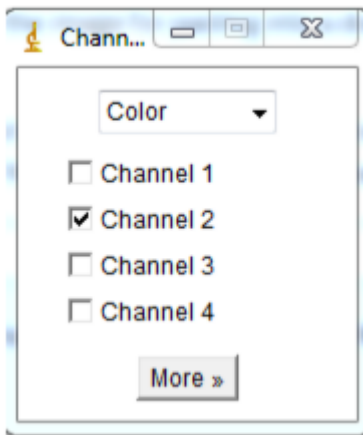
It is then a simple matter to use the middle slider bar to select the best field and then create Regions of Interest (ROIs) to copy a part of the image for pasting into a document report or Powerpoint presentation.

### 3.3 The F12 Key

In order to copy a region of interest you can use the F12 key. This will present you with a dialogue that allows you to create 4 sizes of ROI: small (s), medium (m), large (l) and very large (v).



Once you have created the ROI you can then use the mouse to position it where you want it (see above hyperstack example) and then use the F1 key to copy the image to the clipboard for subsequent pasting into another document. The other important Image J internal function is the channel manager which is also opened by our hyperstack build function. With this menu you can switch between channels (you can also do this with the first slide bar in the hyperstack) and more importantly you can toggle between individual channels and a colour composite.



Our program automatically assigns the channel 1 to blue, channel 2 to green, channel 3 to red and channel 4 to magenta. You can change these default settings using the channel manager if so desired. Once you have the appropriate colour composite use the F12 key to create appropriate ROIs and the F1 key to copy the information to another program.

There is also another set of useful shortcut keys which are enabled by our addin and described below.

### 3.4 The R Key

Brings up a dialogue box so you can rename an image or hyperstack.

### 3.5 The F2 Key

Allows multiple stacks to be combined into a single stack (short cut key for the menu command Image/Stacks/Tools/Concatenate).

### 3.6 The F3 Key

Combines a stack into a hyperstack (short cut key for the menu command Image/Hyperstacks/Stack to Hyperstack).

## 4 Conclusion

I hope if you are a company or academic organisation that is using a GE Incell Analyser you might find this little program useful in helping you deal with GE images. If you do find the program useful why not tell your colleagues about it and point them to the website ([www.bensonium.com](http://www.bensonium.com)) where they can download it. You might also like to check out my other programs which are also available on the website [here](#).