Counting objects by template matching (simple "machine vision") in ImageJ

(by: Vytas Bindokas, University of Chicago Light Microscopy Core Facility; Oct 2008)
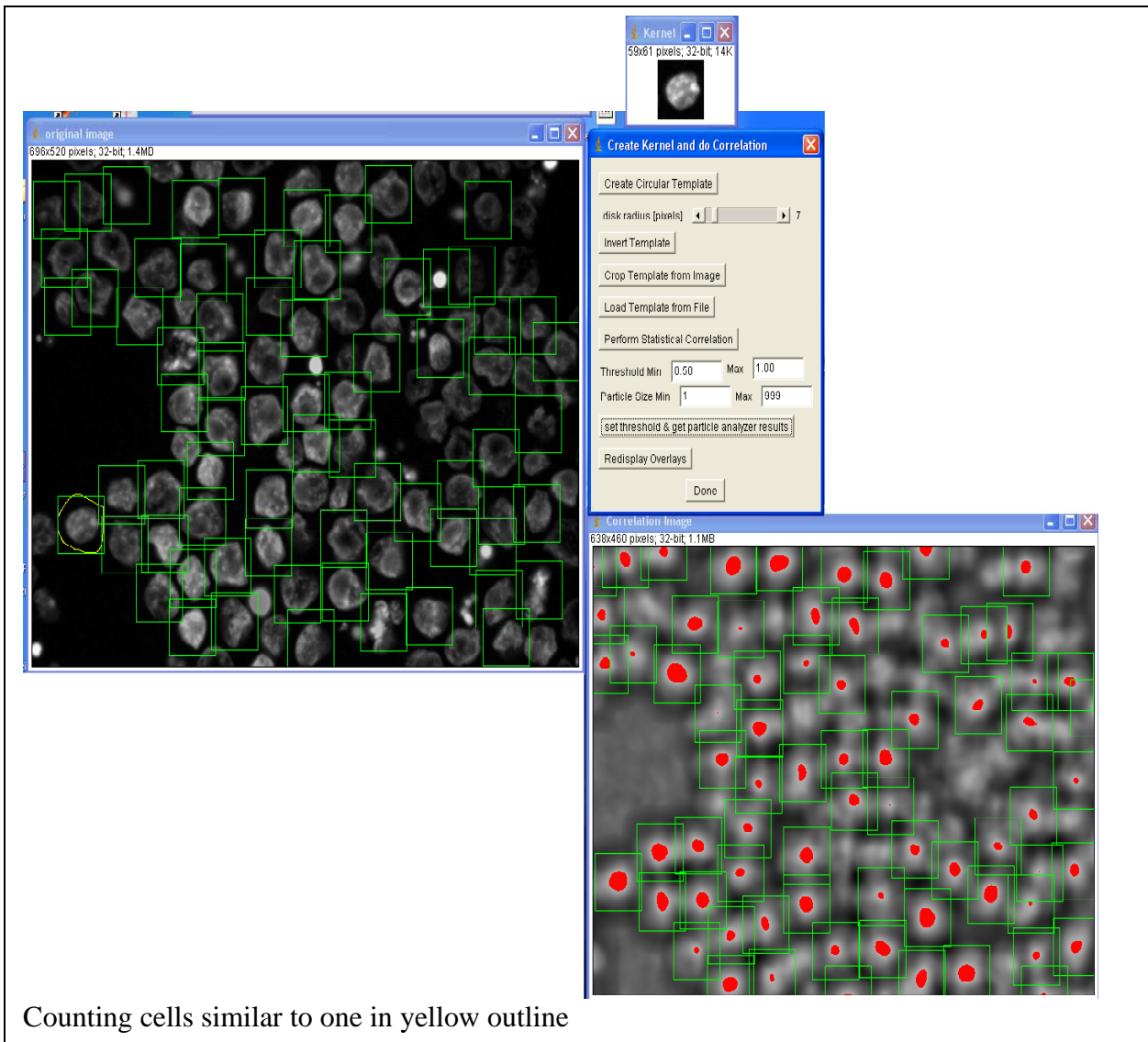
You need to download the Create_Template.jar plugin into your IJ plugins folder and open an image. It appears the plugin accepts all image types, but will silently convert it to a single 32-bit image (stacks become the current slice, RGB becomes 32bit gray, etc); best to work on copies vs originals…
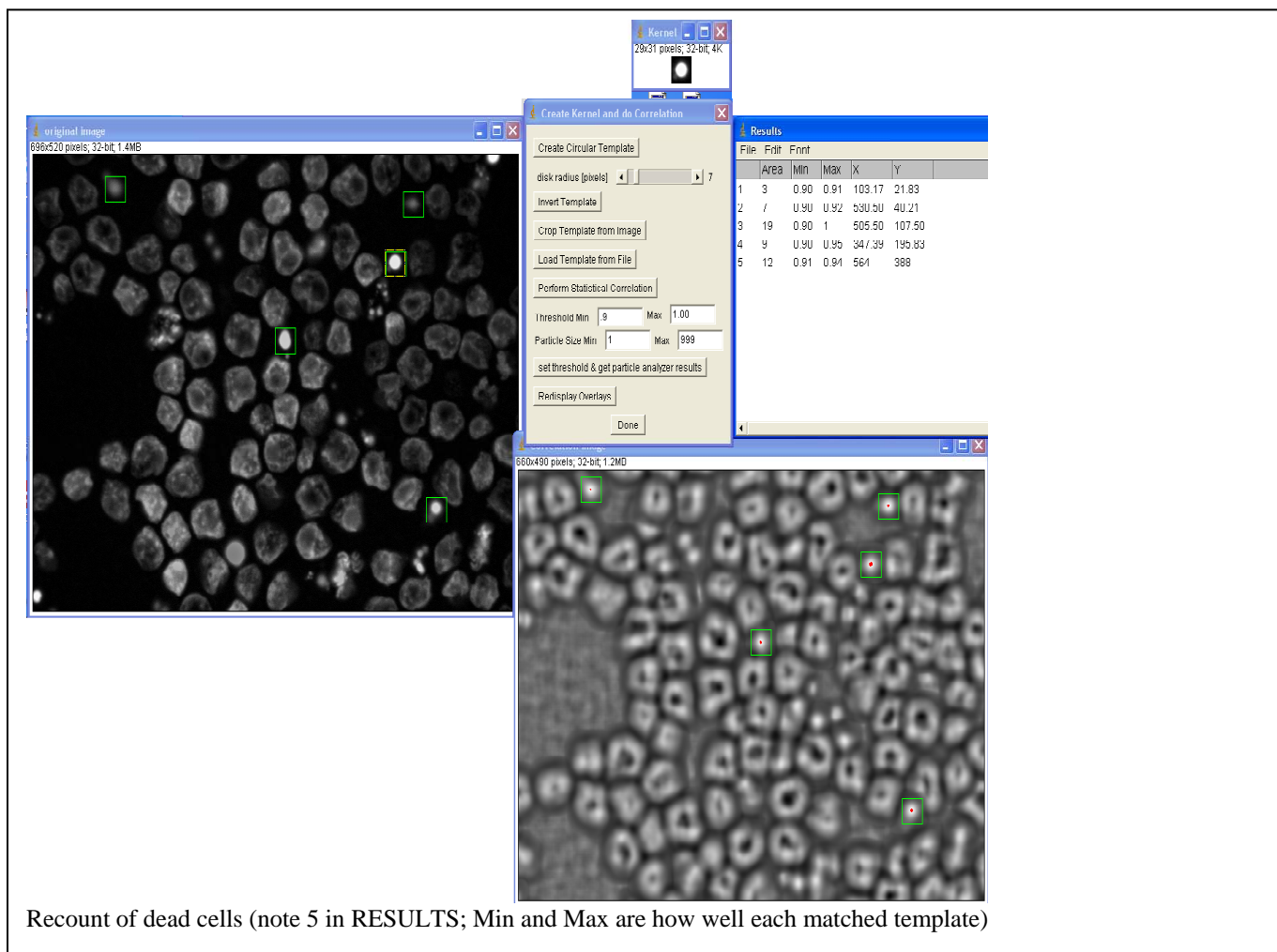
1) You can use the built in circular template generator (circle radius slider and invert buttons) to count round objects, or draw a region of interest and use the "Crop Template from Image" button to copy the structure of interest. The template image can be a separate image file that you can load in via the button provided.

2) Press the "Perform Statistical Correlation" button and wait until it creates a pixel map of how well the template matches the pixels in the entire image. (The threshold and size ranges are used in the next step, not the correlation mapping)

3) The threshold range and sizes will feed into the built in Particle Analyzer routine to produce object measurements/counts. The 0.50 means that the image is at least 50% similar to the template. The higher the Min Threshold, the tighter the match to the template. The routine outputs a results table and overlays green boxes over the objects that were matched. You can set different thresholds and recount, or you can create a new template reference and count other structures, if desired.

4) Press the "Done" button to quit the routine. It will ONLY run on the image that was current when launched, so you need to relaunch the plugin on each new input image.

Counting cells similar to one in yellow outline

Create Kernel and do Correlation

Create Circular Template

disk radius [pixels] 7

Invert Template

Crop Template from Image

Load Template from File

Perform Statistical Correlation

Threshold Min 9   Max 1.00

Particle Size Min 1   Max 999

set threshold & get particle analyzer results

Redisplay Overlays

Done

Results

File Edit Font

	Area	Min	Max	X	Y
1	3	0.90	0.91	103.17	21.83
2	7	0.90	0.92	530.50	40.21
3	19	0.90	1	505.50	107.50
4	9	0.90	0.95	347.39	195.83
5	12	0.91	0.94	564	388

original image
696x520 pixels; 32-bit; 1.4MB

Correlation Image
660x490 pixels; 32-bit; 1.2MB

Recount of dead cells (note 5 in RESULTS; Min and Max are how well each matched template)

original image
738x299 pixels; 32-bit; 862K

You·need·to·download·the·Create_Template.jar·plugin·into·your·IJ·plugins·folder·and·open·an·image.·It·appears· the·plugin·accepts·all·image·types,·but·will·silently·convert·it·to·a·single·32-bit·image·(stacks·become·the·current· slice,·RGB·becomes·32bit·gray,·etc);·best·to·work·on·copies·vs·originals…¶

→ 1)·You·can·use·the·built-in·circular·template·generator·(circle·radius·slider·and·invert·buttons)·to·count· round·objects,·or·draw·a·region·of·interest·and·use·the·"Crop·Template·from·Image"·button·to·copy·the·structure· of·interest.·The·template·image·can·be·a·separate·image·file·that·you·can·load·in·via·the·button·provided.¶

→ 2)·Press·the·"Perform·Statistical·Correlation"·button·and·wait·until·it·creates·a·pixel·map·of·how·well·the· template·matches·the·pixels·in·the·entire·image.·(The·threshold·and·size·ranges·are·used·in·the·next·step,·not·the· correlation·mapping)¶

→ 3)·The·threshold·range·and·sizes·will·feed·into·the·built-in·Particle·Analyzer·routine·to·produce·object· measurements/counts.·The·0.50·means·that·the·image·is·at·least·50%·similar·to·the·template.·The·higher·the·Min· Threshold,·the·tighter·the·match·to·the·template.·The·routine·outputs·a·results·table·and·overlays·green·boxes· over·the·objects·that·were·matched.·You·can·set·different·thresholds·and·recount,·or·you·can·create·a·new· template·reference·and·count·other·structures,·if·desired.¶

→ 4)·Press·the·"Done"·button·to·quit·the·routine.·It·will·ONLY·run·on·the·image·that·was·current·when· launched,·so·you·need·to·relaunch·the·plugin·on·each·new·input·image.·¶

Create Kernel and do Correlation

Create Circular Template

disk radius [pixels] 7

Invert Template

Crop Template from Image

Load Template from File

Perform Statistical Correlation

Threshold Min 0.8   Max 1.00

Particle Size Min 1   Max 999

set threshold & get particle analyzer results

Redisplay Overlays

Done

Correlation Image
716x287 pixels; 32-bit; 803K

.gif

Results

File Edit Font

	Area	Min	Max	X	Y
2	1	0.81	0.81	644.50	81.50
3	1	0.81	0.81	544.50	99.50
4	1	0.81	0.81	704.50	118.50
5	1	0.81	0.81	121.50	136.50
6	1	0.81	0.81	203.50	136.50
7	1	0.81	0.81	587.50	136.50
8	1	0.81	0.81	701.50	136.50